Autumn 2017

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Master of Science in Molecular Medicine
Thesis/Project Catalogue

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Faculty of Medicine

NTNU

Master Thesis in Molecular Medicine

Name of Group: **Oksenych/Bjørås**

Name of Research Community: **IKM**

Role of Histone acetyltransferases Gcn5 and PCAF in B lymphocyte development

DNA in our cells is constantly damaged by internal and external factors. There are multiple pathways that detect and repair the damaged DNA after the lesions occur. In addition, during the DNA damage response (DDR), posttranslational modifications of multiple DNA repair factors occur [1]. Acetyltransferases Gcn5 and PCAF modify histones and other proteins in response to DNA damage [2,3]. Higher vertebrates employ DNA repair for DNA recombination in developing lymphocytes. In particular, to ensure rearrangement of V, D and J gene segments of heavy and light chains of immunoglobulin variable regions during the V(D)J recombination in developing B cells. In response to antigens, mature B lymphocytes perform Class Switch Recombination (CSR). This process is also mediated by programmed DNA breaks and repair, and results in changes in constant regions of immunoglobulins [1].

The key DNA repair pathway responsible for both V(D)J recombination and CSR is Non-Homologous End Joining (NHEJ) [1]. In addition to the NHEJ, we have demonstrated that several DDR proteins (protein kinases ATM and DNA-PKcs, histone H2AX and scaffold protein 53BP1) regulate some aspects of the V(D)J recombination, including the joining itself and the DNA end protection [1,4]. Our preliminary data demonstrate that Gcn5 and PCAF, DDR proteins [2,3], function in B cell development.

The goal of the current project is to find specific role of Gcn5 and PCAF in B lymphocyte development and DNA repair. The project includes following steps:

Aim 1. Using Cas9/CRISPR approach, to inactivate *Gcn5* and *PCAF* genes in stable pro-B cell lines (Abelson murine leukemia virus kinase transformed) and B cell lines (CH12F3). To verify gene inactivation by PCR and/or Western Blot.

Aim 2. To perform functional V(D)J recombination assay using Southern Blot, and to determine the CSR efficiency using *Fluorescence activated cell sorting* (FACS), with cell lines generated in Aim 1 and wild type controls.

Aim 3. To characterize sensitivity to DNA double strand breaks and proliferation efficiency of human cells deficient for Gcn5, PCAF and Gcn5/PCAF.

This project requires to learn and to use the following techniques*: PCR*, *molecular cloning*, *mammalian cell culture*, *Cas9/CRISPR genome editing*, *Western Blot*, *Southern Blot*, *FACS*

During this project, new high impact knowledge on the role of acetyl transferases Gcn5 and PCAF in immune system development and DNA repair will be obtained.

1. Kumar, V., F.W. Alt, and V. Oksenych, Functional overlaps between XLF and the ATM-dependent DNA double strand break response. DNA Repair (Amst), 2014. 16: p. 11-22.
2. Lee et al., A cooperative activation loop among SWI/SNF, γ-H2AX and H3 acetylation for DNA double-strand break repair. EMBO J. 2010 Apr 21; 29(8): 1434–1445.
3. Li et al., Cell Rep. 2013 Nov 14;5(3):702-14. Combinatorial H3K9acS10ph histone modification in IgH locus S regions targets 14-3-3 adaptors and AID to specify antibody class-switch DNA recombination.
4. Oksenych, V., et al., Functional redundancy between the XLF and DNA-PKcs DNA repair factors in V(D)J recombination and nonhomologous DNA end joining. Proc Natl Acad Sci U S A, 2013. 110(6): p. 2234-9.

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Master Thesis in Molecular Medicine

Name of Group: **Oksenych/Bjørås**

Name of Research Community: **IKM**

Impact of DNA damage response factor MDC1 in early lymphocyte development

DNA in our cells is constantly damaged by internal and external factors. There are multiple pathways that detect and repair DNA after the lesions occur. In addition, there is a DNA damage response (DDR) pathway that includes posttranslational modifications of multiple factors in response to DNA damage. Evolutionary, higher vertebrates employed DNA repair for DNA recombination in developing lymphocytes. In particular, to ensure rearrangement of V, D and J gene segments of heavy and light chains of immunoglobulin variable regions during the V(D)J recombination in developing B cells. In response to antigens, mature B lymphocytes perform Class Switch Recombination (CSR). This process is also mediated by programmed DNA breaks and repair, and results in changes in constant regions of immunoglobulins.

The key DNA repair pathway responsible for both V(D)J recombination and CSR is Non-Homologous End Joining (NHEJ) [1]. In addition to the NHEJ, we have demonstrated that several DDR proteins (protein kinases ATM and DNA-PKcs, histone H2AX and scaffold protein 53BP1) regulate some aspects of the V(D)J recombination, including the joining itself and the DNA end protection [2-4]. Our preliminary data demonstrate that MDC1, a key DDR protein [5], functionally overlaps with the NHEJ factor XLF in mouse development.

The goal of current project is to generate cell lines deficient for XLF, MDC1 or both XLF and MDC1, using Cas9/CRISPR approach, and to characterize the role of MDC1 in early B lymphocyte development. The project includes following steps:

Aim 1. Using Cas9/CRISPR approach, to inactivate *XLF* and *MDC1* genes in stable pro-B cell lines (Abelson murine leukemia virus kinase transformed) and B cell lines (CH12F3). To verify gene inactivation by PCR and/or Western Blot.

Aim 2. To perform functional V(D)J recombination assay using Southern Blot, and to determine the CSR efficiency using *Fluorescence activated cell sorting* (FACS), with cell lines generated in Aim 1 and wild type controls.

Aim 3. To characterize sensitivity to DNA double strand breaks and proliferation efficiency of available human cells deficient for XLF, MDC1 and XLF/MDC1.

This project requires to learn and to use the following techniques*: PCR, molecular cloning, mammalian cell culture, Cas9/CRISPR genome editing, Western Blot, Southern Blot, FACS*

During this project, new high impact knowledge on the role of MDC1 factor in immune system development will be gained.

1. Kumar, V., F.W. Alt, and V. Oksenych, Functional overlaps between XLF and the ATM-dependent DNA double strand break response. DNA Repair (Amst), 2014. 16: p. 11-22.

2. Oksenych, V., et al., Functional redundancy between repair factor XLF and damage response mediator 53BP1 in V(D)J recombination and DNA repair. Proc Natl Acad Sci U S A, 2012. 109(7): p. 2455-60.

3. Oksenych, V., et al., Functional redundancy between the XLF and DNA-PKcs DNA repair factors in V(D)J recombination and nonhomologous DNA end joining. Proc Natl Acad Sci U S A, 2013. 110(6): p. 2234-9.

4. Zha, S., et al., ATM damage response and XLF repair factor are functionally redundant in joining DNA breaks. Nature, 2011. 469(7329): p. 250-4.

5. Lou, Z.K., et al., MDC1 maintains genomic stability by participating in the amplification of ATM-dependent DNA damage signals. Molecular Cell, 2006. 21(2): p. 187-200.

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Master Thesis in Molecular Medicine

Name of Group: Regulation of cellular stress and genome dynamics

Name of Research Community: IKM, Molecular biology, DNA repair and Genome stability

My group is doing research on the scaffold proteins XRCC1 and PCNA and their role in genome stability. We are studying multiple interactions partners of XRCC1 and PCNA and multiple repair pathways associated with them. For more information see https://www.ntnu.no/ansatte/marit.otterlei. Our projects are associated with drug development (anti-cancer and anti-bacterial) as well basal science related to genome stability, cellular signalling and DNA repair. Projects are available within all these fields. The master student will be working on sub-projects of current PhD, postdoctor or researcher projects, and will be co-supervised by these in addition to prof. Otterlei.

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Personalized cancer treatment by developing integrated MR methods and data analysis tools for functional and molecular assessment of tumors.

The MR Cancer group (http://www.ntnu.edu/isb/mr-cancer) studies functional and metabolic properties of cancer using both MR imaging and MR spectroscopic technologies. Our long-term objective is to improve and individualize cancer treatment by developing integrated MR methods and data analysis tools for functional and molecular assessment of tumors. We use a broad spectrum of systems for cancer studies, spanning from cultured cancer cells to humans, and have access to dedicated MR equipment for in vitro, ex vivo, preclinical and clinical research.

We have several large ongoing research projects supported by The Research council of Norway, the Norwegian Cancer Society and regional health authorities, and we can offer students tailored projects in line with their main interests.

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Master Thesis in Molecular Medicine

Name of Group: **Myeloma group (Cell biology group)**

Name of Research Community: **Cancer Research and Molecular Medicine (Gastrosentert 2nd floor)**

Interactions between genetic aberrations and growth of multiple myeloma cells

Multiple myeloma is a neoplasm of the plasma cells in the bone marrow. In Norway, 300 are diagnosed with the decease every year. The cancer is non-curable and the overall survival is five years.
All MM patients are cytogenetically abnormal. MM can be divided in two groups based on chromosome numbers (hyperdiploid myeloma, HRD) and on immunoglobulin heavy chain translocations (non-hyperdiploid myeloma, NHRD). Doing basic myeloma research usually means doing experiments with myeloma cell lines (HMCLs). A lot of conclusions have been drawn based on observations from experiments with these HMCLs grown in monocultures in the laboratories. There are more than a hundred HMCLs around the world, and they are important tools in the study of MM. Virtually all HMCLs used in laboratories are derived from NHRD tumors. HMCLs do not fully represent MM, since only approximately half of the MM patients have a NHRD tumor. The bias that HMCLs are from NHRDs has to be considered when HMCLs are used as models of the human disease. The reason for the lack of HRD HMCLs is perhaps that HRD tumors are strictly dependent on mitogenic cytokines and the bone marrow microenvironment. It appears that NHRDs more likely become independent of their microenvironment compared to HRDs, and this confers a more aggressive disease allowing some NHRDs to grow *in vitro* and become HMCLs. However, we have been able to establish two HMCLs from HRD myeloma patients. We now want to use these to find one or a few factors that enable the HRD cells to grow *in vitro,* and hopefully have the key to be able to establish long sought for HRD HMCLs. These undetermined factors might also be important in understanding the HRD MM, and be potentially drug targets.

We would like the student to test the effect of the microenvironment on the different HMCLs and primary cells from patients by using different growth media (human, cow, goat, rabitt ect.). Both look at the growth itself (proliferation /apoptosis) and the expression of different genes and proteins varying with the growth and growth conditions (qPCR/sequencing/Western Blots).

Name of Contact Person: Thea Kristin Våtsveen (post doc)

Master Thesis in Molecular Medicine

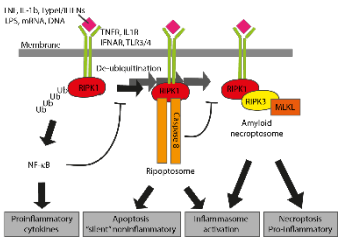
Name of Group: **Centre of Molecular Inflammation Research (CEMIR)**

Name of Research Community: **Department of cancer and molecular medicine**

CRISPR/Cas9-mediated screening for novel regulators of inflammatory cell death

The Receptor Interacting Protein Kinase 1 (RIPK1)-controlled pathway is a central regulator of inflammation and cell death in macrophages (Figure 1).

Our group is investigating whether RIPK1-signaling can be a potential target in cancer therapy. However, much is still unclear about how RIPK1 directs different inflammatory outcomes.



We have used mass-spectrometry to identify a set of novel RIPK1 interaction partners. We are currently working on establishing a CRISPR/Cas9-mediated knockout-screen to identify which of these factors are functionally important for RIPK1 signaling and cell death.

In this assignment the student will generate knockout clones of one or more RIPK1-interaction partners, and test these for their involvement in RIPK1-dependent inflammation and cell death in human macrophage systems.

The assignment will use cutting-edge gene editing techniques (CRISPR/Cas9) as well as standard cell biology and immunology methods (monocyte/macrophage cell culture, ELISA and immunoblotting, cell death assays). The student will work closely with members of the Autophagy and Oxidative Stress Defence Group (CEMIR). CEMIR is a Norwegian Centre of Excellence situated at the new Kunnskapssenteret, Øya Campus/St. Olavs Hospital. We offer a dynamic environment and a nationally leading infrastructure for cell biology and inflammation studies.

Supervisor: Kristian K. Starheim, PhD, Researcher CEMIR.
Co-supervisor: Geir Bjørkøy, PhD, Professor CEMIR.

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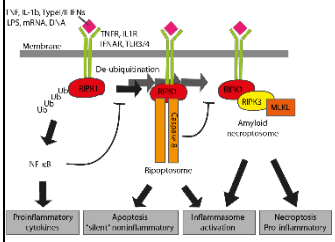
Master Thesis in Molecular Medicine

Name of Group: **Centre of Molecular Inflammation Research (CEMIR)**

Name of Research Community: **Department of cancer and molecular medicine**

Dissecting inflammatory cell death in macrophage subtypes.

The Receptor Interacting Protein Kinase 1 (RIPK1)-controlled pathway is a central regulator of inflammation and cell death in macrophages (Figure 1).



Macrophages differentiate into a variety of functional states. While some subtypes are associated with fighting infection, others are associated with clearing up dead cells and wound healing. These subtypes will differ in their inflammatory status and signaling.

Preliminary data indicate that RIPK1-dependent cell death is involved in several types of cancer development. Our group is investigating whether RIPK1-signaling can be a potential target in cancer therapy. However, much is still unclear about how RIPK1 directs different inflammatory outcomes in macrophage subtypes. As the signaling dynamics of RIPK1 in tumor-associated macrophages, and thus their contribution to tumor development and therapeutic potential, remains unknown.

In this assignment, the student will investigate the RIPK1 signaling dynamics in different human macrophage subtypes. Are some macrophage types more prone to cell death than others, and will they differ in their inflammatory response? We will also investigate whether pharmaceutical manipulation may change cell death outcome and inflammatory profile.

The assignment will use cell biology and immunology methods (monocyte/macrophage cell culture, ELISA and immunoblotting, cell death assays). The student will work closely with members of the Autophagy and Oxidative Stress Defence Group (CEMIR). CEMIR is a Norwegian Centre of Excellence situated at the new Kunnskapssenteret, Øya Campus/St. Olavs Hospital. We offer a dynamic environment and a nationally leading infrastructure for cell biology and inflammation studies.

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Master Thesis in Molecular Medicine

Name of Group: **Collaboration between Centre of Molecular Inflammation Research (CEMIR) and the DrugLogics (NTNU Health).**

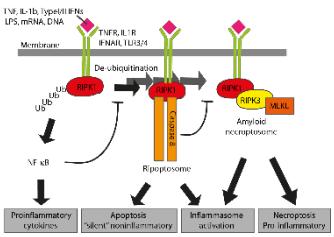
Name of Research Community: **Department of cancer and molecular medicine**

Using Boolean modeling to identify multitarget drug treatments in macrophages.

The Receptor Interacting Protein Kinase 1 (RIPK1)-controlled extrinsic cell death pathway is a central regulator of inflammation (Figure 1).

Several works have shown the potential of targeting the RIPK1-pathway in in e.g. cancer therapy. We are investigating whether the RIPK1 signaling system can be pharmaceutically manipulated to dampen the tumorigenic effect of tumor-associated macrophages (TAMs).

A major obstacle in pharmacological targeting of signaling pathways is redundancy. Many pathways are structured to neutralize or counteract perturbations, and this can be circumvented through combination treatment that takes out several cellular backup systems. However, identifying how to combinatorially target signaling pathways relies on a systems understanding of the pathway organization.



In our group we aim to use both wet-lab experiments and computational modeling to develop multi-target cancer treatment strategies.

In this assignment the student will use the GINsim computational modeling software (http://ginsim.org/) to build a Boolean model of RIPK1 signaling in macrophages. This model will be used to predict combination treatment strategies in TAMs with respect to anti-inflammatory signaling and cell death. The model predictions will form the basis of experimental wet-lab testing of drug-treatments by the student or others in the group. The assignment will mainly be computer modeling.

Supervisor: Kristian K. Starheim, PhD, Researcher CEMIR
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Master Thesis in Molecular Medicine

Name of Group: **Experimental Surgery and Pharmacology**

Name of Research Community: **Unit of Molecular Biology, Department of Cancer Research and Molecular Medicine**

Project title: Potential new treatment for type 2 diabetes: Peptide tyrosine–tyrosine (PYY)

The increasing worldwide prevalence of type 2 diabetes (T2D) has raised concerns about increasing health care and financial burden. Thus, it is an ever-growing and an urgent need to improve the management of this disease. The challenges occurring in the treatment of T2D are mainly attributed to the complex heterogeneous nature of the disease and its close association with a wide variety of metabolic, cardiovascular and neurological disorders. To overcome these challenges, "diabetes surgery" such as gastric bypass has been suggested as promising treatment strategies.

Improved understanding of the shared pathogenesis and common molecular denominators involved in the aetiology of T2D and associated comorbidities will aid management and treatment of this disease. It has been suggested that the increase of glucagon-like peptide-1 (GLP-1) secretion in the hindgut after gastric bypass improves the control of glucose regulation in diabetic individuals. This might explain why, in several studies, patients have improvement of T2D after gastric bypass before they have any significant weight loss 5. In our own studies, we have found increased GLP-1 productions not only in the ileum but also in the pancreatic islets after the surgery. We have subsequently tested our hypothesis that following gastric bypass surgery, GLP-1 in the pancreatic islets plays an important role in the regulation of insulin secretion probably via paracrine pathway. Recently, in collaboration with the Oxford Centre for Diabetes, Endocrinology & Metabolism, University of Oxford, we have obtained strong evidence supporting a role for peptide tyrosine-tyrosine (PYY) in the modulation of both insulin and glucagon release after gastric bypass.

Primary goal of this project is to develop a non-surgical T2D treatment by elucidating the mechanism by which gastric bypass leads to a resolution of T2D.

Secondary goals are 1) to evaluate the potential trophic effects of PYY in pancreatic islets and the stem cells according to our hypothesis that PYY and its analogue could be eventually used as a potential drug not only in remission of T2D but also as a cure for the disease; and 2) to conduct preclinical trial in rats and phase I clinical trial of PYY administration in humans for the treatment of T2D.

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Master Thesis in Molecular Medicine

Name of Group: **Experimental Surgery and Pharmacology**

Name of Research Community: **Unit of Molecular Biology, Department of Cancer Research and Molecular Medicine**

Project title: ReBOO project (Repurposing of Endoscopic Injection of Botulinum Toxin A for Obesity Treatment): A study of the mechanism-of-action

Summary
This proposal in its full length was submitted to Horizon 2020 in April this year and is currently under review. We aim to provide a new treatment for the huge number of adolescents with severe obesity who do not respond to current best practice in paediatric obesity treatment - lifestyle treatment (LST). In H2020 proposal, we want to fill this treatment gap by testing the efficacy of a novel treatment - Endoscopic Injection of Botulinum toxin A for Obesity (EIBO) - in a multinational, randomised, double blind, placebo-controlled phase II clinical trial.

During the past 5 years of the European FP7 research project entitled "Understanding food-gut-brain mechanisms across the lifespan in the regulation of hunger and satiety for health" (Full4Health; www.full4health.eu/), we have been particularly investigating the role of the vagus nerve in gut-brain signalling. In this study, we plan to undertake 3 experiments including brain signalling, profiling with RNA Sequencing, secretome with MS, and brain activity using fMRI in both animal models and patients in order to understand how the satiety signals are sent from the stomach via the vagus nerve directly to the hindbrain, and how signals from the hindbrain then feed forward to the hypothalamus which also monitors and integrates metabolic and blood borne homeostatic signals in connection with EIBO.

Primary goal: To understand the mechanism-of-action (MOA) of EIBO

Secondary goals:
- Signalling profiling of brain anorexigenic and orexigenic neuropeptides in response to EIBO in animal model of obesity
- Secretome analysis of the serum from the obese animals and the obese patients in response to EIBO
- Functional magnetic resonance imaging (fMRI) of the obese patients in response to EIBO

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Master Thesis in Molecular Medicine

Name of Group: **Experimental Surgery and Pharmacology**

Name of Research Community: **Unit of Molecular Biology, Department of Cancer Research and Molecular Medicine**

Project title: Development of new medicine for pancreatic cancer

Summary:

Unlike other cancer entities such as breast cancer and colon cancer, the therapies in pancreatic ductal adenocarcinoma (PDAC) have largely failed to positively impact patient survival after a decade of effort.One of the reasons why the standard chemotherapy, immunotherapy and targeted therapies failed is that PDAC contends with particularly severe metabolic stress within the tumor. Thus, we propose the tumor microenvironment to be the potential targets for therapeutic interventions.

Recently, we and others have obtained strong evidence that neuronal signaling plays an important role in tumorigenicity in general, which is probably via the Wnt-signaling pathway. We have also shown that denervation in combination with chemotherapy or metabolic therapy could represent a new approach for gastric cancer and possibly for other solid malignancies. Our preliminary results showed a 2-fold increase in therapeutic response to typical chemotherapy by adding targeted therapy against WNT/β-catenin signaling pathway. In this project, we plan to develop a new treatment by targeting the nerves for advanced-stage PDAC.

Main objective: PDAC usually lies directly against the perineurium, and grows internally and externally along the nerve. Thus, we plan to test our hypothesis that combination of local denervation and local chemotherapy will suppress the locally advanced PDAC.

Secondary objectives:

1. Establishment of therapeutic methods in the mouse model.
2. Completion of preclinical trials of the new treatments in animal models of PDAC.
3. Completion of a small clinical trial in patients with PDAC.
4. Completions of MSc theses and a PhD thesis.

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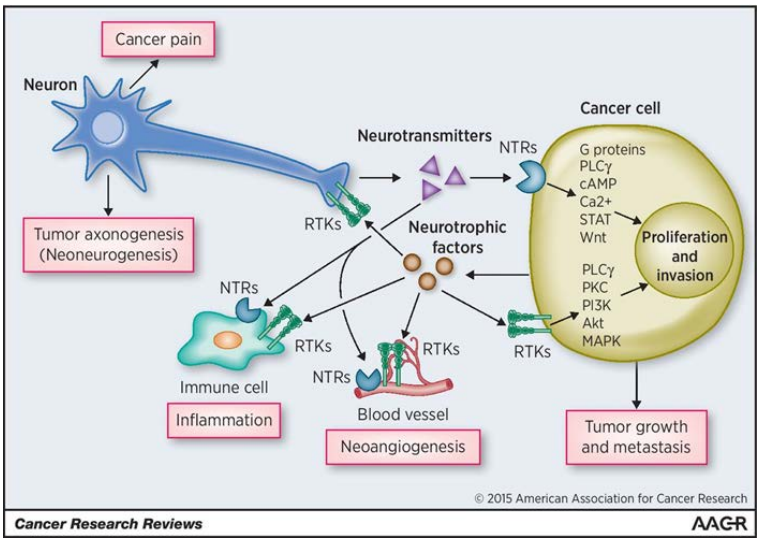
Master Thesis in Molecular Medicine

Name of Group: **Experimental Surgery and Pharmacology**

Name of Research Community: **Unit of Molecular Biology, Department of Cancer Research and Molecular Medicine**

Project title: Targeting signalling pathways in nerve-cancer cell cross-talk: A study of gastric cancer

Background: One hallmark of cancer cells, including gastric cancer cells, is the loss of homeostatic regulation and – sustained or uncontrolled – proliferation. The dysregulated cancer cell proliferation and growth manifest in a number of ways, including an increase in growth factor produced by the cancer cells, an increased growth factor receptors on the cancer cells, altered structure of growth factor receptors, dysregulated downstream of growth factor receptors, and inappropriate response to growth factors produced in the tumour microenvironment. Based on the findings reported by us and other research groups showing that innervation plays an important role in the tumorigenesis , a novel concept of "nerve-cancer cell cross-talk" in the tumour microenvironment has been recently proposed (Fig. 1).



Primary (long-term) goal: To develop the precision treatment targeted toward the nerve-cancer cell crosstalk within the tumour microenvironment.

Secondary (short-term) goals: 1) to study nerve growth factor (NGF) signalling and Hippo-YAP-TAZ signalling using in vitro and ex vivo models, animal models of gastric cancer, and tumours of gastric cancer patients; and 2) to publish the results of this project in the prestigious scientific journals.

Study design, choice of methods and analyses include in vitro and ex vivo experiments (cancer cell lines), in vivo experiments (mice), and gastric cancer tissues from human patients.

In our ongoing projects as well as this project, the world leading expert groups have been involved, including Columbia University, MIT, and The University of Tokyo.

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Master Thesis in Molecular Medicine

Name of Group: **Mycobacteria Group**

Name of Research Community: **CEMIR**

Keap1 in regulation of bacteria-induced innate immune responses.

1. ***Cytokine responses and sepsis:*** Previous studies show that the stress sensor, Keap1, negatively regulates TNF-induced NF-κB signaling in cancer cell lines and inflammatory signaling induced by mycocbacteria. Proteasomal degradation of IKKβ mediated by Cul3 or degradation by autophagy and prevention of phosphorylation of IKKβ seems to be the mechanism behind this. *Mycobacterium avium* induces various inflammatory cytokines and type I IFNs. This motivates the hypothesis that Keap1 may also be important in the regulation or outcome of sepsis. The extent to which Keap1 regulates inflammatory signaling initiated by bacteria, the mechanisms employed and the impact on bacterial survival will be addressed. We would use siRNA knockdown of Keap1 and associated partners (p62, Nrf2, Cul3 and Rbx1) in primary human macrophages and macrophage cell lines subsequently infected with *M. avium* and other bacteria that frequently cause sepsis. Activation of TLR downstream components and induction of classical NF-κB target genes and type I IFNs will be assessed by western blotting, real-time PCR and ELISAs; survival of bacteria by colony plating, PCR or luciferase assays. Overexpression of tagged Keap1 constructs (intact, deleted or mutated in central interaction domains) will be done to assay for ubiquitination of target proteins, pull-down of binding partners, and used with inhibitors of proteasomal degradation and autophagy. **Sub-project: Making different tagged Keap1 deletion constructs and testing effects on cytokine release.**

2. ***Inflammasome activation:*** Several proteins have been shown to interact with Keap1 including PGAM5, p62, Nrf2, and IKKβ and compete for binding to the same domain, Kelch, on Keap1. We hypothesize that Keap1 may also regulate inflammasome activation and subsequent IL1b release during infection through the recently shown phosphatase, PGAM5, that regulates inflammasome inflammation. We have preliminary evidence that Keap1 is recruited to PGAM5 punta, accompanied by some receptors of inflammasome complexes in both primary cells and cell lines. Here we will first examine how these proteins are recruited to inflammasome complexes and which inflammasome complexes maybe relevant, and then the outcome of the interaction of these proteins with inflammasomes during infection. This will be done by western blot analysis, immunoprecipitation assays, ELISAs and confocal imaging of macrophages as they are infected to follow the kinetics, and by transfecting cell lines with fluorescent mutant or deletion constructs of Keap1 and PGAM5. Through siRNA mediated knockdown of Keap1/PGAM5 in primary macrophages, THP-1 macrophages cell lines we can evaluate if and how Keap1/PGAM5 influences inflammasome activation and IL-1b release, and if this impacts intracellular survival of the bacteria. We aim to verify findings using relevant bacteria including *M. tuberculosis* (Mtb) in the long run. **Sub-project: Making different tagged PGAM contructs with both phosphatase and non-phosphatase activity and testing for interactions with inflammasome components.**

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DrugLogics: Anti-cancer drug combinations and personalized medicine

The DrugLogics initative aims to integrate different technologies and analyses providing a test for cancer patients to tailor their treatment to their disease (personalized medicine or precision medicine). This initiative comprises of different subprojects.

- 'Crossover Research 2.0 – Well constructed Knowledge Commons' (www.ntnu.edu/crossover-research) the domain of precision medicine is explored as a key visionary driver for developing the Knowledge Commons and the enabling of Systems Biology approaches to innovate health care.
- 'Rational development of anti-cancer combinations' focuses on precision medicine for cancer by pursuing novel insight into cancer disease mechanisms.
- 'Using computer models to predict drug resistance in colon cancer' (www.colosys.org) we will develop a deeper understanding of colon cancer networks and convert them into computer models with which we will be better capable to predict response to treatment.

The Master student will be able to work in the subproject 'Rational development of anti-cancer combinations'. This subproject aims to develop and integrate computational, experimental and analytical approaches to predict and validate anti-cancer drug combinations and to produce a pipeline for rational screening of synergistic drugs and for clinical decision support in precision medicine. Researchers have previously shown that logical (Boolean) multiscale models can describe molecular mechanisms underlying cellular decision making (Abou-jaoudé et al., 2016) and our group has accurately predicted the effect of 20 out of 21 drug combinations with only one false positive combination (Flobak et al., 2015). The network model presented in (Flobak et al., 2015) will now be extended to represent several different cancer cell lines and tumor types. The model will be tailored by integrating data about tumor heterogeneity, patient variation and molecular mechanisms in the different cancer cell lines in response to perturbations. Screening for synergistic drug combinations and validation of the model will be performed using high throughput cell line drug response screens by SINTEF. Promising drug combinations will be further tested in low throughput experiments and xenograft models.

The Master project will focus on wet-lab experience generated from ongoing drug combinations screening and data modelling. The methods will include western blot, RT-PCR, xCELLigence for Real-time cell proliferation measurements and cell viability assays. The project will be discussed in detail with the candidate.

More information about the DrugLogics project can be found on the website http://www.druglogics-ntnu.org/about.html.

References: Abou-jaoudé, W., Traynard, P., Monteiro, P. T., Rodriguez, J. S., Helikar, T., Thieffry, D., & Chaouiya, C. (2016). Logical modeling and dynamical analysis of cellular networks. *Frontiers in Genetics*, *7*(May), 1–20. http://doi.org/10.3389/fgene.2016.00094.

Flobak, Å., Baudot, A., Remy, E., Thommesen, L., Thieffry, D., Kuiper, M., & Lægreid, A. (2015). Discovery of Drug Synergies in Gastric Cancer Cells Predicted by Logical Modeling. *PLoS Computational Biology*, *11*(8), 1–20. http://doi.org/10.1371/journal.pcbi.1004426.

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Master Thesis in Molecular Medicine

Name of Group: **Bone disease group**

Name of Research Community: **CEMIR/ Department of Cancer Research and Molecular Medicine**

The role of adipocytes in multiple myeloma bone disease

Background
Multiple myeloma is a cancer caused by accumulation of malignant plasma cells in the bone marrow. Most patients develop an osteolytic bone disease caused by a misbalance between the bone making cells, osteoblasts, and the bone resorbing cells, osteoclasts.

It is well known that the bone marrow microenvironment plays a crucial role in supporting survival of the myeloma cells. Recently, attention has been given to the role of adipocytes (fat cells) in multiple myeloma. Traditionally, adipocytes have been considered inactive "space-fillers", but recent advances are revealing an important role for adipocytes in inflammation and cancer progression. The role of adipocytes in multiple myeloma is scarcely studied.

Goal
In this project we will define a role for adipocytes in multiple myeloma. Specifically, we will study factors produced by the adipocytes that might play a role in the differentiation of osteoblasts and osteoclasts. The role for adipocytes in terms of supporting myeloma cells will also be investigated.

Methods: Cell culture/differentiation of adipocytes, bone cells and cancer cells, co-cultures, isolation of blood cells/ adipocytes from blood/bone marrow, cDNA isolation, RNA production, qPCR, western blot, histological stainings, ELISA, confocal imaging, miscellaneous viability assays,

The specifics of this project will be made according to progression of the project and the students' area of interest.

Paper of interest: Falank, C., Fairfield, H., & Reagan, M. R. (2016). Signaling Interplay between Bone Marrow Adipose Tissue and Multiple Myeloma cells. *Frontiers in Endocrinology*, *7*, 67. http://doi.org/10.3389/fendo.2016.00067

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Master Thesis in Molecular Medicine

Name of Group: **Centre for Myeloma Research**

Name of Research Community: **IKM**

Bioassays for bone morphogenetic protein activity

Multiple myeloma is a cancer arising in the antibody-producing plasma cells that are located in the bone marrow. The malignant cells disturb the balance between bone forming and bone degrading cells, leading to bone disease in most patients. Despite recent advances in drug development, myeloma is regarded incurable, thus there is a need for novel treatments.

Bone morphogenetic proteins (BMPs) are potent inhibitors of multiple myeloma cells and may potentially be exploited to develop treatment for myeloma patients. We want to find potential drugs that could enhance BMP activity in the bone marrow. By using cell lines that are sensitive to BMPs we can easily detect changes in BMP activity. We want to modify these cells in a way that makes it easier and less expensive to detect BMP effects and use this to screen for BMP-potentiating compounds. In parallel, we want to generate a bioassay for detecting biologically active BMPs in serum and cell supernatants. Also here, BMP-sensitive myeloma cell lines will be used as tools.

The project requires much laboratory work and results will depend on high accuracy. Methods that will be used include cultivation of myeloma cell lines, lentiviral transduction of cells, flow cytometry, as well as assays for cell proliferation and cell death.

This project is suitable for one master student.

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Master Thesis in Molecular Medicine

Name of Group: **Hereditary cancer**

Name of Research Community: **Medical genetics**

Genetic risk factors in hereditary colorectal cancer

Introduction

Colorectal cancer (CRC) is one of the most common cancer types worldwide. 5-10 % of CRC patients belong to high-risk families suffering from a hereditary CRC syndrome where we know the associated genes, and Lynch syndrome is the most common of these syndromes. In addition, about 20-25 % has a higher risk of CRC than rest of the population. In this group, there is less knowledge about genetic risk factors. These families have heterogenic phenotypes. The hypothesis is that the cause of increased cancer risk in this group is high penetrant genes yet to be identified in some families, while several low penetrant genes which together increase CRC risk (polygenic inheritance) may be the cause in other families.

It is important to identify inherited risk factors for CRC because they can be used to find high-risk individuals, which can be offered intensive surveillance programs. Thereby the mortality rate of CRC may decrease by early detection or by preventing cancer to arise by removing precancerous tissue.

Aim

The aim of the project is to uncover genetic causes and risk factors for hereditary colorectal cancer.

We focus both on known syndromes like Lynch syndrome, in addition to search for new genes as cause of hereditary CRC.

Methods

We use high throughput sequencing technology (Next generation sequencing, NGS) and bioinformatics in order to map gene variants in high-risk families. New variants may need to be further characterized by functional analyses in cell line systems.

Ongoing projects

Lynch syndrome and Lynch syndrome like: Mutations in mismatch repair (MMR) genes are known to cause Lynch syndrome, and we work to find new mutations causing Lynch syndrome. Not all families showing a Lynch syndrome phenotype actually have a mutation in the MMR genes. We search to find which genes that are causing the high risk of CRC in these families.

Functional studies: Sometimes we find new gene variants which are difficult to interpret whether they give an increased risk of CRC or not. These variants of uncertain clinical significance, we aim to characterize by doing bioinformatics prediction and functional analyses.

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Master Thesis in Molecular Medicine

Name of Group: **Humangenetikk, LBK**

Name of Research Community: **Virus in immunity and disease**

"Immune reprogramming by respiratory viruses"

Scope:

Airway viral infections have been found to reprogramme the immune responses of the host having both short-term and long-term consequences. The effect of this can be to overcome the infection, but it can also lead to increased susceptibility e.g. to secondary bacterial infections and or it may promote chronic airway diseases, such as asthma. We seek to understand how viral infections reprogramme immune responses and how anti-inflammatory and antiviral drugs intervene with immune factors that drive airway disease. Successful research in this area is necessary to develop antiviral therapeutics, vaccines, and novel preventive strategies for asthma and allergic disease.

Approach:

Step 1: Investigate immune reprogramming, e.g. of antiviral and inflammatory responses, in vitro in cells relevant for respiratory infections with clinically relevant viruses, e.g. human metapneumovirus (hMPV).

Step 2: Based on results, test potential intervention strategies in cellular models.

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Master Thesis in Molecular Medicine

Name of Group: **Microbiology Group**

Name of Research Community: **LBK, DMF**

Project title: Molecular mechanisms of intrinsic beta-lactam resistance in *Staphylococcus aureus*

Background: *Staphylococcus aureus* is an important human and animal pathogen, giving rise to a wide variety of infections. Beta-lactam antibiotics are the first line of defence against a number of bacterial pathogens, including *S. aureus*. However, resistance to beta-lactams is increasing and disseminating, and so has become an important global health problem causing problems in treatment of infections. Beta-lactam antibiotics act by inhibiting a step in the cell wall synthesis of bacteria, by binding to the active site of enzymes called penicillin-binding proteins (PBPs). *S. aureus* are known to become resistant to beta-lactams either by expressing a beta-lactamase enzyme or by acquiring an alternative PBP gene (called mecA or mecC). Studies have furthermore indicated that mutations in the native PBP proteins of *S. aureus* can cause resistance to some beta-lactams, giving rise to so-called borderline oxacillin-resistant *S. aureus* (BORSA) or intrinsically resistant *S. aureus*.

Aim of study: To identify the molecular basis for borderline oxacillin resistance in S.aureus.

Materials and methods: In this project we will perform whole genome sequencing of selected borderline oxacillin-resistant *S. aureus* from the collection of the Norwegian MRSA reference laboratory at St. Olavs Hospital. We will characterize potential mutations giving rise to the resistance phenotype in these strains, and introduce selected mutations in a sensitive strain in order to establish the causative mutation of intrinsically resistant *S. aureus*.

The student will learn fundamental methods in molecular microbiology, such as working with bacterial cultures, PCR and cloning, and will in addition become familiar with high-throughput sequencing and genome data analysis using bioinformatics methods.

Researcher Christina Gabrielsen at the MRSA laboratory, St Olavs Hospital will be main supervisor and prof. Jan Egil Afset will be responsible-supervisor.

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Master Thesis in Molecular Medicine
Name of Group: **Prof. Jan Egil Afset**
Name of Research Community: **LBK, DMF**

Project title: Characterization of new adhesion factors in Shiga toxin-producing *Escherichia coli*
Main supervisor: Prof. Jan Egil Afset
Co-supervisors: researchers Christina Gabrielsen, Yi-Qian Sun

Background. Diarrheagenic *E. coli* is a major contributor to diarrheal illness in children under the age of five worldwide, especially causing high mortality in developing countries[1]. Gastrointestinal infection with Shiga toxin-producing *E. coli* (STEC), one of six major *E. coli* diarrheagenic pathotypes, usually causes a range of diseases, from uncomplicated infection to hemorrhagic colitis, and the worst scenario hemolytic uremic syndrome (HUS)[2]. HUS is characterized by thrombocytopenia, nonimmune hemolytic anemia, and acute renal failure. It is still unknown why some patients only get mild disease and others will develop HUS. The key virulence factors in STEC are the Shiga toxins, and most STEC have the ability to adhere to intestinal epithelial cells by forming attaching and effacing lesions, a property encoded by genes located on the locus of enterocyte effacement (LEE) pathogenicity island in the chromosome[1]. In addition other adhesins have been described which may influence on the virulence of *E. coli*. However, in STEC, especially in non-O157 STEC, the genes involved in adhesion and colonization have not been investigated thoroughly.

Aim of the project. The aim of this project is to characterize new adhesins as potential virulence factors in STEC.

Materials and Methods. Based on preliminary results of global transcriptional profiles in HUS and non-HUS STEC, we have identified 13 genes upregulated in HUS strains which may be involved in cell adhesion in non-O157 STEC[3]. Among them there are five genes located in Type 1 fimbriae operon and a number of not-well characterized putative fimbrial genes. Interestingly, two genes in a putative fimbrial operon are not similar to any previously characterized fimbrial genes. These two genes will be investigated in this project. For biosafety reasons we will instead of STEC use *Citrobacter rodentium* as model organisms in this project, to investigate the functions of these two genes on adhesion to intestinal epithelial cells[4]. *C. rodentium* is a natural mouse pathogen and it also carries LEE that is essential for the pathogenesis of attaching/effacing bacterial pathogens, but lack the stx-genes. The two putative adhesion genes will be amplified and cloned into expression plasmids. The putative adhesins will then be expressed in *C. rodentium* carrying the expression plasmids[5]. Polarized cell lines, such as Caco-2 will be used to investigate the effect of these genes on adhesion as *in vitro* infection model. Alternatively, STEC with knockout of Shiga-toxin genes can also be used as model bacterium.

Through this project we expect to get new knowledge about adhesins as potential virulence factors in STEC. The master student will learn the fundamental techniques in molecular microbiology, such as cloning, construction of bacterial mutants, heterologous gene expression, as well as phenotypic analysis.

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Master Thesis in Molecular Medicine

Name of Group: **Group Van Loon – "DNA Base Damage and Chromatin Integrity"**

Name of Research Community: **Unit for Molecular Biology, Department of Molecular Cancer Research, Faculty of Medicine**

Living organisms are constantly exposed to alkylating agents. These agents can be found in our environment, water, food, inside cells as products of our metabolism, and are one of the most frequently used type of cancer chemotherapeutical drugs. Alkylating compounds can damage all essential biological molecules, including lipids, proteins, RNA and DNA. Accumulation of DNA damage, miscoding mRNA transcripts, malfunctional proteins and, or membranes can have a detrimental effect on cellular homeostasis. In case cells cannot repair induced damage they will undergo cell death by apoptosis or necrosis. However, if they manage to escape death mechanisms and to maintain DNA lesions in their genome irreversible mutations will be formed, directly leading to genomic instability and onset of different human pathologies, such as cancer. To counteract detrimental effects of alkylating agents, cell response mechanisms have to be activated. Though extensive research has been done to identify individual response mechanisms, such as DNA damage responses, our current knowledge is limited about how are those mechanisms coordinated and controlled, as well as which signals are generated and how are they transduced upon active damage response.

The aims of this thesis are to: (a) determine modulators of survival upon alkylation stress; (b) define proteins that specifically associate with alkylated DNA base lesions; and (c) identify factors that modulate sensitivity to alkylation treatment through recognition of alkylated DNA base lesions.

To determine modulators of alkylation stress response a highthroughput genome-wide screen based on recently published CRISPR-Cas9 and a short-guided RNA library will be used. This approach coupled to deep sequencing analysis represents a powerful new tool for genome engineering allowing simultaneous targeting of numerous different genes in wide variety of cell types. The proteins that specifically associate with alkylated DNA base lesions will be determined by using basic molecular biology approaches in combination with protein mass spectrometry. To address the third aim and identify factors that modulate sensitivity to alkylation treatment through recognition of alkylated DNA base lesions, cell biology and survival assays, together with analysis of nucleic acids will be performed.

Taken together, by combining the modern genome engineering approaches and basic molecular and cell biology techniques, this master thesis will shed more on understanding of the cellular factors and networks that determine biological outcome in response to alkylation damage. This is particularly important for both cancer prevention and therapy.

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Master Thesis in Molecular Medicine

Name of Group: **Bioinformatics and Gene Regulation**

Name of Research Community: **Dep. of Cancer Research and Molecular Medicine; Unit for Molecular Biology**

The Bioinformatics and Gene Regulation research group is developing and using software for analysis of data from molecular biology. The group is mainly doing research on eukaryote gene regulation, using computational data analysis together with experimental data generation and verification. Active research areas are in particular transcription factors, non-protein-coding RNAs, genome structure (including epigenetics), and the molecular biology of cancer. The group is also involved in projects on prokaryote genomics.

Transcription factors are proteins that bind to regulatory regions in the genome and control gene expression. Binding sites for transcription factors are described by motifs representing short fragments of DNA. Binding site motifs may be determined experimentally or by modelling, but modelling gives several false hits in a typical genomic sequence. We are working on improved modelling and analysis of transcription factor-based gene regulation by using a combination of large experimental data sets and better prediction methods. This can be used to analyse regulatory relationships between genes. In addition to binding sites for traditional transcription factors the genome also contains binding sites for structural proteins that define the physical organisation of the genome and demarcate functional subunits within the genomic sequence. This is an important aspect of gene regulation, and detailed analysis of such regions can give us valuable information about the interplay between genome organisation and gene regulation. *(Main contact person: Finn Drabløs)*

Non-protein-coding RNAs, or non-coding RNAs (ncRNAs) are important in many aspects of gene regulation. An important sub-group is the microRNAs (miRNAs), which are approximately 22 nucleotide long RNA genes that regulate protein coding genes. MicroRNAs constitute about 2% of the currently known human genes, but these miRNAs may regulate more than 60% of the human protein coding genes. Many miRNAs have been implicated in developmental disorders and diseases such as cancer. MicroRNAs regulate protein coding genes by binding to the genes' transcribed RNA and preventing the RNA from being translated into protein. How a miRNA recognizes its target genes is still a focus of intense research. Our group develops and uses computational and experimental tools to study how miRNAs individually and cooperatively recognize and regulate target genes, how miRNAs and transcription factors jointly regulate gene expression, and how genomic alterations such as single nucleotide polymorphisms (SNPs) affect gene regulation. We are particularly interested in how miRNAs and transcription factors regulate and affect processes such as DNA repair, cell proliferation, cell differentiation, and virus infection. *(Main contact person: Pål Sætrom)*

Personalized medicine aims to separate patients having a specific disease into different groups based their individual disease characteristics. Cancer is a genomic disease, and profiles of DNA, RNA and epigenetic alterations are increasingly used to characterise and diagnose individual cancers. However, there are several challenges for the application of these genomic markers for personalized clinical use. Using transcriptomic data (RNA) as a starting point, we are using bioinformatics to characterise clinical tissue sample from prostate cancer, with the aim to identify the individual genomic cancer profile for each sample. Our approaches include integration of transcriptomic data with data from DNA, metabolomics and epigenetics, meta-analysis and integration of publicly available data on prostate cancer, pathway and network analysis for integrated data, computationally purifying cancer profiles signals in heterogeneous tissue samples, identification of gene-sets and features characteristic for specific tumours. Our overall aim is to identify features which can be used as biomarkers for aggressive/indolent disease and drug-targets. *(Main contact person: Morten Rye)*

Our available projects range from pure experimental work to pure bioinformatics analyses. For the latter projects, some programming experience may be required. Interested students should contact Finn Drabløs, Pål Sætrom or Morten Rye to discuss project-specifics.

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Master Thesis in Molecular Medicine

Name of Group: **Cancer - molecular mechanisms and genetic risk factors**

Name of Research Community: **Unit for Laboratory Medicine, Department of Laboratory Medicine, Children's and Women's Health**

Omega-3 polyunsaturated fatty acids (PUFAs) are normal constituents of the diet and have essential roles in maintaining important cellular functions. Especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to have anticancer activity, both in vitro and in human tumor xenografts. Clinical studies have shown that patients with different types of cancer may have beneficial effects of omega-3 PUFAs alone or in combination with chemo- and radio therapy. We have been studying signalling pathways affected by PUFAs in human cancer cells for several years. Our results show that DHA and EPA induce stress in the endoplasmic reticulum (ER) of cancer cells. The ER stress is a part of the "Integrated stress response", which also includes golgi stress and autophagy. Lately the project has been focusing on the importance of the basal level of autophagy and the level of "autophagic flux" in colorectal cancer cells stimulated with DHA. DHA-sensitive cancer cells tend to have higher basal autophagy level compared to less sensitive cells. The student will participate in the ongoing project, focusing on in vitro analyses of colon cancer cells treated with DHA in order to further explore the importance of autophagy – and other pathways of the integrated stress response in DHA-sensitivity. Methods will include cell culturing, survival assays, flow cytometry, western blotting and confocal imaging.

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Master thesis project: Activation of TLR9 in inflammatory response by intracellular relocalization

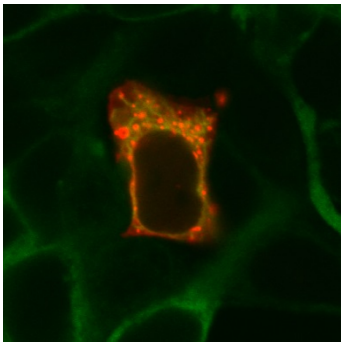

Figure 1. Rab7 (red) TLR9 (green). Astrid Skjesol

We are looking for a highly motivated and talented master student for our project on regulation of TLR9 in inflammatory response.
Background: Pattern recognition receptors (PRR) are important in recognition of pathogens and induction of the immune responses. Precise regulation of these receptors is important during infection, but dysregulation can also be involved in autoimmune diseases and in cancer. An important family of PRRs is the Toll-like receptors (1). Toll-like receptor 9 (TLR9) is a PRR able to recognize bacterial DNA and, as recently described, viral DNA:RNA hybrids. In response to binding of these pathogenic patterns, TLR9, through its adaptor molecule MyD88 activates pro-inflammatory cytokines and anti-viral responses. Binding of nucleic acid to TLR9 takes place on intracellular endosomes. As TLR9 normally resides in the endoplasmic reticulum (ER), this activation requires internalization of ligand and relocalization of TLR9 to endosomes from the ER compartment (2).
Practical: This project is about discovering how the TLR9 travels from the ER to late endosomes. This is important if we want to understand how TLR9 contributes to cancer. We will make use of the latest imaging technology to study the path taken by this receptor, and the mechanisms involved. Conventional methods such as western blotting, ELISA, RT-PCR, cell culture work and flow cytometry will be implemented. The project will be carried out at the new Centre of Excellence, Centre of Molecular Inflammation Research (CEMIR) at the Department of Cancer Research and Molecular Medicine. The project will be supervised by Researcher Lene M. Grøvdal and Researcher Harald Husebye.

For more information please contact Lene Grøvdal at lene.m.grovdal@ntnu.no

Reference List

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Master Thesis in Molecular Medicine

Name of Group: **The Centre of Molecular Inflammation Research (CEMIR)**
Name of Research Community: **Instituttgruppe for Cellebiologi, IKM**

THE ROLE OF PATTERN-RECONITION RECEPTORS IN CANCER PROGRESSION

Our central hypothesis is that low-grade chronic inflammation mediated by pattern recognition receptors (PRR)s may have important effects on the development and progression of cancer. Our main goal in this project is to unveil how activation of different PRRs affects cancer progression.

Background- Inflammation is an immune response which normally protects during the early phases of infection. Dysregulated inflammation can, however, cause chronic inflammation and disease. Pattern recognition receptors (PRR)s are highly expressed on immune cells. These receptors are important for sensing infection and danger by recognizing certain molecular structures expressed by microorganisms or associated with stress or injury. Upon recognition of such components, PRRs mount an inflammatory immune response.

Certain PRRs are considered promising targets in cancer immunotherapy because they can trigger an anti-cancer immune response. Some cancer cells express PRRs, and PRR activators may impair the growth and survival of these cancer cells. PRRs may, however, play detrimental roles in the development and progression of cancer by mediating chronic inflammation when dysregulated. The association between inflammation and cancer is evident in certain cancers like colon cancer, where chronic inflammation in the bowel is a major risk factor for developing cancer. Once formed, tumors may also manipulate PRR expression and signaling to their advantage. Cancer cells can also produce PRR activating factors that activate other cells in the tumor microenvironment and create tumor-promoting inflammation. Thus, the outcome of PRR activation in cancer progression is complex and still poorly understood.

This project seeks to determine how PRR expression and signaling in the tumor microenvironment affects the progression of the bone-marrow cancer Multiple Myeloma. We aim to determine PRR expression and signaling in the tumor microenvironment at different cancer stages. We also seek to identify new PRR activating components produced by cancer cells that may drive tumor-promoting inflammation. The projects should provide new insight on how PRR expression and activation may affect, and possibly predict cancer progression. This knowledge may aid early diagnosis and the development of optimized treatment

Our main goal in this project is to unveil how expression and activation of different PRRs in the tumor microenvironment affects the progression of multiple myeloma (MM).
In particular, we will;
1. Assess PRR expression and signaling in tumor cells
2. Identify PRR-expressing cells in the tumor microenvironment that mediate signals that affect cancer progression
3. Identify new PRR ligands in the tumor environment that may promote cancer progression.

Overall, we aim for novel understanding of PRR-mediated mechanisms that affect cancer progression.

Methods- *In vitro* experiments on MM cancer cell lines and patient cells. Peripheral blood mononuclear cells, particularly monocytes will also be isolated and differentiated to macrophages or dendritic cells. Methods will include sterile technique, cell culturing, monocyte isolation and stimulation assays. Enzyme-linked immunosorbent assay (ELISA) will be applied for cytokine detection in serum and for cytokine release. Flow cytometry and western blot may also be applied for protein detection, as well as reverse transcriptase-PCR for analysis of mRNA levels. Additional methods such as confocal microscopy may be selected depending on the student's findings and interests.

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Master Thesis in Molecular Medicine

Name of Group: **DNA repair and adaptive immunity (Kavli/Krokan group)**

Name of Research Community: **IKM**

The role of AID in B-cell cancer development

Activation-induced cytidine deaminase (AID) is an enzyme that actively mutates antibody genes to yield highly specific antibodies with specialized effector functions. Although AID is essential for antibody-mediated immunity, its mutagenic nature also represents a threat to human health. Indeed, recent studies have shown that DNA mutational signatures from AID and similar cytidine deaminases are present in several cancers. We study the roles of AID and related proteins in both adaptive immunity and cancer development.

Possible Master's projects would study the regulation of antibody maturation in B-cells and/or the role of AID and related proteins in cancer development. Depending on the student's existing skillset and his/her learning goals, a wide range of methodologies may be used, including analytical biochemistry (mass spectrometry), work with a knockout mouse model, next-generation sequencing, and more traditional molecular biology techniques (PCR, blots, etc).

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Master Thesis in Molecular Medicine

Name of Group: **Humangenetikk, LBK**

Name of Research Community: **Virus in immunity and disease**

"Host factors exploited by hepatitis C virus to promote viral replication"

Hepatitis C virus (HCV), a human oncogenic, blood-borne virus, first described in 1989, infects mainly hepatocytes, the chief parenchymal cell of the liver. Like all other positive-stranded RNA viruses HCV induce massive rearrangements of intracellular membranes, called membranous web, that provide a scaffold for the assembly of the HCV replication complex and protection from host immune defences. The HCV membranous web has a complex morphology consisting of clusters of single-, double- and multi-membrane vesicles, which probably include autophagosomes and lipid droplets. Numerous host factors have been shown to be exploited by HCV to promote its replication, e.g proteins that regulate the catabolic process autophagy. These proteins may be targets for host-directed therapeutics.

Autophagy is a cellular process by which cytoplasmic components are sequestered in double membrane vesicles and degraded to maintain cellular homeostasis. It has also been implicated as an important component of the innate and adaptive immune response against a variety of viral and bacterial pathogens. Autophagy plays a positive role in HCV replication and infection, but detailed knowledge of the molecular mechanisms involved is lacking.

Scope:
We will explore the mechanisms by which HCV infection exploits host factors leading to establishment of efficient HCV replication and development of liver pathology.

Approach:
The project requires extensive laboratory work. Methods that will be used include cultivation of hepatocytes cell lines, viral infections, siRNA transient transfection, Western blot and qPCR analysis.

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Master Thesis in Molecular Medicine

Name of Group: **Humangenetikk, LBK**

Name of Research Community: **Virus in immunity and disease**

"Regulation of lipid metabolism by hepatitis C virus"

Hepatitis C virus (HCV) is a small positive-sense single-stranded RNA virus that can cause both acute and chronic hepatitis infection resulting in serious disease and death from cirrhosis and liver cancer. A hallmark of HCV infection is the extraordinary ability of the virus to cause chronic infections. No effective vaccine against HCV is available. Treatment with new HCV antiviral therapeutics are encouraging, but these medicines are cost-prohibitive to the patient and do not achieve sustained virologic response in all patients. Thus, a need for continued development of effective and affordable therapy for HCV treatment is required. In patients persistently infected with HCV, chronic inflammation typically lasts for decades. It frequently leads to progressive liver disease that ranges from mild inflammation to severe fibrosis that can culminate in life-threatening disease, such as hepatic cirrhosis and hepatocellular carcinoma and thus poses a significant public and global health threat.

Scope:
The HCV lifecycle is closely tied to lipid metabolism in infected cells. Abnormal accumulation of fatty deposits in the liver, termed steatosis, is a frequent phenotype in HCV infected patients. HCV-mediated dysregulation of lipophagy; a selective degradation of lipids by autophagy, may cause accumulation of lipids, leading to steatosis and liver pathology. We aim to determine in detail how HCV modulates lipid metabolism through selective autophagy

Approach:
The project involves extensive laboratory work. Methods that will be used include cultivation of hepatocytes cell lines, viral infections, siRNA transient transfection, Western blot and qPCR analysis.

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Master Thesis in Molecular Medicine

Name of Group: **Molecular Regeneration group (Oudhoff Lab)**

Name of Research Community: **Centre of Molecular Inflammation Research (CEMIR), IKM**

Title: Regulation of Hippo and Wnt pathways during regeneration and inflammation by epigenetic modifiers.

Project:
The overarching goal of research in the Oudhoff lab is to define the cellular and molecular mechanisms that control regeneration and inflammation in the digestive system. The digestive tract is responsible for absorption of nutrients and water, but at the same time it has a crucial role in acting as a barrier to the external environment. The barrier function is complicated by the requirement to simultaneously be able to respond appropriately to dangerous pathogens, and remain tolerant to innocuous antigens like commensal organisms and food.

The Hippo and Wnt pathways are known to regulate various aspects of biology such as organ size, but also regenerative processes require optimal Hippo and Wnt signalling. This is established by a complex interplay of signalling pathways that rely on various cues such as cell-cell contact, but also extracellular signals. We have recently found that methylation of the Hippo transducer YAP plays an important role during intestinal infection, inflammation, regeneration, and tumorigenesis.

In the Oudhoff lab we combine mouse animal work with intestinal organoid cultures, as well as standard biochemical and cellular biology. The MSc student, depending on his/her interest, will either focus on organoid work and testing various compounds in this system, or will focus on more biochemical-based assays to study aspects of Wnt and Hippo signalling. The student will have direct supervision from Menno Oudhoff (group leader) and Rosalie Zwiggelaar (PhD student), and will be an important part of the research team. For further information, please contact menno.oudhoff@ntnu.no

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Master Thesis in Molecular Medicine

Name of Group: **Regeneration group**

Name of Research Community: **Centre of Molecular Inflammation Research (CEMIR), IKM**

Title: Trafficking of Fc receptor gamma chain-associated innate immune receptors

Project description:
We are looking for a highly motivated and talented master student for our project on trafficking of Fc receptor gamma chain-associated innate immune receptors.
The Fc receptor gamma chain (FcRγ) is a component of multiple multimeric immune receptor complexes, inducing signaling via its Immunoreceptor tyrosine-based activation motif (ITAM) and recruiting kinases for downstream signaling. The requirement of FcRγ for surface localization and signal transduction of innate immune receptors was first shown for the IgE receptor FcεRI, FcRγ is furthermore part of the FcαRI (only human), FcγRI, FcγRIIIA (mouse and human) and FcγRIV (only mouse) complex. C-type lectin receptors (CLR) have emerged as important class of pattern recognition receptors in the last 15 years; several CLR such as Dectin-2, Mcl and Mincle associate with FcRγ to induce inflammatory signalling. These receptors detect e.g. fungal pathogens and mycobacteria.
Whereas FcRγ-dependent Syk recruitment to Fc receptors is essential for phagocytosis induction, ligation of FcRγ-coupled C-type lectin receptors (CLR) leads to inflammatory signaling dependent on Syk without regularly inducing phagocytosis. This leads to the question why, despite of overlapping signaling pathways, some FcRγ-associated receptors in innate immune cells act as phagocytic receptors while others seem to not initiate endocytosis. Furthermore the project aims to investigate the trafficking of FcRγ. Given its abundance in innate immune cells it is intriguing to study where FcRγ is localized under physiological conditions and how assembly of FcRγ with its associated receptors and trafficking of the FcRγ-receptor complex is controlled. It is not yet clear whether different FcRγ-associated receptors undergo different fates after ligand binding. We will address these questions in both cell lines and primary cells, combining molecular biology techniques, flow cytometry and confocal microscopy.
The project will be carried out at the new Centre of Excellence, Centre of Molecular Inflammation Research (CEMIR) at the Department of Cancer Research and Molecular Medicine. The project will be supervised by Jenny Ostrop and Menno Oudhoff. Please contact Jenny Ostrop for further information: jenny.ostrop@ntnu.no

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Master Thesis in Molecular Medicine

Name of Group: **Center for myeloma research**

Name of Research Community: **Unit for Cell Biology**

Multiple Myeloma (MM) is an incurable cancer disease, although new treatment introduced the last decade has improved the outcome substantially. Usually, the drugs works well on newly diagnosed patients, but unfortunately the disease will come back and the drugs are not as efficient later in the disease course. After rounds of treatments, the patients often becomes resistant towards the drugs. Drug resistance is a major and unsolved issue in MM. In our MM group in Trondheim, we have done much research the last years on proteasome inhibitor (PI) resistance. Proteasome inhibitors, such as Bortezomib (Velcade), are a much used class of drugs in MM. We have recently published a paper showing that oxidative stress is important for the effects of Bortezomib.
In this thesis the candidate will continue working with proteasome inhibitors and resistance mechanisms. One obvious model system will be a cell line we have established in our lab, that is resistant towards Bortezomib, and another one being resistant towards another PI, Carfilzomib.

Methods that will be used in this work: Cell Culture, Viability Assays, PCR, Western Blot.

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Master Thesis in Molecular Medicine

Name of Group: **Systems Inflammation Group**

CEMIR/IKM: Dissecting the role of a novel phosphosite on viral RNA sensor LGP2 in antiviral immune response

Dissecting the role of a novel phosphosite on viral RNA sensor LGP2 in antiviral immune response

The host evokes innate immune responses to eliminate invading pathogens by detecting the presence of infection. Cells in the innate immune system, such as macrophages and dendritic cells (DCs), express a limited number of germline-encoded pattern-recognition receptors (PRR) that specifically recognize pathogen-associated molecular patterns (PAMPs) within microbes, which are unique to these microbes and not found in the host. At least three main classes of PRRs have been implicated in the detection of viral nucleic acid: (i) Toll-like receptors (TLRs), which sense incoming virions in endolysosomes by binding to viral RNA (TLR3 and -7/8) or CpG-containing DNA (TLR9), (ii) viral DNA sensors, including cGAS, IFI16, and DAI, and (iii) RIG-I-like receptors (RLRs), that are essential for the detection of viral RNA in the cytoplasm of most cell types.
RLRs are members of the RNA helicase family and are responsible for cytoplasmic virus recognition ensuing signaling leading to type I interferon (IFN) and inflammatory cytokine production. RLRs, comprising RIG-I, MDA5, and LGP2, are characterized by a conserved domain structure, consisting of a central DExD/H-box helicase domain and a C-terminal domain (CTD), both responsible for binding viral RNA. In addition, RIG-I and MDA5 harbor two N-terminal caspase activation and recruitment domains (CARDs) which, upon virus sensing, initiate downstream signaling, leading to type I IFN gene expression. In contrast, LGP2 lacks the CARD signaling module and cannot initiate downstream signaling. However, its ability to recognize dsRNA allows it to modulate the signaling capacity of RIG-I and MDA5. LGP2 down-regulates signaling by RIG-I. In contrast, LGP2 enhances MDA5 signaling. The molecular mechanism of this enhancement remains unclear, but LGP2 appears to facilitate recognition of viral RNA by MDA5 through interactions between the LGP2 CTD and RNA. Despite the fact that evidence accumulates for the opposing roles of LGP2 on RLR signaling, the emerging perspective is that LGP2 can control the balance between RIG-I and MDA5 responses during viral infection. Phosphorylation is emerging as an important regulatory mechanism for RLR signaling. We have recently identified a novel phosphosite in mouse macrophages LPG2 protein using global phosphoproteomics approach. This specific phosphosite is also conserved in humans.
Our main objective is the functional characterization of this novel phosphosite on LGP2 and elucidate its biological relevance during antiviral immune response. To this aim, we have made CRISPR/Cas9-based knockouts in mouse macrophages to study LPG2 function during virus infection using different strategies. In this proposed project, we will study the LGP2 interaction with others RLRs proteins and cellular partners and the implication of its phosphorylation site using LPG2 mutant proteins. Biological relevance and antiviral responses will be monitored at an early and late stage of infection by measuring: (1) the expressions of antiviral cytokines, IFN-β, CXCL10, CCL5, and CCL20 by RT-qPCR; and (2) the nuclear levels of transcription factors (NF-κB, IRF-1, and IRF-3) by western blot.

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Master Thesis in Molecular Medicine

Name of Group: **Scheffler/Bjørås**

Name of Research Community: **IKM**

Role of Neil3 in Alzheimer's disease

Genomic DNA is continuously challenged by endogenous and exogenous oxidative agents, thus preserving genomic integrity is a key for development and health. The brain is particular vulnerable to oxidative stress due to its large consumption of oxygen and high metabolic rate. Base excision repair (BER) is the major pathway for removal of oxidized bases in the genome. BER is initiated by DNA glycosylases, recognizing and excising a broad range of base lesions (1). The Neil-type of DNA glycosylases (Neil1, Neil2 and Neil3) show significant sequence homology and overlapping substrate specificity. However, Neil3 is exceptional among the three Neil homologs due to its distinct expression in proliferative tissue (2). Interestingly, Neil3 is highly expressed in cells in the subgranular and subventricular zones, the neurogenic niches in the brain, suggesting a role in regulating neurogenesis (3, 4). Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder clinically characterized by a progressive loss of memory and deterioration of cognitive abilities (5). Alterations of neurogenesis occur at very early stages of AD progression indicating that it represents an important part of AD pathology (6). The goal of the current study is to identify the role of Neil3-dependent BER in the progression of AD by characterizing newly developed AD mouse models defcient for Neil3.
The project includes the folling steps:

1. Characterize AD progression in transgenic mice at different disease stages by immunohistochemical staining of histopathological hallmarks of AD (β-amyloid plaques, microglia, astrocytes)
2. Identify alterations in neurogenesis first by immunohistochemical staining of mouse brain sections with neural stem cell specific marker and second by cultering *in vitro* freshly isolated neural stem cells (neurospheres) to analyze their proliferation and differentiation capacity
3. Quantify DNA damage in transgenic mouse tissue by real-time quantitative PCR-specific methods and mass spectrometry-based detection of DNA base lesions.

During the project, the following techniques will be applied: mouse genotyping by PCR, neural cell culture, immunohistochemical stainings, isolation of DNA and RNA from mouse tissue, real-time quantitative PCR

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2. Liu M, *et al.* (2010) The mouse ortholog of NEIL3 is a functional DNA glycosylase in vitro and in vivo. *Proc Natl Acad Sci U S A* 107(11):4925-4930.
3. Regnell CE, *et al.* (2012) Hippocampal adult neurogenesis is maintained by Neil3-dependent repair of oxidative DNA lesions in neural progenitor cells. *Cell Rep* 2(3):503-510.
4. Sejersted Y, *et al.* (2011) Endonuclease VIII-like 3 (Neil3) DNA glycosylase promotes neurogenesis induced by hypoxia-ischemia. *Proc Natl Acad Sci U S A* 108(46):18802-18807.
5. Selkoe DJ (2001) Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev* 81(2):741-766.
6. Verret L, Jankowsky JL, Xu GM, Borchelt DR, & Rampon C (2007) Alzheimer's-type amyloidosis in

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Master Thesis: Stiffness vs. Cell response in artificial extra cellular matrixes.

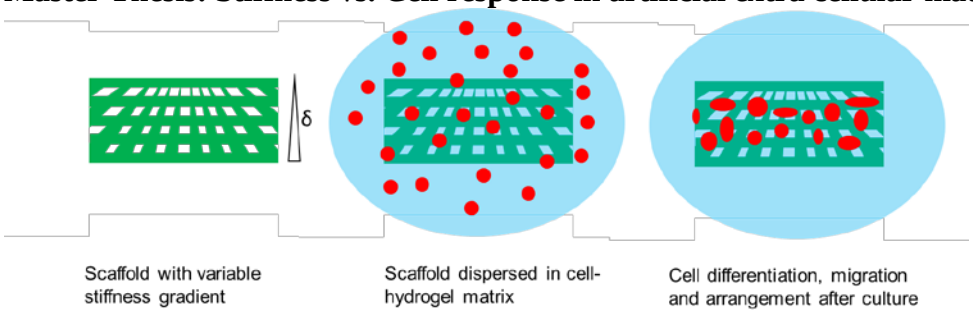

Figure 1: Scaffold design, seeding and cell culture

Cells regulate their fate binding to and contracting against their natural surroundings, the Extracellular Matrix (ECM). The interaction of cells to this matrix is complex. It is believed that cells respond differently depending on matrix geometry, stiffness, type of material and adhesive proteins. For example, stem cells show an increased differentiation to bone and cartilage on 3D cell culture than on 2D substrate. 3D substrate are hence a better resemblance to the *in vivo* condition. Despite the intensive research on understanding the interplay between the natural ECM and cells, the knowledge is still limited and results sometimes controversial. In a recent study on the interplay between matrix stiffness and protein tethering in stem cell differentiation, for example, researchers showed that only the stiffness of matrices regulate stem cell differentiation and that it is independent of porosity, so far understood as being decisive for proper cell adhesions and transport of nutrients (Wen et al., 2014). However, due to the lack of a well-defined artificial cell scaffold, they did not link the exact stiffness values to distinct cell behaviour.
We want to build upon this study and deepen our understanding of stiffness vs cell response. We want to deconstruct the natural ECM landscape to the point of only varying one variable, the stiffness, while leaving all other parameters constant and beneficial for cell differentiation. We hence need a replicable, and fully characterized artificial ECM construct that allows us:

(a) to perform 3D cell culture
(b) resemble the natural ECM and
(c) construct a defined stiffness gradient.

In this master thesis, you will use additive manufacturing to create tissue-engineering scaffolds from bonelike material. These scaffolds have a defined homogeneous porosity that does not change in 3D. However, the geometry is adapted such that the lower part of the scaffolds has thicker beams for increased stiffness. To better mimick the natural ECM, you will encapsulate cells in alginate hydrogels according to an established protocol. You will disperse the scaffolds in a cell-hydrogel solution and equip your tissue engineering constructs with growth factors. Depending on the stiffness, you will then characterize their differentiation, migration and spreading. You will obtain an exact model of stiffness vs. cell response in 3D, in a condition that is close to the *in vivo* scenario.

Research community: CEMIR/ Department of Cancer Research and Molecular Medicine/ Department of Engineering Design and Materials

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Literature

Wen, J.H., Vincent, L.G., Fuhrmann, A., Choi, Y.S., Hribar, K.C., Taylor-Weiner, H., Chen, S., and Engler, A.J. (2014). Interplay of matrix stiffness and protein tethering in stem cell differentiation. Nat. Mater. *13*, 979–987.

Master Thesis in Molecular Medicine

Name of Group: **Mycobacteria group**
Name of Research Community: **Centre of Molecular Inflammation Research (CEMIR)**

Hide and seek: Intracellular dynamics during mycobacterial infections

Tuberculosis, caused by infection with *Mycobacterium tuberculosis*, is a major global health challenge. Other non-tuberculosis mycobacteria such as *M. Avium* and *M. Abscessus* also cause a variety of serious diseases and are a rising threat. Common among mycobacteria is their ability to evade host immune defenses by surviving and replicating inside macrophages, the front line cells of the innate immune system.

Macrophages use pattern recognition receptors (PRRs) to detect pathogens and mount appropriate defenses. Among these, the toll-like receptors (TLRs) 2 and 8 detect mycobacterial surface ligands and RNA respectively, and are a vital part of the macrophage defense upon mycobacterial infections. However, these defenses are insufficient, as at some point after phagocytosis by the macrophages the mycobacteria escape both lysosomal degradation and detection by PRRs. Instead, the bacteria establish latent infections in a little characterized intracellular "stealth" compartment.

In this project, we seek to learn more about how the phagocytosed mycobacteria evade the macrophage PRRs and degradation pathways, and the nature of the "stealth" compartment where the mycobacteria reside. To study the detailed spatial and temporal dynamics of intracellular mycobacterial infections, we have engineered macrophages to express fluorescent proteins coupled to e.g. TLRs. Important methods could include state-of-the-art high resolution single-cell microscopy, combined with classical immunological methods such as ELISA, Western blots and qPCR, cell culture and microbiology work, as well as preparing samples for modern high throughput proteomics methods.

For this project, we seek a highly motivated and talented student who is interested in learning and performing some of the methods mentioned above, and contributing to a better understanding of the underlying molecular mechanisms of mycobacterial infections. The project will be defined in further detail depending on the research interests of the student. The work will be performed at CEMIR, a national center of excellence in inflammation research, with access to state of the art facilities and a dynamic international environment.

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Name of Research Community: **The Ohio State University, Columbus, OHIO (USA)**

Dissecting the role of AMC12, a novel protein controlling Complex I biogenesis

Complex I (CI) is a mitochondrial multimeric enzyme which actively transports protons from the matrix to the intermembrane space and thereby contributes to the electrochemical gradient needed for ATP production during aerobic respiration. During assembly of CI, the mitochondrion has to coordinate the integration of more than 40 subunits originating from both cytosolic and mitochondrial ribosomes as well as a number of cofoactors (8 iron-sulfur clusters and 1 flavin mononucleotide). This coordination is facilitated by proteins termed biogenesis or assembly factors, of which only fifteen have been identified in humans. CI deficiency is a major contributor to mitochondrial disease and has been implicated in severe human disorders including Leigh Syndrome, Mitochondrial Encephalomyopathy Lactic Acidosis and Stroke-like Episodes (MELAS), and Parkinson Disease, among others. However, in only 40% of patients does the mutation occur in a gene encoding a CI subunit or known CI biogenesis/assembly factor. In the remaining 60% of patients, the molecular basis is unknown. In such cases, it is expected that the CI deficiencies are the result of mutations in yet-to-be identified biogenesis factors. Our lab has previously established a method for identifying potential genes encoding CI biogenesis factors using the photosynthetic green alga *Chlamydomonas reinhardtii*.

C. reinhardtii is an established model system for investigating CI biogenesis owing largely to two characteristics. First, its CI subunit composition is similar to that of the human counterpart and second, CI mutants are still viable in contrast to mammalian models, due to the existence of biochemical by-passes for CI. Such mutants display a characteristic *S*low growth *I*n the *D*ark (*SID*) phenotype due to decreased respiration. This *SID* phenotype was used as a basis to conduct a forward genetic screen, via insertional mutagenesis, through which 13 CI mutants, *amc1-amc13* were isolated. The goal of this project is to characterize *amc12*, an insertional mutant that contains decreased levels of a fully formed CI and severely reduced CI activity. Genetic analysis showed that the insertional cassette in *amc12* is linked to the CI deficient phenotype and complementation of *amc12* with the WT copy of the disrupted gene restores WT-like growth in the dark. Additionally, the abundance of CI is rescued to WT levels and enzymatic activity of CI is also improved in complemented strains. Specifically, the cassette disrupts a gene of unknown function, which has not previously been associated with Complex I function. The predicted gene encodes a large, low-complexity protein (LCP) which is predicted to go to the mitochondria. AMC12 shares several similarities with AMC11, another large low-complexity protein that has been shown to translocate to the mitochondria and regulate the abundance of several mitochondrial-encoded CI subunit transcripts. The student working on the project will perform molecular genetics and biochemical experiments in order to understand the role of AMC12 in CI biogenesis. Specifically, our objectives are to test the mitochondrial localization of AMC12 and analyze the abundance of mitochondrial transcripts encoding CI subunits in the *amc12* mutant.

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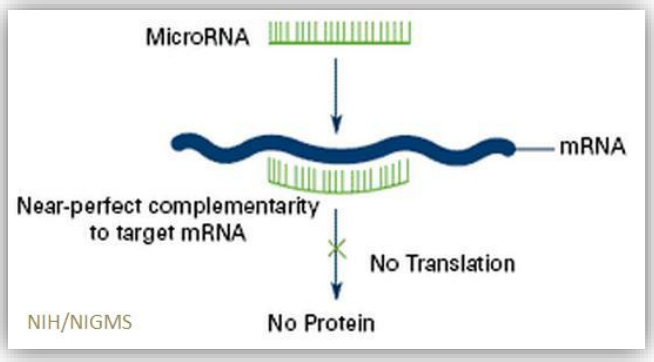
Master Thesis in Molecular Medicine

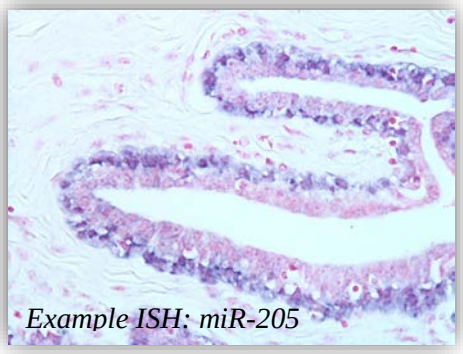
Name of Group: **Biobank1**

Hunting early prognostic biomarkers in prostate cancer

Prostate cancer is the most common cancer among men. Risk factors are older age, a family history of the disease and lifestyle. The 5 year survival rate is 99 %, and the cancer is usually slow growing. However some individuals develop a more aggressive type of cancer which metastasizes to other parts of the body, preferably bone.

As of today, PSA is the screening marker used to detect prostate cancer, but it is not very accurate. It would be of interest to find more potential screening markers in blood, which not only detects cancer, but also can predict the seriousness of the disease. We would like to have a look at miRNAs as a possible new and early marker for prostate cancer. MicroRNA is small non-coding RNA which regulates the gene expression by binding to messenger RNA.




Example ISH: miR-205

At our laboratory at Biobank1, we have established a method for staining miRNAs in formalin fixated paraffin embedded tissue, ISH. This allows us to have a closer look at miRNAs in cancer cells. We want to combine the findings of differentially expressed miRNAs in serum of prostate cancer patients, with the findings of miRNAs in cancer tissue from the same patients.

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Biobank1®

Master Thesis in Molecular Medicine

Name of Group: **Group Van Loon – "DNA Base Damage and Chromatin Integrity"**

Name of Research Community: **Unit for Molecular Biology, Department of Molecular Cancer Research, Faculty of Medicine**

Molecular pathomechanisam of X-linked intellectual disability

Intellectual disability (ID) is a complex neurodevelopmental condition that results in arrested or incomplete development of the mind. It manifests before the age of 18, is characterized by impaired cognitive, motor, language and social abilities, and has an estimated 2% prevalence in the western world population. X-linked ID (XLID) is a subset of ID associated with alterations in over 100 X-chromosome genes. Due to large number of genetic alterations associated with XLID, the symptoms of this disease are multifaceted and vary extensively between different syndromes. Though different mutations are clearly implicated in XLID, currently much is unknown about the pathomechanisms that underlie XLID. To determine how genetic alterations cause onset of XLID and affect human brain development in this master thesis cerebral organoids in combination with CRISPR/Cas9 technology will be used. Cerebral organoids represent a unique system to study human neurodevelopment and by using XLID-patient derived human induced pluripotent stem cells allow identification of pathomechanisms that underlie XLID.

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