List of groups at Karolinska Institutet which express interest to host a CSC funded scholar from 2020

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The project descriptions follow the index.
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Group/ Supervisor name	Given Name	Level of recruitment	Department at KI	Project title
Andersson	Emma	Visiting doctoral students/Post-docs/Visiting researchers	Dept of Cell and Molecular Biology	Modelling cholestasis in vivo and in vitro
Antovic	Jovan	Postdoc	Dept of Molecular Medicine and Surgery	Cardiovascular risk and the role of microparticles in aging hemophilia A patients - New challenges with new treatments
Catrina	Sergiu-Bogda	postdoc/visiting researcher	Dept of Molecular Medicine and Surgery	Dissecting the mechanisms behind the inadequate hypoxia signalling in diabetes
Chang	Zheng	visiting phd student/ postdoc/visiting researcher	Dept of Medical Epidemiology and Biostatistics	Medication for mental disorders and risk of somatic health problems
Coquet	Jonathan	doctoral student	Dept of Microbiology, Tumor and Cell Biology	Exploring the interaction between the environment and immune system
Genander	Maria	doctoral student	Dept of Cell and Molecular Biology	Screening for self-renewal in esophageal progenitor subpopulations
Girnita	Leonard	doctoral student	Dept of Oncology-Pathology	Insulin/Insulin-like growth factors biased signaling: from autoimmnune diseaseas to cancer treatment
Götherström	Cecilia	postdoc	Dept of Clinical Science, Intervention and Technology	Biological signature and efficacy of mesenchymal stem cell-based therapies
Hassan	Moustapha	posdoc/visiting researcher	Dept of Laboratory Medicine	Personalized medicine and multimodal Imaging in Cancer

Group/ Supervisor name	Given Name	Level of recruitment	Department at KI	Project title
Holmberg	Johan	doctoral student	Dept of Cell and Molecular Biology	RNA fusion transcripts in neurodegenerative disease
Holmdahl	Rikard	postdoc/visiting researcher	Dept of Medical Biochemistry and Biophysics	Autoantibodies in rheumatoid arthritis with focus on cartilage oligomeric matrix protein
Holmdahl	Rikard	postdoc/visiting researcher	Dept of Medical Biochemistry and Biophysics	Oxidative regulation of autoreactive B cells
Jagodic	Maja	postdoc	Dept of Clinical Neuroscience	Utilizing cell-free DNA methylome as a biomarker for early detection of brain damage in Multiple Sclerosis patients
Kutter	Claudia	visiting phd student/postdoc/visiting researcher	Dept of Microbiology, Tumor and Cell Biology	Gene regulation and transcriptional control in liver metabolism
Lagergren	Jesper	doctoral student	Dept of Molecular Medicine and Surgery	Prevention of oesophageal adenocarcinoma
Lagergren	Jesper	postdoc	Dept of Molecular Medicine and Surgery	Prevention of gastric cancer
Li	Nailin	postdoc/visiting researcher	Dept of Medicine, Solna	Platelet-regulated inflammatory mechanisms in atherosclerosis
Marklund	Ulrika	doctoral student/postdoc	Dept of Medical Biochemistry and Biophysics	Function and differentiation of newly discovered cell types in the enteric nervous system
Masucci	Maria Grazia	visiting researcher	Dept of Cell and Molecular Biology	Role of bacterial co-infection in the immune regulation of Epstein-Barr virus oncogenesis
Matussek	Andreas	postdoc/visiting researcher	Dept of Laboratory Medicine	Pathogenesis study of haemorrhagic uremic syndrome causing Shiga toxin-producing Escherichia coli
McInerney	Gerald	doctoral student	Dept of Microbiology, Tumor and Cell Biology	Application of Cellular Thermal Shift Assay for the study of responses to virus infection
Nilsson	Ida	Doctoral student/visiting Phd/	Dept of Molecular Medicine and Surgery	The Neurobiology of anorexia nervosa
Okret	Sam	postdoc	Dept of Biosciences and Nutrition	Endocrine regulation of lymphomas
Pan-Hammars	Qiang	doctoral student/visiting Phd	Dept of Biosciences and Nutrition	Regulation of immunoglobulin class switch recombination in human B cells

Group/ Supervisor name	Given Name	Level of recruitment	Department at KI	Project title
Pan-Hammars	Qiang	postdoc/visiting researcher	Dept of Biosciences and Nutrition	Discovery of therapeutic targets in B cell lymphoma /Regulation of immunoglobulin class switch recombination in human B cells
Parameswarar	Lalitkumar	doctoral student	Dept of Women's and Children's Health	Autologous transplantation of endometrial stem cells and effect of niche factors in endometrial regeneration
Pasetto	Anna	postdoc/visiting researcher	Dept of Laboratory Medicine	Identification of the neo-antigenome in Hepatocellular Carcinoma to guide T cells against relevant targets for successful immunotherapy
Pelechano Gar	Vicente Jose	doctoral student/visiting Phd/postdoc	Dept of Microbiology, Tumor and Cell Biology	Transcriptional complexity and RNA metabolism as a readout for personalised medicine
Pivarcsi	Andor	postdoc	Dept of Medicine, Solna	Investigation of the role of non-coding RNAs in epidermal differentiation and cancer
Qian	Hong	doctoral student	Dept of Medicine Huddinge	The contribution of the bone marrow niche to the development and treatment response of myeloid leukemia
Rothfuchs	Antonio	postdoc	Dept of Microbiology, Tumor and Cell Biology	Defining the transport of BCG through lymphatics in priming T cells
Rudd	Sean	postdoc	Dept of Oncology-Pathology	Exploiting cancer cell metabolism to enhance current cancer therapies
Schlisio	Susanne	doctoral student	Dept of Microbiology, Tumor and Cell Biology	Exploring tumor heterogeneity in sympatho-adrenal malignancies to identify new therapeutic targets
Sonkoly	Enikö	postdoc	Dept of Medicine, Solna	Investigation of non-coding RNAs in inflammatory skin diseases
Sällberg	Matti	postdoc	Dept of Laboratory Medicine	Curing hepatitis B
Tapia Paez	Isabel	doctoral student	Dept of Medicine, Solna	Genetic and functional studies of skin disorders
Teixeira	Ana	Postdoc	Dept of Medical Biochemistry and Biophysics	DNA nanotechnology to investigate the roles of the spatial organisation of membrane receptors in cell signalling
Treuter	Eckardt	Postdoc	Dept of Biosciences and Nutrition	Role of macrophage epigenome alterations in linking type 2-diabetes and atherosclerosis

Group/ Supervisor name	Given Name	Level of recruitment	Department at KI	Project title
Xu Landén	Ning	doctoral student/postdoc/visiting phd/visiting researcher	Dept of Medicine, Solna	Investigation the role of regulatory RNAs in human skin wound healing
Zaphiropoulos	Peter	Postdoc	Dept of Biosciences and Nutrition	Circular RNAs in cancer development

Interested in recruiting a Postdoc, Visiting researcher or Visiting doctoral student

Project title
Modelling cholestasis in vivo and in vitro

Supervisor
Emma R. Andersson, Assistant Professor, Dept of Cell and Molecular Biology
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Type of recruitment and qualifications of applicant
- Visiting doctoral students (6 month - 1 year),
- Post-docs (6 month - 2 years), and
- Visiting researchers (6 month - 1 year)

Background
Cholestatic liver disease arises when either the biliary system or hepatocytes do not function. This can result in chronic liver disease, and/or ultimately be a risk factor for developing liver cancer. Many genes have been identified that lead to cholestatic disease in humans. Our lab works on Alagille syndrome, a disease caused by mutations in the Notch ligand JAGGED1 or in the receptor NOTCH2. In children with Alagille syndrome, bile ducts fail to develop adequately, leading to liver disease and sometimes organ transplant. We aim to both understand liver development and develop therapies for Alagille syndrome, using mouse models, patient samples, and 3D organoid culture.

Research project description
This project aims to characterise mouse models of cholestatic disease, and compare phenotypes with experiments in 3D organoids derived from human patients and mouse models. The researcher, post doc or student would perform live imaging experiments, single cell RNA sequencing, western blot and immunofluorescence with confocal imaging. The organoids will also be used to test therapeutic interventions to rescue pathological phenotypes.

Research group
The Andersson group at KI consists of 5 PhD students, 2 post docs, 1 senior scientist and 1 technician. The group covers various aspects of developmental biology including liver biology, vascular biology, and molecular biology. We aim to perform research that will ultimately be of use to patients.

Key words Liver, Organoids, Notch, bile ducts, Alagille syndrome

Karolinska Institutet

Interested in recruiting a Postdoc

Project title

CARDIOVASCULAR RISK AND THE ROLE OF MICROPARTICLES IN AGING HEMOPHILIAA PATIENTS – NEW CHALLENGES WITH NEW TREATMENTS

Supervisor

Jovan Antonio, Associate professor,
Department of Molecular Medicine and Surgery
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Type of recruitment and qualifications of applicant

Postdoc (6 - 24 months)

Educational background: biomedicine/life sciences/biotechnology/molecular biology /biology/ pathology/hemostasis

Skills: essential: fluorescent microscopy, experience working with mice including application of injections and surgery. Desirable: histopathology, flow-cytometry, protein chemistry, western-blot, intravital microscopy, ELISA, basic biostatistics, at least one first author publication in the peer-reviewed journal, ability to independently carry out experimental research projects.

Languages: fluency in written and oral English.

Other: communicative with good intrapersonal communication skills, ability to work in the team.

Background

Elderly patients with hemophilia A may be under the risk of cardiovascular diseases while microparticles may have role in the pathogenesis of atherothrombosis. Some, although limited knowledge about use of standard factor concentrates in the ageing patients exists, but effects of novel therapies including monoclonal antibody–emicizumab, in the older patients with hemophilia A are almost completely unknown. Considering that emicizumab practically transforms severe hemophilia to mild/normal phenotype it is of great importance to perform pre-clinical translational research based on in-vitro and animal study of the effects of microparticles and emicizumab on global hemostasis.

Research project description

Adequate treatment enables patients with hemophilia A to have normal lives and gives them a possibility to reach normal life expectancy including the risk of suffering from cardiovascular diseases. The risks of atherosclerosis and atherothrombosis have been described to increase with elevated microparticles. Additionally, treatment of hemophila A has been changed a lot during recent years due to the novel factor concentrates. However, a real paradigm shift has been made with the introduction of specific monoclonal antibody - emicizumab which

was first approved for the treatment of the patients with hemophilia with inhibitors but now is also approved for the treatment of hemophilia without inhibitors. It has been shown that a combination of emicizumab with bypassing agents (aPCC) may be prothrombotic in some patients. Increased thrombin generation has been shown after addition of both emicizumab and aPCC in-vitro. Our group has also recently shown that combination of aPCC and emicizumab generated clots with the highest density formed from very thin fibers and small intrinsic pores. In the light of such findings, issue of potential cardiovascular diseases (CVD) in older patients treated with emicizumab (which practically would have hemostatic level as in mild hemophilia/normal individuals) may be of particular interest.
Using a translational approach, we would like to perform a study in both in-vitro and an animal model. The focal goal of the study is to investigate if microparticles (MPs) play a role in the development of atherothrombotic complications in ageing patients with hemophilia A and if MPs can be used as atherothrombotic biomarkers.

Specific goals
We will investigate ho w in vitro addition of MPs together with emicizumab to the hemophilic plasma alters global hemostasis.We will study how the injection of MPs together with emicizumab to the hemophilia mice improves their bleeding phenotype and if it is potentially prothrombotic. Additionally, we will compare those effects to those induced by standard FVIII concentrate. We will visualize MPs in fibrin clot preparations and investigate how they may regulate fibrin clot structure and spatial organization. We will investigate the amount and characteristics of MPs in murine models predisposed to both hemophilia and thrombosis. We will study how the emicizumab change ApoE/hemophilia A mice bleeding phenotype and if it could potentially be prothrombotic and if there is any difference in comparison to standard treatment with factor VIII concentrate.

We propose a project to study the potential prothrombotic effects of microparticles in addition to standard factor VIII concentrates and in combination with emicizumab. Together with the clinical study which is on-going we would like to investigate if microparticles may be used as an atherothrombotic biomarker in hemophilia A.

Appropriate therapy based on prophylactic treatment with factor concentrates enables patients with hemophilia A to live normal lives and gives them a possibility to reach normal life expectancy suffering from CVDs. It is therefore of great importance to investigate whether MPs contribute to the CVD in ageing hemophilia A patients. In that respect, MPs may be used as biomarkers for the diagnosis of atherothrombotic changes in hemophilia A, indicating the potential need for a decrease of treatment intensity in older patients, more prone to atherothrombosis.

Introduction of new treatment - monoclonal antibody emicizumab practically transform severe hemophilia to mild/normal phenotype and therefore may be of particular importance in this context. Since it is very novel treatment it will take time to collect experience in older patients particularly those with CVD. Therefore, a translational approach based on in-vitro and experimental animal studies is of highest priority currently.

Research group

PI is associated professor and senior consultant in coagulation. One basic research post-doc, two clinical post-docs, one basic research PhD student (CSC, one clinical PhD student and one part-time biomedical scientist belong to the group.

All equipment for global coagulation assays and flow cytometry are available in the laboratory. State-of-the art confocal and intravital microscopy belong to the group also. PCR, protein chemistry and western blot equipment are also available in the group premises.

Key words

hemophilia A, microparticles, atherosclerosis, knockout mice, emicizumab, global hemostasis, flow-cytometry, confocal microscopy, electron microscopy, intravital microscopy

Interested in recruiting a Postdoc and a Visiting researcher

Project title

Dissecting the mechanisms behind the inadequate hypoxia signalling in diabetes.

Supervisor

Sergiu-Bogdan Catrina, Associate professor
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Type of recruitment and qualifications of applicant

- Postdoc (24 months)
- Visiting researcher (12 months)

The applicant must be talented, well-organized, highly motivated and enthusiastic for science, have good communication skills and the ability to interact in an international and dynamic team. The applicant should have innovative thinking and be eager to solve problems. A documented practical experience in animal studies, cell culture, molecular biology and biochemistry is meritorious. Good knowledge in written and spoken English is a requirement.

Background

Diabetes is reaching epidemic proportions and is predicted to affect 300 million people worldwide by 2025. Chronic complications of diabetes represent the main concern for modern diabetes therapy, and it has become a priority to further characterise the pathophysiological mechanisms of these complications to ensure the development of novel rational therapeutic strategies.

Although the prolonged exposure of tissues to hyperglycaemia is the primary causative factor for chronic diabetes complications, it has recently become increasingly evident that hypoxia also plays an important role in all diabetes complications. Compelling evidence has accumulated over the last decade indicating that the cellular reaction to hypoxia is impaired in diabetes and is a central pathogenic mechanism for diabetes complications. It is represented by a complex repression of hypoxia-inducible factor (HIF), which is the main regulator of the adaptive response to hypoxia. The exact mechanisms by which hyperglycaemia has a repressing effect on HIF are still not completely unravelled.

Research project description

The project proposes to investigate the pathways that are relevant for this repression in order to identify new potential therapeutic targets for complications of diabetes. The work will involve investigation in vitro but also confirmation in vivo in animal models for diabetes complications. Unique patient material that can generate hypothesis to be confirmed experimentally is available. If successful, the work will provide the chance to tailor new therapy for complications of diabetes.

Research group
https://ki.se/en/mmk/growth-and-metabolism

Key words
Diabetes Complications Treatment Hypoxia Biomarkers
Mechanisms of disease

Interested in recruiting a Visiting doctoral student, a Postdoc, a Visiting researcher

Project title

Medication for mental disorders and risk of somatic health problems

Supervisor

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Type of recruitment and qualifications of applicant

- Visiting doctoral student (6-12 months)
- Postdoc (12-24 months)
- Visiting researcher (6-12 months)

We look for a highly motivated student/researcher with a background in epidemiology, biostatistics, public health, psychiatry or other relevant field. Experience with statistical software (e.g., SAS, STATA, or R) or programming languages is preferred.

Background

Management of the rising prevalence of chronic disorders is the main challenge facing health-care systems worldwide. Mental disorders often coexist with chronic somatic conditions, leading to mental-somatic multimorbidity. Despite the growing evidence that multimorbidity was associated with greater symptom burden and functional impairment, poorer quality of life, and excess mortality, very few studies have investigated the how preventable these outcomes are and how to intervene to minimise them.

Research project description

The aim of the project is to provide real-world evidence regarding somatic risks and benefits associated with pharmacological treatment for mental disorders. We will investigate the short-term and long-term effects of psychotropic medications (e.g., antidepressants, ADHD medication, and antipsychotics) on somatic health problems (e.g., metabolic, cardiovascular, and chronic inflammatory diseases), and risk of adverse events from polypharmacy. Data are available through linkage of national registers in Sweden, which provide longitudinal information on disease diagnoses, drug prescriptions, medical and functional outcomes.

Research group

Our research group is interested in understanding the causes and consequences of psychiatric disorders, as well as the risks and benefits associated with pharmacological treatments for these disorders. We have an interdisciplinary research team from various backgrounds, including epidemiology, biostatistics,

sociology, and psychiatry. We also collaborate with a number of Swedish and international research groups.

Key words
Mental disorders, psychiatry, pharmacoepidemiology, real-world evidence

Interested in recruiting a Doctoral student

Project title
Exploring the interaction between the environment and immune system

Supervisor
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Type of recruitment and qualifications of applicant
Doctoral student, 48 months

We are recruiting a doctoral student for a period of up to 4 years. It is important that the applicant be able to communicate well in English, be highly motivated and be able to work well in a team environment.

The applicant is to have completed a master's degree, specialising in Immunology. It is important that the applicant have an interest or documented work experience in T helper cell biology and cytokine biology. Preferably, the applicant should also have experience working with mice and have knowledge of tissue processing techniques, flow cytometry and analysis of flow cytometric data. Experience with molecular biology techniques such as real time PCR, cloning and western blotting is a plus. Experience with single cell RNA- Sequencing and associated analysis is also preferred but not required.

Background
Inflammatory diseases are known to have markedly increased in incidence over the past hundred years. These include allergies such as asthma and eczema, inflammatory bowel diseases and a range of autoimmune disorders. At the heart of many of these diseases are CD4 T cells (also called T helper cells). T helper cells secrete cytokines that promote our body's health and function. However, these cells can sometimes get out of control and cause pathological disorders, such as the ones listed above.

In the past hundred years, changes to our environment are thought to have led to the rapid increase in inflammatory diseases. These changes include rapid urbanisation, the introduction of new toxins into the environment, eradication of some pathogens, altered air pollution, vastly different diets and many other factors.

Although the epidemiological evidence clearly suggests that environmental changes have triggered a sharp rise in inflammatory diseases, the mechanism(s) by which

this has occurred remains contentious. In particular, it is unclear how environmental changes have impacted on T helper cell function and balance. In this project, we aim to uncover the impact of different environments on T helper cells and their associated inflammatory disorders.

Research project description

In this project, we will try to decipher how different environmental factors impact on T helper cell responses to allergens and pathogens. We aim to understand how nematode infection, the microbiome, diet and exposure to plastics and other factors impact on the function of T helper cells.

To come to this understanding, we will work largely in mouse models. Mice will be exposed to the environmental factors stated above, such as nematodes (H. polygyrus), different types of microbiotas, different diets, and chemicals in plastics. Thereafter, mice will be analysed for their responsiveness to allergens such as house dust mites, or pathogens such as viruses and bacteria. The responsiveness of mice to allergens and pathogens will be assayed by performing bronchoalveolar lavage and quantifying the levels of cellular infiltrates. Cytokine responses will also be gauged using a combination of ELISA and flow cytometry. Gene expression profiling at single cell or population levels will be performed to understand how different environments impact on gene expression in T helper cells and other cells within the lung. T cell receptor transgenic cells (for instance to house dust mites or to the model antigen OVA) will be transferred to mice and their activation and differentiation in different environments will be analysed.

Other cells apart from T helper cells, in the lung environment will also be analysed. The impact of environmental changes to the epithelial cell profile and innate cell infiltrates will be analysed and correlated with the impact on allergen- or pathogen-driven inflammation. Analysis on these cells will comprise of scRNA-Seq experiments and other molecular assays.

In vitro differentiation assays of T helper cell will also be important and help to identify the mechanisms by which the environment affects T helper cell functions.

We expect our results to shed light on the mechanism(s) by which alterations to our environment impact on T helper cells in our body, and how that in turn makes us more or less susceptible to allergic and inflammatory diseases.

Research group

My research group is comprised of two post-doctoral scientists and two PhD students, and we often have one or two master's and undergraduate students in the lab. It is important that we work well together since we tend to perform very large experiments, and it is handy to have help from multiple members of the lab to perform our experiments well.

The focus of our group's work is on allergy and T helper cell biology, although we also have considerable work ongoing in the field of cancer immunology. In the

cancer projects, we also focus on the role of T cells (both CD4 and CD8 T cells) in cancer immunity. All group members are experienced in animal handling, organ harvest and processing, flow cytometry, ELISA, sterile work and other techniques. Some members are also adept in scRNA- Seq technologies and analysis, cloning, transfection and transduction, and CRISPR-Cas9 technology.

Our group is situated at the Biomedicum, which houses several core facilities and expertise of around 100 independent research groups. Thus, there is a broad range of expertise within my group, and nearby, which will facilitate the aims of our project.

Key words
Allergy, T helper cells, environment, cytokines, scRNA-Seq

Interested in recruiting a Doctoral student

Project title

Screening for self-renewal in esophageal progenitor subpopulations.

Supervisor

Maria Genander, Doctor, Department of Cell and Molecular Biology
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Type of recruitment and qualifications of applicant

Doctoral student (48 months)

We expect that the student has a background in Biomedicine or other relevant area. Previous research experience is a strong merit. We particularly look for students with previous experience working with mouse models, organoids or Crispr/Cas9 technology.

The working language in the lab is English and the student is expected to be able to socialize and communicate science in English.

Background

This project aims to delineate esophageal self-renewal programs in distinct progenitor cell subpopulations using mouse and human organoid Crispr/Cas9 screening methodology.

The incidence of squamous cell carcinoma of the esophagus, one of the most common fatal cancers worldwide, is constantly increasing. Understanding how normal tissue stem cell fate is regulated is of considerable importance in defining and treating cancer. Whereas oncogenic lesions acquired by cells that are undergoing terminal differentiation have relatively little impact on a tissue because the cells are rapidly lost, stem cells are permanent tissue residents, and therefore have the potential to acquire further deleterious changes and form a tumor.

In the esophagus, a single progenitor cell population has been proposed to maintain the epithelia during homeostasis and to be the cell-of-origin in squamous cell carcinomas. The studies from which these hypotheses arose are based on lineage tracing data generated from randomly recombined progenitors at single cell density, and do not formally exclude the existence of functionally distinct subpopulations of esophageal progenitor cells. Although this minimalistic, single progenitor model is very attractive and potentially able to explain maintenance of the esophageal epithelia and initiation of cancer, defining functional subpopulations of progenitor cells would infer the possibility of designing new targeted therapeutic strategies in esophageal squamous cell carcinomas.

My group have recently identified heterogeneity within the esophageal progenitor population in homeostasis. Using genetic labelling and lineage tracing we demonstrate that a subset of esophageal cells comprises a distinct, slow cycling, progenitor population with the ability to contribute to the esophagus long-term, challenging the current progenitor cell model proposed for the esophagus in homeostasis and tumor initiation.

Research project description

We hypothesize that identifying mechanisms to differentially deplete subpopulations of normal esophageal progenitors will be imperative to eventually target and reduce tumor growth. This project aims to do this by understanding mechanisms required for self-renewal of normal esophageal epithelial progenitor cells.

We will isolate progenitor subpopulations from healthy human and mouse esophagus by fluorescence activated cell sorting (FACS). To identify genes required for self-renewal of these subpopulations, primary progenitor cells will be directly transduced with a CRISPR lentiviral barcoded and puromycin-expressing gRNA library containing multiple gRNAs for each candidate gene. Viral titers will be calculated so that each progenitor only gets infected with one gRNA. Single transduced cells will be suspended in Matrigel and cultured for 10 days, during which time organoids are formed. Uninfected progenitors will be selected against using puromycin. After 10 days, organoids will be dissociated and subpopulations of progenitor cells re-isolated and re-plated for a total of five passages.

To determine the contribution of the gRNAs to each progenitor pool at each passage, genomic DNA will be isolated from dissociated organoids derived from each subpopulation followed by Solexa deep sequencing using bar-coded primers. Comparative analysis will then reveal which gRNAs are selectively under/overrepresented at each passage. Progenitor cells expressing a gRNA that is deleterious for the cell, i.e. inhibiting self-renewal or pushing cells towards differentiation, will be underrepresented in the comparison. gRNAs overrepresented will be suggestive of a role in promoting self-renewal and proliferation.

Interesting gene candidates will be tested individually both in human organoids or in vivo mouse models. The lab uses traditional genetic mouse models, as well an in utero lentiviral injections allowing us to transduce esophageal progenitor cells in vivo during early esophageal development. We expect that the results from this screen will be highly relevant for the stem cell field at large, since genetic programs regulating epithelial stem cell self-renewal is still largely undescribed.

The student working in this project will gain knowledge in human and mouse stem cell biology, Crispr/Cas9 technology, organoid culturing as well as DNA sequencing methodology and analysis. Previous expertise in any of these areas is a strength for the applicant.

Research group

The Genander lab is working on understanding general mechanisms in epithelial stem cell establishment and specification, linking these processes to tumor initiation and development. The lab has recently received ERC funding for the project described here and will therefore be able to provide sufficient means for the student to carry out the proposed project. The lab currently consists of two registered PhD students and three postdoctoral fellows. We are situated in a new research building, Biomedicum D5, sharing lab space with several other strong research groups working in stem cell and tumor biology. We actively collaborate with other research groups inside and outside of Biomedicum for bioinformatical expertise or technological knowledge transfer. The Genander lab is part of several seminar series where the student will learn about ongoing stem cell research at Karolinska and will be asked to present their work independently.

Key words

Stem cell, cancer, Crispr/Cas9, screen, organoid, self-renewal, epithelium, esophagus, sequencing

Karolinska Institutet

Interested in recruiting a Doctoral student

Project title

Insulin/Insulin-like growth factors biased signaling: from autoimmune disease to cancer treatment.

Supervisor

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Type of recruitment and qualifications of applicant

Doctoral student (48 months)

The doctoral education project and the duties of the student

The supervisors represent expertise in tumor pathology, molecular pathology endocrinology and tumor biology. The translational approach is aided through access to clinical material relevant for the project and close clinical collaborations. The overall objective of this PhD program is to investigate, and to use the generated knowledge of, the β-arrestin (β-arrs) signaling complexes involved in the GPCR/RTK crosstalk for improved management of cancer patients. The successful candidate will investigate the mechanisms controlling the biased-signaling at the IGF-1R in thyroid-associated ophthalmopathy, an autoimmune component of Graves' disease. The generated knowledge will be used to develop new strategies for targeting the same type of signaling for cancer treatment.

Entry requirements at KI: http://ki.se/en/phd/entry-requirements-eligibility-for-doctoral- education

Skills and personal qualities

We are looking for an ambitious student with strong enthusiasm towards science to complement our interdisciplinary team. A successful candidate holds a recent MSc degree in translational medicine, (bio)medicine or similar topic and strong background in molecular cell biology, biochemistry, genetics and/or cancer biology. Practical experience in cell culture, protein analytic techniques, basic or advanced molecular biology techniques, and analysis of cancer cell phenotypes using immunofluorescence and immunohistochemistry methods are expected from a highly qualified applicant. Knowledge in cell signaling, membrane trafficking, cytoskeleton and metabolic pathways is a plus. The candidate must have good communication skills and is expected to be able to work as part of a team, in a very competitive research area. The project will be run in collaboration with Prof. Terry Smith, University of Michigan Medical School, Ann Arbor, USA and Prof. George Calin at MD Andersson Cancer Center, Texas, USA (MDACC).

Background

The tyrosine kinase receptors (RTKs) are a related family of 59 cell surface receptors with similar structural and functional characteristics. Although few compared with the large number of GPCRs, RTKs receive particular interest as therapeutic targets in cancer. Among them, owing its key roles in fundamental biological processes, it is not surprising that IGF1R it is frequently found to be hijacked by awry oncogenic processes. As such, the IGF system has emerged as an obvious target for cancer therapy, fuelling development of several anti-IGF1R drugs - kinase inhibitors and targeting antibodies (AB). The most effective in preventing IGF1R kinase activation were selected to be tested in clinical settings, yet in spite of promising preclinical data, clinical trials did not deliver the expected results. This failure has led the field to question: is the inconsistency between outcomes in clinical and experimental settings a result of drug ineffectiveness or ultimately the wrong target?

Our studies revealed the GRK/β-arrestin system, as a critical regulator of antibodies targeting the IGF1R. These results have led us to propose a paradigm shift in which targeting AB act as biased agonists for the IGF1R. Intriguingly, agonistic properties were recently revealed for the IGF1R autoantibodies (AAB) developed in thyroid-associated ophthalmopathy (TAO), an autoimmune component of Graves' disease. The largest trial for TAO ever initiated in USA disclosed unprecedented clinical response when the biased signaling activated by TAO IGF1R AAB was inhibited by anti-IGF-IR therapeutic AB.

The overall goal of this study is to investigate the biased signaling generated by IGF1R AAB vs biased signaling triggered by IGF-IR therapeutic AB. Defining and controlling such biased signaling represents a practical therapeutic strategy to enhance response to anti-IGF-1R therapies for all types of cancer relying on the IGF-1R.

Research project description

The overall objective of this PhD program is to investigate and to use the generated knowledge of the β-arrs signaling complexes downstream IGF1R, triggered by therapeutic antibodies (AB) or autoantibodies (AAB), for improved management of cancer patients. This is based on the underlying hypothesis that the signaling complexes coordinated by β-arrs and involving kinases, ubiquitin ligases and ncRNAs, contribute to tumorigenesis and the progression of cancer, and could therefore be targeted in therapies.

Specific Objectives
WP1. Investigate the mechanisms controlling the kinase/β-arrestin/GRK-dependent signaling at the IGF-1R triggered by AB or AAB.

WP2. Evaluate different strategies for targeting β-arrestin/GRK-biased agonism at the IGF-1R for cancer treatment.

IGF1R is classified as an RTK and accordingly, tyrosine phosphorylation was considered to be the central process governing IGF1R signaling. However, during the last decade, we challenged this view by demonstrating the involvement of

GPCRs components in IGF-1R function [PMID: 24276851, 23188799, 22509025]. Recently, we discovered the opposing regulatory roles of the β-arr1/2 isoforms and their preferential partnership with GRKs on expression and function of the IGF-1R [PMID: 28581517, 28092675]. The new perspective for an RTK opened by our latest studies and largely accepted for GPCRs is that the conformation of a receptor that activates the kinase cascade can be distinct from that initiating non- canonical signaling. This model is validated by studies demonstrating that IGF-1R signaling could be generated in a "biased manner" via β-arr1 by targeting antibodies and most importantly, controlled therapeutically.

The thyroid-associated ophthalmopathy (TAO) is a potentially blinding autoimmune component of Graves' disease. Central to TAO are the anti-IGFIR AAB which sustain the pathogenic process through kinase-independent, biased IGF1R activation. Unlike cancer treatment, therapeutic anti-IGF1R AB were proven very effective in clinical settings to cure or delay TAO (PMID: 28467880).

The PhD student will investigate the signaling activated by IGF1R-AAB vs IGF1R-AB seeking the answer why the AB are effective in TAO and not in malignant processes. As preliminary data indicate that AAB render IGF1R responsive to AB, we will use this paradigm to develop a novel strategy for cancer treatment.

WP1 We will investigate the interplay between β-arr/GRK isoforms as mediators of IGF-1R activities in response to AB and/or AAB. We will also investigate the potential competition between different ligands (IGF1, IGF2, AB, AAB) as well as competition between different substrates (β-arrs, IGF-1R, GRK2) for the same ligase. As non-canonical/canonical-signaling processes, regulates ncRNA expression, the ncRNA profile could be used as an indicator of biased/balanced signaling, thus predicting response to anti-IGF-1R therapy [PMID: 31434680]. Accordingly we will evaluate the ncRNA expression profiles as indicators of balanced/biased IGF1R signaling following various ligand activation with focus on AB vs AAB.

WP2. We demonstrated that overexpression of β-arr2 or inhibition of β-arr1 considerably increases the inhibitory effects of chemotherapeutic drugs. AAB or activated GPCRs, through a regulatory negative feedback, target the β-arr2 into proximity of its membrane receptor substrate. In this project the PhD studnet will further investigate the biological effects of IGF-1R inhibition following direct (transgenic modulation, pharmacological inhibition, AAB) or indirect (GPCR-cross-targeting) induced perturbations of β-arr/GRK function with the final goal to identify drugs that could render the cancer cells more sensitive to IGF-1R AB.

Taken together these studies will test the proof of concept for selectively targeting components of the RTK/GPCR crosstalk complex in tumor cells.

Research group

The project will bring together internationally renowned groups at Karolinska Institute, Ann Arbor (AA), Michigan, USA and MD Andersson Cancer Center, Texas, USA with longstanding records in both translational and basic research in RTK/GPCR signaling and ncRNAs in cancer and endocrine disorders. The student will become part of a team of scientists trained in tumor pathology, genomics and signaling to exchange expertise and perform the complementary experiments.

LG's group at KI has pioneered the work of biased signaling from the IGF-1R. LG group (one senior researcher, 2 postdocs and 4 PhD students) use a range of experimental models from normal and malignant cell lines, genetically modified to express or not the proteins of interest, to animal models and studies on tumor samples, to cover the gap between the basic experimental research and clinical practice. The student will benefit from state-of-the art facilities and expertise to perform molecular and cellular pathology studies that include a unique platform combining confocal microscopy and ELISA to analyze protein-protein interactions, in real-time, in living cells by powerful FRET-BRET and fluorescence polarization studies. KI PI's is additionally an active pathologist and has access to clinical samples, as well as ethical permission to perform the proposed research. TS, postdoctoral researcher will introduce the student to the lab techniques and data collection.
At AA, the student will learn about autoimmune diseases and RTK/GPCR cross-talk. At MDACC, the student will work with identification of ncRNA changes associated with biased signaling by using ncRNA microarray, subsequent validation of ncRNA targets that will lead to mechanism elucidation. The outlined project is based on already established research and network of collaborations and the student will become part of it. The combined expertise of the KI, AA and MDACC groups would be a clear benefit for the student's doctoral education.

Key words

Personalized cancer treatment; Receptor Tyrosine Kinase; Signaling; G protein-coupled receptors; RTK, GPCR, cancer, ubiquitination, arrestin, GRK, cancer, signal transduction, IGF-1R; IGF1R; Insulin; ncRNA; microRNA; progession; metastasis; melanoma; sarcoma; ALL; IGF1.

Supplementary information

This is a collaborative project between Karolinska Institute, University of Michigan Medical School, Ann Arbor, Michigan, USA and MD Andersson Cancer Center, Texas, USA. Depending on the project progress, the prospective student might have to get some training in the partners' labs.

Karolinska Institutet

Interested in recruiting a Postdoc

Project title
Biological signature and efficacy of mesenchymal stem cell-based therapies.

Supervisor
Cecilia Götherström, Associate professor,
Department of Clinical Science, Intervention and Technology
Email: cecilia.gotherstrom@ki.se Phone: +46 704 712300
Home page: http://ki.se/en/people/cecgot

Type of recruitment and qualifications of applicant
Postdoc (12 months)

The applicant should have a PhD in relevant area, for e.g. cell biology, immunology, regenerative medicine, transplantation. You have an interest in developing new methods and to further develop your research skills in the expanding regenerative medicine field. You are flexible and self-going and can carry out tasks independently and responsibly. You must have a very good ability to express yourself in speech and writing in English and can utilize computer program such as Word, Excel and PowerPoint, and have a good knowledge in statistical analysis.

Background
Vast efforts have been put towards developing mesenchymal stem cell (MSC) therapy for various disorders. However, obstacles exist due to lack of consistent MSC-potency across patients, undefined mechanisms of action as well as difficulties in large scale manufacture of uniform MSC. We have developed the highly bone-forming fetal MSC for clinical use, with initial patient data showing promising results for treatment of the inherited brittle bone disease Osteogenesis Imperfecta (OI). The approved clinical trial Boost Brittle Bones Before Birth (BOOSTB4) will evaluate transplantation of fetal MSCs before and/or soon after birth as a treatment for OI. The clinical trial is open in Sweden and will provide unique insight into MSC therapy for inherited disorders. Following intravenous infusion, fetal MSC migrate to the bones, partially engraft and form bone cells. Their clinical effect (growth and reduction of fractures) is transient, suggesting that they also act through triggering the body's own regenerative machinery via extracellular vesicles (EVs), which are vital in intercellular communication.
The purpose of this study is to improve the clinical efficacy of MSC-therapies. We will characterize the biological signature of fetal MSC and also investigate additional mechanisms of action of the cells. Accordingly, this will improve the manufacturing and help predict therapeutic outcome.
Successfully treating children early in their life will result in greater health benefits, transforming the future care of the pediatric population where few therapies currently are developed.

Research project description

The objective of the study is to improve our understanding of the clinical efficacy of MSC-based therapies, using the brittle bone disease Osteogenesis Imperfecta (OI) as the model disease. For this we will use fetal mesenchymal stem cells (MSCs) manufactured under Good Manufacturing Practice (GMP) and systematically testing the MSCs and Extracellular vesicles (EVs) derived from the fetal MSC in in vitro bone model systems. To further interrogate the obtained results, we will utilize biological samples from patients with OI treated with fetal MSCs collected in the BOOSTB4 trial.

We will further explore additional mechanisms of action of fetal MSCs by applying two in vitro human bone model systems for OI and healthy controls; the bone-on-a-chip model and 3D bone cultures. Using the models we will be able to determine the direct effects of fetal MSCs and indirect effects of EVs from fetal MSCs on OI and healthy controls. In the bone-on-a-chip model co-cultures of MSC or active and inactive EVs in different ratios with bone cells will be investigated and the interactions of fetal MSCs and EVs with bone cells will be traced using dyes. The cells/EVs can be kept in culture for up to four weeks in the models. The bone-on-a-chip model (gel and matrix containing the cells, supernatant, EVs) will be examined for viable cells, bone mineralization (Alizarin Red S) and with immunohistochemistry, PCR, cytokine multiplex assays and mass spectrometry-based proteomic analysis of bone growth, metabolic and pro- angiogenic factors, healthy/mutated collagen, osteocalcin, osteonectin, osteopontin, osterix, BSP, ALP, vW f, HGF, VEGF, PDGFR, G-CSF, IL 6, IL 8 and cell stress.

The second model, 3D bone cultures, will be established from fresh bone samples (OI and healthy control). Leftover bone chips from surgery are embedded in fibrin matrix and cultured for up to three weeks in media as described above. Tissue engineering experiments with similar methods have been performed in our laboratory. Outgrowth of cells is followed microscopically over time and the metabolic activity is followed with alamarBlue™ test for cell health. The bone is analyzed as described above for bone-on-a-chip with the addition of analysis of tissue integrity with HTX/Eosin.

Research group

The research group belong to Department of Clinical Science Intervention and Technology, Division of Obstetrics and Gynecology and the laboratories and offices are located at the newly constructed ANA Futura (https://staff.ki.se/about-ana-futura) at Karolinska Institutet, Campus South. We are working closely with physicians in the Karolinska University hospital.

Cecilia Götherström, PhD, Associate Professor and research group leader, with the main research interest to evaluate the clinical potential and significance of mesenchymal stem cells. Is the consortium leader for the European BOOSTB4 project (www.boostb4.eu).
Lilian Walther Jallow, PhD, expert in drug development, especially quality and regulatory documentation of advanced therapy medicinal products (ATMP).
Annika Goos, lab manager and responsible for the production of clinical grade fetal MSC.

Åsa Ekblad-Nordberg, PhD, postdoc and part of the production team of clinical grade fetal MSC, has recently defended her thesis on regenerative medicine and tissue engineering.
Fawaz Abomaray, PhD, postdoc, has recently defended his thesis on the role of MSC in endometriosis. Is currently evaluating the biosignature of MSC.

Key words
Mesenchymal stem cells, Mesenchymal stromal cells, Cell therapy, Cell biology, Advanced therapy medicinal products (ATMP), GMP production, Regenerative medicine, Transplantation, Bone tissue engineering, Extracellular Vesicles, Exosomes.

Interested in recruiting a Postdoc and a Visiting researcher

Project title

Personalized medicine and multimodal Imaging in Cancer.

Supervisor

Moustapha Hassan, Professor, Department of Laboratory Medicine
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Home page: https://staff.ki.se/people/mouhas,
https://ki.se/en/labmed/experimental-cancer-medicine-ecm

Type of recruitment and qualifications of applicant

- Postdoc (24 months)
- Visiting researcher (12 months)

Specific requirements are:

1) PhD in the field of pharmacology/medicine and/or medical sciences;
2) Research experience in cell biology and animal models;
3) Excellent written and oral English skills;
4) Documented experience in writing and publishing scientific papers.

Other meritorious competences include:

1) Research skills: FACS; cytotoxicity assay; immunohistochemistry; chromatography and mass spectrometry; fluorescence microscopy.
2) Research experience: pharmacokinetics/pharmacodynamics; preclinical in vivo imaging; animal models of leukemia, lymphoma, myeloma and GVHD

Background

Moustapha Hassan, Professor of Transplantation Research at the Department of Laboratory Medicine, Karolinska Institutet. Professor Moustapha Hassan has several years of experience in chemotherapy and stem cell transplantation research. His research focuses on personalized medicine, nanomedicine and multimodal imaging of cancer. He is also the Director of the Preclinical Laboratory at Karolinska University Hospital-Huddinge.

Research group Experimental Cancer Medicine (ECM) headed by Prof Moustapha Hassan aims to improve the survival and quality of life in cancer patients treated with cytostatics and SCT through increased treatment efficacy and decreased or eliminated side effects.

Our projects are divided into the following categories:
We have developed a transplantation model which enables us to study the mechanisms of the side effects (GVHD and arterial damage) and contributing factors in order to improve prophylactic treatment. Moreover, in the last few years we have developed reproducible models of hematological malignancies in order to

facilitate understanding of the mechanisms behind the graft-versus-leukemia (GVL) effect. We are investigating the effect of genetic variations in individual patients on the metabolism of cytostatics and thereby on the outcome of SCT. The goal of these studies is to develop reliable methods for dose individualization based on the patient's individual genome. We are investigating whether new cytostatics that block cell division and metastasis of cancer cells could prevent relapse after SCT. We are studying a number of cytostatics on a cellular and molecular level. We have been also working on molecular imaging in combination with nanomedicine to build a number of different carriers which can be loaded with cytostatics and imaging agents.

Research project description

We want to recruit one-two postdoc /visiting researcher in the following research projects:

Personalized medicine during stem cell transplantation:
Although stem cell transplantation (SCT) is a curative treatment for many children suffering from hematological or solid tumors as well as for children with metabolic or genetic disorders, the results are often far from satisfactory. After SCT, Complications including liver and lungs toxicity; infections, rejection and graft-versus- host disease (GVHD) deteriorate the life quality of the child and may lead to morbidity, mortality, high economic costs for the society and less successful clinical results. Recently, studies showed a higher incidence of cardiovascular complications in adults who received stem cell transplantation as young children and were treated with high doses of chemotherapy. Our aim in the present project is to elucidate the mechanisms underlying cardiotoxicity, to develop prophylactic treatment and to personalize the cytostatics treatment prior to SCT in order to minimize acute and long-term side effects. A personalized therapy based on the patient´s gene expression and relevant prophylactic treatment, certainly will increase the treatment efficacy and diminishes the risk of acute and late side-effects. This results most of all in lesser suffering, greater survivability and increases the quality of life for SCT children, and also diminishes the cost to society.

Molecular imaging and theranostics in cancer:
We aim to develop new imaging/contrast agents together with new molecular imaging strategies to offer tools for the need to address accurate diagnosis and personalize medicine of cancer. In the project we will design and validate multimodal imaging agent which can be applied in optical imaging, ultrasound imaging, photoacoustic imaging, MRI, CT and magnetic particle imaging. The goal is to combine the advantages of different imaging techniques, i.e. sensitivity of detection and resolution of the image. The integration of multimodal imaging technologies would therefore provide complementary and complete information for subsequent decision-making. We will also further improve the specificity and sensitivity of the imaging agents by utilizing antibodies targeting tumor specific markers. Such multi-target imaging agent will provide the opportunity to early diagnose, stage and predict chances of recurrence for cancer patients. The third strategy is to develop the nanoparticles to form theranostics agents by loading imaging contrasts and cytostatic. Such multifunctional NPs will allow us to perform

early diagnostics, follow drug delivery and to monitor the treatment efficacy and hence improve the clinical outcome for cancer patients.

Research group

Division of Experimental Cancer Medicine (ECM) at LABMED is composed of one professor, Prof Moustapha Hassan, four senior researchers and three PhD students.

Key words

Personalized medicine, GVHD, multimodal imaging, Stem cell transplantation, theranostics, nanomedicine

Karolinska Institutet

Interested in recruiting a Doctoral student

Project title

RNA fusion transcripts in neurodegenerative disease.

Supervisor

Johan Holmberg, Associate professor, Department of Cell and Molecular Biology
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Home page: https://ki.se/en/cmb/johan-holmbergs-group

Type of recruitment and qualifications of applicant

Doctoral student (48 months)

Given that the doctoral research project contains both experimental and computational aspects, the applicant should have a research profile/interest that encompasses molecular biology as well as bioinformatics. See below for a more detailed description of skills that will be considered as valuable for the applicant. Thus, an educational background in basic biomedicine is suitable. Research experience with different experimental techniques and computational analysis in diverse international research environments is considered a substantial advantage. Participation in research projects at Karolinska Institutet, is also considered a merit. In addition, experience of research in different biological systems will be valued as a merit.

Relevant skills: Protein analysis (e.g. Western blot), PCR, immunofluorescent staining, confocal analysis, cell culturing techniques, FACS, usage of R for bioinformatic and statistical analysis, analysis of differential gene expression (e.g. through DESeq2).

The applicant must be able to communicate well English, both verbally and in text.

Background

Parkinson's disease (PD) is a neurodegenerative disease characterized by a loss of midbrain dopamine cells causing debilitating movement symptoms. Most cases of PD are sporadic, however rare hereditary forms have provided insights into the mechanisms of the disease. Several findings suggest that defective mitochondria are critically involved in PD pathogenesis. The two most commonly mutated factors in autosomal recessive PD are the PARKIN and PINK1 genes, both of which are key players in mitochondrial function and homeostasis. A specific mode of generating alternative gene products, distinct from DNA-changing mutations, is represented by cis-spliced RNA fusion transcripts. This type of transcripts has recently been identified in tumors as well as in healthy tissues and are characterized by fusion of neighboring RNA transcripts. We have recently identified a large array of splicing sensitive RNA fusion transcripts in neuroblastoma, a disease that like PD exhibits a lack of recurrent mutations. Among the fusion transcripts we detected was the ZNF451-BAG2 fusion, which generates a truncated BAG2 protein (ΔBAG2). The wild-

type BAG2 protein acts as a co-chaperone for several substrates, including PINK1 which is stabilized by BAG2 resulting in a PINK1-mediated translocation of PARKIN to mitochondria allowing for increased neuronal survival upon exposure to oxidative stress in a model of PD. We have now identified the ZNF451-BAG2 fusion as well as several additional interesting fusion transcripts through analysis of sequenced PD-patient tissue samples. Through in vitro experiments we have been able to show that the fusion protein ΔBAG2 fails to stabilize PINK1 and does not promote mitochondrial translocation of PARKIN. Thus, expression of the ZNF451-BAG2 fusion transcripts disrupts key PINK1/PARKIN-dependent processes previously established to be central in the pathophysiology of PD.

Research project description

Firstly, we will determine whether the fusion transcripts we previously identified, indeed are PD specific and whether there is regional specificity. We intend to perform paired-end RNA sequencing on NeuN-stained and fluorescent cell sorted neuronal nuclei from both the substantia nigra and the neocortex of PD-patients as well as age-matched controls followed by bioinformatic analysis to detect PD-specific fusion transcripts. Given that we already have identified that expression of the ΔBAG2 fusion product abrogates PINK1 stability and PARKIN mitochondrial translocation, we will in parallel investigate whether the ZNF451-BAG2 is indeed selectively expressed in PD-patients through PCR and Western blot of midbrain tissue from PD-patients and healthy controls. We will also explore if ZNF451-BAG2 exhibits region dependent expression in PD-patients. Functional characterization of both the wild type BAG2 transcript and the ZNF451-BAG2 fusion as well as additional selected candidates will be performed in a transgenic mouse model that express the mCherry reporter under Cre- dependent control of the dopamine transporter DAT specifically in midbrain dopamine neurons. Wherein we will perform unilateral stereotactic injection of AAV-virus vectors into the midbrain encoding Cre-dependent PD- specific fusion transcripts. We will explore whether expression of single or multiple PD-specific fusion transcripts in dopamine neurons predispose for neurodegenerative changes akin to PD or if it will increase sensitivity toward 6-OHDA induced cell death in the substantia nigra. Histological alterations in nigrostriatal neurons as well as dopamine dependent behaviors related to fine movements, postural control and locomotion will also be studied in mutants both as in ground state and challenged with intracranial injections of 6-OHDA or with systemic delivery of methamphetamine. Analysis will include genome wide transcriptome/chromatin changes analysis after isolation of midbrain nuclei according to protocol recently developed by our research group as well as viral tracing to reveal possible loss of connectivity between the substantia nigra and the striatal target area. Given the potential dominant negative effect of ΔBAG2 on BAG2 function we will perform loss-of function experiments targeting wild type BAG2 in the substantia nigra through Crispr/Cas9. In addition, we will in collaboration with the group of Prof. Johan Ericson investigate the role of PD-specific fusions in ES-cell derived human dopamine neurons.

Initially, we will overexpress ZNF451-BAG2 and explore the impact of ΔBAG2 on key processes affecting PINK1/PARKIN and mitochondrial stability in differentiated

human dopamine neurons. Similar gain-of-function experiments will be performed for other valid candidates. Our preliminary data furthermore show that ΔBAG2 efficiently abrogates the capacity of wild type BAG2 to clear phosphorylated TAU from microtubuli potentially affecting microtubuli stability and increasing the risk of neurofibrillary tangle formation a well-known component of neurodegenerative disease. Thus we will in our ΔBAG2 gain-of-function experiments continously monitor for signs of TAU-fibrils. In a parallel effort we aim to explore whether the expression of RNA fusion transcripts in the healthy central nervous system increases with aging and if this is correlated with loss of neuronal function.

Taken together, our approach will not only explore a possible role for ΔBAG2 in PD but also the possibility that the lack of mutations in PD is counterbalanced by the presence of additional fusion transcripts capable of generating altered gene products impinging on dopamine neuron function and survival.

Research group

Principal Investigator: Johan Holmberg, PhD, PI, Senior Research Fellow, funded by The Strategic Research Programme in Cancer (StratCan, SFO), Dept of Cell and Molecular Biology (CMB), Karolinska Institutet (KI). Expertise: Mouse genetics, developmental biology, stem cell biology and tumor biology.
Post-Doc: Yao Shi, Expertise in tumor models, bioinformatics and biochemistry. Yao is responsible post-doc for the project regarding fusion transcripts.
Post-Doc: Juan Yuan, Expertise: Crispr/Cas9, xenografts, molecular biology, neuroscience, drug delivery, cell biology.
Graduate Student: Konstantinos Toskas, Expertise: In utero electroporation, intracranial injections, orthotopic xenografts and microdissection. One or two additional post-docs will be recruited during 2019-2020.
Master students: Hanna Schwärmle, Mingzhi Liu.

Key words

RNA-fusion transcripts, Parkinson's disease, Chaperones, Mitochondria, Protein stability, Mouse transgenics, Neurodegeneration, Mitophagy, Transcription, Splicing.

Interested in recruiting a Postdoc and a Visiting researcher

Project title

Autoantibodies in rheumatoid arthritis with focus on cartilage oligomeric matrix protein.

Supervisor

Rikard Holmdahl, Professor, Department of Medical Biochemistry and Biophysics
Email: Rikard.Holmdahl@ki.se Phone: +46 705424607
Home page: http://ki.se/en/mbb/research-division-of-medical-inflammation-research

Type of recruitment and qualifications of applicant

- Postdoc (24 months)
- Visiting researcher (12 months)

PhD or comparable exam. Knowledge in immunology and immunological/cell biology techniques, biostatistics, animal experiment technology. Fluent in English with certificate such as TOEFL.

Background

Autoimmune diseases, such as rheumatoid arthritis (RA), develop in three distinct stages: priming, onset and chronicity (Ge and Holmdahl, Nature Reviews of Rheumatology 2019). Most likely, the same type of development stages occurs in different types of autoimmune diseases.

In most of these diseases, priming is characterized by activation of B cells to produce disease-specific IgG autoantibodies that may appear in the blood several years before clinical onset. In the case of RA these include antibodies directed towards modified immunoglobulin and citrullinated protein antigens (ACPA). However, how this priming stage becomes an inflammatory attack on the joints, leading to clinical onset, remains unknown.

Research project description

We have made efforts to isolate antibodies that crossreacts with joint cartilage and isolated their targeted epitopes. For studies of RA we have identified relevant epitopes on 5 selected joint proteins (JP1-5) and made a Luminex based multiplex test. This test can be used to detect peptides that could be used for improved diagnosis and understanding of RA.

On protein of particular interest is Cartilage oligomeric matrix protein (COMP), a non-collagen glycoprotein produced primarily by the cartilage, as well synovium, tendon, meniscus and even vascular smooth muscle cells. COMP is traditionally considered as an important regulator of assembly and maintenance of the fibrillar collagen I and II networks. We have previously shown that the immune system is not completely tolerant of proteins associated with cartilage proteins, which induce

arthritis in mice. COMP, as well as antibodies to COMP, induces arthritis in mice.

In the project Luminex will be used to perform the diagnostic multiplex test on already established cohorts of RA as well as serum from individuals with emerging RA. Antibodies specific for these epitopes will be characterized both functionally and to be used as standard antibodies in the assay.

The results will be analysed by biostatistically both in our laboratory and together with cooperating clinicians.

In the next round of analysis we will identify new peptides, that comes from work in the animal models. We have established additional anti-COMP monoclonal antibodies that have not yet been characterized. The first goal will be characterized these regarding function and epitope specificity. Functional studies is done by injection of antibodies in the mouse and study effects on arthritis, bone erosion and pain. The antibodies will also be used to characterize the expression of COMP in different tissues of the mouse (skin, lung, vessels, joints). They will also be used to establish an assay with which circulating COMP fragments can be detected in sera (in both mouse and human sera). To help in the characterization we will compare with COMP deficient mice available in the lab. For the studies we have available unique humanized mouse strains available in the laboratory (expressing DR*0401, DR*0405 associated with rheumatoid arthritis as well as arthritis in the mouse models) and analyse the peptides specific response both for B cells and T cells. We also have series of CRISP mutated and conditional mouse strains to analyse the functional role of the anti-COMP antibodies and the immune response to COMP.

Research group

The Medical Inflammation Research (MIR) laboratory (http://ki.se/en/mbb/research-division-of-medical-inflammation-research) is located within the Karolinska Institute, within the Biomedicum building, with full access to core facilities, collaboration, research courses and seminars. The laboratory is fully equipped for the project and has 20 members from PhD students to professors. We work closely with many research groups in China and perform several projects (both analysis of human samples and animal experimental technologies) in tight collaboration with them.

Key words

Immunology, Rheumatoid arthritis, Cartilage, Autoimmunity

Interested in recruiting a Postdoc and a Visiting researcher

Project title

Oxidative regulation of autoreactive B cells.

Supervisor

Rikard Holmdahl, Professor, Department of Medical Biochemistry and Biophysics
Email: Rikard.Holmdahl@ki.se Phone: +46 705424607
Home page: http://ki.se/en/mbb/research-division-of-medical-inflammation-research

Type of recruitment and qualifications of applicant

- Postdoc (24 months)
- Visiting researcher (12 months)

PhD or comparable exam. Knowledge in immunology and immunological/cell biology techniques, biostatistics, animal experiment technology. Fluent in English with certificate such as TOEFL.

Background

The immune system is selected on endogenous self-structures. Both T cells and B cells are selected in central lymphoid organs (thymus and bone marrow) to respond to self, both self-antigens and costimulatory molecules and antigen receptors. This stimulation leads to rescuing of the developing lymphocytes but also different types of differentiation. It is well known that thymus produces both anergic effector T cells but also regulatory T cells but it is less clear whether similar cell types are produced in the bone marrow.

Our group has been interested in how cartilage antigens, such as type II collagen (CII) selects the immune system (see Raposo et al Nat Com 2018 and Ge et al Nat Rev Rheumatology 2019). Lymphocyte recognition of cartilage plays an important role in the development of autoimmune arthritis and the collagen induced arthritis model is the most commonly used model for rheumatoid arthritis. Rheumatoid arthritis is genetically dependent on MHC class II molecules presenting self-peptides from autoantigens such as CII in the development of the disease. It leads to a prominent CII specific T and B cell response that cause development of arhtriits.

Remarkably the epitopes on CII, recognized by both B and T cells, are conserved between mouse and humans. This allowed us to develop a unique and diseases relevant system for studies of CII autoreactivity. We have so far developed unique tools with mouse strains humanised with MHC molecules and knock in strains with clonal T and B cells specific for CII. We have now made some remarkable findings that address fundamental qualities of the immune system.

Research project description

In the present project we are interested in studies on how oxidation of B cells affects selection and regulation and also how this will affect autoimmune disease development. The basis for this is that we I (see Holmdahl et al Immunol Rev 2016) identified the Ncf1 gene to be the most important polymorphic gene regulating both animal models abut also some major autoimmune diseases, including RA. We have made unique conditional mouse strains inducing the expression of Ncf1 allowing to study a dominant influence of the NOX2 complex that is a non-redundant source of induced peroxide. This mouse models will be studies using specific Cre leading to NOX2 functional expression in specific B cell types and their development will be followed using VDJ knock in CII reactive B cells. The B cells at different functional stages, determined by in vivo unique expression, will be studied on a single cell stage using high throughput RNA sequencing combined with proteomics and detailed immune functional assays.

Research group

The Medical Inflammation Research (MIR) laboratory (http://ki.se/en/mbb/research-division-of- medical-inflammation-research) is located within the Karolinska Institute, within the Biomedicum building, with full access to core facilities, collaboration, research courses and seminars. The laboratory is fully equipped for the project and has 20 members from PhD students to professors. We work closely with many research groups in China and perform several projects (both analysis of human samples and animal experimental technologies) in tight collaboration with them.

Key words

Immunology, B cells, Ncf1, Peroxide, Rheumatoid arthritis, Cartilage, Autoimmunity

Supplementary information

Qualifications of the applicant: PhD or comparable exam. Knowledge in immunology, immune-assays, biostatistics and animal experiments. Fluent English with TOEFL certificate.

Interested in recruiting a Postdoc

Project title

Utilizing cell-free DNA methylome as a biomarker for early detection of brain damage in Multiple Sclerosis patients

Supervisor

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Home page: http://ki.se/en/cns/maja-jagodics-research-group

Type of recruitment and qualifications of applicant

Postdoc (24 months)

We are looking for a dedicated postdoctoral researcher with expertise in molecular biology in general and DNA methylation in particular. An ideal candidate would have biomedical/molecular biology/life sciences degree. The successful candidate will perform cutting-edge techniques, therefore, theoretical and practical knowledge in DNA methylation sequencing is a plus. Bioinformatics skills would be an advantage. The applicant should also have good interpersonal skills and the ability to work both independently but also as a part of a large team. The successful applicant will be involved in collaborations within and outside the Unit. To be eligible, an applicant must have obtained a PhD within five years prior to the application deadline.

Background

Multiple Sclerosis (MS) is a leading cause of non-traumatic progressive disability in young adults with recent rise in incidence. This heterogeneous and yet-incurable disease is characterized by autoimmune destruction of myelin and neurons in the central nervous system, leading to various debilitating neurological symptoms. Importantly, the choice of treatment strategy, some of which are connected to adverse side effects, highly depends on prognosis in relation to disease severity, progression and treatment response. Hence, development of accurate MS prognostic markers appears crucial to better guide personalized therapeutic intervention, in particular for patients with severe progressive MS. While the methods for monitoring and prognosis of MS patients are primarily based on neuroimaging methods, the use of molecular biomarkers have the potential to reflect ongoing rather than delayed events occurring in the brain of MS patients. An emerging approach based on detection of cell-free circulating DNA (cfDNA) released from damaged/dying cells into body fluids, originally designed for cancer and graft failure detection, has recently been proposed as a promising biomarker candidate for autoimmune disease as well (Lehmann-Werman et al.2016).
The objective of this project is to develop and validate methylation-based sensitive biomarkers for early and quantitative detection of demyelination and

neurodegeneration in MS.

Research project description

The successful candidate will be responsible for the investigation and validation of the biomarker in biofluid samples, i.e. cfDNA from cerebrospinal fluid (CSF) and plasma, from unique cohorts of MS patients and controls.

The most competitive clinical material worldwide. cfDNA will be isolated using serial CSF and plasma samples from MS patients, with the prospect to estimate the relative proportion and phenotype of CNS cell types that die at different stages of disease. It is of vital importance to combine biofluid analysis with detailed long-term follow-up of disease evolution. For a majority of patients followed at the Karolinska University Hospital, we have access to CSF (n=500) and plasma (n=500) samples with detailed clinical follow-up from the MS registry, along with samples from patients affected by other neurological diseases (n~600). Additionally, we benefit from collaboration with Prof. F. Piehl who has recently initiated the largest real world pragmatic drug trial, CombatMS (n=3350), which includes regular and more detailed neurological disability ratings, cognitive testing, yearly blood sampling and MRI using a national automated protocol. Within the MultipleMS effort (co-coordinated by the host applicant), an additional 100 patients will be recruited in Stockholm and followed prospectively over four years with regular biosampling and an extensive MRI protocol. We will isolate circulating cell-free DNA from plasma and CSF using the QIAamp Circulating Nucleic Acid Kit (Qiagen).

A recognized DNA methylation platform. We have experience with various state-of-the-art molecular approaches. For this project, DNA methylation levels will be quantified using both targeted sequencing and whole genome-wide bisulfite sequencing (WGBS). The later might require optimization for such low-input cfDNA samples.

Research group

A successful applicant will work in the Epigenetic group headed by Assoc. Professor Maja Jagodic at the Department of Clinical Neuroscience (CNS). Her group, currently comprising 4 post-docs, 3 PhD students and 1 bioinformatician, has been supported by Horizon2020 (European Research Council), Swedish Research Council (2008, 2012, 2014 and 2019), Wallenberg Foundation (2019) and the Swedish Brain Foundation, among others. The group is a part of the prominent Neuroimmunology unit, that gathers ~50 researchers working on different aspects of pathogenesis of MS, including genetics, epidemiology, immunology and epigenetics, with excellent clinical cohorts and experimental models. M. Jagodic is also a co-founder of the Karolinska Neuroimmunology & MS centre (KNIMS), which is a close network of established PIs committed to working collaboratively within the field of neuroimmunology.

The group is physically located at the Center for Molecular Medicine (CMM, www.cmm.ki.se), which brings together leading experts in basic and clinical research on common diseases from Karolinska Institutet and University Hospital

(~500 researchers) and is today an internationally recognized leader at the forefront of translational research (latest Cf = 2.4). Besides being a stimulating intellectual environment, CMM offers access to many state-of-the-art facilities, which M. Jagodic has been in charge of as a steering group member since 2017, e.g. Clinical Single-cell facility (10X Genomics and liquid handlers/dispensers), Flow Cytometry facility, the IT & Computing infrastructure, among others.

The CNS Department, the second-largest department at KI, conducts research and education in the field of neuroscience from the molecular level to the society level. The clinical research and education is conducted in collaboration with other research groups from the Karolinska Institutet, with other universities as well as the Stockholm County Council.

Key words
DNA methylation Neuroinflammation Neurodegeneration Sequencing Translational Research Multiple Sclerosis.

Interested in recruiting a Visiting doctoral student, a Postdoc, a Visiting researcher

Project title
Gene regulation and transcriptional control in liver metabolism

Supervisor
Claudia Kutter, Principal Investigator/Assistant Professor, Department of Microbiology, Tumor and Cell Biology (MTC)
Email: claudia.kutter@ki.se Phone: +46 852482412
Home page: https://ki.se/en/mtc/claudia-kutter-group

Type of recruitment and qualifications of applicant

- Visiting doctoral student (6-12 months)
- Postdoc (12-24 months)
- Visiting researcher (12 months)

Profile: Bioinformatics, genomics and/or transcriptomics, chromatin and/or RNA biology
Educational background:

- Postdoc: PhD in bioinformatics, computational biology or genomics with programming documented by scientific publications.
- Visiting doctoral student: currently enrolled in a PhD program in bioinformatics, computational biology or genomics.
- Visiting researcher: Master's degree in bioinformatics, biomedicine, genomics, molecular biology or equivalent with training in computational and/or experimental methods.

Research experience and skills:

- Proven experience in working with eukaryotic gene regulation, transcriptome-wide studies and/or RNA biology.
- Demonstrated familiarity with sequencing data analysis and/or genome assembly/comparison as well as statistics and modelling approaches.
- Excellent skills in computer programming (primarily Python, R, UNIX) and knowledge of biological database systems.
- Wish to integrate computational and experimental approaches.
- Enthusiastic, highly motivated, collaborative, scientifically adventurous, curiosity-driven candidate Bringing independent and original ideas into the project are welcome.
- Previous records of independent research as well as productive interactions within a multi-disciplinary team environment (e.g. first- and/or co-author publications in high profile journals) are beneficial Good communication skills (proficiency in spoken and written English).

Background

Over 200 highly specialized cells with diverse morphologies and functionalities exist in the human body, yet virtually every cell in the body contains the same genetic information. To exert cell-specific functions high fidelity mechanisms evolved to restrict the synthesis and processing of discrete sets of regulatory RNA molecules.

Abnormal cell behaviour as seen in many fatal human diseases is often the consequence of aberrant transcripts formation. The missing key in attempts to understand the functional variation is that 96-98% of the genome is not protein coding. Research in the Kutter lab focuses on identifying and characterizing the regulatory interdependencies of protein-coding and noncoding RNAs (long noncoding, transfer and small RNAs) in diseases, organ development and species evolution.

Our goal is to gain mechanistic insights into transcriptional and post-transcriptional gene regulation during cell differentiation and disease progression. We are particularly interested in revealing the origin and disease association of ncRNAs deciphering the molecular mechanism underpinning regulation by ncRNAs and implementing ncRNAs in biomedical research for diagnosis, prognostics and therapeutics.

Our approaches include developing and deploying powerful high-throughput RNA sequencing methodologies and epigenetic profiling coupled to hypothesis- and data-driven computational analysis, phenotypic characterization using CRISPR/Cas9 genome editing tools in cell lines and tissues detailed biochemical and immunological assays.

Research project description

Liver cells ensure metabolic homeostasis by regulating synthesis and breakdown of nutrients. Activating specific gene regulation programs facilitates homeostasis. Deregulation of genes has been observed upon increased and unbalanced food consumption and is linked to developing liver diseases. Hormonal treatment strategies have been effective to prevent liver diseases but the underlying molecular mechanisms that facilitate this outcome remain unknown. The aim of this project is to:

- identify how transcriptional programs are regulated to maintain cellular plasticity by using state-of-the-art chromatin- and transcriptome-sequencing techniques in human and mouse primary hepatocytes and multiple liver cancer cell lines (comprehensive dataset already available),
- investigate how gene regulatory programs get unhinged during high- and low-fat diet and measure the effect upon applying hormonal treatments in human and mouse by using comparative genomics determine predictive measurements in gene deregulation by using machine learning approaches.

This project contributes to our understanding of the molecular mechanisms employed in liver metabolism. Since deregulation of liver metabolism can lead to severe diseases, the aim is to benefit patients by providing novel prognostic and therapeutic markers.

Research group

The successful candidate will be part of a multidisciplinary and collaborative

research team active within the fields of functional and comparative genomics, chromatin and RNA biology. The Kutter lab explores the molecular mechanism by which noncoding RNAs regulate protein-coding genes and genome structure in mammalian cell lines, healthy and diseases somatic tissue. The roles of noncoding RNAs are interrogated genome- and transcriptome-wide by employing a combination of next generation sequencing technologies and high-throughput genetic screening approaches, developing computational methods, along with additional biochemical, molecular and cell biological methods (Kutter Mobile DNA, 2018; Ernst NatComm, 2017; Rudolph PLoS Genetics, 2016; Schmitt Genome Research, 2014; Kutter PLoS Genetics, 2012; Kutter Nature Genetics 2011). The group uses an integrative and collaborative approach and works closely with experimental and computational groups. The Kutter lab has daily interactions, weekly joint group meetings and journal clubs with the labs of Vicent Pelechano, Marc Friedlander and Erik Sonnhammer. Thereby the groups have created a larger constellation of 30+ researchers with an exceptional presence of knowledge and expertise. The group owns a Next Generation sequencer facilitating data provision in a quickly manner. Frequent courses are provided on campus. Additional professional training is available through summer schools and workshops. The work will be conducted at SciLifeLab in Stockholm, a national center for large-scale life science research with an advanced technological infrastructure. SciLifeLab performs multidisciplinary research based on DNA sequencing, gene expression analysis, proteomics, bioinformatics, biostatistics and systems biology. Bioinformatics and systems biology are significant key activities within the center. The medical link is provided by strong interaction with the Karolinska Institute.

Key words
Genomics, transcriptomics, evolutionary biology, CRISPR technology, RNA binding proteins, RNA biology, transposon biology, long noncoding RNAs, transfer RNAs, piwi-interacting RNAs.

Interested in recruiting a Doctoral student

Project title

Prevention of oesophageal adenocarcinoma.

Supervisor

Jesper Lagergren, Professor, Department of Molecular medicine and Surgery
Email: jesper.lagergren@ki.se Phone number: +46 85177 6012
Home page: https://staff.ki.se/people/jeslag

Type of recruitment and qualifications of applicant

Doctoral student (48 months)

Qualifications:

- Documented interest in cancer research, ideally with a specific interest in upper gastrointestinal cancer
- Medical education, typically a medical degree
- Interest, knowledge and skills in epidemiological methods and biostatistics
- Documented research experience, preferably within the field of causes, prevention and treatment of cancer diseases
- Excellent knowledge in English, both orally and written
- Friendly and open-minded personality with good skills in communication and collaboration.

Background

Oesophageal adenocarcinoma (OAC) is characterised by increasing incidence, strong male predominance, extensive treatment and poor prognosis, highlighting the need for preventive actions. OAC is preceded by the metaplastic condition Barrett's oesophagus (BO), readily identified at endoscopy and biopsy. With a limited prevalence of BO in adults in Western populations (1%), individuals with BO constitute a target group for preventive actions of OAC.

Obesity is more strongly associated with OAC than other cancer. Yet, it is unknown if weight loss prevents OAC. Obesity surgery is a useful means of assessing the effects of weight loss because the weight loss starts at a specific date and is substantial and persistent. If obesity surgery prevents OAC is unknown. Existing studies have had too few OAC cases after obesity surgery to examine this question.

The male-to-female ratio in OAC is 6-to-1 (up to 9-to1). The reasons for this striking male predominance are unknown, but differences in sex hormone levels, particularly oestrogens, are likely involved. Limited data indicate that oestrogen-containing medications, e.g. menopausal hormone therapy and oral contraceptives decrease OAC risk.

The bacterium Helicobacter pylori (HP) colonises the stomach in >50% of the global population. Interestingly, individuals with HP have 36-44% decreased risk of OAC. HP might cause gastric atrophy, lowering acid production, and counteracting BO

development. No study has examined if eradication of HP (with antibiotics and proton pump inhibitor) increases OAC risk.

There is a great need for very large cohort studies with long and complete follow-up to understand if OAC is prevented by weight loss (here studied using obesity surgery), sex hormonal therapy (here studied using menopausal hormone therapy and oral contraceptives) or avoidance of HP eradication in high-risk people. The answers to these questions may lay the ground for research assessing tailored preventive strategies of OAC in individuals with BO.

Research project description

Study 1: Obesity surgery and risk of oesophageal adenocarcinoma (OAC)

Design: This will be a population-based cohort study in all Nordic countries of adults with obesity diagnosis in the patient registries. Operated (obesity surgery) and non-operated patients are compared for OAC risk (from the cancer registries). The study period 1980-2019 allows up to 40 years of follow-up.

Novelty: The first sufficiently powered study to assess obesity surgery and OAC risk.

Analysis: First, the incidence of OAC in the obesity surgery cohort will be compared with the incidence in the corresponding background population, providing standardised incidence ratios (SIRs) with 95% confidence intervals (CI). Second, Cox regression will compare OAC incidence in operated and non-operated obese patients, providing hazard ratios (HRs) with 95% CIs, adjusted for confounders, including comorbidity. All analyses will assess changes in risk over various lengths of follow-up: 1-5 years, 6-10 years, 11-15 years, 16-20 years and >20 years.

Power: With >700,000 individuals in the obesity cohort and >70,000 having undergone obesity surgery, the study has ≥80% power to verify HRs ≤0.8.

Experience: We have published on obesity surgery in renowned journals (e.g. Gastroenterology and Ann Surg).

Status: We lead a group of researchers in all Nordic countries and have collected an obesity cohort with data up to year 2012 with 506,826 participants, of whom 57,283 had bariatric surgery. This now planned update of the cohort will add data to 2019.

Studies 2 + 3: Menopausal hormone therapy and oral contraceptives and OAC risk

Design: Swedish population-based cohort study in 2005-2019, based on data from the Swedish Prescribed Drug Registry and other relevant national registries (for cancer, patients, death). Users and non-users of menopausal hormone therapy and oral contraceptives will be compared for OAC risk.

Novelty: The largest study to date to assess menopausal hormone therapy and oral contraceptives and OAC risk.

Analysis: Cox regression will be used to provide HRs with 95% Cis, adjusted for confounders, including comorbidity.

Power: With >9 million individuals in the cohort the study has ≥80% power to verify HRs ≤0.6.

Experience: We have published original studies on sex hormones and OAC and reflux disease in renowned journals (e.g. JAMA, Gastroenterology, Gut).

Status: We have a cohort of 8.4 million participants (PMID: 30696673), and will now increase the number of participants further and also increase the length of follow-up until 2019.

Study 4: Helicobacter pylori eradication and risk of OAC.

Design: Population-based Nordic cohort study based on national medication registries. HP-eradicated and non- eradicated participants will be compared for OAC risk. The study period 1994-2019 allows for up to 26 years of follow-up. The study exposure is HP-eradication treatment using a proton pump inhibitor) in combination with at least two of the antibiotics clarithromycin, amoxicillin or metronidazole.

Novelty: The first ever study assessing OAC risk after HP-eradication. Analysis: Cox regression will provide HRs with 95% CIs, djusted for several potential confounders, including comorbidity.

Power: With >15 million individuals in the cohort and >400,000 having had eradication therapy, the study has ≥80% power to verify HRs ≥1.2.

Experience: We have published on eradication therapy and gastric cancer (e.g. Gut, JNCI).

Status: We will establish this new cohort for the period 2005-2019.

Significance
There is a great need for valid research to clarify if obesity surgery, female sex hormone medication or HP eradication influence OAC risk. There is a need for very large cohorts with long and complete follow-up. We have excellent possibilities to do this by merging nationwide datasets from the Nordic countries. This will bring new and extremely valuable information for the possibilities of preventing OAC in studies that are possible to conduct only in the Nordic countries.

Research group

Our research group was rated ‘Outstanding’ (highest) by the last external assessment of all research groups at Karolinska Institutet (“ERA2010”). We are 30 members: 8 PhD students (2 with CSC scholarship), 1 professor, 1 executive assistant, 2 biostatisticians, 2 assistant professors, 1 medical student and 15 affiliated researchers (mainly clinicians). We share office space with another research group of 18 people (including 2 with CSC scholarship). Thus, many people are in the office every day, meaning that the PhD student will have excellent opportunities for academic interactions and discussions with people with various expertise. Our PhD education is well-organised. We have weekly meetings with the supervisors and monthly written reports to the supervisors. We have bi-weekly ‘Research Seminars’, monthly ‘Journal Clubs’ for PhD students (together with a teacher), and monthly ‘Methods Club’. Each study is carefully planned. We use a ‘study protocol approach’ where each new study is planned in detail before it is

started. The protocols are revised and discussed at study protocol meetings several times where all authors participate. As first author, the PhD student drafts and revises the study protocol. We have shared 'Planning Days', 'End-of-Term Seminars' and 'Brainstorming Meetings' for planning of all our activities.

The research group scores high on employee surveys. In the last survey, the index scores were 90 (out of 100) for 'working climate' and 91 for 'leadership', which could be compared with the corresponding average scores at Karolinska Institutet of 71 and 75, respectively. The supervisors have expertise in oesophageal cancer research, with many papers published in the world leading journals of general medicine, oncology, surgery and gastroenterology. They have completed projects of similar design as in this and supervise other PhD students from China funded by CSC. Thus, they have the know-how to complete the project and supervision.

Keywords

Cancer; Neoplasm; Oesophageal; Adenocarcinoma; Aetiology; Epidemiology; Prevention; Cohort; Clinical research; Gastroenterology; Oncology; Surgery; Obesity surgery; Bariatric surgery; Sex; Oestrogen; Helicobacter pylori; eradication.

Supplementary information

This project is based on data from nationwide registries in Sweden and the other four Nordic countries. Thus, experience and interest in modern epidemiological methods and biostatistics is very helpful.

The student will read and assess literature, produce study protocols, collect and manage data, perform analyses and write manuscripts. The PhD courses in e.g. epidemiology, statistics and study design will provide valuable knowledge.

The supervisors include the main supervisor Jesper Lagergren who is professor of surgery and international authority in oesophageal cancer and has supervised 39 PhD students to successful thesis defence.

Giola Santoni has a PhD in computer engineering and also a PhD in epidemiology. She is experienced biostatistician with specific knowledge in analyses of large cohort studies.

Shaohua Xie is assistant professor in cancer epidemiologist. He is originally from China and has with excellent knowledge in oesophageal cancer, study design and pharmaco- epidemiology.

Interested in recruiting a Postdoc

Project title
Prevention of gastric cancer

Supervisor
Jesper Lagergren, Professor, Department of Molecular medicine and Surgery
Email: jesper.lagergren@ki.se Phone number: +46 85177 6012
Home page: https://staff.ki.se/people/jeslag

Type of recruitment and qualifications of applicant
Postdoc (24 months)

The successful candidate should have the following qualifications:

- Documented experience in cancer research, ideally with a specific knowledge in upper gastrointestinal cancer
- Medical education, e.g. a medical degree
- Clinical experience from cancer patients is qualifying
- Interest and documented knowledge and skills in epidemiological methods and biostatistics
- Documented research experience, preferably within the field of causes, prevention and treatment of cancer diseases
- Good track record in writing scientific manuscripts in English
- Excellent knowledge in English, both orally and written
- Friendly and open-minded personality with good skills in communication and collaboration

Background
Gastric adenocarcinoma (GAC), including gastric cardia and non-cardia adenocarcinoma, is responsible for 783,000 deaths worldwide (1 in 12 deaths globally), making it the 3rd leading cause of cancer death. The treatment of GAC is extensive and the prognosis is poor. These facts highlight the great need for preventive actions. Obesity is associated with a moderately increased risk of non-cardia adenocarcinoma and a strongly increased risk of gastric cardia adenocarcinoma. Yet, it is unknown if weight loss counteracts GAC. Obesity surgery is a useful means of assessing effects of weight loss because it results in substantial and persistent weight loss, which starts from a specific date. If obesity surgery prevents GAC is unknown.

The male-to-female ratio in GAC is 2-to-1, and as high as 6-to-1 for cardia adenocarcinoma. The reasons for this male predominance are unknown, but differences in sex hormone levels, particularly oestrogens, may be involved. Limited data indicate that menopausal hormone therapy and oral contraceptives decrease GAC risk, but larger and valid studies are needed. The bacterium Helicobacter pylori (HP) is the major causal risk factor for GAC. HP colonises the stomach in

>50% of the global population, with wide variations across regions. The prevalence of HP is much higher in Eastern compared to Western populations. Yet, only two studies from Western populations have examined if eradication of HP (with antibiotics and proton pump inhibitor) prevents GAC development.

There is a great need for large cohort studies with long and complete follow-up to better understand if GAC is prevented by weight loss, sex hormonal therapy or avoidance of HP eradication in Western populations. The answers to these questions could lay the foundation for research assessing tailored preventive strategies of GAC.

Research project description

Obesity surgery and gastric adenocarcinoma (GAC)

Design: Population-based cohort study in all Nordic countries of adults with obesity diagnosis in the patient registries. Operated (obesity surgery) and non-operated patients will be compared for GAC risk (from the cancer registries). The study period 1980-2019 allows up to 40 years of follow-up.

Novelty: The first sufficiently powered study to assess obesity surgery and GAC risk.

Analysis: GAC risk will be analysed using two approaches. First, the incidence of GAC in the obesity surgery cohort will be compared with the incidence in the corresponding background population, providing standardised incidence ratios (SIRs) with 95% confidence intervals (CI). Second, Cox regression will compare GAC incidence in operated and non-operated obese patients, providing hazard ratios (HRs) with 95% CIs, adjusted for confounders, including comorbidity. All analyses will assess changes in risk over various lengths of follow-up: 1-5 years, 6-10 years, 11-15 years, 16-20 years and >20 years.

Power: With >700,000 individuals in the obesity cohort and >70,000 having undergone obesity surgery, the study has ≥80% power to verify HRs ≤0.9.

Experience: We have published on obesity surgery in renowned journals (e.g. Gastroenterology and Ann Surg).

Status: We lead a group of experienced researchers in all Nordic countries and have collected an obesity cohort with data up to year 2012 with 506,826 participants, of whom 57,283 had obesity surgery. This now planned update of the cohort will add data to 2019.

Female sex hormone medication and GAC

Design: Swedish population-based cohort study in 2005-2019, based on data from the Swedish Prescribed Drug Registry and other relevant national registries (for cancer, patients, death). Users and non-users of menopausal hormone therapy and oral contraceptives will be compared for GAC risk.

Novelty: The largest study to date to assess menopausal hormone therapy and oral contraceptives and GAC risk.

Analysis: Cox regression will be used to provide HRs with 95% CIs, adjusted for confounders, including comorbidity.

Power: With >9 million individuals in the cohort the study has ≥80% power to verify HRs ≤0.8.

Experience: We have published original studies on sex hormones and GAC in renowned journals (e.g. Int J Cancer, Eur J Cancer).

Status: We have a cohort of 8.4 million participants (PMID: 30696673), and can now increase the number of participants further and also increase the length of follow-up until 2019.

Helicobacter pylori eradication and GAC

Design: Population-based Nordic cohort study based on national medication registries. HP-eradicated and non- eradicated participants will be compared for GAC risk (retrieved from the cancer registries). The study period 1994-2019 allows for up to 26 years of follow-up. The study exposure is HP-eradication treatment using a proton pump inhibitor) in combination with at least two of the antibiotics clarithromycin, amoxicillin or metronidazole.

Novelty: The largest study assessing the risk of GAC after HP-eradication and the third from a Western population.

Analysis: Cox regression will be used, providing HRs with 95% CIs. The HRs will be adjusted for several potential confounders, including comorbidity.

Power: With >15 million individuals in the cohort and >400,000 having had eradication therapy, the study has ≥80% power to verify HRs ≤0.9.

Experience: We have published on eradication therapy and GAC (e.g. Gut, JNCI).

Status: We will establish this new cohort for the period 2005-2019.

Significance
There is a great need for valid research to test if obesity surgery, sex hormone medication, or HP eradication influences GAC risk. This requires very large cohorts with long and complete follow-up. We have excellent possibilities to do this by merging nationwide data in Sweden and the other Nordic countries. This will bring novel possibilities of preventing GAC in studies that are possible to conduct only in the Nordic countries.

Research group

Our research group was rated ‘Outstanding’ (highest possible) in Cancer Research by the last external assessment of all research groups at Karolinska Institutet (“ERA2010”). The group has 30 members: 1 professor, 2 assistant professors, 1 executive assistant, 2 biostatisticians, 8 PhD students, 1 medical student and 15 affiliated researchers (mainly clinicians). We share office space with another research group of 18 people. Thus, many researchers are in the office every day, meaning that the postdoc will have excellent opportunities for academic interactions and discussions with researchers with various expertise. Our postdoc education is well-organised, including weekly meetings with the supervisor, bi-weekly ‘Research Seminars’, monthly ‘Journal Clubs’, and monthly ‘Methods Club’.

Each study is carefully planned. We use a 'study protocol approach' where each new study is planned in as much detail as possible before it is started. The protocols are revised and discussed at study protocol meetings several times where all authors participate. As first author, the postdoc drafts and revises the study protocol. We have shared 'Planning Days', 'End-of-Term Seminars', and 'Brainstorming Meetings' for planning of future activities.

The research group scores very high on employee surveys. In the last survey, the index scores were 90 (out of 100) for 'working climate' and 91 for 'leadership', which could be compared with the corresponding average scores at Karolinska Institutet of 71 and 75, respectively.

The supervisor (Professor Jesper Lagergren) is a highly internationally recognised in the field of gastric cancer research, with publications in all the world leading journals of general medicine, oncology, surgery and gastroenterology. He has led and completed several projects of similar design as in this now planned and has supervised 18 postdocs before. Thus, he has the know-how to complete the project and supervision of the postdoc.

Keywords

Cancer; Neoplasm; Gastric; Stomach; Adenocarcinoma; Aetiology; Epidemiology; Prevention; Cohort; Risk factor; Clinical research; Gastroenterology; Oncology; Surgery, Hormone; Obesity surgery; Bariatric surgery; Sex; Oestrogen; Menopausal hormone therapy; Hormone replacement therapy; Oral contraceptive; Helicobacter pylori; Eradication.

Supplementary information

This project is based on data from nationwide registries in Sweden and the other four Nordic countries. Thus, experience and interest in epidemiological methods and biostatistics is very helpful for the postdoc to be recruited. The postdoc will read and assess literature, produce study protocols, collect and manage data, perform analyses and write manuscripts. The supervisor is Jesper Lagergren who is professor of surgery and international authority in oesophageal and gastric cancer research.

Karolinska Institutet

Interested in recruiting a Postdoc

Project title

Prevention of gastric cancer

Supervisor

Jesper Lagergren, Professor, Department of Molecular Medicine and Surgery
Email: jesper.lagergren@ki.se Phone number: +46 85177 6012
Home page: https://staff.ki.se/people/jeslag

Type of recruitment and qualifications of applicant

Postdoc (24 months)

The successful candidate should have the following qualifications:

- Documented experience in cancer research, ideally with a specific knowledge in upper gastrointestinal cancer
- Medical education, ideally a medical degree
- Clinical experience from cancer patients
- Interest and documented knowledge and skills in epidemiological methods and biostatistics
- Documented research experience, preferably within the field of causes, prevention and treatment of cancer diseases
- Good track record in writing scientific manuscripts in English
- Excellent knowledge in English, both orally and written
- Friendly and open-minded personality with good skills in communication and collaboration

Background

Gastric adenocarcinoma (GAC), including gastric cardia and non-cardia adenocarcinoma, is responsible for 783,000 deaths worldwide (1 in 12 deaths globally), making it the 3rd leading cause of cancer death. The treatment of GAC is extensive and the prognosis is poor. These facts highlight the great need for preventive actions. Obesity is associated with a moderately increased risk of non-cardia adenocarcinoma and a strongly increased risk of gastric cardia adenocarcinoma. Yet, it is unknown if weight loss counteracts GAC. Obesity surgery is a useful means of assessing effects of weight loss because it results in substantial and persistent weight loss, which starts from a specific date. If obesity surgery prevents GAC is unknown.

The male-to-female ratio in GAC is 2-to-1, and as high as 6-to-1 for cardia adenocarcinoma. The reasons for this male predominance are unknown, but differences in sex hormone levels, particularly oestrogens, may be involved. Limited data indicate that menopausal hormone therapy and oral contraceptives decrease GAC risk, but larger and valid studies are needed. The bacterium Helicobacter pylori (HP) is the major causal risk factor for GAC. HP colonises the stomach in >50% of the global population, with wide variations across regions. The prevalence of HP is much higher in Eastern compared to Western populations. Yet, only two

studies from Western populations have examined if eradication of HP (with antibiotics and proton pump inhibitor) prevents GAC development.
There is a great need for large cohort studies with long and complete follow-up to better understand if GAC is prevented by weight loss (obesity surgery), sex hormonal therapy (menopausal hormone therapy and oral contraceptives) or avoidance of HP eradication in Western populations. The answers to these questions could lay the foundation for research assessing tailored preventive strategies of GAC in high-risk individuals, e.g. those with heredity for this devastating cancer.

Research project description

Obesity surgery and gastric adenocarcinoma (GAC)
Design: Population-based cohort study in all Nordic countries of adults with obesity diagnosis in the patient registries. Operated (obesity surgery) and non-operated patients will be compared for GAC risk (from the cancer registries). The study period 1980-2019 allows up to 40 years of follow-up.

Novelty: The first sufficiently powered study to assess obesity surgery and GAC risk.

Analysis: GAC risk will be analysed using two approaches. First, the incidence of GAC in the obesity surgery cohort will be compared with the incidence in the corresponding background population, providing standardised incidence ratios (SIRs) with 95% confidence intervals (CI). Second, Cox regression will compare GAC incidence in operated and non-operated obese patients, providing hazard ratios (HRs) with 95% CIs, adjusted for confounders, including comorbidity. All analyses will assess changes in risk over various lengths of follow-up: 1-5 years, 6-10 years, 11-15 years, 16-20 years and >20 years.

Power: With >700,000 individuals in the obesity cohort and >70,000 having undergone obesity surgery, the study has ≥80% power to verify HRs ≤0.9.
Experience: We have published on obesity surgery in renowned journals (e.g. Gastroenterology and Ann Surg).

Status: We lead a group of experienced researchers in all Nordic countries and have collected an obesity cohort with data up to year 2012 with 506,826 participants, of whom 57,283 had obesity surgery. This now planned update of the cohort will add data to 2019.

Female sex hormone medication and GAC

Design: Swedish population-based cohort study in 2005-2019, based on data from the Swedish Prescribed Drug Registry and other relevant national registries (for cancer, patients, death). Users and non-users of menopausal hormone therapy and oral contraceptives will be compared for GAC risk.

Novelty: The largest study to date to assess menopausal hormone therapy and oral contraceptives and GAC risk.

Analysis: Cox regression will be used to provide HRs with 95% CIs, adjusted for confounders, including comorbidity.

Power: With >9 million individuals in the cohort the study has ≥80% power to verify HRs ≤0.8.

Experience: We have published original studies on sex hormones and GAC in renowned journals (e.g. Int J Cancer, Eur J Cancer).Status: We have a cohort of 8.4 million participants (PMID: 30696673), and can now increase the number of participants further and also increase the length of follow-up until 2019.

Helicobacter pylori eradication and GAC

Design: Population-based Nordic cohort study based on national medication registries. HP-eradicated and non- eradicated participants will be compared for GAC risk (retrieved from the cancer registries). The study period 1994-2019 allows for up to 26 years of follow-up. The study exposure is HP-eradication treatment using a proton pump inhibitor) in combination with at least two of the antibiotics clarithromycin, amoxicillin or metronidazole.

Novelty: The largest study assessing the risk of GAC after HP-eradication and the third from a Western population.

Analysis: Cox regression will be used, providing HRs with 95% CIs. The HRs will be adjusted for several potential confounders, including comorbidity.

Power: With >15 million individuals in the cohort and >400,000 having had eradication therapy, the study has ≥80% power to verify HRs ≤0.9.

Experience: We have published on eradication therapy and GAC (e.g. Gut, JNCI).

Status: We will establish this new cohort for the period 2005-2019.

Significance
There is a great need for valid research to test if obesity surgery, sex hormone medication, or HP eradication influences GAC risk. This requires very large cohorts with long and complete follow-up. We have excellent possibilities to do this by merging nationwide data in Sweden and the other Nordic countries. This will bring novel possibilities of preventing GAC in studies that are possible to conduct only in the Nordic countries.

Research group

Our research group was rated ‘Outstanding’ (highest possible) in Cancer Research by the last external assessment of all research groups at Karolinska Institutet (“ERA2010”). The group has 31 members: 1 professor, 2 assistant professors, 1 executive assistant, 2 biostatisticians, 8 PhD students, 1 medical student and 16 affiliated researchers (mainly clinicians). We share office space with another research group of 18 people. Thus, many researchers are in the office every day, meaning that the postdoc will have excellent opportunities for academic interactions and discussions with researchers with various expertise. Our postdoc education is well-organised, including weekly meetings with the supervisor, bi-weekly ‘Research Seminars’, monthly ‘Journal Clubs’, and monthly ‘Methods Club’.

Each study is carefully planned. We use a ‘study protocol approach’ where each new study is planned in as much detail as possible before it is started. The protocols are revised and discussed at study protocol meetings several times where all authors participate. As first author, the postdoc drafts and revises the study protocol. We have shared ‘Planning Days’, ‘End-of-Term Seminars’, and ‘Brainstorming Meetings’ for planning of future activities.

The research group scores very high on employee surveys. In the last survey, the index scores were 90 (out of 100) for 'working climate' and 91 for 'leadership', which could be compared with the corresponding average scores at Karolinska Institutet of 71 and 75, respectively.

The supervisor (Professor Jesper Lagergren) is a highly internationally recognised in the field of oesophageal cancer research, with publications in all the world leading journals of general medicine, oncology, surgery and gastroenterology. He has led and completed several projects of similar design as in this now planned and has supervised 18 postdocs before. Thus, he has the know-how to complete the project and supervision of the postdoc.

Key words

Cancer; Neoplasm; Gastric; Stomach; Adenocarcinoma; Aetiology; Epidemiology; Prevention; Cohort; Risk factor; Clinical research; Gastroenterology; Oncology; Surgery, Hormone; Obesity surgery; Bariatric surgery; Sex; Oestrogen; Menopausal hormone therapy; Hormone replacement therapy; Oral contraceptive; Helicobacter pylori; Eradication.

Supplementary information

This project is based on data from nationwide registries in Sweden and the other four Nordic countries. Thus, experience and interest in epidemiological methods and biostatistics is very helpful for the postdoc to be recruited. The postdoc will read and assess literature, produce study protocols, collect and manage data, perform analyses and write manuscripts. The supervisor is Jesper Lagergren who is professor of surgery and international authority in oesophageal and gastric cancer research.

Interested in recruiting a Postdoc and a Visiting researcher

Project title

Platelet-regulated inflammatory mechanisms in atherosclerosis.

Supervisor

Nailin Li, Associate professor, Department of Medicine, Solna
Email: nailin.li@ki.se Phone: +46-8-51773996

Type of recruitment and qualifications of applicant

- Postdoc (24 months)
- Visiting researcher (12 months)

Applicants to this position must have a doctoral degree in medicine, biomedicine, or life sciences conferred during last five years. The applicant should be self-motivated and wish to conduct research independently. The successful applicant should have expertise in cell culture, flow cytometry, and basic cellular and molecular biology techniques. Previous research experience in T cell and macrophage immune/inflammatory responses and/or animal studies of atherosclerosis is an advantage. Good communication skill in English is a prerequisite. Teamwork spirit is also an important qualification.

Background

Atherosclerotic cardiovascular diseases are the leading cause of morbidity and mortality in humans. Atherosclerosis is an inflammatory and thrombotic disease. Platelets not only dominate thrombotic mechanisms but also closely regulate inflammatory mechanisms in atherosclerosis. Hence, antiplatelet treatment has been established as the cornerstone for prevention and treatment of the diseases. Current antiplatelet treatment targeting platelet activation per se has reached its therapeutic plateau, which can only provide ~1/3 cardiovascular protection. Thus, there is a need for optimizing antiplatelet treatment. Intervention of platelet-regulated vascular inflammation and vessel remodelling in atherogenesis may be a promising strategy for future therapeutic developments.

Research project description

We and others have recently shown that platelets distinctly regulate immune responses of different CD4+ T cell subsets, and that platelets closely regulate macrophage functions. Aim of the project is thus to elucidate the impact of platelet-regulated vascular inflammation on the development of atherosclerotic lesions. We are investigating the mechanisms underlying platelet regulation of CD4+ T effector responses of T helper (Th1 and Th17) cells and regulatory T (Treg) cells. We will study platelet influence on M1 and M2 polarization and plasticity. Using various murine models, we are studying how platelet deficiency of specific inflammatory mediators affects CD4+ T effector responses and M1-M2 balance in vivo and how the deficiency influences atherosclerotic lesion formation in a pro-atherosclerotic mouse model. The work may lead to novel therapeutic

developments for atherosclerotic disease management.

Research group

The supervisor has extensive research experience in pre-clinical and clinical research on thrombotic mechanisms in atherothrombotic diseases, as well as evaluation of antiplatelet drugs. The research group currently focus on research of thrombosis-inflammation crosstalk in atherogenesis. The group used to maintain a team of 4-5 researchers.

Key words

Thrombosis, inflammation, atherosclerosis, platelets, CD4 T cells, macrophages

Interested in recruiting a Doctoral student and a Postdoc

Project title

Function and differentiation of newly discovered cell types in the enteric nervous system.

Supervisor

Ulrika Marklund, Doctor, Department of Medical Biochemistry and Biophysics
Email: ulrika.marklund@ki.se Phone number: +46702931870
Home page: https://ki.se/en/mbb/ulrika-marklund-group

Type of recruitment and qualifications of applicant

- Doctoral student (48 months)
- Postdoc (24 months)

Applications are invited for both a PhD student and a Postdoctoral Fellow to work on the described project. All projects require collaboration between members of the lab and other labs, hence the applicants (both for the PhD and postdoc positions) need to be good team-players and understand the value of constructive interactions. Good English skills is a requirement. The applicants are expected to have a high degree of motivation and work in a structured manner.
The Postdoctoral Fellow must hold a PhD degree (or expected to hold in 2020; not older than 3 years) in the fields of neurobiology, gastroenterology or developmental biology. Experience in relevant techniques including transgenic mouse models, histochemical methodologies, cell dissociation, cell tracing, bioinformatics and/or gut physiology assays are particularly meritorious but not a prerequisite.

The applicant for the PhD position must hold (or expected to hold in 2020) a master's degree in molecular biology/equivalent or Degree in Medicine. Previous experience in lab work including basic molecular biology methods and animal work are particularly meritorious. Prior knowledge in computing is advantageous for the project but not a must.

Background

The gastrointestinal tract is the only visceral organ with an own intrinsic nervous system. This enteric nervous system (ENS) is classically known to regulate basic gut physiology (peristalsis), but a broader functional significance of the ENS is apparent by its recently demonstrated communication with the immune system. Neuropathy of the ENS contributes to congenital, degenerative and inflammatory disorders that lack satisfactory treatment. While bowel segments are completely devoid of ENS neurons in patients with Hirschsprung disease, only subsets of neurons are affected

in other ENS disorders (e.g. diabetic gastroparesis). The diversity of enteric neuron subtypes is thus critical for normal gut function. How ENS is affected in Irritable Bowel Syndrome (IBS) and Inflammatory Bowel Disease (IBD) has yet to be addressed.

We surmise that a clear categorization of ENS neuron components and their range of functions is imperative to further understand abnormalities within the ENS and its contribution to disease. The host lab has recently identified and mapped ENS neuron subtypes based on transcriptome analysis of single cells in the outer layer gut (Cell, 2018). Using the same technology, also the inner-most ganglia (manuscript) have been analyzed. The proposed project builds on the transcriptome resources coupled with new methodologies allowing selective targeting of adult ENS neurons and aims to determine the functional role of individual ENS neurons, their interaction with other cell types and how they link to inflammation.

A second research focus of the lab is the developmental formation of the ENS neuron subtypes. The proposed project will utilize already obtained data sets (transcriptomes, histochemical resource) and tools (viral gene- targeting of the embryonic ENS) to decipher gene regulatory programs that could be employed to manipulate cell fate determination in the developing and adult ENS.

Research project description

The project aims to achieve a conceptually new understanding of the function (A) and development (B) of neuronal constituents of the enteric nervous system (ENS). The overall project design is comprehensive but modulatory and can be adapted to fit only one new member or alternatively one PhD student and one Postdoctoral Fellow. The unique data sets and techniques recently established in the host lab offers new members fruitful research avenues.

Project A. Determining enteric neural cell function in the healthy and inflamed gut. The host lab has generated a novel genetic classification of the outer (myenteric; Cell, 2018) and inner (submucosal; manuscript) layer gut neurons based on gene expression profiles of single cells. To decipher the function of these neuron types, Cre-driver mouse lines enabling cell-specific targeting have been acquired. Constructs delivered to these mice by a specific type of AAV-virus will achieve class-specific visualization, ablation/silencing and gene-editing. The new members will use these tools to determine:

I. the connectivity patterns of newly discovered enteric neuron subtypes. Neurons and their projections will be visualised by introducing Cre-dependent fluorescent viral reporters into class-specific Cre-mice. By additional histochemical detection of target cell types (endothelial cells, glands, muscle cells, immune cells) physiological functions and neuro-immune units will be assigned.
II. how neuro-immune units are affected in mouse model of IBD. Single ENS and immune cells from a chemical mouse model of IBD and wildtype mice will be isolated, and transcriptomes established. Computational analysis will reveal

vulnerable ENS cell populations and determine inflammation-induced signaling in the neuro-immune interface.

III. how enteric neuron types contribute to the immunological homeostasis. Using the AAV-mediated targeting, specific enteric neuron types will be silenced or ablated. The susceptibility of the animals to spontaneous microbiota-driven inflammation and experimental IBD will be assessed. Functional assays will reveal affects in gut physiology (secretion, peristalsis).

Impact: The project will enable a better understanding of the functional cellular units within the gut's own nervous system. In particular, it has potential to discover ENS-immune interplay that maintain or break immunological homeostasis, a steppingstone for further translational studies of Inflammatory Bowel Disease.

Project B. Determining differentiation programs of enteric neuron subtypes as basis to manipulate neuron identity in the adult ENS.
Neuropathy affects specific neuron subtypes in several gut disorders. Recent progress in enteric stem cell research has incited hope for cell-based therapies, but challenges remain for their implementation. To achieve functional recovery, a balanced cellular constitution needs to be re-created - this stresses the importance of defining the developmental pathways of enteric neuron subtypes. Towards this end, the host lab has recently identified a new principle of enteric neuron diversification through transcriptome analysis of the developing ENS (manuscript) and a histochemical expression atlas (Gastroenterology, 2018). These gene expression resources coupled with new gene-manipulation technologies forms the basis for the proposed project.

I. First, specific gene regulatory programs of enteric neuron subtypes in the developing gut will be determined. This will be achieved by systematic assessment of candidate gene function by in utero ultra-sound guided transduction, a methodology developed in a collaborator lab at KI.

II. Secondly, using the molecular insights obtained above, we will attempt to modify cell identities in adult ENS using AAV-mediated gene-expression described in project A.

Impact: The outcome of these experiments might provide a basis for cell engineering strategies for the future purpose of restoring a balanced enteric cell composition in gut neuropathies.

Research group

Dr. Marklund has a long-standing interest in understanding the developmental, molecular and functional foundation of neural cell types. At the doctoral level, she discovered key molecular developmental mechanisms, which was reconstructed in stem cell cultures to derive midbrain neurons of relevance for Parkinson's disease (Cell, 2006). She then identified the Enteric Nervous System (ENS) as fundamentally under-investigated and collected post-doctoral training in the UK. Focusing on the differentiation and function of ENS neuron subtypes, her group has contradicted developmental dogmas set in the ENS field and provided a new ENS

classification. Marklund's lab is situated in a highly stimulating unit of 7 research groups conducting research in neurobiology using state-of-the-art technologies. Having a collaborative spirit, we share laboratory equipment but also exchange scientific ideas at unit seminars, retreats and daily interactions. We are located within Biomedicum (https://ki.se/en/about/biomedicum-laboratory-of-the-future), a newly opened research center at Karolinska Institute, giving in-house access to a range of facilities and a unique environment for research exchange with complementary competencies. The building is in direct connection with the Karolinska Hospital facilitating translational research and at close distance to the SciLifeLab providing transcriptome-based analysis. Marklund lab participate in several local and international seminar series where the new members will create wider networks and receive project inputs. International meetings is seen as a very important part of the research period in the lab. The Marklund group currently consist of a PhD student and a postdoctoral fellow. The PhD student performs the bioinformatic analysis of the projects, while the postdoctoral fellow with immunological background has set up the methodologies needed for detailed imaging of enteric neuron subtypes and their involvement in neuro-immune networks.

Key words

Enteric Nervous System, Nervous System, Neuron Subtypes, Gastrointestinal Tract, Gut, Intestine, Neuron, Cell Differentiation, Developmental Biology, Inflammatory Bowel Disease, Hirschsprung Disease, Gastroparesis, Neurobiology, single cell transcriptome, transcriptomics, histochemistry, transcription factor, AAV, Regeneration Gene-manipulation

Interested in recruiting a Visiting researcher

Project title

Role of bacterial co-infection in the immune regulation of Epstein-Barr virus oncogenesis.

Supervisor

Maria Grazia Masucci, Professor, Department of Cell and Molecular Biology
Email: maria.masucci@ki.se Phone: +46705448690

Type of recruitment and qualifications of applicant

Visiting researcher (12 months)

- Location and Functional Study of Conserved Amino Acids in Newcastle Disease Virus HN Protein. Site- directed mutations were used to construct the mutants of high conserved amino acids in HN head.
- Biological function of mutations was detected by FACS, IFA and Co-IP assays to study the roles of key amino acid in HN head.
- Phylogenetic Analysis and Full-length cDNA Construction of NDV BJ Strain. A wild virus strain was isolated from dead chickens. We named it NDV-BJ strain and it was purified by limited dilution method. Virulence was detected by mean death time in 8 days embryo eggs, intracerebral pathogenicity index in 1day chicks and hemagglutination test, respectively. The virus was amplified in chicken primary fibroblasts and purified by ultracentrifugation. The NDV polyclonal antibody was obtained by inoculation virus into 1- month old chickens. Complete genome sequencing and phylogenetic analysis of NDV BJ strain were confirmed. Full-length cDNA was constructed into plasmid vector to rescue the NDV BJ strain.
- Research in enterovirus 71 isolation and purification. The wild virus originated from children infected with EV71 virus with severe neurological complications. The purified wild strains were sequenced and inoculated into the brain of suckling mice for virulence test. And wild EV71 was rescued in vitro. After graduation from doctoral, I still studied the oncolytic effect and mechanism of NDV. The aim of the study is to introduce exogenous cytokines genes into the genome of NDV to obtain recombinant viruses with higher oncolytic effect.
- Experimental skills: Cell culture; Primary cell culture; Virus isolation; Virus purification; Virus rescue; Molecular cloning technology; Transfection; ELISA; RT-qPCR; Antibody preparation; Protein function research; Cell proliferation assay; Eukaryotic expression of protein; Prokaryotic expression of proteins; Protein purification; Flow cytometry; Western bolt; Animal experiments(Mouse, Guinea Pig, RabbitChicken Embryo).

Background

My research uses EBV as a model to study the interaction of oncogenic viruses with the infected host with focus on both the immunological control of viral infection

and vial strategies of immune escape, and the contribution of viral products to the molecular events of malignant transformation. We aim to understand how viral products reprogram the cell and host environment to allow the establishment of persistent infections and promote malignant transformation. Post-translational modifications play critical roles in guiding the function and turnover of cellular proteins. The covalent attachment of small peptides, including ubiquitin (Ub) and ubiquitin like (UbL) molecules such as SUMO, NEDD8, ISG15, is a key event in the regulation of virtually all cellular and immune functions. Viruses hijack the Ub and UbL signaling cascades to promote their own replication and escape from immune control. Viral proteins that mimic the activity of cellular enzymes are particularly interesting since viral homologues often exhibit unique features that render them particularly suitable for drug discovery. we have discovered that the EBV large tegument protein, BPLF1 serves as a Ub and NEDD8 specific deconjugase. Using co-immunoprecipitation and mass spectrometry we found several members of the 14-3-3 proteins family amongst the top hits of the BPLF1 interactome. BPLF1 promotes the assembly of a tri-molecular complex that includes, in addition to 14-3-3, the ubiquitin ligase TRIM25 that regulates the innate immune response via ubiquitination of the pattern recognition receptor, RIG-I. The viral enzyme functionally inactivates the RIG-I signalosome and inhibits the IFN response. The large tegument proteins are expressed during virus replication and packaged into virus particles. Thus, the enzyme may contribute to extend the time of unchecked productive infection and participate in the reprogramming of newly infected cells that allows the establishment of latent infections.

Research project description

This research proposal aims to identify new biomarkers of infection-associated oncogenesis using as model Epstein-Barr virus (EBV), a widely spread human herpesviruses that causes clinically silent primary infections in childhood followed by the establishment of a life-long carrier state. EBV infects normal B lymphocytes in vitro, leading to the establishment of autonomously growing lymphoblastoid cell lines (LCLs). In contrast, very little is known about the interaction of the virus with epithelial cells. This is partly explained by the difficulty to infect cultured epithelial cells, and partly by the rapid loss of the viral genome upon in vitro explant of biopsies from EBV positive NPC and gastric carcinoma. Thus, the infection of epithelial cells appears to be dependent on environmental factors that are not reproduced in conventional tissue cultures. EBV carrying epithelial tumors arise in the nasopharynx and stomach that are colonized by a highly diverse bacterial microflora. In order to explore whether co-infection with oral pathogenic bacteria may affect the outcome of EBV infection in epithelial cells we have established a tissue culture model where EBV negative and EBV carrying epithelial cell lines are cultured in the presence of live bacteria or secreted bacterial metabolites. We could confirm previous observations on the capacity of bacterially produced small chain fatty acids to induce reactivation of the productive virus cycle in EBV positive cells. In addition, exposure to the cytolethal distending genotoxin (CDT) that is produced by the common oral pathogen Aggregatibacter actynomicetemcomitans (Aa) and other Gram- negative bacteria that colonize the gastrointestinal tract, caused virus reactivation via a new mechanisms involving the induction of DNA

damage and activation of the Ataxia Telangectasia Mutated (ATM) kinase (Frisan et al. Int.J.Cancer, 2018). These findings delineate a scenario where, in addition to inducing virus reactivation, certain bacteria species can directly contribute to oncogenesis by causing genomic instability, establishing thereby a pre-malignant phenotype that may, together with viral infection, progress to malignancy. We shall assess the validity of this hypothesis and explore in detail the effect of bacterial co-infection on the susceptibility of epithelia cells to EBV infection and oncogenesis. in vitro models that mimic the microenvironment of the nasopharynx and gastric epithelia will be developed to address the following issues:

1. Effect of the CDT on the susceptibility of epithelial cells to EBV infection
   Experimental design: (i) monitor the efficiency EBV infection in epithelial cell lines and primary cells exposed to different doses of bacteria or CDT in conventional 2D cultures with or without addition of selected cytokines and growth factors; (ii) develop 3D culture models to test the effect of cell differentiation on the susceptibility of epithelial cells to bacterial and viral infection; (iii) identify CDT-induced cellular changes that determine susceptibility to infection.

2. Synergy between bacterial and viral products in the induction of malignant phenotypes
   Experimental design: (i) dissect the combined effect of CDT and viral proteins on the induction of DNA damage and activation of different branches of the DNA damage response (DDR); (ii) examine the effect of long-term exposure of EBV positive cells to low doses of CDT with focus on the activation of signaling pathways that regulate cell survival and the acquisition of phenotypic properties of malignancy including anchorage independent growth, epithelial to mesenchymal transition and invasiveness, expression of markers of immunoregulation including the checkpoints PD1/PDL1.

Research group

Noemi Nagy - PhD, research assistant. Immunology
Päivi Ylä-Anttil - PhD, postdoctola fellow. Cell biology, autophagy Jinlin Li - PhD, senior postdoc. Molecular virology
Jiayu Zhang - PhD, postdoc. Cell biology, oxidative stress.

Key words

Virus Bacteria, Co-infection, viral pahogenesis

Interested in recruiting a Postdoc and a Visiting researcher

Project title

Pathogenesis study of haemorrhagic uremic syndrome causing Shiga toxin-producing Escherichia coli.

Supervisor

Andreas Matussek, Associate professor, Department of Laboratory Medicine
Email: andreas.matussek@ki.se

Type of recruitment and qualifications of applicant

- Postdoc (12 months)
- Visiting researcher (12 months)

Background

The applicant should be highly motivated, independent, inventive and communicative with good English skills and with a PhD in microbiology, molecular biology or related discipline, and should have solid knowledge in bacteriology, more specifically on enteropathogenic bacteria. In addition, experience in bioinformatics and omics analysis is considered as a significant advantage.

Applicants must have completed their PhD by the start of the appointment and within the last 3 years.

Applicants should submit their curriculum vitae, including date of the thesis defence, title of the thesis, previous and current academic positions, academic title, academic distinctions, field experience, publication list, and e-mail address of two references.

Research project description

Shiga toxin-producing Escherichia coli (STEC), also known as enterohemorrhagic E. coli (EHEC), is an emergent food-borne pathogen that causes a wide range of clinical symptoms including haemorrhagic colitis (HC) and life- threatening haemorrhagic uremic syndrome (HUS). Serotype O157: H7 is one of the predominant serotypes responsible for sporadic STEC cases and outbreaks worldwide. However, there is a growing number of non-O157 STEC in humans that have been isolated from patients with HUS and from outbreaks worldwide including the Nordic countries. Remarkably, in a recent study, non-O157 serogroups were found to dominate incidence of STEC-gastroenteritis and contribute significantly to STEC-associated HUS in Germany, which might apply to many other EU countries considering European surveillance data on HUS.

The main virulence factors of STEC are the phage-encoded Shiga toxins (Stx) and the intimate attachment to host cells, which are highly associated with the development of HUS. However, Stx and intimin are necessary but not sufficient to cause severe clinical outcomes, such as HUS. The clinical significance of STEC for

humans is determined by the production and interplay of various bacterial factors, as well as host-related factors.

The objective of this project is aimed at identifying biomarkers to distinguish between strains with the potential to cause HUS and less virulent strains by comparative genomics and proteomics through a Nordic collaboration.

Moreover, the association between disease severity with Shiga toxin production level, stx subtypes, the structure of Stx-converting phage will be investigated. With the goal of gaining these insights, our study could aid to improve diagnostics, predict public health risk, improve infection control measures and make better clinical intervention strategies for HUS. The purpose of recruitment of a CSC post-doc/visiting researcher, who has solid experience on STEC study, is to perform the experimental and bioinformatic work within this project.

Research group

The research group consists of two research teams: Antimicrobial Resistance (AMR) team, headed by the research group leader Professor Christian Giske MD/Ph.D., and the intestinal pathogen team, headed by Associate professor Andreas Matussek, MD/Ph.D. The research group contains 4 Ph.D. students and 4 post-doc researchers currently, who work on multidrug- and extensively drug-resistant gram-negative bacilli, and pathogenic E. coli especially Shiga-toxin producing E. coli.

Key words

Shiga toxin-producing Escherichia coli; Haemorrhagic uremic syndrome; Pathogenicity; Whole-genome sequencing; Genetic marker; Toxin production.

Karolinska Institutet

Interested in recruiting a Doctoral student

Project title

Application of Cellular Thermal Shift Assay for the study of responses to virus infection

Supervisor

Gerald McInerney, Associate professor,
Department of Microbiology, Tumor and Cell Biology
Email: gerald.mcinerney@ki.se Home page: www.alphavirus.org

Type of recruitment and qualifications of applicant

Doctoral student, 48 months

We are looking to recruit a person to join as a PhD student in the group of Associate Professor Gerald McInerney. The applicant shall be a highly motivated person with an interest in virology, cell biology and immunology and with some laboratory experience with biochemistry and molecular biology. The candidate will be expected to have very good communication skills in written and spoken English.

The project aims at unravelling the changes in the interaction states of the proteome of cells during the early hours of alphavirus infection. We are using innovative mass-spectrometry based techniques to get a comprehensive picture of the events that occur early in infection with RNA viruses. In the project, the student will also utilize well established methods such as recombinant DNA technology, eukaryotic cell culture, protein biochemistry and confocal microscopy to validate interesting findings from the mass-spectrometry experiments.

The work in this project will provide unique perspectives on the cell-intrinsic response to viral infection, an area of utmost importance in antiviral immunology. In-depth knowledge into virology, molecular and cell biology, proteomics, and innate immunology will be attained. We believe therefore that it constitutes an excellent doctoral project.

Background

The complexity of the living cell has confounded attempts to map interactions of functional importance between macromolecules. Although many methods exist to study protein function and mechanism in cells and tissues, they are often indirect and correlative to the physiological situation. A major factor determining protein function is the interactions made by a specific protein to other molecules in the cell. Each protein will have a limited set of “protein interaction states” (PRINTS), where each state defines an activation form of the protein. In a specific cell state, a

population of multiple PRINTS for a given protein might be occupied and one or more of these PRINTS can change occupancy in a transition between two cell states, for example upon infection with a virus.

Recently, the Cellular Thermal Shift Assay (CETSA) was introduced for direct studies of PRINTS in intact cells (Martinez Molina et al, Science 2013). CETSA is based on the discovery that even when heated in cells, many proteins unfold in a manner similar to purified proteins, and the unfolding is often followed by rapid precipitation. Thus, after the heating step, the remaining soluble protein correlates to the amount of residual folded protein in the cell. Therefore, by isolating and quantifying the amount of soluble protein, and subsequently plotting this against temperature, a CETSA melting curve can be generated. Due to the biophysical origin of CETSA, measurements are highly stringent, reproducible and not prone to false positives. CETSA now constitutes an impressive discovery method for studies of whole-proteome PRINTS changes across cell states. In a study of the cell cycle, our collaborators recently showed that unprecedented information on the modulation of cellular protein machineries can be directly extracted (Dai et al. Cell 2018). With this application, we intend to recruit a student to apply CETSA to study changes in pathways utilised by cells in their intrinsic resistance to infection

Research project description

Overall aim

Eukaryotic cells rapidly react to virus infection, activating cell-intrinsic stress response and innate immune pathways. Because these changes are so extensive and complex, it has been challenging to get a comprehensive picture of the events that occur early in infection. In this project, we will apply CETSA to study changes in pathways utilised by cells in their intrinsic resistance to infection with alphaviruses. We will study two different cellular states associated with cell-intrinsic responses to infection. Both have been shown to be activated by different virus infections and both are targeted for inhibition by diverse viruses, affirming their importance in broad antiviral defence.

WP1. Conserved stress response pathways to virus infection.

Cells subjected to adverse conditions, including virus infection, inhibit translation initiation leading to the accumulation of stalled translation initiation complexes that trigger the formation of membraneless organelles known as stress granules (SGs). It has been shown that a majority of viruses encode mechanisms to inhibit the induction of the SG response.

The student will use CETSA to map the stress-dependent protein interactome in sodium arsenite- treated HOS cells. Arsenite is a mitochondrial poison that leads to induction of SGs within 10 minutes post treatment. Sodium arsenite will be added to cell culture medium and cells will be harvested for CETSA analysis at 10 min and 60 min post treatment. The CETSA shifts will be analysed and systematically compared to existing data. This approach will allow to i) better define the succession of events during SG assembly, ii) differentiate between early and later

factors of SG assembly and iii) potentially identify novel proteins involved in SG assembly that could not be identified in affinity based, pull-down experiments. Once promising candidates are identified, we will make use of the GM lab's toolbox of SG-related antibodies, plasmids, cell lines and more, for verification of interactions and determination of their relevance for SG assembly and function in stressed and virus-infected cells.

WP2. Mapping the protein-interaction network of type I interferon-stimulated genes.
The IFN system is central in antiviral defence and represents one of the greatest obstacles for a virus to overcome in order to establish a productive infection. In IFN stimulated cells, hundreds of interferon-stimulated genes (ISG) are upregulated. Functional understanding for the overwhelming majority of ISGs is still lacking.

The student will use CETSA to map the PRINTS changes in cells after treatment with recombinant type I IFN. Our human osteosarcoma (HOS) cell line for CETSA experiments are IFN-competent, meaning that they can produce and react to IFNs. We will take timepoints for the CETSA experiment at 1 h post IFN treatment to detect IFN- dependent PRINTS which are not dependent on transcription of new genes, and at 24 hours post treatment, when we expect a robust IFN response to be established. Well characterized proteins of the JAK-STAT pathway and ISGs will serve as valuable controls in this experiment. Proteins that show significant CETSA changes will be further analysed using various techniques such as fluorescence microscopy, immunoprecipitation in cells treated with IFN or infected with virus. To test the relevance of especially interesting hits, knockout cell lines will be generated using CRISPR/Cas9. Taken together, this approach may give important insights into the type I interferon induced proteome dynamics.

In both WPs, data will be collected and analysed using the CETSA pipelines maintained in our collaborator's group. Once novel candidate proteins are identified, mechanistic studies towards functional understanding will be performed in the McInerney lab. These will include generation of KO cell lines, reconstitution with tagged and mutated variants, pulldowns and MS analyses to identify binding partners and other techniques.

Research group

In this project a student will undertake his/her PhD studies in Gerald McInerney's research group at the Dept of Microbiology, Tumor and Cell Biology, situated in the Biomedicum building. Biomedicum is an advanced research environment that promotes interdisciplinary cooperation and research. The GM group consists of the PI, two postdocs, two PhD Students and a number of short-term MSc students. We have several ongoing collaborations with other groups at KI, and internationally at Harvard Medical School, UT Southwestern, Imperial College London and others. We have weekly group meetings and fortnightly journal clubs at which all group members are expected to present and contribute. GM is heavily involved in education at KI and so members of the lab are encouraged to participate in

undergraduate seminars and lab courses as well as research lab supervision. The work in the laboratory is funded by project grants from the Swedish Research Council and Swedish Cancer Society as well as several other sources.

The project is part of a bigger collaboration with Pär Nordlunds lab at Bioclinicum, directly opposite Biomedicum across Solnavägen. We have larger consortium grants in review which would serve to significantly expand the collaboration, if awarded. We will hold regular joint group meetings to discuss the projects, ensuring a free flow of ideas and expertise between the 'wet' lab and computational arms of the collaboration.

Key words
Virus, cell biology, biochemistry, mass spectrometry

Interested in recruiting a Doctoral student or a Visiting doctoral student

Project title
The Neurobiology of anorexia nervosa.

Supervisor
Ida Nilsson, Doctor, Department of Molecular Medicine and Surgery
Email: ida.nilsson@ki.se Phone number: +46 708631361
Home page: https://staff.ki.se/people/idanil

Type of recruitment and qualifications of applicant
Doctoral student (48 months) or a visiting doctoral student (12 months)

- Master in Biology/Medicine or equivalent required
- Good cooperative and social skills required
- Fluency in written and spoken english required
- Interest in psychiatric research
- Interest in neuroscience
- Interest in eating disorders
- Applicants with experience with histochemical techniques, microscopy, statistical programming in R, cell work, cryosectioning, animal work and gene expression will be prioritized.

Background
Anorexia nervosa (AN) is a severe psychiatric disorder affecting women and men of all ages, even though a majority of the once affected are young females. The costs for the individual, the family, and the society are substantial. Moreover, AN has the highest mortality rate of all the psychiatric disorders, with estimates as high as 10%. Complete recovery, within 5-10 years of presentation, is seen in only around 60% of cases, and treatments are unacceptable to patients leading to premature withdrawal. Despite significant morbidity and mortality associated with AN, is the evidence base for its treatment, especially in adults, weak. No medications exist that effectively target the core biology of the disorder, in part, because the biology of the illness has been inadequately investigated. Twin studies have identified a strong genetic contribution, i.e., 58-70% of variance in liability is due to additive genetic factors. One central and unexplained feature of AN is the paradoxical response to negative energy balance. While most people cannot lose a few kilos, these individuals stay in an emaciated and starved condition commonly for many years. Even after therapeutic renourishment, their bodies commonly revert to what appears to be a negative set point, i.e. the inverse of happens for obese individuals. AN GWAS have yielded significant negative single-nucleotide-based genetic correlations with BMI and other anthropometric measures, which indicates a genetic predisposition for a lower body weight set point in AN. However, we do not

understand the (neuro)biological underpinnings of such a set point nor the mechanisms explaining the contradictory response to underweight in AN.

Research project description

We have explored neurobiological mechanisms related to anorexia in an anorectic mouse called the anx/anx mouse. This is a unique spontaneous genetic model mimicking some of the core features of AN; elective starvation, emaciation and premature death. A mouse model is not able to capture all aspects of AN; however, mirroring the core and deadly features of the disorder is important and has the potential to elucidate pathways that are central in the development and maintenance of the starved condition. We have shown that the anorexia of this mouse is related to a dysregulation of the hypothalamic systems central for regulation of food intake, and this is associated with inflammation and degeneration in the same brain region. A key finding is the selective activation of microglia cells in the hypothalamus of the anx/anx mouse. It appears as if the aberrances in the food intake regulating systems of the hypothalamus makes the anx/anx mouse, in common with AN patients, respond paradoxically to negative energy balance.The overarching hypothesis of the project is that neuroinflammation and- degeneration, in particular in the hypothalamus, are central in the development and maintenance of AN. The studies that are planned in order to explore the hypothesis are:

-NfL in AN CSF
In collaboration with the teams of Proffessors Zetterberg, Blennow (both at GU), Landen (KI/GU) and Bulik (KI) we have shown that neurofilament light chain (NfL), a fluid marker of axonal injury, is significantly increased in plasma from females with AN, and to a lower degree also in females recovered from AN, when compared to healthy normal weight female controls. The levels in AN are comparable to those seen in boxers and in older patients with multiple sclerosis. In order to confirm that this injury is localized in the brain, and not the periphery, we plan to evaluate the levels of NfL in CSF samples from individuals with AN and the same two control groups.

-Regenerative treatment of anx/anx
The Fasudil derivative FSD-C 10 has neuroprotective, -regenerative and -inflammatory effects. FSD-C 10 will be administered intranasally, max ten times between postnatal day (P) 3 and 19, to the anx/anx mouse. We will subsequently by immunohistochemistry evaluate if we can prevent the neuroinflammatory and neurodegenerative signs of the anx/anxhypothalamus, which without treatment are visible from around P10. In addition to this, will we monitor their growth by regular body weight measurements. Thus, a rescue of the body weight phenotype, and prevention of the hypothalamic inflammation/degeneration of the anx/anx mouse, would prove a causal link between these mechanisms and the anorexia of this mouse.

-iPSCs

Reprogramming of human somatic cells to patient-specific iPSC is a powerful tool to study the mechanisms of disease and ultimately to develop new treatments. Protocols for differentiation of iPSC into different neuronal types have been established, including protocols for deriving hypothalamic-like neurons which we will utilize. Fibroblast cultures will be generated from skin biopsies collected from patients with AN and healthy controls. The fibroblasts will subsequently be used to generate iPSC and thereafter be exposed to established protocols for hypothalamic differentiation. At this step we will compare if there is a difference in the maturation to hypothalamic-like phenotype between AN and controls. We will thereafter utilize the hypothalamic-like neurons generated from individuals with AN and CTRLS for comparative studies of the effects of exposure to; food intake regulating hormones such as leptin and ghrelin, oxidative stress, anti-inflammatory drugs, induction of inflammation etc. The read outs include e.g. electrical activity, gene-expression profiling, and migration assays. Thus, generation of patient specific hypothalamic-like neurons will be an invaluable tool for molecular studies evaluating the dysregulated food intake in AN.

Research group

The project is situated in a research group belonging to Dept. of Molecular Medicine & Surgery and situated at the Center for Molecular Medicine (CMM). The group called Translational Psychiatry is headed by Assoc. Prof.

Catharina Lavebratt and Prof. Martin Schalling, and within the group there are several smaller teams on different psychiatric disorders; bipolar disorder, schizophrenia, major depressive disorder and anorexia nervosa, all headed by different PIs. The PI for the research on anorexia nervosa is Assist. Prof. Ida Nilsson, whom will be the main supervisor of the doctoral student. The anorexia nervosa team is also affiliated with the Centre for Eating Disorder Innovations (CEDI) headed by Prof. Cynthia Bulik, and collaborate closely with the clinic Stockholm Center for Eating Disorders.

At the moment there are 1 technical assistant, 4 PhD-students, 2 Postdocs and several senior researchers in the group. In addition, there are several clinical researchers / clinicians affiliated to the group..

Key words

psychiatry eating disorders, anorexia nervosa neuroscience hypothalamus food intake neuropeptides neurotransmitters microglia, short chain fatty acids

Supplementary information

Ethical permits for taking skinbiopsies from patients, as well for breeding of and administering regenerative substance to the anx/anx mouse has been granted. We have access to blood and CSF samples from patinets with AN via collaboration with Prof. Cynthia Bulik and Prof. Mikael Landen.

Interested in recruiting a Postdoc

Project title
Endocrine regulation of lymphomas

Supervisor
Sam Okret, Professor, Department of Biosciences and Nutrition
Email: sam.okret@ki.se Phone: ++46852481069
Homepage: https://ki.se/en/bionut/effects-of-glucocorticoids-and-estrogens-on-cells-and-functions-of-the-immune-system-sam

Type of recruitment and qualifications of applicant
Postdoc (24 months)

The applicant should have theoretical and on-hand practical experience in the research area of signal transduction and gene expression. Experience of cancer research e.g. in the area of hematological malignancies, immunology and/or endocrinology, particularly on steroid hormone action is an advantage.

In silico bioinformatic knowledge in the analysis of global gene expression is of great advantage. Experience of cell culture work, flow cytometry, immunostaining techniques, western blotting, virus work or rodent experimental experience are also favourable.

The applicant should be highly motivated, creative, have the ability to work rather independently and should easily interact with other group and lab members. The candidate should also be proficient in English.

Background
I'm a professor of Molecular Endocrinology mainly in the area of steroid hormone action (glucocorticoids and estrogens) and the effects on cells of the immune system, inflammation and cancer.

Previous work has mainly focused on glucocorticoid receptor signaling as well as on extraadrenal glucocorticoid synthesis. During the last 8-9 years we have more focused on the endocrine regulation of lymphomas with a particular interest in sex differences and the role of sex hormones on lymphoma initiation and progression.

Lymphomas show a sex- and age dependent difference in incidence and prognosis according to several epidemiological studies. For example, the lowest male/female incidence ratio is seen in premenopausal women copared to men of the same age. Furthermore, women generally have a better prognosis. The reason and mechanism for this is not clear but epidemiological and our experimental studies suggest that estrogens may play a role in this difference with estrogens having a protective effect. For example, increased number of pregnancies have been suggested to be protective and some epidemilogical studies show that women on hormone replacement therapy (HRT) have a lower incidence of lymphomas

compared to women who are not on HRT. Furtermore, we have shown that lymphomas grafted to mice grow faster in male vs. female mice and that the difference disappear following ovariectomy. Furthermore, administration of estrogen receptor beta (ERβ) selective agonists decreases lymphoma progression in grafted mice. This involves e.g. decreased tumor cell proliferation and tumor angiogenesis.

Contrary, blocking estrogen synthesis using an aromatase inhibitor enhances tumor progression. These studies suggest that lymphomas indeed are under hormonal control despite not generally considered as endocrine-related malignancies. It may not be unlikely that this could at least partially contribute to sex and age dependent differences in lymphoma development and prognosis in patients.

Research project description

The candidate may join the following projects:

Aim 1

a) Expression analysis using RNAseq/microarray data. In order to identify age- and sex specific differences of gene expression in clinical and experimentally treated lymphoma, In addition, publicly available microarray data sets where age and sex information is available will be used to validate the results. Particularly, differences between pre- and postmenopausal women will be analyzed in order to pinpoint genes and signaling pathways that might be regulated by estrogens in order to identify possible mechanisms for the protective role of estrogens on lymphoma development as indicated by epidemiological and our previous experimental studies. To further analyze if the pre- vs. postmenopausal differentially expressed genes are under direct or indirect control by estrogens, we will examine ER binding to genes by Chromatin-immunoprecipitation (ChIP), either experimentally or by analyzing publically available global ChIP data sets. As far as we are aware, almost no studies have in depth addressed changes in gene expression in lymphomas in general from a sex- and age dependent perspective.

(b) Validate differences in gene expression on protein level by immunohistochemistry (IHC). In order to validate differences in expression of sex- and age associated genes derived from results above (a) on protein level, we will stain lymphoma tissue microarray using IHC.

(c) Validate estrogen regulation of genes differentially expressed in pre- and postmenopausal patients in mouse models. In order to confirm estrogen regulation of the genes identified to be differentially expressed in pre- and postmenopausal lymphomas, we will xenograft human lymphoma cell lines to female immunocompromised mice that either have been ovariectomized or will be treated with an aromatase inhibitor to block estrogen synthesis. Differences in tumor gene expression analyzed by RNAseq/qPCR in comparison to intact and vehicle treated mice will verify estrogen regulation of genes identified in (a) above.

Aim 2). Are the effects on lymphoma progression by estrogens mediated by estrogen receptor b (ERb) in the lymphoma cells? ERb is the main ER expressed in lymphoma cells. In studies in our group we have noticed that administration of ERb agonists or inhibition of estrogen synthesis only have a limited effect on lymphoma

cells in culture in contrast to the effects seen in vivo. This might suggest that the estrogen effects on the tumor cells are indirect e.g. involving effects on the tumor microenvironment. Furthermore, estrogens do not only act via ER but also through other signaling pathways e.g. the G-protein-coupled estrogen receptor-1 (GPER1). In order to evaluate if the estrogen effects are mediated via a direct effect on ERb in the lymphoma cells, we will delete or inactivate the ERb gene in lymphoma cells, where after we will graft the cells to mice and test for estrogen response in vivo. For deletion or inactivation of the ERb gene we will use the CRISR/Cas9 technology.

Significance:
With this research we hope to explain mechanisms for age- and sex differences of lymphomas. This may also contribute to the development of new and more personalized treatment strategies taking sex and age into account.

Research group

The group presently consists of one principal investigater (Sam Okret), one senior researcher, one researcher, one postdoc and one PhD student.

Key words

- Hematological malignancies Lymphoma
- Endocrine regulation Sex difference
- Age differences Sex hormones Estrogen
- Signal transduction Gene expression Bioinformatics

Interested in recruiting a Postdoc and a Visiting researcher

Project title

1. Regulation of immunoglobulin class switch recombination in human B cells;
2. Discovery of therapeutic targets in B cell lymphoma

Supervisor

Qiang Pan-Hammarström, Professor, Department of Biosciences and Nutrition (BioNut)
Email: qiang.pan-hammarstrom@ki.se Phone: +46 703884943
Home page: https://ki.se/en/bionut/research-group-qiang-pan-hammarstrom

Type of recruitment and qualifications of applicant

- Postdoc (12-24 months)
- Visiting researcher (12 months)

Postdoc (12-24 months) or Visiting researcher (12 months)
The applicant is eligible to apply if he or she has obtained a PhD in the fields of Medicine, Biology, Genetics, Oncology, and Immunology, or related fields. The applicants for all positions should be talented and highly motivated students or researchers who are able to work within a team environment. The candidates are expected to possess a strong background in immunogenetics or cancer genetics and master several molecular biology techniques, equivalent to their carrier ages. Good knowledge of molecular biology, cell culture, FACS, the CRISPR/Cas 9 technology or skills in analysing large-scale data, is an advantage. Furthermore, the candidate should possess excellent communicating and writing skills in English.

Background

B-cells play an important role in adaptive immunity against pathogens through the production of antibodies. B- cells develop from hematopoietic stem cells in the bone marrow in a stepwise process during which they generate a broad repertoire of unique B-cell receptors through a somatic recombination process referred to as V(D)J recombination. Two additional Ig gene diversification processes occur in peripheral lymphoid organs: class switch recombination (CSR) and somatic hypermutation (SHM).

Maintenance of genome stability depends on an appropriate response to DNA damage and may, when insufficient, lead to development of neoplasia. Double-strand break (DSB) is considered to be one of the most severe forms of DNA damage. There are two general types of DSB repair: homologous recombination (HR) and nonhomologous end-joining (NHEJ). The classical NHEJ machinery is also required during V(D)J recombination and CSR.

Primary immunodeficiencies (PID) are inherited disorders of the immune system that predispose affected individuals to an increased rate and severity of infections, immune dysregulation, autoimmune manifestations and malignancy1. To date, more than 300 different types of PIDs have been described and most of these

disorders are rare and due to a monogenic defect. The category of antibody deficiencies comprises the largest category within the total group of PID and these patients are expected to have defects/blocks in various stages of B cell development.

Lymphomas account for approximately 5% of all cancers worldwide and in western populations, B-cell lymphomas comprise over 90% of all lymphomas. The most common subtypes of B-cell lymphomas are of GC origin: follicular lymphoma and diffuse large B-cell lymphoma. Linking potentially oncogenic DNA breaks to CSR and SHM may explain the genesis of these B-cell malignancies.

Research project description

Project I: Regulation of immunoglobulin class switch recombination in human B cells

The project is aimed at understanding the complex molecular mechanisms involved in DNA editing, repair and recombination during immunoglobulin class switch recombination (CSR) and somatic hypermutation (SHM) and their involvement in the pathophysiological processes leading to immunodeficiency, genome instability and cancer development in humans.

We have developed a series of PCR-based assays to study the pattern of in vivo generated V(D)J recombination coding joints, switch recombination junctions and mutations introduced in the VH and JH4 intronic region genes in human B cells. Using these methods, we have studied the V(D)J recombination, CSR or SHM processes in a number of diseases. These studies have helped us to gain insights into the molecular mechanism of the Ig gene diversification processes.

To further dissect the repair pathways in V(D)J recombination and CSR, we are developing several deep sequencing strategies to simultaneously characterize the V(D)J recombination junctions, the VH repertoire and SHM pattern in the VH regions genes in human B cells. Furthermore, we have adapted a modified version of his high-throughput, genome-wide, translocation sequencing (HTGTS) method by linear-amplification-mediated PCR (LAM-PCR) to human cells and have designed a system where switching to all human Ig isotypes can be analyzed simultaneous in a high-throughput, unbiased fashion. A similar assay for analyzing V(D)J recombination will also be developed.

We have already collected DNA samples from patients suffering from PID, from a large number of consanguineous, multi-case as well as single-case families. Cells from some of these patients show an increased level of radiosensitivity, suggesting a defect in DSB repair. Exome or whole genome sequencing will be performed in samples from these patients. Functional studies will be performed in selected patients representing novel genetic disease models or with interesting biological questions raised from the clinical and genetic phenotypes.

Project II: Discovery of therapeutic targets in B cell lymphoma

The project is aimed at identifying potentially treatable molecular targets in mature B cell lymphomas (with focus on diffuse large B cell lymphoma, follicular lymphoma and mantle cell lymphoma) by next generation-sequencing (whole genome and exome sequencing, RNA-seq) and other high-throughput technologies such as proteomic analysis and genome-wide CRISPR/cas9 loss- or gain-of-function

screening.
We plan to sequence the whole genome of altogether 500 follicular lymphoma and diffuse large B cell lymphoma samples and will systematically characterize the driver mutations, mutation signatures and their correlation with DNA repair gene mutations, viral infection status, clinical data, as well as gene expression profiles. We will also systematically characterize the somatically occurring structural variants, especially translocations, in the tumors and their association with NHEJ gene defects. We plan to validate all the translocations identified by whole genome sequencing, using recombination breakpoint-specific PCR and Sanger sequencing. On the validated data set, we will further characterize the sequences flanking the breakpoints and investigate if these translocations are associated with AID activation sites, or additional fragile sites in the genome.

Based on these analyses, we hope to further dissect the role of DNA repair deficiency and resulting aberrant SHM activity as well as chronic viral infection in B-cell mutagenesis and lymphomagenesis. We hope also to discover novel therapeutic targets for lymphoma patients.

Research group

My group is currently composed of 1 laboratory manager, 1 senior researcher, 2 postdoctoral fellows, 3 PhD students and 1 visiting scientist, who collectively have expertise in molecular biology, genetics, hematology, immunology and bioinformatics. For details please visit our website:
https://ki.se/en/bionut/research-group- qiang-pan-hammarstrom

Key words

human, immunodeficiencies, B cell lymphoma, immunoglobuline gene, cancer, cancer genetics, immunogenetics.

Interested in recruiting a Doctoral student and a visiting doctoral student

Project title

Regulation of immunoglobulin class switch recombination in human B cells

Supervisor

Qiang Pan-Hammarström, Professor, Department of Biosciences and Nutrition (BioNut)
Email: qiang.pan-hammarstrom@ki.se Phone: +46 703884943
Home page: https://ki.se/en/bionut/research-group-qiang-pan-hammarstrom

Type of recruitment and qualifications of applicant

- Doctoral student (48 months)
- Visiting doctoral student (12 months)

PhD students (4 years) or visiting students (1 year)
The applicant is eligible to apply if he or she has obtained a master's degree in the fields of Medicine, Biology, Genetics, Oncology and Immunology, or related fields, and fulfils all academic entry requirements set by the Karolinska Institutet. Good knowledge of molecular biology, cell culture, FACS, the CRISPR/Cas 9 technology or skills in analysing large-scale data, is an advantage. Furthermore, the candidate should possess excellent communicating and writing skills in English.

Background

B-cells play an important role in adaptive immunity against pathogens through the production of antibodies. B- cells develop from hematopoietic stem cells in the bone marrow in a stepwise process during which they generate a broad repertoire of unique B-cell receptors through a somatic recombination process referred to as V(D)J recombination. Two additional Ig gene diversification processes occur in peripheral lymphoid organs: class switch recombination (CSR) and somatic hypermutation (SHM).

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disorders are rare and due to a monogenic defect. The category of antibody deficiencies comprises the largest category within the total group of PID and these

patients are expected to have defects/blocks in various stages of B cell development.

Research project description

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Research group

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https://ki.se/en/bionut/research-group- qiang-pan-hammarstrom

Key words

human, immunodeficiencies, B cell lymphoma, immunoglobuline gene, cancer, cancer genetics, immunogenetics.

Interested in recruiting a Doctoral student

Project title

Autologous transplantation of endometrial stem cells and effect of niche factors in endometrial regeneration

Supervisor

Lalitkumar Parameswaran, Doctor, Department of Women's and Children's Health
Email: Lalit.kumar@ki.se Phone number: +46704757457

Type of recruitment and qualifications of applicant

Doctoral student, 48 months

Educational background:
Master degree in Biomedical Sciences or MD. Preference will be given to candidates with special training in Gynaecology, Training in Clinical trial or clinical research, GLP or GMP will be added advantage.

Research experience
Laboratory research experience is essential. Candidate should have working experience and knowledge in Cell culture and molecular biology techniques such as DNA/RNA extraction, PCR, Flow cytometer.

Other skills
Good communication skills in English (both written and verbal communication) is mandatory. Should have good knowledge in literature search and scientific writing. Should be a team player. Should be good at Microsoft office and statistical program.

Background

Our experimental work focuses on understanding the molecular mechanism involved in endometrial receptivity and human embryo implantation in human. We have studied the role of different molecules involved in human embryo implantation in vitro, using a 3D human embryo implantation model. Using this model, we have studied the effect of different doses of progesterone receptor modulators, antiprogestins and cytokines like LIF on human embryo implantation process. Currently, we are pursuing research on identifying embryonic signals that are secreted out into culture media by different grades of embryos using RNAseq technology with the objective of identifying non-invasive marker for good quality embryos. Similarly, we are also looking into the molecular profile of endometrial secretion of receptive human endometrium.

We have studied the pheno-typical characteristics of endometrial mesenchymal stem / stromal cells of women with endometrioma after expanding them in vitro. This has given us a good understanding about endometrial stem / stromal cells in healthy endometrium. Further, we would be using this knowledge to expand the above cells for therapeutic purposes in women with thin endometrium.

Research project description

Endometrial regeneration is essential for human embryo implantation and maintenance of pregnancy. Adequate thickness of the endometrium is essential to accomplish a successful pregnancy. Several studies show that endometrial thickness matters for successful pregnancy. A non-responsive endometrium resulting in “endometrial infertility” may be caused by radiation, endocrine, genetic, inflammatory disease or trauma caused by surgery.

Surgical trauma or severe infection may result in damaged endometrial and adhesions (Asherman’s syndrome). In either case, a very thin endometrium will be found which lack sufficient glandular structure and stroma.

Studies show that 43% of women with Asherman's syndrome suffer from infertility or repeat pregnancy loss. One of the underlying reasons is lack of endometrial tissue sufficient enough to support the implantation or development of the placenta. The rate of miscarriage in these patients is about 40%. They also suffer from other obstetric complications such as preterm delivery (23%), placenta accreta (13%), and ectopic pregnancy (12%).

1. This project deals with endometrial regeneration using autologous cell transplantation. There is no proven effective treatment for infertile women with endometrium atrophy (EA) or Asherman syndrome (AS). We hypothesize that dysregulation of endometrial mesenchymal stem cells(eMSCs) and/or their surrounding environment (or niche cells) disturbs endometrial regeneration and causes EA and AS, which can be cured by transplantation of eMSCs. To determine the defects of eMSCs and their niche cells from EA and AS patients
2. To compare the secretome of endometrial cells in normal endometrium, EA and AS
3. To study the actions of niche factors on eMSCs.
4. To establish an efficient protocol for expansion of eMSCs in vitro

Niche signals are crucial to adult stem cell activation and differentiation. A universal challenge with in vitrostem cell expansion is to prevent spontaneous differentiation. Maintaining the stem cell population requires activities of specific regulators involving in self-renewal and proliferation while preventing differentiation. Wnt/β signaling mediates proliferation of stem cells by retaining its stemnes. Knowledge of endometrial niche is important for regulation of proliferation of undifferentiated eSCs in vitro. The overall purpose of the project isto optimize a GMP protocol for in vitro expansion of eMSCs using endometrial niche factors in xeno-free environment. The established protocol will be used in a subsequent randomized clinical trial on using eMSCs for endometrial repair in women with EA or AS.

The specific goals are: About 3-5% of women in general population suffer from endometrial infertility, which is a challenge in the treatment of infertility. Till now, there is no effective cure for the problem. With the advancement of cancer treatment, the survival rate of childhood cancer has gone up to 80%. Techniques are available to preserve the ovaries/eggs of the female survivors. However, most women after radiotherapy have thin endometrium, which is non-receptive to implanting embryos. This makes the whole effort of fertility preservation in this

group of women futile. Importantly, these women after having trauma with cancer would have greater disappointment when they find that their fertility treatment is unsuccessful.
Findings from this study will provide a novel eMSC based approach for infertility treatment. Other potential outcomes are possible use of niche factors or their associated signaling molecules for treatment of women with EA and AS by stimulating regrowth of their endometrium. Understanding the mechanisms involved in stem cell maintenance will provide new advances in treatment of other endometrial disorders.

Research group

The applicant Dr Lalit Kumar is part of the larger research group headed by Professor Kristina Gemzell Danielsson. The group has three major research fields – Clinical research, Global reproductive health and Experimental research. The research group is based at the WHO collaborating centre for research in human reproduction and reproductive health. The research group has GCP trained staff; clinical co-ordinator, research midwives with experience of clinical trials. Experimental research is conducted in the newly built wet lab Bioclinicum were the group collaborates with several other groups. At the moment our group has two postdoctoral fellows and several PhD students working in the lab. The group has international research collaboration with China, Hong Kong, Germany, UK, US, Estonia and Spain. The research work is supported by National, international and EU research grants. The research group has strong ties with China with an on-going research project Sunming with Shenzhen Medical center and Hong Kong University. Professor Kristina Gemzell Danieslsson is also Honorary Clinical Professor at Hong Kong university.

Key words

Infertility, Endometrial mesenchymal stem cells, Autologous stem cell transplantation. stem cells, niche factors.

Interested in recruiting a Postdoc and a Visiting researcher

Project title

Identification of the neo-antigenome in Hepatocellular Carcinoma to guide T cells against relevant targets for successful immunotherapy.

Supervisor

Anna Pasetto, Assistant professor, Department of Laboratory Medicine
Email: anna.pasetto@ki.se Phone: +46 852483857
Home page: https://staff.ki.se/people/annapa1

Type of recruitment and qualifications of applicant

- Postdoc (24 months)
- Visiting researcher (12 months)

We are seeking a highly motivated early-career scientist with skills and expertise in cell culture and immunology. Applicants need to demonstrate their experience in running projects independently but also have a strong attitude for cooperation and teamwork. Excellent written and oral communication skills in English are needed, as the group and work environment are international.

We also required three or more of the following skills:

- experience with cell culture work and processing of blood and human tissue samples
- experience with immunological methods including functional assays (ELISA, ELISpot)
- experience with advanced multicolour flow cytometry acquisition and data analysis
- experience with flow cytometry cell sorting, including single-cell sorts
- experience with processing cells for RNA sequencing and bioinformatical analysis
- experience with viral vector production and transduction of human lymphocytes
- experience with molecular cloning of immune receptors.

Background

Our research is focused on developing immunotherapies for cancer. Increasing evidence is pointing at a key role of the immunological response against neoantigens in succesful immunotherapy. Immune check point inhibitors, like anti-PD-1 and anti-CTLA-4 are examples of succesful immunotherapy for an increasing number of solid cancers. The mechanism behind this success is not clearly understood but a link between the presence of neoantigens in the tumor and infiltrating lynphoocytes is now evident. Our hypothesis is that tumor-specific and neoantigen-specific T cells play a central role in attacking the cancer cells, and clinical trials of neoantigen- specific adoptive cell transfer conducted by Dr. Steven

Rosenberg at the NIH constitute the proof of principle for this hypothesis. Dr. Anna Pasetto trained for 5 years in Dr. Rosenberg's group and she is now further developing the concept of adoptive cell transfer of neoantigen-specific T cells at the Karolinska Institutet.

Research project description

Summary
Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death in the world and the current available treatments, like surgery or ablative therapy, are effective only at an early stage of the disease. For advanced HCC the only approved therapy is Sorafenib (a multi kinase inhibitor) which has low response rate (about 3%) and prolongs the life expectancy for only few months. However, new immunotherapy approaches, like antibodies against PD-1, can instead achieve a higher objective response rate, but still only a minority of HCC patients (20%) respond to the therapy. To boost the objective response rate in the setting of immunotherapy, it would be critical to guide the immune response in subjects towards relevant antigens expressed by all tumor cells.

Genome-wide sequencing of HCC indicates that unique non-synonymous mutations are present in tumors and theoretically could give rise to neoantigen-specific T cell responses. However, the presence of these type of immune responses has not been investigated yet. A focused approach would be to aim at mutations that are expressed on all cancer cells, like mutations in driver oncogenes. The central tenet to this project proposal will be to identify the neo-antigenome in HCC and evaluate the presence of an immunological response to HCC-specific neo-antigens with particular attention to hot-spot mutations in driver oncogenes. While performing the functional characterization, T cell receptors (TCRs) recognizing these mutations will be collected and made available to generate “off-the shelf” reagents that can be utilized for patient treatment.

Purpose and Aims
Cancer care has moved into a new age with the emergence of treatments that boost the immune system. Particularly, immune checkpoint blockade (ICB) of inhibitory receptors (such as PD-1 and CTLA-4) has revolutionized the treatment agenda in the cancer field and can induce long-term remission and even “cure” metastatic disease of multiple cancers. Despite preliminary evidence suggesting that ICB can induce a much higher objective response rate than Sorafenib, only a minority (20%) of patients with HCC respond to ICB. New immunotherapy modalities are therefore needed to improve the objective response rate. T cells are central targets for ICB and other immunotherapeutic interventions. High levels of infiltrating cytolytic T cells (CTL) in tumors have been associated with favorable prognosis in multiple malignancies, probably as a direct consequence of CTL recognition of mutated antigens displayed by tumor cells. In this project proposal, we want to develop a library of anti-HCC T cell clones that can be used in combination therapy to eradicate the tumor.

The main objective will be to study the T cell response directly from HCC tumors and draining lymph nodes (LNs) and sequence hotspot regions of HCC tumors to identify neoantigens that exist in the patients. This study will teach us how the

immune response can be harnessed to fight HCC. This study will be divided into three specific aims:

- Identify the neo-antigenome from HCC cells.
- Identify neo-antigen-specific T cells from HCC tumors and draining LNs and generate a library of neo- antigen-specific TCRs for future immunotherapy.
- Genetic transfer of the neo-antigen-specific TCRs to recipient lymphocytes to evaluate the feasibility of future immunotherapy.

Project overview

1. Collection of HCC tumors and draining lymph nodes from HCC patients that undergo liver transplant and isolation of lymphocytes present in these specimens.
2. Genetic analysis of cancer cells to identify non-synonymous mutations in relevant genes for HCC.
3. Generation of immunological targets for the functional screening.
4. Functional screening of the lymphocytes after co-culture with target cells.
5. TCRs from reactive cells will be isolated and cloned into expression vectors and collected in a library.

Different recipient lymphocytes types will be evaluated.

Research group

Prof. Matti Sällberg: Head of department of Laboratory Medicine, Karolinska Institutet. Lars Frelin, PhD: Associate Professor in Virology, Karolinska Institutet. Programme Director for the Study Programme in Biomedical Laboratory Science and the Master´s Programme in Diagnostic Cytology, Karolinska Institutet. Member of the Infrastructure Committee at Karolinska Institutet. Anna Pasetto, PhD: Senior Lecturer. Managing Director of the pre-GMP facility at ANA Futura, Karolinska Institutet. Gustaf Ahlén, PhD: Project leader Panagiota Maravelia, MS: PhD. student Michael Chrobok, PhD: research assistant.

Key words

HCC, immunotherapy, cancer, T cells, tumor infiltrating lymphocytes (TIL), TCR-T cells, neoantigens.

Karolinska Institutet

Interested in recruiting a Doctoral student, Visiting doctoral student or Postdoc

Project title
Transcriptional complexity and RNA metabolism as a readout for personalised medicine

Supervisor
Vicente Jose Pelechano Garcia, Doctor, Department of Microbiology, Tumor and Cell Biology (MTC)
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Home page: pelechanolab.com

Type of recruitment and qualifications of applicant

- Doctoral student (48 months)
- Visiting doctoral student (6-12 months)
- Postdoc (12-24 months)

We aim to recruit either a PhD candidate or a postdoctoral researcher, depending the qualification and match to the proposed project. The candidate will have the opportunity to learn and develop a variety of computational and experimental genome-wide tools. Applicants to this position should possess adequate experience (according to their respective education level) in computational biology, molecular biology, genomics or RNA biology.

Preferred experience also includes familiarity with NGS data handling and technology development, eukaryotic transcription, ribosome-profiling, RNA degradation and quantitative trail loci analysis. A strong interest in interdisciplinary technology development, and novel and creative thinking abilities are essential. The successful candidate is expected to be highly motivated and take a strong lead on his/her project and start to develop independent ideas. The candidate should be able to communicate scientific results by writing up scientific papers and attending scientific meetings in English. The ideal candidate is also expected to participate in the general duties of the team and to effectively communicate with scientists of very diverse backgrounds in a highly interdisciplinary and international environment. Applicants wishing to integrate computational and experimental biology approaches are especial encouraged to apply.

Background
One of the biggest challenges in biology is to understand how identical genetic information encoded in the genome generates diversity between cells and tissues. Gene expression is the fundamental process whereby genetic information is expressed to control cellular identity and plasticity; defects in this process have been associated with numerous diseases. To adapt to changes in the environment cells and organism must alter their gene expression program, often involving

changes in RNA abundance. However, in recent years, our view of RNA has markedly changed, from regarding these molecules solely as intermediates of genetic information to appreciating their variety of functions that are independent of their protein-coding potential. The development of high-throughput approaches has revealed pervasive transcription in all genomes that have been investigated so far. This has uncovered a highly interleaved transcriptome organization that involves thousands of coding and non-coding RNAs and has challenged our traditional definitions of genes and functional regions of the genome.

Research project description

Our group has developed a variety of genome-wide approaches to study gene expression, and to improve clinical analysis. We investigate the complexity of overlapping human transcript isoforms simultaneously sequencing both the 5' and 3' ends of each RNA molecule (TIF-Seq, Nature 2013). We have now improved our published approach to make it suitable for the study of complex mammalian genomes (TIF-Seq2). Our work in Chronic Myeloid Leukaemia (CML) has identified many "transcriptionally fused" transcripts (conjoined) with potential to produce fused proteins and to rewire gene expression regulation. Those novel transcripts are present both in commonly used cell lines (K562) and patient cohorts. However, we do not know up to what degree those conjoined isoforms are associated with disease progression and could be use as biomarkers. In additions to the study of the functional consequences of transcriptional complexity, our group is also interested in understanding the control of RNA degradation. We have previously showed the widespread existence of co-translational mRNA degradation in eukaryotes and how that process allows studying ribosome dynamics by sequencing mRNA degradation intermediates (5P-Seq, Cell 2015).
As starting point for this project, we hypothesize that signatures of full-length and "in degradation" mRNA would be useful indicators to report the physiological status of the cells, and thus potentially help with patient stratification. The selected candidate will combine the use of TIF-Seq2 with long-read sequencing approaches and RNA degradome sequencing to investigate the functional consequences of transcriptome complexity. The candidate will study both cellular systems as well as clinical samples. Special focus will be placed on the development of novel computational tools to investigate the transcriptional complexity in human cells.

Research group

Our lab is located at the Science for Life Laboratory (https://www.scilifelab.se). SciLifeLab is equipped with state- of-the-art instrumentation and core facilities for NGS and high-throughput biology, and thus is the ideal place to develop the project we propose. Our lab is composed by 3 PhD students at different stages of their education that combine experimental and computational work, 6 postdoctoral researchers and a technician with expertise on RNA biology, bioinformatics, yeast biology, clinical genomics and epigenetics. This will provide the candidate with additional support, practical supervision and opportunities for collaboration. To increase our critical mass, and to contribute to our interdisciplinary environment, we perform weekly joint group meetings and journal clubs with the groups of other 2 SciLifeLab Fellows: Claudia Kutter (KI-MTC; focused on lncRNA and tRNA biology) and Marc Friedlander (SU; Computational RNA biology).

Key words

Transcription, RNA, NGS, bioinformatics, computational biology, mRNA isoforms, RNA degradation, genomics.

Supplementary information

We aim to recruit either a PhD candidate (or visiting PhD student) or a postdoctoral researcher, depending the qualification and match to the proposed project.
The candidate will work in strong collaboration with the group of our colleague Prof. Wei Wu (CAS Key Laboratory of Computational Biology and Shanghai Institutes for Biological Sciences, http://www.picb.ac.cn/weiLab/index.php). The candidate is expected to visit the Wei Lab in the context of an ongoing collaboration funded by STINT and NSFC: “Joint China-Sweden Mobility Programme: Transcriptome complexity in human disease”.
Group home page: http://pelechanolab.com/

Karolinska Institutet

Interested in recruiting a Postdoc

Project title

Investigation of the role of non-coding RNAs in epidermal differentiation and cancer

Supervisor

Andor Pivarcsi, Associate professor, Department of Medicine, Solna
Email: andor.pivarcsi@ki.se Phone: +46 738330057

Type of recruitment and qualifications of applicant

Postdoc (6 - 24 months)

The applicant should have a PhD within the area of biomedical research and have an interest in exploring molecular mechanisms underlying human diseases. The applicant should have a strong interest in exploring and understanding the molecular basis of cell differentiation and cancer.

The applicant should have a documented experience in molecular biology techniques and have good communication skills in English both in oral and written form. The successful applicant is a team player, who can learn from and teach other members of the team and who can smoothly interact with other researchers within and outside of the team.

Previous experience in techniques relevant to non-coding RNA-research is an advantage. Experience with in vitro and in vivo models of cancer, CRISPR-based gene editing, chromatin immunoprecipitation, or bioinformatics are meritorious.

Background

Long non-coding RNAs are a heterogenous group of ncRNAs, which are transcribed and processed similar to mRNAs and are operationally defined based on their size (> 200 nucleotides) and apparent lack of capability to encode for proteins. LncRNAs can act at the transcriptional, posttranscriptional or at the posttranslational level and have been implicated in key biological processes including cell-cycle regulation, apoptosis, the control of pluripotency and lineage specification. Although functional studies indicate important roles for several lncRNAs, the biological significance of the vast majority of them is still unknown.

Cutaneous Squamous Cell Carcinoma (cSCC) is the most common and fastest-increasing cancer with metastatic potential. cSCC accounts for 20% of all skin cancer-related deaths and patients with advanced tumors - especially organ-transplanted patients with multiple and aggressive cSCCs - lack efficient treatment options. Thus, there is an urgent medical need to find novel therapeutic approaches. Despite progress in our understanding about the environmental factors and genetic mutations in skin carcinogenesis, our understanding about the role of lncRNAs in the malignant transformation is limited.

To identify clinically relevant lncRNAs in cSCC, we performed RNA sequencing in human cSCCs, which allowed us to detect cSCC-associated lncRNAs with previously unparalleled sensitivity. This proposal focuses on the understanding of the regulation and role of selected differentially expressed lncRNAs in skin carcinogenesis using a translational approach which combines the use of patient material with experimental cancer models. We will explore the role of specific lncRNAs in cancer initiation and progression and systematically characterize their transcriptional regulation by oncogenic pathways. Moreover, we analyze the role of skin-cancer-associated lncRNAs in in epidermal differentiation, which is one of the key processes that are impaired in cancer.

Research project description

In this project we aim to explore the role and mechanism of action of lncRNAs in skin carcinogenesis using a translational approach, which combines the use of patient material with experimental cancer models. The proposal is built on a solid amount of preliminary data and experience from previous research on ncRNAs in skin cancer. Recently we performed RNAseq on cSCC and healthy skin and identified a set of lncRNAs differentially expressed in cSCC. Results obtained with CRISPR-SAMbased overexpression and GapmeR-mediated knockdown of lncRNAs demonstrated that several of our identified lncRNAs are important regulators of processes related to tissue homeostasis and malignant transformation. We aim to expand on these observations and comprehensively investigate the function, mechanism of action and transcriptional regulation of cSCC-associated lncRNAs.

The postdoctoral researcher will join to this ongoing study and work with one of the selected noncoding RNAs. He or she will perform mechanistic studies aiming at the identification of the mode of action of non-coding RNAs, and functional experiments using in vitro and in vivo experimental models of cancer and differentiation alongside the biochemical characterization of binding partners by RNA pulldown and RNA-immunoprecipitation. Specifically, the postdoctoral researcher will explore the transcriptional regulation of skin-cancer-associated lncRNAs during epidermal differentiation and carcinogenesis identify the direct interacting partners of skin-cancer-associated lncRNAs by biochemical approaches including RNA-immunoprecipitation (RIP) establish the role of skin-cancer-associated lncRNAs in the regulation of epidermal differentiation using a loss-of function and gain-of-function approaches in 3D organotypic skin models participate in identifying the putative tumor suppressor effect of skin-cancer-associated lncRNAs in cSCC in in vitro and in vivo experimental models of cancer Results from this project will increase knowledge about epidermal development and carcinogenesis and may be utilized in the future in the therapy of skin cancer.

Research group

The research group is located in CMM (Center for Molecular Medicine) at the Karolinska Hospital Solna area, which allows collaboration with other research groups located at CMM, access to core facilities in CMM and other locations at KI. The proximity to the Karolinska Hospital which is advantageous for sample collection and clinical collaboration. The team consist of experienced postdocs,

physicians and PhD students as well as master students and freshly starting PhD students.
Andor Pivarcsi, PhD - Associate professor, project leader
Warangkana Lohcharoenkal, PhD – lab manager, responsible for in vitro and in vivo methods (tumor xenograft and metastasis models)
Ankit Srivastava, Postdoctoral researcher, responsible for molecular biology methods, functional assays
Kunal Das Mahapatra– PhD student, responsible for functional assays, molecular biology methods, signaling studies
Li Chen, CSC PhD student, start in the group in 2019 November Mohan Krishna, visiting PhD student with FEBS fellowship Nicole Hemmer, master student
Enikö Sonkoly, MD PhD - Associate professor, responsible for ethical permits, collection of patient material
Jan Lapins, MD PhD – Physician; responsible for collecting skin biopsies from patients with skin cancer
We collaborate with other research groups with expertise in RNA-biology and skin-research within and outside of Sweden.

Key words

Cancer, development, non-coding RNA, microRNA, differentiation, skin, RNAseq, CRISPR, lncRNA, lincRNA, gene regulation, cell biology.

Karolinska Institutet

Interested in recruiting a Doctoral student

Project title

The contribution of the bone marrow niche to the development and treatment response of myeloid leukemia

Supervisor

Main supervisor: Hong Qian Senior researcher, Department: Department of Medicine Huddinge. Co-supervisors: Dr. Sören Lehmann, for his clinic expertise in AML and Dr. Julian Walfridsson, for his expertise in molecular biology, gene-editing
Email: hong.qian@ki.se Phone: +46 8-52483453
Group website: https://ki.se/en/medh/hong-qian-group

Type of recruitment and qualifications of applicant

Doctoral student (48 months)

We are looking for a highly self-motivated person with a background in cellular and molecular biology with a keen interest in bone marrow microenvironment. Experience in working with molecular and cellular biology techniques, multi-color flow cytometry, mice and bioinformatics is highly desired; and good ability to communicate in English (written and verbal) is required.

Background

The main challenge for successful treatment of myeloid leukemia, particularly, acute myeloid leukemia (AML) has been drug resistance resulting in treatment failure, relapse and leukemia progression, which ultimately leads to death. Recent studies have suggested that the drug resistance is attributed to the persistence of leukemia-initiating stem cells (LSCs). Thus, there is a great need for more effective therapies. To develop such therapies a crucial step is to understand how LSCs become resistant to the current therapies and outcompete normal hematopoiesis. There is increasing evidence indicating that LSC persistence is due to the protection by bone marrow (BM) microenvironment, the so-called niche (Beaulieu et al., 2011; Xu et al., 2016; Zhang et al., 2012; Zhang et al., 2013). Therefore, targeting the CML niche may offer new treatment options to eradicate the LSC and to cure the disease. The BM niche consists of multiple types of stromal cells including endothelial cells, mesenchymal stem cells (MSCs) and their progeny, as well as cytokines and extracellular matrix molecules. The dynamic interactions between leukemic cells and the BM niche play an important role in the niche-remodeling and subsequent loss of normal hematopoiesis. However, little is known about the key niche factors contributing to LSC resistance and relapse in patients. Deciphering this may help us identify new niche-based therapeutic targets for developing better treatment options.

Research Project description

The primary aim of our research is to understand the role of BM microenvironment/niche in the development and treatment response of myeloid malignancies including chronic myeloid leukemia (CML) and AML. The long-term goal is to identify new niche factors that mediate molecular interactions between leukemia and their niche, therefore can serve as novel therapeutic targets for better treatment of the leukemia. There are several research questions we aim to answer in the future, 1) What characterizes the BM niche in CML or AML? 2) How is the niche feature related to the disease progression and treatment response? 3) What are molecular pathways mediating the interactions between the LSCs and their niche? 4) Can we restore the leukemic niche by editing the niche factors to make LSC susceptible to chemotherapy?

We plan to answer these questions by first characterizing BM mesenchymal niche in patients and mice with CML and AML and functional evaluation of the functional impact and therapeutic potential of the candidate niche factors on LSCs by gain and loss of functional strategies.

Research group

This project will be performed at Center for hematology and Regenerative Medicine (HERM) at Karolinska Institute. Currently, the group consists of the following members:

1. Hong Qian, group leader, hong.qian@ki.se
2. Lakshmi Sandhow, currently a doctoral student.
3. a postdoc will start in November 2019.
4. master students: Leah Hernandez-Muñoz, Moeen Ud-din, Sanchari Choudhury.
5. Anne-Sofie Johansson, only about 20% works in this group, help with lab infrastructure work like ordering and invoice-handling.
6. Marja Ekblom, Professor, Emerita in Hematology in the Department of Molecular Hematology, Lund University, associated to the group.

In addition, Monika Jansson, a lab manager, 50% works for our center HERM to help with introducing new persons, ordering commonly used reagents, plastic supplies, maintaining well-functioning infrastructure at HERM, and so on.

Key words (add relevant key words for finding the project in database)

Myeloid leukemia, bone marrow, niche/microenvironment, leukemic stem cells, mesenchymal stem cells, mouse models, flow cytometry, transplantation, CRISPR, RNA sequencing

Karolinska Institutet

Interested in recruiting a Postdoc

Project title
Defining the transport of BCG through lymphatics in priming T cells.

Supervisor
Antonio Rothfuchs, Associate professor, Dept of Microbiology, Tumor and Cell Biology
Email: antonio.rothfuchs@ki.se Phone: +46-8-5248 5252
Home page: http://ki.se/research/rothfuchs

Type of recruitment and qualifications of applicant
Postdoc (12 – 24 months)

The applicant should have a PhD in Immunology or Infection Biology. Previous experience in mouse models and in performing experimental procedures in mice is important. Previous experience in flow cytometry, tissue culture work, handling mycobacteria or other microbiological agents is a merit. Previous experience in cryo- sectioning and confocal microscopy is also a merit. The applicant must be fluent in English, have good communication skills and be able to work as part of a team.

Background
Naïve T cells are activated or primed in lymph nodes by Dendritic cells. This fundamental process that arms T cells to fight-off pathogens and tumors is poorly understood during infection with Mycobacterium bovis Bacille Calmette-Guérin (BCG), the only available but ineffective vaccine against tuberculosis. In this regard, litte is known about T-cell priming in the lymph node that grains the BCG infection (vaccination) site in the skin, and in particular how BCG bacilli relocate from the infection site in the skin to the lymph node. We have been addressing these questions in a BCG infection model in mice. Using a fluorochrome-based migration assay developed in our laboratory (Bollampalli et al J Vis Exp 2016) we have discovered a migratory skin Dendritic cell sub-population that actively transports BCG to the lymph node; in doing so this Dendritic cell subset helps prime CD4+ T cells to BCG (Bollampalli et al PLOS Pathog 2015). However, our studies also show that BCG may drain to the lymph node in the absence of Dendritic cell transport (Bollampalli et al PLoS Pathog 2015) but the contribution of the latter transport to priming T cells in the lymph node is unknown. Available methods have so far been inadequate to investigate this. We have therefore set up a novel technique for intralymphatic injections to address this issue.

Research project description
AIM
The overall goal of this project is to investigate T-cell priming to BCG. The specific aim of this project is to investigate how lymphatic drainage of BCG in the absence of Dendritic cell transport affects the outcome of T-cell priming.

APPROACH

Our experiments rely on a BCG infection model in mice, transgenic tools, multi-color flow cytometry, high- resolution confocal microscopy and the ability to deliver BCG and Dendritic cells to the lymph node via intralymphatic injections. Briefly, we have established a simple but robust BCG mouse model that mimics the human intradermal route of BCG vaccination. In this model BCG is injected in the footpad skin and the priming of BCG-specific CD4+ T cells studied in the draining, popliteal lymph node. We have previously reported on the priming of CD4+ T cells in this model. It relies on the adoptive transfer of BCG-specific P25 T-cell receptor transgenic (TCRTg) cells and the use of flow cytometry to monitor their activation (Bollampalli et al PLOS Pathog 2015; Obieglo et al J Immunol 2016; Nasi et al Sci Rep 2017).

By cannulating the lymphatic vessel upstream of the popliteal lymph node, Dendritic cells and BCG can be directly delivered to the popliteal lymph node by intralymphatic injection. This unique approach allows us to investigate T-cell priming in our model following the arrival of "free" BCG bacilli that have gained direct access to lymphatic vessels without becoming associated to Dendritic cells. The outcome of T-cell priming will then be compared to BCG delivered inside Dendritic cells via this same route. To this end, BCG expressing red- fluorescent protein (RFP) will be used to infect Dendritic cells in vitro and infected (RFP+) Dendritic cells will be separated on a cell sorter. Infected Dendritic cells will then be transferred intralymphatically to naïve recipients and the outcome of T-cell priming studied via the adoptive transfer of P25 TCRTg cells. Protocols to remove extracellular bacilli from infected Dendritic cells and to control for antigen leakage from infected Dendritic cells have been designed but are omitted here for the sake of brevity.

In parallel, we will perform confocal microscopy to evaluate the distribution in the draining lymph node of BCG delivered inside Dendritic cells relative to lymph-borne bacilli. We have already established the feasibility of delivering bone marrow-derived dendritic cells and BCG-RFP via the intralymphatic route, confirming the arrival of both Dendritic cells and BCG to the lymph node port-of-entry, the subcapsular sinus (Wowk and Krmeská, unpublished results). Through the execution of this project, the candidate will learn or consolidate the following: techniques in laboratory animal science including intralymphatic injections; basic cellular immunological methods including flow cytometry; confocal microscopy; cultivation of mycobacteria and working under Biosafety Level-2 conditions.

SIGNIFICANCE

Our experiments will unravel the contribution of BCG that drain directly to the lymph node in the absence of Dendritic cell transport, in priming CD4+ T cells therein. Our experiments add as such to the general knowledge of how an immune response to bacteria unfolds in vivo. Since intralymphatic delivery of BCG formally bypasses the skin and its Dendritic cells during priming, these experiments also provide novel insights on the relative role played by migratory skin Dendritic cells in priming T cells to BCG. Finally, our studies may offer new avenues for manipulating

Dendritic cells for clinical benefit and for improving the efficacy of BCG and other vaccines of low-to-modest efficacy that rely on CD4+ T-cell responses.

Research group

We are a compact team of 4 researchers working at the Department of Microbiology, Tumor and Cell Biology (MTC), Biomedicum, Karolinska Institutet. The team is composed of group leader and Associate Professor Dr. Antonio Rothfuchs and 3 young scientists: 1 PhD student, 1 visiting PhD student from Oswaldo Cruz Foundation (Brazil) and 1 research assistant. We will be joined in January 2020 by a Masters student from France. A CSC postdoctoral fellow just returned to China after completing 2 years of research training in the lab.

The group leader Dr. Rothfuchs has guided 1 PhD student in the group to completion of her thesis (2017) and as co-supervisor has guided another 2 PhD students to completion (2017 and 2018, both CSC scholars). Dr. Rothfuchs is currently main supervisor to 1 PhD student working in a project strongly aligned to the current proposal, and co-supervises 2 PhD students at MTC, Biomedicum, in related areas of infection biology and immunology. As main supervisor, the group leader has coached to date 4 postdocs (2015-2019), 2 visiting PhD students (2014, 2019), 3 research assistants (2011-2019), 6 Masters students (2011-2019) and 2 Bachelors students (2015-2019). The group is part of a larger constellation at MTC of researchers with similar interests in tuberculosis infection biology and immunology (5 PhD students, 2 postdocs, 1 research assistant) at Biomedicum C 9 quarter, and an even larger group of tuberculosis scientists under the Center for tuberculosis research at Karolinska Institutet. All the necessary infrastructure and equipment for the proposed project is available within the laboratory of the group leader or his collaborators, or within core facilities at Biomedicum including the Biomedicum Biosafety Level-2 animal facility (KM-B ABSL-2), Biomedicum Imaging Facility (BIC) and Biomedicum Flow cytometry Core (BFC).

Key words

Dendritic cells, T cells, mycobacteria, tuberculosis, lymph node, immunology, mouse models

Karolinska Institutet

Interested in recruiting a Postdoc

Project title
Exploiting cancer cell metabolism to enhance current cancer therapies

Supervisor
Sean Rudd,: Assistant Professor, Department of Oncology-Pathology
Email: sean.rudd@scilifelab.se
Home page: https://staff.ki.se/people/searud

Type of recruitment and qualifications of applicant
Postdoc (24 months)

The applicant should have a PhD in biochemistry / molecular biology / biomedicine or a related field, ideally a recent graduate. A keen interest in cancer biology and translational cancer research is essential. Previous research experience with chemotherapeutics, assay development, and anti-cancer drug discovery would be highly desirable. The applicant would need to be familiar with standard biochemical, molecular and cell biology techniques. Experience working both with recombinant proteins and cell culture would be ideal but not a requirement. Previous experience with microtiter plate-based high-throughput assays would be a strong merit, such as those used for enzymology experiments or high-throughput drug screening. A basic understanding of bioinformatics and statistical methods would be a plus. Fluency in spoken and written English is a must. Previous publications in internationally recognized journals would be a strong merit. A critical attribute of the applicant is the ability to work in a team and conduct collaborative research projects.

Background
Cancer is one of the leading causes of death worldwide. Over one fifth of cancer patients will receive treatment containing one or more of a class of chemotherapy called antimetabolites, which provide an extremely important treatment option, regardless of whether the patient is a child or adult, or whether the malignancy is solid or haematological. Antimetabolites are synthetic analogues of endogenously occurring metabolites, such as DNA precursors like nucleobases and nucleosides, which compete with their endogenous counterparts to exert their anti-cancer activities, often exploiting the rapid proliferation of cancer cells. Despite being cornerstones in many standard-of-care treatments, their efficacy can vary greatly between patients within the same diagnostic group, and treatment failure can occur through lack of response or relapse. Currently, our understanding of the metabolism of these drugs cannot fully account for, or predict, patient response. Thus, there is a clear need to identify additional factors involved in the metabolism of these drugs, and to utilise these for the development of new strategies to improve treatment of cancer in adults and children.

Our research identified the deoxynucleoside triphosphate (dNTP) triphosphohydrolase SAMHD1 as a cytarabine (ara-C) resistance factor in acute myeloid leukaemia (AML), which we reported in 2017 in Nature Medicine. In short, we demonstrated that SAMHD1 converts the active metabolite of this drug back to its prodrug form, thereby controlling the therapeutic response of AML to ara-C. Continuing from this study, we demonstrated that many other clinically used nucleoside analogues are subject to SAMHD1 control in cancer cells. Thus, SAMHD1 could be a promising biomarker to predict patient response to a variety of antimetabolite-based cancer therapies, not only ara-C in AML, and furthermore, SAMHD1 could be a therapeutic target to potentiate the efficacy of these drugs in adults and children with cancer.

Project description

Manipulation of DNA precursor pools to influence genome integrity has long been exploited in cancer therapy (1), a prime example of which is a group of chemotherapies called antimetabolites. However, despite these drugs remaining standard-of-care for most common cancers, a frequent problem is that patient response can vary, and the underlying mechanisms often remain unclear (2). Research by Dr Rudd and collaborators at Karolinska Institutet identified the enzyme SAMHD1 as a critical modulator of these therapies, in particular cytarabine therapy for treatment of acute myeloid leukaemia (3, 4), and a subsequent study implicated SAMHD1 in the control of several other antimetabolites active in a range of malignancies (5). Building upon these studies, the successful candidate will aim to further define this role of SAMHD1 as a drug resistance factor and develop strategies to inactivate this enzyme in cancer cells, principally developing small molecule inhibitors, thus providing a potential means to overcome this barrier to therapeutic efficacy. Practically, this project entails working in a small interdisciplinary team with close collaboration with research physicians at Karolinska Hospital, and employs an array of different techniques, including biochemical, biophysical, and cell-based assays.

References:

1. Rudd SG, Valerie NC, Helleday T. Pathways controlling dNTP pools to maintain genome stability. 2016. DNA Repair; 44:193-204. PMID: 27311542.
2. Tsesmetzis N, Paulin CBJ, Rudd SG, Herold N. Nucleobase and Nucleoside Analogues: Resistance and Re-Sensitisation at the Level of Pharmaco-kinetics, Pharmacodynamics and Metabolism. 2018. Cancers; 10, 240. PMID: 30041457.
3. Herold N, Rudd SG, Ljungblad L, Sanjiv K, Myrberg IH, et al. Targeting SAMHD1 wit the Vpx protein to improve cytarabine therapy for hematological malignancies. 2017. Nature Medicine; 23(2):256-263. PMID: 28067901.
4. Rudd SG, Schaller T, Herold N. SAMHD1 is a barrier to antimetabolite-based cancer therapies. 2017. Molecular and Cellular Oncology; 4(2):e1287554. PMID: 28401188.
5. Herold N, Rudd SG, Sanjiv K, Kutzner J, Bladh J, et al. SAMHD1 protects cancer cell from various nucleoside-based antimetabolites. 2017. Cell Cycle; 16(11):1029-1038. PMID: 28436707

Research group

The research group of Assistant Professor Sean Rudd (www.ki.se/en/people/searud) was established in January 2019 and currently consists of one senior Postdoctoral Research Fellow, Dr Si Min Zhang, and one Biomedicine MSc thesis student. Dr Zhang is an expert in drug metabolism and cancer biology and has more than five years post-PhD research experience in academic and industry labs both in Sweden and abroad. The Rudd Group is placed within the laboratory of Professor Thomas Helleday (www.helleday.org), a multidisciplinary group consisting of over 40 scientists from different nations covering all the necessary disciplines required for conducting translational cancer research, from molecular biologists and medicinal chemists to pharmacists and research physicians. Members of the Rudd Group are well integrated within Professor Helleday's laboratory, attending weekly lab meetings and journal clubs in addition to collaboration on research projects. The Rudd Group is affiliated with the Department of Oncology-Pathology at Karolinska Institutet, which has a clear focus upon basic and translational cancer research, whilst the group is physically located at Science For Life Laboratory (www.scilifelab.se), a national hub for molecular biosciences in Sweden containing a plethora of high-throughput instrumentation and expertise.

Key words

Cancer, Precision Medicine, Drug Discovery, Chemotherapy, Genome Stability, Nucleotide Metabolism, Drug metabolism, Antimetabolites, SAMHD1, Cytarabine, Ara-C, Drug combinations, Drug synergy, DNA repair, DNA replication, High-throughput, Screening, Small molecule, Chemical probe.

Interested in recruiting a Postdoc

Project title
Curing hepatitis B

Supervisor
Matti Sällberg, Professor, Department of Laboratory Medicine
Email: Matti.Sallberg@ki.se Phone number: +46 706082101

Type of recruitment and qualifications of applicant
Postdoc (24 months)
The applicant should have extensive experience in molecular immunology and flow cytometry. The applicant should be able to work relatively independently within the project. The applicant should know cloning, ELISA, ELISpot, FACS analysis, and western blot. It is an advantage if the applicant has experience with animal work (mice). The applicant should have a PhD in a relevant area of virology or immunology.

Background

Despite the availability of preventive vaccines and antiviral therapies, chronic hepatitis B virus (HBV) infection currently affects over 250 million people across the globe. As many as one million chronic carriers die every year due to the liver related complications caused by the virus, such as liver cirrhosis and eventually hepatocellular carcinoma (HCC). The hepatitis D virus (HDV), an RNA satellite virus to HBV that “steals” the surface antigen from HBV (HBsAg), co-infects 15-25 million of HBV carriers globally and worsens disease progression. Till now, there is no effective functional cure for chronic HBV or HDV infection. The current standard of care therapy for HBV consists of nucleoside analogues (NAs) that inhibit the reverse transcriptase (RT) function of the HBV polymerase. This prevents viral maturation by blocking the synthesis of the partially dsDNA inside the capsid. Hence, NAs effectively suppress the viral replication as long as the treatment lasts, however sustained offtherapy responses are rare. This blocking of RT neither affects protein production (including HBsAg) and release, nor synthesis of the covalently closed circular DNA (cccDNA), the main cause for HBV persistence. A life-long NA therapy reduces, but does not eliminate, the risk of HCC. HBV infected cells overproduce sub-viral HBsAg particles mainly composed of the small (SHBsAg) protein encoded by the S-gene to block the neutralizing antibody population directed to SHBsAg. This ensures survival of viral particles whose surface are more dense in the middle HBsAg (MHBsAg; containing S and PreS2) and large (LHBsAg; containing S, PreS2 and PreS1) proteins. Thus, an obvious way to target infectious HBV particles and prevent the infection of new hepatocytes, would be to raise antibodies to the PreS1 domain of the virus, and use T cells to kill HBV and HDV infected cells.

Research project description

AIM
The project aims at developing new therapies for chronic HBV and HDV infections. The focus is immunotherapies that utilize neutralizing antibodies and T cells. Thus, the tools that will be developed are vaccines, monoclonal antibodies (mAbs), chimaeric antigen receptor (CAR) T/NK cells, and T cell receptor (TCR) modified T/NK cells.

METHODS
The applicant will participate in optimizing a newly developed HBV/HDV vaccine with respect to delivery, adjuvants and dosing. From vaccinated mice mAbs will be generated and used for neutralization studies and to generate novel CAR gene constructs. Similarly, novel TCRs will be generated from immunized HLA-A2 transgenic mice. These mAbs, CARs, and TCRs are then tested for recognition of HBV and HDV by ELISA, ELIspot and FACS to select those that will be tested further. The best candidates are then analyzed for neutralization/killing of HBV/HDV in tissue culture using NTCP expressing HepRG cells (collaboration with Prof Urban, Univ Heidelberg). Those that neutralize/kill in vitro will then be tested for neutralization) killing in vivo using mice repopulated with human hepatocytes (collaboration with Prof Meulemann, Univ Gent).

PROJECT PLAN
The applicant will participate in immunizations of wildtype and various transgenic mice and in the analysis and selection of the most potent immunogens. The optimal immunogen will be used to generate mAbs and TCRs in wild type and transgenic mice. Significant work will include cloning and expression analysis of immune genes. During the second year the focus will be the testing and analysis of the cloned immune genes by the in vitro and in vivo assays described above.

The successful applicant will participate in most tasks in the proposed project. This will include the possibility to travel to partner laboratories in Germany and Belgium, and present data at national and international conferences.

Research group

The research group consists of professor Matti Salllberg, with a more than 25 years experience in viral hepatitis and vaccine development. Professor Sallberg holds multiple grants for the proposed research and for development of new advanced therapies. He has supervised more than 25 PhD students and more than 20 post docs.
Assistant professor Anna Pasetto has been recruited from Dr Steven Roesenbergs laboratory at the NIH and has a unique hands-on knowledge in isolation, cloning and expression of immune genes. Associate professor Lars Frelin has an extensive experience in generating transgenic animals and vaccine development. Dr Gustaf Ahlen is heading the clinical development of a Horizon 2020 funded project on vaccine development against Crimean Congo hemoragic fever virus. A new post doc has been recruited that will participate on isolation, cloning and expression of immune genes with a focus on cancer. Finally, Panagiota Maravelia is a highly

experienced PhD student focusing on vaccine development. Overall, the group has expertise in all areas within the proposed project.

Key words
Immunotherapies, viral hepatitis, CAR-T, CAR-NK, monoclonal, vaccine.

Interested in recruiting a Doctoral student

Project title

Exploring tumor heterogeneity in sympatho-adrenal malignancies to identify new therapeutic targets.

Supervisor

Susanne Schlisio, Doctor,
Department of Microbiology, Tumor and Cell Biology
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Type of recruitment and qualifications of applicant

Doctoral student (48 months)

We seek a PhD student to work on the analyses of a large-scale single-cell transcriptome project on childhood neuroblastoma and pheochromocytoma to understand heterogeneity and drug resistance of this malignancy.

Position-specific requirements: MSc.
Furthermore, the student should have educational background in cell biology, molecular biology, oncology, medicine. Previous experience in in vitro study including all cell-based methods is required. Other skills in using programming language for data analysis and experience with animal experiments will be considered as a merit. Furthermore, fluent English skills and team spirit are essential as well.

Background

Purpose: Heterogeneity of tumors including the presence of resistant clones represents a great challenge for high risk neuroblastoma (NB). Currently, there are no treatments that address cancer heterogeneity. Classification of bulk tumors and corresponding predictions are poor and too general. Understanding heterogeneity will provide critical insight for the development of targeted therapeutic strategies. Here we elucidate the origin and heterogeneity of sympathoadrenal malignancies, such as childhood neuroblastoma and pheochromocytoma. Discovering the pathways and target genes that can drive tumors into less metastatic and differentiated states will provide important clues for the development of new therapies that are currently missing in high risk neuroblastoma and malignant pheochromocytoma.

Background: Neuroblastoma (NB) is the most common pediatric solid tumor and represents 15% of all childhood cancer deaths. High cellular heterogeneity is a hallmark of NB, which may account for the wide range of clinical presentations and non-uniform response to treatment. In preliminary single cell analysis, we observed that neuroblastoma is highly heterogeneous in itself and resemble some stages of neural crest differentiation into sympathetic neuroblast. Creating “comparative

identity maps" using single cell analyses of tumors and healthy progenitor populations will allow us to identify the precise developmental and adult origin cell types and corresponding differentiation status for different subtypes of NB. The methodology we propose involves mapping individual tumor cells onto developmental or homeostatic trajectory to reveal the molecular processes leading to NB. Identifying cell of origin of these sympathoadrenal malignancies will reveal the causes of disease heterogeneity and clinical behavior.

Research project description

Rational: The neuroblastoma cell of origin is thought to be a developing sympathoblast, however, its precise cell of origin remains unknown. Single cell transcriptomics will identify gene expression clusters that are prominent in specific embryonic sympathoadrenal developmental stages in NB. Identifying the cell(s) of NB origin and their temporal development will provide mechanistic insight into: disease initiation, heterogeneity, progression, clinical behavior and spontaneous NB regression.

Questions:

- Understanding the clinical heterogeneity of neuroblastoma: What distinguishes the "favorable" vs the "unfavorable" neuroblastoma?
- What unique signatures can be found in the spontaneously regressing neuroblastoma ?
- Do neuroblastoma types represent regulatory patterns in early or late embryonic sympathoblast/crest populations (SCP, Bridge, sympatho-verus immature chromaffin)?
- Can we identify cell-of-origin subpopulations for neuroblastoma initiation?

AIM1: Profiling tumor heterogeneity and immaturity on single cell resolution of human and mouse neuroblastoma and pheochromocytoma tumors:

We will analyse primary neuroblastoma tumors provided by the Karolinska Hospital that are characterized by stage, outcome, MYCN and 1p-status as well as genomic profile group using single nuclei transcriptomics to investigate heterogeneity, maturation and the identification of sub-groups within a heterogenic tumor. In the beginning, we will profile multiple frozen tumor samples from single nuclei RNA sequencing technique and in parallel with 2D transcriptomics analysis performed on the same tumors (targeted in situ sequencing (ISS) in collaboration with Mats Nilsson at SciLife).Single cell transcriptomics provides us with completely new insights into the molecular diversity of different cell types within the tumor. The major advantage of single nuclei RNA sequencing is that it allows to work with any frozen tissue samples, including those massively stored in biobanks. Rare tumors such as NB and PGL/PCC including relapsed tumor pairs are mostly available as frozen samples in biobanks, and it is rather infeasible to chase few patients for fresh material to perform whole cell analysis.

Furthermore, we will focus on the analysis of spatial information within tumors by performing 2D transcriptomics (targeted in situ sequencing (ISS)with the extensive sets of probes (100-200 probes/genes) that will be aim to identify diverse clones

deduced from Single Nuclei Sequencing and also all ligands that can bind to the receptors expressed by these diverse tumorigenic clones.

Aim 3: Understanding drug resistance in high risk neuroblastoma and malignant pheochromocytoma.

Our tumor-bearing mice (aim 2) allows us to monitor tumor dynamics, evolution of tumor clonally and microenvironment composition during the development of benign to malignant state in a single cell mode. Next, we will move to experimental pre-clinical validations using human cells lines in vitro and grafted into experimental animals as well as different genetic animal models described above. We will manipulate experimentally the pathways inferred from aim 1 and 2 in the appropriate models as outlined above. For instance, the analysis of switching receptors in developing sympatho-adrenal lineage stage by stage should indicate the pathways that are sequentially activated by signal-emitting cell types within the microenvironment or as a result of autocrine stimulation. An important central emphasis will address whether some cell trajectories are reversible and may thus lead to plasticity which is very probably a key phenomenon to account for tumor resistance, for instant will test if the MES component is much more resistant to current chemotherapies than the ADR component. Single cell analyses and/or in situ imaging approaches will be used to investigate tumors before and after treatment to evaluate cell selection induced by the experimental pharmacological or genetic manipulation

Research group

PI: Dr. Susanne Schlisio, Associate Professor, Karolinska Institutet, MTC
PhD student: Wenyu Li. Wenyu's project involves the characterization of neuroblastoma tumor mouse models and investigate hypoxia signaling in sympathoadrenal malignancies.
PhD student: Maria Arceo, computational biologist
Post docs: Dr Monika Plescher: in vivo characterization of KIF1Bb in the mouse sympathetic nervous system and characterization of neuroblastoma mouse model including single cell sequencing of these mouse tumors. Dr. Petra Bullova: Biochemist. Petra´s investigate the role of SDHx loss in sympathoadrenal malignancies. Dr. Shuijie Li: Dr. Li is investigating the impact of cancer metabolism in neuroblastoma as outlined in aim 3 and identified new pathogenic targets in neuroblastoma. Dr. Oscar Bedoya Reina. Oscar is a bioinformation analyzing single cell data and mapping individual tumor cells onto developmental or homeostatic trajectory to reveal the molecular processes leading to neuroblastoma as outlined in aim 1 and 2.

collaboration:

(1) Professor's Per Kogner and Tommy Martinson, Pediatric Oncology at Karolinska Institutet and University of Gothenburg, Sahlgrenska: providing neuroblastoma tumor material and genomic characterization of the tumors.
(2) Professor's Q. Deng and R. Sandberg, CMB, Karolinska Institutet, and the Ludwig Institute will provide bioinformatics help with the single cell analysis of primary neuroblastoma tumors.

(3) Prof. I. Adameyko, Department of Physiology and Pharmacology at Karolinska, providing single cell analysis of normal mouse embryonic cells from different sympathetic developmental stages (E9.5-E13.5).
Prof. W.G. Kaelin, Jr., M.D.; Dana-Farber Cancer Institute/Harvard Medical School, USA, expert in oxygen sensing and its implication in cancer. For his break through discovery he was awarded last year with the US most prestigious biomedical award, the Lasker award.

Key words

neuroblastoma, tumor heterogeneyity single cel ananlysis Hypoxia tumor mouse models sympatho-adrenal development

Karolinska Institutet

Interested in recruiting a Postdoc

Project title
Investigation of non-coding RNAs in inflammatory skin diseases

Supervisor
Enikö Sonkoly, Associate professor, Department of Medicine, Solna
Email: eniko.sonkoly@ki.se Phone: +46730770374

Type of recruitment and qualifications of applicant
Postdoc (6 - 24 months)

The application is open of a highly motivated scientist with a strong interest in human biology and medicine. The applicant should have a PhD within the area of biomedical research and have an interest in exploring molecular mechanisms underlying human diseases.

The applicant should have a documented experience in molecular biology techniques and statistics and should demonstrate ability to simultaneously participate in several research projects. Experience with animal handling, in particular disease models of inflammation and/or cancer is an advantage. Competence in bioinformatics including analysis of large-scale gene expression data, and experience with immunology techniques is meritorious. Previous experience with non-coding RNA research is an advantage.

The applicant needs to have excellent communication skills in English, both in oral and written communication. Also, the applicant should demonstrate ability to smoothly interact and collaborate with others within the research group. Previous experience with supervision of students is an advantage.

Background
The main research focus of my group is the investigation of non-coding RNAs in inflammation, in particular the most common chronic inflammatory skin disease in adults, psoriasis.

In our research, we integrate data from human patient samples, in vitro models of monolayer cell culture and three-dimensional epidermal models, and animal models of skin inflammation such as the IL-23- and imiquimod- induced animal models.

In 2007, we described deregulation of miRNAs in psoriasis skin for the first time and since then my group has been investigating the function of non-coding RNAs in psoriasis in mechanistic studies, and their therapeutic and biomarker potential. We have identified several miRNAs (miR-203, miR-21, miR-125b, miR-146a, etc) that not only have altered expression in psoriasis skin but can also contribute to the disease pathogenesis by altering cellular functions including T cell apoptosis,

keraitnocyte proliferation, and responses of keratinocytes to inflammatory cytokines.

As the skin is a complex tissue, using full-depth biopsies to generate mRNA and non-coding RNA experssion data can mask the cell-specific gene expression changes. Therefore, in the past years we have moved towards a more cell-specific approach, and characterized the coding and noncoding transcriptome of keratinocytes, the skin epithelial cells in psoriasis. Investigation of cell-specific transcriptomes by single-cell RNAseq is ongoing.

Research project description

The aim of this project is to explore the roles of non-coding RNAs in inflammation, with a focus on the inflammatory skin disease psoriasis, in which a disturbed interaction between keratinocytes(skin epithelial cells) and immune cells leads to chronic inflammation. Non-coding RNAs, including among others long non-coding RNAs (lncRNAs) and microRNAs (miRNAs), have emerged as important regulators of gene expression, and their deregulation can contribute to diseases.

Recently we analyzed the coding and non-coding transciptome of keratinocytes in psoraisis and healthy skin, using a cell-specific approach. We have identified several non-coding RNAs with differential expression between keratincytes of psoriasis and healthy skin. In the proposed project the postdoc will investigate the regulation and function of a selected lncRNA identified in our screen, using patient material, in vitro monolayer and 3D cell cultures, and in vivo disease models. The postdoc will

1) identify the cellular and subcellular localization of the lncRNA by single-molecule in situ hybridization
2) investigate the regulation of the lncRNA by psoriasis-associated cytokines in 2D and 3D models, and explore its upstream regulation using specific pathway inhibitors
3) identify downstream signaling pathways/targets of the lncRNA by plasmid- or CRISPR-based overexpression and inhibition of the lncRNA in primary cells followed by gene expression anaysis by RNAseq
4) explore the function of the lncRNAs in vitro, by plasmid- or CRISPR-based overexpression and inhibition of the lncRNA in primary cells followed by relevant functional assays (cytokine/chemokine production, proliferation etc)
5) investigate the function and therapeutic potential of the lncRNA in vivo, using animal models of psoriasis combined with local delivery of the lncRNA inhibitor, followed by analysis of macrosopic and histological characteristics, immunohistochemistry, and gene expression by RNAseq

We have preliminary data and all methods inculded in this project (including primary cell clulture and 3D models, single-molecule in situ hybridization, lncRNA overexpression/inhibition, CRISPRa, functional assays, animal models) are established in the laboratory wihch allows for the successful realization of the project within the time frame.

The proposed project allows the postdoc to learn state-of the art in vitro and in vivo models of inflammation and techniques used in non-coding RNA research. The project is anticipated to identify novel regulatory pathways in epithelial cells which may have implications not only for psoriasis but also for other inflammatory diseases and skin biology.

Research group

Members of the research group:
Group leader: Enikö Sonkoly, senior lecturer at KI and dermatologist, leading the research group in miRNAs and inflammation.

Members:

- Ankit Srivastava, and experienced postdoc, who defended his thesis in 2018. Expertise in non-coding RNA techniques, invitro and in vivo models of inflammation. Experience in supervising students.
- Lorenzo Pasquali, PhD student in his 3rd year, experience in non-coding RNA research and special interest in bioinformatics analyses.
- Einar Rosén, research assistant, MD. Assists in research projects and human sample collection.
- Longlong Luo, PhD student, registered in August 2019. He will start his PhD education durign October/November 2019, on non-coding RNAs in psoriasis.
- Chenying Gao, PhD student at the Xian Jiao Tong University, China. She will join the group from 4 November 2019 for one year, as a visiting PhD student, to perform research on miRNAs in psoriasis.
- Warangkana Lohcharoenkal, research coordinator shared with two other research groups. Responsible for introduction of new members, lab safety etc.
- Research nurse Helena Griehsel assists in collection of human samples.

We also have a close collaboration with other research groups within dermatology which allow for synergistic interactions. The research group is located in CMM (Center for Molecular Medicine) at the Karolinska Hospital Solna area, and this allows collaboration with other research groups in inflammation research located at CMM, access to core facilities in CMM and other locations at KI, and proximity to the Karolinska Hospital which is advantageous for sample collection and clinical collaboration.

Key words

non-coding RNA, lncRNA, long non-coding RNA, miRNA, inflammation, skin, psoriasis, epithelial cells, keratinocytes, gene regulation, RNAseq, CRISPR, 3D model, animal model

Interested in recruiting a Doctoral student

Project title

Genetic and functional studies of skin disorders.

Supervisor

Isabel Tapia Paez, PhD, Department of Medicine, Solna,
Email: isabel.tapia@ki.se Phone number: + 46 739226453

Type of recruitment and qualifications of applicant

Doctoral student (48 months)

We are a laboratory working in genetics of atopic dermatitis at the Department of Medicine in the unit of Dermatology and venereology. Our lab is located at the newly built BioClinicum laboratory at the Solna campus of Karolinska Institutet in Stockholm, Sweden.

We are looking for a doctoral student that is highly motivated and enthusiastic towards science, with strong interest in learning and creative thinking abilities, who has degree in Biology, Bio(medicine), biochemistry or similar. The student should have proven experience in laboratory work and should have basic or advanced understanding of genetics, cell biology, gene regulation, molecular biology and/or molecular genetics. Laboratory skills such as cell culture, cloning, genotyping, sequence analysis or work with zebra fish are particularly advantageous. The successful candidate should be able to work as part of a team and also in an independent manner, good communications skills are important.

The applicant must be fluent in written and spoken English. Your application should contain a personal letter and a CV. The applicant should have documents certifying the general eligibility to become a graduate student at Karolinska Institutet: Entry requirements (eligibility) for doctoral education.

Background

Atopic dermatitis (AD) is a complex inflammatory skin disease with characteristically features. The prevalence of AD varies greatly between populations. In the Nordic countries, at least 20% of all children and 3-5% of all adults are affected with AD (1). Evidence from twin studies where a concordance rate of 80% has been observed in monozygotic twins, indicate that AD is a highly heritable disease. However, since the concordance rate is not 100% in monozygotic twins, it is believed that environmental factors also contribute to disease susceptibility. Until today, many genes have been found to be associated to AD. Among them the Filaggrin (FLG) gene in chromosome 1, is the main susceptibility gene (2). FLG is involved in the development of keratinocytes to maintain epidermal integrity and it is an important marker of keratinocyte differentiation. Loss-of-function mutations in FLG are found in up to 10% of northern Europeans and up to 40% of the patients with moderate to severe AD carry the 4 most

common FLG mutations. The frequency of these mutations in other populations seems to be very different, in studies in African population the FLG known mutations are present in less than 1% (3)(4).
Genome wide association studies (GWAS) to AD have been performed and more than 30 loci in different chromosomes have been identified. As in most of the genetic complex disorders, the majority of the AD associated variants are in intergenic regions and the target genes are difficult to recognize.
Our group is interested in the studies of AD, see: https://ki.se/en/meds/research-group-maria-bradley

Research project description

The overall aim is to increase the understanding of the etiology of skin disorders by genetic and functional molecular studies.
Specific aims: 1) To understand the effects of the intergenic AD associated SNPs in gene regulation. 2) To study new candidate susceptibility genes arising from the Hi-cap studies performed by our group. 3) To study the genetics of AD in the African (Ethiopian) population in which despite the high burden no mutations have been found.

Studies planned and Methods:
For specific aim1: Identification of keratinocyte-specific regulatory interactions using high throughput chromosome conformation and sequence capture (HiCap). This method is used in our laboratory for mapping cis- acting regions that interact during the 3D conformation of the chromatin. These interactions bring together regulatory regions influencing gene regulation. Using HiCap in normal differentiating keratinocytes we were able to identify these regions and we focus our results on regions containing variants associated to AD (manuscript in preparation). We are performing this method in collaboration with Pelin Sahlen's (Science for life laboratories) (5).

For specific aim 2: Using the method described above we have identified several genes which are potential targets of variants associated to AD. For example, using Hi-cap we found that a GWAS SNP rs10995251 in the 3D genome is in close proximity to a gene that has not been associated to AD previously. The gene is important for keratinocyte hydration and is a potential candidate for AD susceptibility. Another gene located 3.3Mb away from a SNP associated to psoriasis rs33980500, is important for mitochondria homeostasis and dysregulated mitochondria may trigger inflammation.

We plan to use cell models to understand the function of these candidate susceptibility genes in human keratinocytes and in relation to AD. We are also aiming to use the zebra fish as model to study the function of the candidate genes. The zebra fish work will be performed in close collaboration with Dr Raquel Vaz.

For specific aim 3: In this project we collaborate with Dr Kassahun Desalegn in Addis Ababa, Ethiopia. Despite the fact that AD in Ethiopian children is around 20% no major risk gene has been found. Therefore, the overall purpose and aim of our project is to try to improve our understanding of the development of AD in the

African population. We aim to perform Whole Genome Sequencing (WGS) on extended AD families collected from Ethiopia. Families will include both AD affected as well as unaffected individuals over several generations.

Significance for doctoral education: AD is a common disorder of mayor impact in society and therefore of great importance for further studies. The graduate student will take active part in the seminars, conferences and courses relevant for the project. The student with the help of the supervisors will master relevant methods, develop scientific critical thinking and gradually become independent in the project planning and execution.

References:

1. DaVeiga SP. Allergy Asthma Proc. 2012;33(3):227-34.
2. Palmer CN, et al. Nat Genet. 2006;38(4):441-6.
3. Thyssen JP, Godoy-Gijon E, Elias PM. Br J Dermatol. 2013;168(6):1155-66. 4. Winge MC, et al. Br J Dermatol. 2011;165(5):1074-80.
4. Sahlen P, et al. Genome Biol. 2015;16:156.

Research group

The main applicant Dr Tapia is a geneticist with long experience in performing functional studies of genes involved in complex diseases. Dr Tapia works in Bradley's lab focused in molecular and clinical research regarding different aspects of atopic dermatitis (AD). The focus is in translational research with pathogenetic, diagnostic and clinical perspective. For more information about the research group see: https://ki.se/en/meds/research-group-maria-bradley

The project for the graduate student will be performed in a close collaboration with scientist that add key expertise to solve our biological questions. For the study 3D genomic landscape of keratinocytes, we collaborate with Assistant professor Pelin Sahlén, PhD, Science for Life Laboratory, school of Biotechnology. Dr Sahlén is expert in Hi-C and data analysis, she has developed the technique and has publish several articles improving the resolution of the Hi-Cap method. For the patient collection in this project we collaborate with Professor Mona Ståhle, MD, PhD. Professor Ståhle is an expert in Psoriasis research. For the genetic studies in different human populations we have a collaboration with Dr Kassahun Bilcha, MD, PhD working at the Skin Department at Black Lion University Hospital in Addis Ababa, Ethiopia. Dr Samina Asad is a postdoc in the group performing genetic analyzes in the Swedish and Ethiopian cohorts.

Key words

Atopic Dermatitis, psoriasis, gene function, cell culture, genetics, gene expression, zebra fish, common variants, rare variants, Hi-C, human keratinocytes, reporter assays, cloning, luciferase assays, genotyping.

Karolinska Institutet

Interested in recruiting a Postdoc

Project title

DNA nanotechnology to investigate the roles of the spatial organisation of membrane receptors in cell signalling

Supervisor

Ana Teixeira, PhD, Department of Medical Biochemistry and Biophysics
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Home page:http:// ki.se/en/mbb/ana-teixeira-group

Type of recruitment and qualifications of applicant

Postdoc (24 months)
To qualify the applicant must have a PhD degree and experience in bioinformatics and computational biology. The applicant should have a strong publication track-record with first author publications in leading peer reviewed journals. The applicant needs to have a strong interest in the development of methods to analyse DNA sequencing data. Experience in mathematical modelling of biological processes is a merit. The candidate has to have a strong drive and self-motivation, have good communications skills and be able to work effectively in an interdisciplinary team.

Background

Many membrane proteins show non-uniform and dynamic spatial distribution patterns at the membrane. However, little is known about the significance of spatial distribution and lateral mobility of membrane proteins to downstream signalling pathways due to difficulties in controlling and analysing membrane protein nanoenvironments. DNA origami is a nanofabrication technology that uses DNA self-assembly to drive the precise formation of 3D nanostructures. We have shown that DNA origami can be used as a scaffold to tailor the spatial distribution of protein assemblies and control cellular behaviours (Shaw, Lundin et al, Nature Methods, 2014). This new tool allows for display of well-defined protein nanoclusters in solution and is therefore amenable to the study of in vitro tissue models and use in animal models. DNA origami will be used in this project to control and detect the nanoscale spatial distributions of proteins at the cell membrane.

Research project description

Individual mammalian cells express thousands of different membrane proteins. Most clinically used drugs target membrane proteins. Membrane proteins often display non-uniform, dynamic spatial distributions. However, we know surprisingly little about how the spatial distribution of proteins at the membrane and their lateral mobility affect their functions. We have developed a method to precisely control the spatial distribution of membrane proteins and study its effects on cellular responses. We use DNA nanostructures as scaffolds to form well-defined

nanoclusters of affinity binders (ligands, antibodies, computationally designed protein binders) for membrane proteins. Using this method, we found that the nanoscale spatial distribution of ephrin-A5 ligands regulates the levels of activation of EphA2 receptors and their ability to inhibit the invasive properties of breast cancer cells.

This project will make use of DNA origami as a tool to address the following specific aims:

Aim 1. Map the relationships between the nanoscale spatial distribution and lateral mobility of ligands and receptor-mediated signalling: Signalling through membrane-bound ligand/receptor pairs, such ephrin/Eph, is often dysregulated in cancer, with tumor suppressing or tumor promoting effects. The molecular bases for the diversity of outcomes of these pathways in normal tissues and in cancer are largely unknown. This project addresses the hypothesis that the nanoscale spatial organization of ligands and receptors at the membrane are important regulators of cellular outcomes of activation of these pathways.

DNA polyhedral nanostructure technology allows the fabrication of nanostructures of any desired shape that are stable in physiological salt conditions. Flat sheets presenting well-defined nanoclusters of ligands will be immobilized on a surface and the spatial distribution of receptors will be analysed by TIRF/STORM superresolution microscopy. Different numbers of ligands per flat sheet and different spatial configurations will be screened. The effects of nanoscale spatial distribution of ligands and lateral mobility on downstream signalling mediated by membrane receptors will be analysed by proteomics and transcriptomic analysis.

Aim 2. Develop new methods to analyse protein nanoenvironments at the cell membrane with super-resolution: Many biological processes elicit dynamic patterns of spatial distribution of proteins at the membrane. An example of such a process is the formation of an immunological synapse upon stimulation of a T-cell by an antigen presenting cell. It is remarkably difficult to detect the spatial distribution of more than a few proteins at the cell membrane simultaneously. This project aims to develop a method to analyse the microenvironments of membrane proteins that has the potential to detect hundreds of proteins simultaneously with super-resolution. The basis of the method is the conversion of spatial information into a DNA sequencing readout.

The researcher will develop bioinformatics tools to model and analyse the biological significance of the nanoscale distribution of receptors on the cell membrane through analysis of DNA sequencing data.

Research group

The research group led by Ana Teixeira combines biophysics and nanotechnology with cell biology to understand how cells communicate with their microenvironment. The research group is currently composed of researchers with expertise in nanotechnology, biophysics, microfluidics and cell biology. The work will be performed at the division of Biomaterials at the department of Medical Biomedical and Biophysics, Karolinska Institutet, Sweden, located at the newly built

Biomedicum, one of Europe's largest facilities for experimental research. The Biomedicum is a diverse research environment that is conducive to collaborative research. The project will benefit from the interdisciplinary environment at the division of Biomaterials, which has a focus on development of nanotechnologies for biomedicine. The division of Biomaterials is composed of approximately 35 researchers in four labs and has all the essential equipment for the project. This includes equipment for cell and molecular biology experiments as well as equipment necessary for nanostructure production, purification and analysis including FPLC, Surface Plasmon Resonance, an Atomic Force Microscopy, super resolution microscopy TIRF/STORM and NextSeq Illumina Sequencing. Importantly, there is ample expertise in the division in using the different pieces of equipment and a culture of adopting new technologies and tailoring them to fit the needs of the project.

Key words
Bioinformatics, computer modelling, DNA sequencing, nanotechnology, membrane biology

Interested in recruiting a Postdoc

Project title

Role of macrophage epigenome alterations in linking type 2-diabetes and atherosclerosis

Supervisor

Eckardt Treuter, Professor, Department of Biosciences and Nutrition
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Home page: http://staff.ki.se/people/ecktre

Type of recruitment and qualifications of applicant

Postdoc (24 months)
Ph.D. or equivalent in a relevant discipline such as molecular medicine, molecular biology or biochemistry. Prior experience in state-of-the-art epigenomics techniques and bioinformatics analysis is required, knowledge of macrophage biology and manipulation is an advantage. Fluency in English is required. We are looking for an excellent team player with interpersonal skills, organized, efficient, and flexible in dealing with unpredictability and changes.

Background

The rapidly increasing prevalence of obesity, type 2-diabetes (T2D) and cardiovascular diseases (CVD) including atherosclerosis is becoming a severe healthcare burden to the modern society (Lyle & Taylor, 2019). Circulating monocytes play a central role in atherogenesis by recruiting to the intima of vascular lesions where they are reprogrammed into metabolically-activated macrophages (Tabas & Lichtman, 2017; Koelwyn et al., 2018). Both monocytes and macrophages have a memory which contributes to disease progression and relapse after treatment. The epigenome (i.e. chromatin/histone/DNA modifications) is probably a key to unravel the mysteries of monocyte/macrophage memory and activation during atherosclerosis progression, because it stores transcriptional and signaling history (Glass & Natoli, 2016, Treuter et al. 2017). Transcription factors (TFs) and coregulators are the key components that control the epigenome (i.e. the chromatin/enhancer landscape) in macrophages and thereby determine gene expression. Our previous work has revealed that a fundamental chromatin-modifying corepressor complex containing GPS2 is a key regulatory component of the epigenome and its alterations in macrophages, particularly at enhancer regions (Fan et al., 2016; Treuter et al., 2017). Obesity/T2D-associated GPS2 alterations in mouse and human macrophages trigger inflammation and insulin resistance (Fan et al., 2016), and they also affect ABCA1/G1-dependent cholesterol efflux, both highly relevant for atherogenesis (Jakobsson et al., 2009; Huang et al., 2019). Since obesity and T2D are risk factors for developing CVD, our hypothesis is that there could be a causal link between obesity/T2D-associated GPS2 alterations and epigenome/enhancer activity in re-programming gene expression to drive atherogenesis.

Research project description

OBJECTIVE: This project will investigate the role of macrophage epigenome alterations in linking T2D and atherosclerosis. Focus will be on two interconnected epigenome components, i.e. (1) a fundamental corepressor complex containing G protein pathway suppressor 2 (GPS2), and (2) selected pro-atherogenic (inflammatory, metabolic) enhancers that are controlled by the GPS2 complex.

Experiments will address the question whether specific macrophage enhancers control atherogenesis and can be manipulated for therapeutic intervention. Both in vitro studies - using cultivated macrophages - and in vivo approaches - using mouse models - will be applied.

Aim 1: Enhancer profiling in WT vs. Gps2 KO macrophages. Circulating blood monocytes, aorta foam cells, and tissue macrophages will be isolated from mice (our lab) and humans (via clinical collaborations). These will be subjected to a full genomic and epigenomic analysis (i.e. ATAC-seq, ChIP-seq H3K27ac, TFs, RNA-seq). Bioinformatics will identify diet- and/or GPS2-dependent alterations at enhancers, which can be compared to published macrophage datasets from obese/diabetic mice and humans.

Aim 2: Characterization and manipulation of atherogenic enhancers. The postdoc will fully characterize the identified atherogenic enhancers in cultured blood monocytes (mouse and human) and primary tissue macrophages. Approaches will include CRISPR/Cas9-mediated enhancer deletions, dCas9-KRAB-mediated enhancer blocking, and antisense (LNA, locked nucleic acids) mediated enhancer RNA (eRNA) depletion. While distinct macrophage subsets show distinct gene 4 / 14 expression patterns and enhancer activities, our data suggest a substantial overlap of the enhancers/genes regulated by the GPS2 complex (Fan et al. 2016, Huang et al. 2019). We expect the identification of GPS2 ‘sensitive’ vs. ‘resistant’ atherogenic enhancers, some of which may be altered in obesity/T2D as well and perhaps contribute to a monocyte/macrophage ‘memory’ towards driving atherogenesis.

Aim 3: Explore atherogenic enhancer-associated proteomes. Signal responses of macrophage enhancers are coordinated by TFs and coregulators such as the GPS2 complex, but the composition of the ‘enhancer proteome’ and changes of this in disease are virtually unknown. To overcome limits of single target ChIP-Seq approaches, we will apply ChIP-MS approaches such has RIME/qPLEX-RIME (Papachristou et al., 2018) to detect genome-wide enhancer proteomes. We plan to detect the chromatin interactome of the active enhancer mark H3K27ac along with the GPS2-associated proteome, which may reveal new candidates that could influence the function of GPS2-regulated enhancers in macrophages and thereby contribute to atherogenic pathways.

SIGNIFICANCE: The project should lead to a better understanding of the atherogenic macrophage epigenome and the epigenetic mechanisms, including ‘memory’, that link obesity/T2D and CVD/atherosclerosis. We believe that our approach is unique as we attempt to characterize enhancers which are altered in obesity/T2D and in CVD/atherosclerosis, thus potentially linking these diseases, and which are controlled by the GPS2 corepressor complex. Thereby, the project may

contribute to the development of epigenome-based therapeutic strategies that target pro-atherogenic - both inflammatory and metabolic - pathways in monocytes/macrophages.

Research group

The position will be placed in the laboratory of Prof. Eckardt Treuter, Department of Biosciences and Nutrition, which conducts pre-clinical research at the crossroads between epigenomics, metabolism, inflammation and disease. The PI has more than two decades research experience in epigenomics, i.e. the study of chromatin components that regulate gene expression, and a particular interest in understanding the role of transcriptional coregulators in disease mechanisms. Recent work in the laboratory aims at understanding the mechanisms underlying of coregulator-dependent epigenome alterations in the context of metabolic-inflammatory diseases such as obesity, type 2 diabetes, fatty liver disease and atherosclerosis.

The Treuter laboratory utilizes a multidisciplinary approach to address basic and disease-relevant questions, including molecular biology, biochemistry, genomics and epigenomics approaches, genetically modified cell, tissue and mouse models, and collaborations with clinicians to study human material. The team also develops proof-of-concept models for the therapeutic targeting of specific epigenome components, such as chromatin-modifying corepressor complexes and enhancers. For key publications see Nature Communications 2019, Cell Reports 2019, 2018, Nature Medicine 2016, J Clinical Investigation 2013, Genes and Development 2010, Molecular Cell 2009.

Current members of the research group by October 2019:
Dr. Rongrong Fan - KO mice, non-alcoholic fatty liver disease pathways
Dr. Oihane Garcia-Irigoyen - KO mice and atherosclerosis models
Dr. Ning Liang* (PhD KI 2018) - next-generation sequencing and bioinformatics
Dr. Zhiqiang Huang* (PhD KI 2019) - Macrophage pathways
PhD student Serena Barilla - adipose tissue pathways
Dr. Anastasios Damdimopoulos (associated, BEA core facility) - bioinformatics

Key words

Epigenome, epigenomics, histone modifications, chromatin, enhancers, macrophages, type 2-diabetes, atherosclerosis, cardiovascular diseases, inflammation, gene regulation, transcription, corepressors

Karolinska Institutet

Interested in recruiting a Doctoral student, a Visiting doctoral student, a Postdoc or a Visiting researcher

Project title

Investigation the role of regulatory RNAs in human skin wound healing

Supervisor

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Type of recruitment and qualifications of applicant

- Visiting doctoral student or visiting researcher (up to 12 months) or postdoc (up to 24 months)
- Doctoral student for 48 months

Visiting researcher and post-doctoral fellow (longer than 12 months):
The candidate should have obtained a Ph.D. degree within the area of molecular or cell biology and have a deep interest in medical and biological problems. The applicant is preferred to have documented the previous experience with RNA research or skin biology. Previous experience with tissue culture, molecular and biochemical techniques is desired. A high level of English, spoken and written, is a requirement.

Doctoral student and visiting doctoral student (longer than 12 months):
The candidate should have a masters degree within the area of medicine or molecular or cell biology and have a deep interest in medical and biological problems. A high level of English documented by an internationally recognized test e.g. TOEFL or IELTS, is a requirement. The successful candidate needs to be very motivated and able to work independently, and at the same time interact with scientists from other areas to coordinate complex projects. Previous experience with RNA research, tissue culture, molecular and biochemical techniques is merit.

Background

The chronic non-healing wound is a common and severe medical problem with unclear pathophysiology, which severely hampers the development of efficient wound treatment. Although constituting the majority of the transcriptional output of the human genome, the functional importance of non-protein-coding RNA (ncRNAs) has only recently been recognized. Without the need for translation, ncRNAs can rapidly carry out their regulatory function, after which they can be quickly degraded. Moreover, compared to protein-coding genes, not only the expression but also the function of ncRNAs are more tissue- and cell type-specific, underscoring their great potential as precise therapeutic and diagnostic entities.

Research project description

The objectives of our research are to reveal the expression and functional

signatures of ncRNAs in human skin wound healing, and to identify ncRNAs that may be targeted in wound therapy. In particular, we aim:

- To establish a gene expression map of human acute and chronic wounds with single-cell resolution, which will significantly advance current knowledge about cell composition and gene expression in human normal and chronic wounds, providing a basis for the development of new diagnosis and treatment.
- To identify the functions of wound-related ncRNAs: We expect to identify the significant ncRNA regulators for each cellular process impaired in chronic wounds; therefore, combination therapy targeting multiple 'master regulators' may be designed.
- To decipher the targets and signaling networks regulated by the wound-related ncRNAs, which will extend our knowledge on regulatory pathways involved in wound healing and may lead to the identification of additional therapeutic targets than ncRNAs.
- To evaluate the therapeutic potential of targeting wound-related ncRNAs. This proof-of-concept study, if successful, will be the first step towards a novel therapeutic approach for chronic wounds.

The immense economic and social impact of chronic wound calls for attention and allocation of resources to develop more effective therapies, which are essentially lacking to date. Investigation of the role of ncRNAs represents an emerging concept and constitutes a promising area for pharmaceutical intervention. The proposed study will, from a new angle, add to our understanding of wound healing biology, but also to the pathogenesis of chronic wounds, which will open new avenues for disease stratification and highlight novel drug targets for clinical studies.

Research group

The wound healing research group consists of:
Associate Prof., Ning Xu landén, Ph.D., group leader
Assistant Prof. Dongqing Li, Ph.D. in skin immunology in 2014. His projects focus on wound-related long non-coding (lnc) RNAs and single-cell transcriptomic analysis of human wounds.
Postdoc Dr. Manika Vij, Ph.D. in biophysics in 2017. She focuses on the development of nanocarrier to deliver therapeutic RNAs for wound treatment.
Postdoc Dr. Zhuang Liu, Ph.D. in bioinformatics in 2019. He develops and implements bioinformatic approaches to analyze data generated with various high-throughput technologies.
PhD student Letian Zhang, Master in Pharmacology, registered in 2018. His projects focus on wound- related lncRNAs in keratinocytes.
Ph.D. student Maria Toma, Master in Pharmacology, registered in 2017. Her project focuses on wound- related circular RNAs and microRNAs.
Project student Kim Pham, Medical student at KI. She works on human in vivo and ex vivo wound healing models.
Lab manager Dr. Warangkana Lohcharoenkal (13% of full time)

Key words

Wound healing, skin, dermatology, RNA, gene regulation, epigenetics, bioinformatics.

Interested in recruiting a Postdoc

Project title
Circular RNAs in cancer development

Supervisor
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Home page: http://ki.se/bionut/zaphiropoulos

Type of recruitment and qualifications of applicant
Postdoc (24 months)
The applicant should be a motivated individual that enjoys the process of obtaining new knowledge via experimental skills at the laboratory bench.
Experience in current molecular biology techniques is required and knowledge of strategies for the analysis of big data, e.g. RNA-seq results, will be a plus.
Additionally, the applicant should have good social and communication skills, and characterized by the willingness to effectively integrate in the research group.

Background
Circular RNAs are a novel class of RNA molecules that are produced during gene expression. Although gene expression is generally associated with the production of linear mRNA molecules that are translated to proteins, recent evidence has convincingly demonstrated that genes can also produce circular RNAs, which in many cases are more abundant than the mRNAs originating from the same gene.
The production of circular RNAs has to do with the process of splicing. Genes in higher organisms are composed of exons that are joined together, i.e. spliced, to produce an mRNA. However, splicing sometimes joins together exons with an order that is different to the one present in the genomic DNA. This back-splicing mechanism produces circular RNA molecules.
When circular RNAs were identified more than twenty years ago they were considered to simply represent rare products of the splicing process, with limited functional significance. However, in recent years it has been established that circular RNAs can be quite abundant. Convincing evidence pinpoints to the fact that circular RNAs act to sequester miRNAs and consequently modulate gene expression. Additionally, circular RNAs have been postulated to interact with proteins and modify their function. Moreover, it is not inconceivable that nucleic acids, apart from miRNAs, may also be found to be regulated by circular RNAs. Consequently, circular RNAs are a new class of biomolecules with increasingly appreciated functional properties.
There is overwhelming evidence that circular RNAs act as oncogenes or tumor suppressor genes to promote/inhibit cancer development. This has been clearly established in diverse tumors, including prostate, breast and liver cancer. The underlying mechanisms in these cases mostly relates to the capacity of circular

RNAs to act as traps of miRNAs, regulating the stability and translation of mRNAs that encode proteins with oncogenic or tumor suppressor activities.

Research project description

The project addresses the role of a novel class of RNA molecules, circular RNAs, in the context of medulloblastoma, the most common brain cancer in children. Specifically, it is aimed to:

1. Characterize the circular RNA transcriptome in human samples of medulloblastoma tumors: We plan to analyze the circular RNA transcriptome of human medulloblastoma tumors. Tumor samples are available via "Barntumörbanken" https://ki.se/forskning/barntumorbanken and via The Children's Brain Tumor Tissue Consortium https://cbttc.org. We aim to analyze tumors from all classes of medulloblastoma (WNT, SHH, group 3 and group 4) aiming at identifying how the circular RNA transcriptome may differ among the various forms of medulloblastoma. We will also compare these data with the circular RNA transcriptome of Daoy medulloblastoma cells, which is considered to be a SHH type of tumor. A total of 40 samples is aimed to be analyzed with the cost of analysis per sample reaching about 5 000kr according to the Bioinformatic and Expression Analysis (BEA) core facility http://www.bea.ki.se.

2. Examine how circular RNAs impact on the growth of medulloblastoma cells:

Depletion

To address the functional impact of a selected circular RNA, small interfering RNA (siRNA) depletion analysis will be performed. This is particular well-suited for circular RNAs, as these, in similarity to mRNAs accumulate in the cytoplasm, which is the site of action of siRNAs. However, it is critical to deplete only the targeted circular RNA and not the corresponding mRNA. This can be achieved by designing siRNAs that target the back-spliced junction, which is present in the circular RNA but absent in the corresponding linear transcripts. To generate clones of Daoy medulloblastoma cells with specific circular RNAs stably depleted, short hairpin RNA (shRNA) approaches using appropriate plasmids targeting the back-spliced junction will be implemented.

Cells with circular RNAs depleted will first be tested for obvious differences in cellular phenotypes, including cellular proliferation, compared to non-depleted cells. We also aim to subject depleted and non-depleted cells to RNA-seq analysis, aiming at identifying global changes in the transcriptome that are elicited by depletion of specific circular RNAs.

Assuming that depletion of a circular RNA functionally impacts on Daoy medulloblastoma cells, then xenograft analysis will be employed to address the effects of circular RNA depletion in the context of a growing tumor.

Overexpression

Similar approaches to the ones described above, with selected circular RNAs overexpressed in Daoy medulloblastoma cells will be implemented. Circular RNAs can be overexpressed using specialized vectors, some of which are freely available from addgene.org.

Circular RNA interactors

Using recently developed techniques, i.e. circular RNA in vivo precipitation, reverse transcription-associated trap (RAT assay) and chromatin isolation by RNA purification (ChIRP), we aim to identify nucleic acids and proteins that interact with the selected circular RNAs, since this will provide clues for their mechanism of action.

Research group

The project leader has been involved in the identification of circular RNAs during gene expression more than twenty years ago. This is highlighted in two publications:

Zaphiropoulos, P.G. (1996) Circular RNAs from transcripts of the rat cytochrome P450 2C 24 gene: Correlation with exon skipping. Proc. Natl. Acad. Sci. USA 93, 6536-6541.

Zaphiropoulos, P.G. (1997) Exon skipping and circular RNA formation in transcripts of the human cytochrome P450 2C 18 gene in epidermis and of the rat ABP gene in testis. Mol. Cell. Biol. 17, 2985-2993.

In recent years the focus of the groups centered on Hedgehog signaling, a major driver of medulloblastoma development.

Currently the group is composed of a doctoral student funded by the Karolinska Institutet Doctoral program (KID) and a postdoctoral fellow funded by the China Scholarship Council.

Key words

Circular RNA, Back-splicing, Medulloblastoma, Hedgehog signalling, Micro RNA.