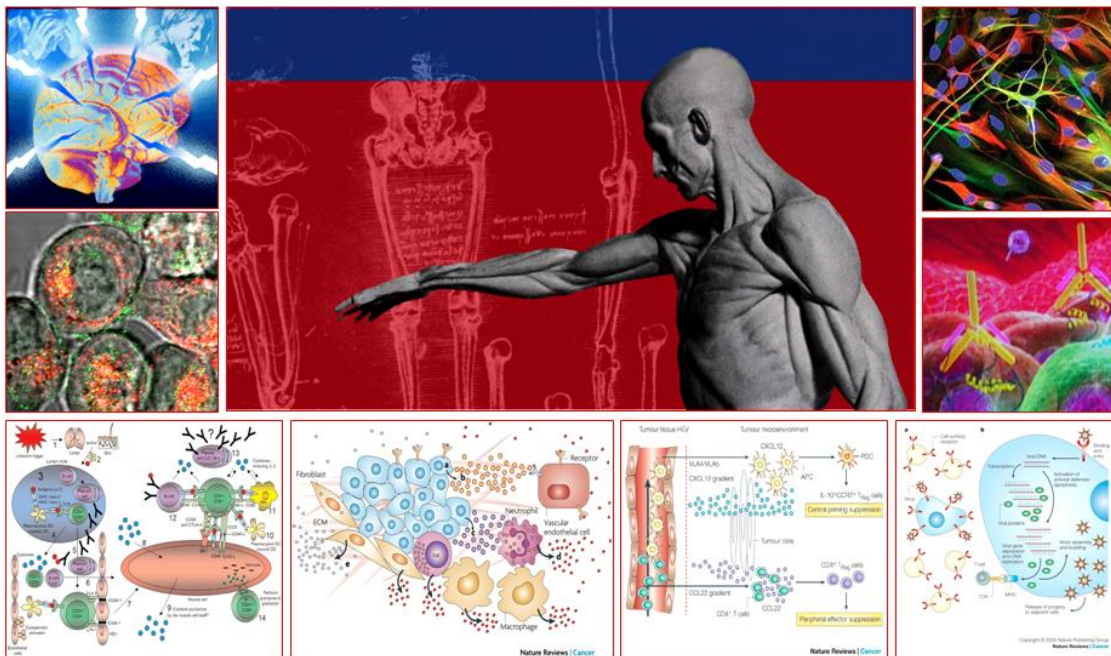


Doctoral School of
General and Clinical Pathophysiology –
Molecular Concepts of Health and Disease

[PATHMED]



Univ.Prof. Dr. Anton Sadjak Univ.Ass. Mag.Dr. Adelheid Kresse
Institute of Pathophysiology and Immunology
Centre of Molecular Medicine
Medical University Graz
Heinrichstrasse 31a, A-8010 Graz
Phone: 0316-3804290
FAX: 0316-3809640
Anton.Sadjak@meduni-graz.at Adelheid.Kresse@meduni-graz.at

Proposal:

Doctoral School of General and Clinical Pathophysiology – Molecular Concepts of Health and Disease

Public health/Socioeconomic Relevance

Throughout history, general concepts of “health” or “disease” in a society have been shaped by its knowledge and beliefs about life, death and disease. In most of the industrialized nations of the world, people are now living longer than ever before which has been made possible largely due to recent advances in biomedical science and technology.

Increased longevity and recent changes in personal lifestyles however, brought about a shift in the age-distribution of the population and sharp increases in age- and stress-related diseases.

Thus, coronary heart disease, stroke and cancer have now replaced pneumonia, tuberculosis and enteritis – the leading causes of death in the last century – and the societal and economic burden of age-related neurodegenerative as well as stress-related illnesses is meanwhile considered enormous.

Although ongoing advances in science and technology continuously are providing new treatments for many diseases, it has become socio-economically apparent that maintaining health is more resource conservative and cost effective than relying on treatment of disease.

However, as of now, there is still plenty to be elucidated concerning the alterations in functional molecular pathways making the difference between ‘healthy physiology’ and pathophysiology leading to a number of diseases compromising public health of the present.

Aims and Goals

The interaction of clinical and preclinical laboratories in order to build up together this doctoral school aims to represent on an educational academic level what is already going on in their current research activities: collaborations in order to join methodological forces as well as theoretical backgrounds to address collaboratively profound questions related to several distinct disease aetiologies.

The pathophysiology of genetic as well as acquired diseases relates not so much to the affected organ system as to the mechanisms of mutation, inheritance and molecular pathways from the genotype via epigenetic influences to the phenotype.

Thus, it seems only natural for established specialists to join forces not only in collaborating on the level of research but also with regard to the education of the next generation of medical professionals with a strong research potential.

The interdisciplinary composition of the teaching faculty of the proposed Doctoral School of General and Clinical Pathophysiology therefore aims at providing the necessary theoretical background for a profound insight into the complex molecular mechanisms that, if perturbed, may give rise to a number of different disease pathways.

Local Relevance

The aims and goals of the proposed Doctoral School of General and Clinical Pathophysiology find themselves in concordance with the recently declared focus of "Sustainable Health Research" at the MUG. Thus, the replenishment of already existing MUG strongholds and programs such as Molecular Medicine and Neuroscience as well as strengthening incoming strongholds such as Cancer Research with the provision of adequate education of doctoral students are reflected as a common theme.

Teaching Faculty

1	Sadjak, Anton, Prof. Ph.D.	Institute of Pathology & Immunology
2	Kresse, Adelheid, Ph.D.	Institute of Pathology & Immunology
3	Ghaffari-Tabrizi, Nassim, Ph.D.	Institute of Pathology & Immunology
4	Pfragner, Roswitha, assoc. Prof. Ph.D.	Institute of Pathology & Immunology
5	Windhager, Reinhard, Univ. Prof. MD	Univ. Clinic of Orthopaedic Surgery
6	Leithner, Andreas, assoc. Prof., MD	Univ. Clinic of Orthopaedic Surgery
7	Samonigg, Hellmut, Prof., MD	Clinical Department of Oncology
8	Moinfar, Farid, assoc. Prof. MD	Department of Pathology
9	Lackner, Karoline, assoc. Prof. MD	Department of Pathology
10	Smolle Jüttner, Freyja-Maria, Prof. MD	Univ. Clinic of Thoracic and Hyperbaric Surgery
11	Wimmer, Gernot, Assoc. Prof. MD	Dental School, Department of Periodontology
12	Speicher, Michael. Prof. MD	Department of Human genetics
13	Holzer, Peter, Prof., Ph.D.	Department of Experimental and Clinical Pharmacology
14	Schwinger, Wolfgang, Prof. MD	Univ. Clinic of Paediatrics, Paediatric Hemato-Oncology

TEACHING – Curriculum

	SWS	ECTS
1st semester:		
Basal Scientific training for biologists: <ul style="list-style-type: none"> ❖ Medizinische Grundlagen für Naturwissenschaftler im Rahmen des Dr. Sci. Med Studiums (bestehende LV) 	4	8
Basal Scientific training for medical students: <ul style="list-style-type: none"> ❖ Methodische Grundlagen für mediziner im Rahmen des Dr. Sci.med Studiums (bestehende LV: Vortragende: Berghold) 		
Presentation of the dissertation thesis topic and work plan to the Doctoral school committee		1
2nd Semester:		
Basal Scientific training II:		4
<ul style="list-style-type: none"> ❖ LV 495.003: Wissenschaftliche Grundlagen im Rahmen des Dr.sci.med Studiums (Mayer, Schaupp, Galistl, Rehak) ❖ Good Scientific Practice (Holzer) ❖ Molecular stress research (Sadjak, Kresse, Ghaffari-Tabrizi,) ❖ Clinical Pathophysiology of bone and soft tissue tumors (Windhager) ❖ Breast cancer biology (Moinfar) ❖ Tumor genetics (Speicher) 	0.34 0.34 0.34 0.34 0.34 0.34	
Thesis seminar: (new) <ul style="list-style-type: none"> ❖ Journal club (all PIs) 		4
Progress report – Interim presentation		1
3rd semester:		
Theoretical background of dissertation topics (new) <ul style="list-style-type: none"> ❖ Signaling pathways in acute and chronic pain (Holzer) ❖ Molecular Pathways in the neuroendocrinology of stress (Sadjak, Kresse) ❖ Clonality assessment in the differential diagnosis of soft tissue tumors (Leithner) 	0.34 0.34 0.067	4

❖ Virus & Cancer: the possible impact of the simian virus 40 (Leithner)	0.067	
❖ The search for potential molecular pathological targets in the therapy of desmoid tumors (Leithner)	0.067	
❖ Bone tumors & genetics: current status and future aspects (Leithner)	0.134	
❖ Genetics of aging (Speicher)	0.34	
❖ Genetic instability syndromes (Speicher)	0.34	
❖ Stress-induced neuro-immunomodulation of inflammatory diseases (Wimmer, Kresse)	0.34	
Thesis seminar (new)		4
❖ Journal club (all PIs)	1	
Courses of free choice from existing curriculum	2	3
4th semester:		
Thesis seminar (new)		4
❖ Journal club (all PIs)	1	
❖ Thesis seminars (all PIs)	1	

Doctoral Dissertation Projects:

- 1) Comparative molecular analysis of colorectal cancer, corresponding metastases, circulating tumor cells and circulating DNA

Principal supervisor: Assoc. Prof. Dr. Karoline Lackner

2nd supervisor: assoc. Prof. Dr. Rudolf Stauber

3rd supervisor: Dr. Jochen Geigl

Invasion of tumor cells into non-neoplastic tissues and the formation of metastases are the hallmarks of malignant neoplasias including cancers. The ability to invade and metastasize is based on changes of invasion- and metastasis relevant gene expression in tumor cells. The most important steps are the induction of angiogenesis as a result of tumor cell growth and the increased need of oxygen. Reduced E-cadherin- and enhanced expression of mesenchymal genes with concomitant decrease of epithelial gene expression (so called epithelial-mesenchymal transition, EMT) enhance tumor cell motility and the invasion into blood vessels (intravasation). In the blood stream tumor cells may either circulate singly (circulating tumor cells, CTCs) or as aggregates consisting of a number of tumor cells (circulating tumor microemboli, CTMs). Recently published data indicate that molecular analysis of CTC/CTMs could provide important information concerning the expression of predictive markers, i.e. molecular markers which are associated with response or non-response to chemotherapy.

- 2) Influence of hyperbaric oxygenation upon immunological parameters of human circulating blood cells – Immune status assessment by FACS analyses.

Principal supervisor: Prof. Dr. Freyja-Maria Smolle-Jüttner

2nd Supervisor: Prof. Anton Sadjak

3rd supervisor: N.N.

Hyperbaric oxygen therapy (HBOT) refers to intermittent treatment of the entire body with 100-percent oxygen at greater than normal atmospheric pressures. While several of the exact mechanisms of action of HBOT are yet to be discovered, it is known that HBOT greatly increases oxygen concentration in all body tissues, even with reduced or blocked blood flow; stimulates the growth of new blood vessels to locations with reduced circulation, thus improving blood flow to areas with arterial blockage, and seems to aid the treatment of infection by enhancing white blood cell action and to potentiate the action of antibiotics. It is yet not clear what exactly is the impact of HBOT, especially since both, immune system enhancing as well as immunosuppressive have been observed so far. We thus intend to investigate in detail the effect of HBOT upon the individual immune status by measuring the expression of a variety of well established cluster of differentiation markers on peripherally circulating immune-competent blood cells via FACS analysis.

- 3) Influence of pressurized air breathing on immunological parameters of human circulating blood cells

Principal supervisor: Prof. Dr. Freyja-Maria Smolle-Jüttner

2nd supervisor: Prof. Dr. Anton Sadjak

3rd supervisor: N.N.

4) The role of proteases in the differentiation between enchondromas and chondrosarcomas

Principal supervisor: Prof. Dr. Anton Sadjak

2nd supervisor: Dr. Nassim Ghaffari-Tabrizi

3rd supervisor: Prof.Dr. Reinhard Windhager

5) Molecular pathological analysis of fibrous dysplasia with special emphasis on ADAMs

Principal supervisor: Prof. Dr. Andreas Leithner

2nd supervisor: Dr. Nassim Ghaffari-Tabrizi

3rd supervisor: Dr. Werner Maurer-Ertl

Fibrous dysplasia (FD) is a benign skeletal disorder which may affect one or multiple bones. The monoostotic form is six times more common than polyostotic fibrous dysplasia. The aetiology is unclear, although activating mutations of the G proteins might play a role in the pathogenesis. By searching the data base of the department of pathology we identified 138 patients with fibrous dysplasia. The aims of the present study are: 1) a retrospective genetic and immunohistochemically analysis using paraffin embedded and/or deep frozen tissue samples. Potential targets for research are: a) evaluation of GNAS mutations (tumour tissue & peripheral blood); a) analysis of the X-chromosome inactivation pattern; b) bone differentiation markers like BMPs and FGFs. 2) The second aim is a prospective follow-up study of these patients using serological, radiological and clinical parameters

6) Neuroendocrine tumors: in vitro models for improvement of diagnosis and therapy

Principal supervisor: Assoc. Prof. Dr. Roswitha Pfragner

2nd supervisor: Prof. Dr. Anton Sadjak

3rd supervisor: N.N.

Tumor cell lines have enormous value for the study of different aspects of cancer biology and have also recently gained great importance in autologous cell-based anti-tumor therapies. However, the use of these cells is still limited because in vitro growth is hampered by suboptimal culture conditions and current media contain fetal bovine serum, which poses serious safety concerns regarding clinical application. To address this drawback, we aimed to develop a strategy for optimization of the culture medium for human medullary thyroid carcinoma (MTC) cell lines as a model system. We combined the general cell screening system which continuously measured the growth behavior of cells in a 96-well plate format, with statistically based experimental designs. This allowed the propagation of two MTC cell lines comparable with conventionally used serum-

supplemented medium. This is of utmost importance because of increasing regulatory requirements. Neuroendocrine regulation of small intestinal (SI) function is poorly understood because pure neuroendocrine cells are unavailable, whereas the biological basis of SI carcinoid tumors is unknown because neoplastic human enterochromaffin (EC) cells are unavailable. Data from previous studies support our quest for novel methodologies to purify live human EC cells for functional characterization and eventual transcriptome assessment, which will allow identification of new targets to control the secretion and proliferation of SI carcinoids.

- 7) Corticotropin-releasing factor (CRF)-mediated stress signaling in neuroendocrine cancer cells

Principal supervisor: Prof. Dr. Roswitha Pfragner

2nd supervisor: Dr. Adelheid Kresse

3rd supervisor: N.N.

Urocortin (UCN) and corticotropin-releasing factor (CRF) are belong to the CRF-neuropeptide family, and bind to their two known receptors, CRF-R1 and CRF-R2. CRF has 10-fold higher affinity for CRFR1 than for CRF-R2, while UCN has equal affinity for both receptors. Both CRF receptors are distributed in the central nervous system (CNS) and periphery, but the two subtypes display quite different pharmacological Profiles. CRFR1 and CRFR2 are expressed in many human cancer and several reports suggest that UCN/CRF have anticancer effects but the mechanisms of these effects may be diverse. UCN and CRF can directly inhibit tumor cell growth via CRF-R1, however, the exact mechanism of this is yet not clear. In the present study, we intend to analyze the potential involvement of MAP-kinase associated signal transduction pathways subsequent to ligand binding to either CRF-R1 or CRF-R2.

- 8) Capture and Characterization of Putative Disseminated Breast Cancer Stem Cells.

Principal supervisor: Prof. Dr. Helmut Samonigg

2nd supervisor: Dr. Marija Balic

3rd supervisor: N.N.

- 9) Role of stromal cells in breast cancer biology and progression.

Principal supervisor: Assoc. Prof. Dr. Farid Moinfar

2nd: Assoc. Prof. Dr. Peter Regitnig

3rd supervisor: N.N.

- 10) Genetic alterations in microenvironment of cervical cancer.

Principal supervisor: Assoc. Prof. Dr. Farid Moinfar

2nd: Assoc. Prof. Dr. Peter Regitnig;

3rd supervisor: N.N.

11) Genetics of aging.

Principal supervisor: Michael Speicher

2nd supervisor: Dr. Jochen Geigl

3rd supervisor: Dr. Karoline Lackner

Recent data in the science of aging suggest that longevity is influenced by both, environmental and genetic factors. Furthermore, aging related degeneration and disease is to a large extent influenced by genetic components. Such genetic factors can be constitutional, i.e. present in the genome of each cell. However, they can be limited to certain cells, as during lifetime mutations may be acquired in a stochastic fashion in different compartment of a body. Here, we will address genetic factors with an impact on the process of aging, which may be hereditary (and such present in all cells) or acquired (and therefore limited to certain fractions of cells as mosaicism). Genome-wide scans and sophisticated technologies for single cell/small cell number analyses will be employed to study both genetic components on aging.

12) Genetic changes in pre-cancerous lesions.

Principal supervisor: Michael Speicher;

2nd supervisor: Dr. Jochen Geigl

3rd supervisor: Dr. Karoline Lackner

The first steps in the process of the transformation of a normal to a malignant cell are for the fast majority of epithelial tumors unknown. Reasons for this lack of knowledge include the limited access to pre-cancerous lesions (i.e. dysplastic lesions, adenomas, etc.) and that naturally only tiny numbers of cells are available for a detailed analysis. Here, we have access to animal models, special *in vivo* imaging approaches for the identification of early changes in cell architecture/appearance and a plethora of technologies to extract from single cells or from small cell numbers a maximum of information about changes in the genome of the respective cells. The aim of this project is to elucidate the very first steps at the beginning of tumorigenesis.

13) Gastrointestinal pain and stress: neurochemical, neuroendocrine and behavioral manifestations

Principal supervisor: Prof. Dr. Peter Holzer;

2nd supervisor: Dr. Adelheid Kresse

3rd supervisor: N.N.

The maintenance of laboratory animals under standard conditions is considered to reflect a psychosocial stress situation that can be ameliorated by maintenance under enriched conditions. Environmental enrichment has been shown to improve a number of brain functions related to learning and memory and to have a beneficial effect on experimentally imposed brain injury and on the behavioral responses to stress. There is, however, little information as to whether environmental enrichment improves the emotionality of laboratory animals, i.e., whether their behavior in experimental paradigms of anxiety and depression is modified. The *first aim* of this project is, therefore, to compare mice kept under standard laboratory conditions and under enriched conditions in their emotional performance in the open field test, the elevated plus maze test, the stress-induced hyperthermia test and the forced swim test, and to examine the impact of

environmental enrichment on the activity of the hypothalamic-pituitary-adrenal axis which is a biochemical stress index. Since the genetic background is an important factor in the expression of the behavioral phenotype, the effect of environmental enrichment will be examined both in an outbred (Him:OF1) and two inbred strains of mice (BALB/c and C57BL/6J) which significantly differ in their emotional behavior. Gender differences will also be addressed. *The second aim* of this project is to examine as to how environmental enrichment affects the gut-brain axis or, in more specific terms, whether environmental enrichment modifies pain and changes in emotional behavior induced by gastrointestinal inflammation. Currently, there is a complete lack of knowledge as to how handling and housing conditions affect the gut-brain axis in health and disease. This aspect is particularly relevant to the establishment of valid animal models of gastrointestinal disease. On the one hand, it has repeatedly been noted that experimentally induced injury and inflammation can vary widely between different laboratories and, over time, even within the same laboratory, for unknown reasons. On the other hand, there is ample evidence that experimentally imposed stress induces or exacerbates injury and inflammation of the gastrointestinal mucosa and can lead to gastrointestinal hyperalgesia. It will, therefore, be tested whether environmental enrichment modifies the induction of experimental gastritis and colitis, whether environmental enrichment modifies the afferent input from the gut to the spinal cord and brainstem as visualized by c-Fos neuroanatomy, and whether environmental enrichment modifies the behavioral changes associated with gastrointestinal inflammation (including possible alterations in the hypothalamic-pituitary-adrenal axis).

14) Effects of systemic immune challenge on neuropeptide Y - corticotropin-releasing factor (CRF) interactions in the limbic system

Principal supervisor: Prof.Dr. Peter Holzer

2nd supervisor: Dr. Adelheid Kresse

3rd supervisor: N.N.

Apart from a change in the activity of NPY neurons, immune challenge might target neuronal circuits connected to the NPY system in the brain. Of particular relevance is the interaction between NPY and CRF. Peripheral immune challenge enhances the expression of CRF and CRF1 receptors in the hypothalamus and stimulates the hypothalamus-pituitary-adrenal (HPA) axis. CRF mRNA expression in the paraventricular nucleus of the hypothalamus is decreased in Y2^{-/-} mice, which may at least in part explain why the LPS-induced rise of circulating corticosterone is attenuated in Y2^{-/-} animals. CRF is in fact widely distributed in the brain including the limbic system, but it is not yet known whether these extrahypothalamic CRF circuits are targeted by endotoxin challenge. There is, however, evidence that, in the basolateral nucleus of the amygdala, CRF and NPY interact with each other in an antagonistic fashion to regulate emotional-affective behavior and stress responses. Against this background, the *first aim* of this project is to explore the impact of peripheral immune challenge with LPS on hypothalamic and extrahypothalamic CRF and NPY systems in the mouse brain. First, *in situ* hybridization will be used to study the expression of CRF and CRF1 receptors as well as of NPY and Y2 receptors in brain areas of interest. Second, immunocytochemistry will be employed to map central neurons that are activated by systemic LPS administration (as visualized by expression of c-Fos) and stain positively for CRF, CRF1 receptors, NPY and/or Y2 receptors. The *second aim* of the work will be to probe the implications of CRF and NPY in the central responses to LPS challenge by pharmacological means. CRF1 and Y2 receptor antagonists will be used to examine whether the impact of LPS administration on the activation of central NPY- and CRF-positive neurons is mediated by CRF1 and Y2 receptors, respectively. In addition, the ability of these receptor antagonists to modify the behavioral responses to LPS challenge will be probed.

15) The potential role of gingival mast cells as a stress target: CRF signaling in neurogenic periodontal inflammation.

Principal supervisor: Assoc. Prof. Dr. Gernot Wimmer;

2nd Supervisor: Dr. Adelheid Kresse

3rd supervisor: N.N.

Pathophysiological changes associated with the body's stress response are misrouted and serve as aggravating or triggering factors in the pathogenesis of many diseases, for example, inflammatory, autoimmune, and allergic diseases. Activation of neurohormones by psychological stress occurs largely via the hypothalamic–pituitary–adrenal (HPA) axis, with subsequent upregulation of key stress hormones, such as corticotropin-releasing factor (CRF), ACTH, and glucocorticoids. Besides the well known immunosuppressive effects of glucocorticoids, relevant examples of proinflammatory actions of CRF — which triggers the release of glucocorticoids — have recently been reported, for example, in inflammatory arthritis, where both CRF and urocortin have been identified. Mast cells are well known for their involvement in allergic and anaphylactic reactions, but recent findings implicate them in a variety of inflammatory diseases affecting different organs, including the heart, joints, lungs, and skin and even specific brain regions. In these cases, mast cells appear to be activated by triggers other than aggregation of their IgE receptors. Instead, anaphylatoxins, cytokines and specific neuropeptides, such as corticotropin-releasing factor (CRF), that are released subsequent to stress exposure may lead to selective release of mast cells contents without involving their degranulation. These findings could explain the neurogenic origin of inflammatory diseases, such as asthma, atopic dermatitis, coronary inflammation, and inflammatory arthritis and periodontitis, all of which worsen by stress. It is proposed that the pathogenesis of these diseases involves mast cell activation by local release of CRF or related peptides. Thus, the combination of CRF receptor antagonists and mast cell inhibitors may present novel therapeutic interventions. The skin and with it mucosal and periodontal tissues have their own neuroendocrine system, which is tightly linked into systemic neuroendocrine axes, probably in order to coordinate peripheral responses to stress and to maintain peripheral and global homeostasis. Strikingly, human skin also expresses CRF and, additionally, urocortin mRNA and peptide. Hence the neural delivery of CRF into the skin must also be considered as a possible means by which CRF peptide can be transported into the skin in a highly localized fashion. Most recently, the expression of urocortin II (stresscopin-related peptide) mRNA in both human and mouse skin could be detected, and corresponding receptors for these ligands were also identified in skin cells. In a similar attempt, we were recently able to show for the first time the presence of CRF peptide and both its receptors in human periododontal tissues, localized there predominantly to specific subsets of mast cells, however with high inter-individual variations as to the specific expression levels and abundance of such mast cells. With regard to the problem of recurrent periodontitis in some patients, it seems of interest to determine whether it is possible to establish a quantitative link between the abundance of CRF-receptor bearing mast cells and occurrence of periodontitis in patients.

16)) Aging of hematopoietic and mesenchymal stem cells

Principal supervisor: Prof. Dr. Wolfgang Schwinger

2nd supervisor: Dr. Ingeborg Stelzer

3rd supervisor: Dr. Nassim Ghaffari-Tabrizi

The clinical application of hematopoietic stem cell (HSC) transplantation has evolved in the absence of rigorous understanding of many aspects of its biology. Only a small fraction of donor's HSCs must suffice to reconstitute the hematopoietic system. Therefore donor's HSCs must

increase their rate of proliferation and shorten in their telomere length with each round of mitosis. The aim of our project is to investigate mechanisms of aging in hematopoietic (HSCs) and mesenchymal stem cells (MSCs). To gain new insights in hematopoietic stem cell transplantation we will analyse generation of DNA damage, reactive oxygen species, primary and secondary granulocyte/macrophage colony-forming unit (GM-CFU) assays and apply molecular biologic methods to characterise different stages of ex vivo expanded mesenchymal stem cells (MSCs) and HSCs.