

## **List of Supervisors and Projects for German Exchange Summer Research Program 2012**

### **1. Dr. Andrea Ewald:**

**Project Title: Cytocompatibility Testing of Reinforced Calcium Phosphate Cements**

#### **Project description:**

Calcium phosphate cement (CPC) has been well-established as a bone graft material. It is highly moldable and can easily be adapted to fit a bone defect, and sets in situ to form solid hydroxyapatite under physiological conditions. CPC has been shown to be osteoconductive and biocompatible, and is eventually resorbed and replaced by original bone at the site of implantation. Unfortunately, CPC has inherently poor mechanical properties under tensile or flexural loading and may fracture under applied stresses. Because of this, it may only be used in non load-bearing locations. Methods of improving its physical properties have been developed to make it suitable for a wider range of applications. CPC composite materials have been developed using various fibers for reinforcement. These composite materials show promising results, however there has been limited testing of the cellular response to these materials.

This project seeks to determine the biocompatibility of fiber-reinforced CPC cements in vitro using MG63 osteoblast-like cells. The attachment of different cell lines (e.g. fibroblasts, osteoblasts, and osteoclasts) and their growth will be studied, first, by using cytocompatibility testing (e.g. by determination of cell proliferation and analyzing cell viability (WST test)). Second, the samples will be examined by SEM as well as by light microscopy. With the latter protein distribution can be analyzed using specific antibodies. Furthermore cells grown on CPC composites will be analyzed for protein expression regarding cell line specific proteins. This will be done by separating the cell protein in gel electrophoresis and subsequent western blotting as well as with PCR-analysis. Additionally the students will have the opportunity to do all the cell culturing needed for the experiments.

**e-mail:** [Andrea.Ewald@fmz.uni-wuerzburg.de](mailto:Andrea.Ewald@fmz.uni-wuerzburg.de)

**phone:** not available

## **2. Dr. Marco Metzger:**

**Project Title: In vitro modulation of epithelial stem cells isolated from murine gastrointestinal tract.**

### **Project description:**

Objectives: (i) establishing intestinal stem cell cultures of mouse gut in vitro, (ii) applying selected recombinant proteins on intestinal stem cells (iii) analyzing in vitro effects on epithelial proliferation, differentiation and apoptosis.

Hypothesis and Rationale: The gut is one of the most regenerative tissues in the body. Thus, the gut epithelium of the adult colon is constantly replaced by multipotent stem cells located at the base of the mucosal crypts. The characteristics of these cells include the ability to proliferate, to replicate themselves and to regenerate the lineage precursors that differentiate into different mature epithelial cell types. Recently, the identification and isolation of epithelial stem and precursor cells have been shown allowing us to develop in vitro models to study stem cell determination and gut diseases. Meanwhile, a number of key molecular pathways influencing cellular determination have been described in the epithelial stem cell niche itself and the surrounding tissue in vivo. These include members of the Wnt, BMP and Notch pathways but also several growth and differentiation factors such as hepatocyte, transforming or keratinocyte growth factor. In addition, components of the basement membrane such as various laminin isoforms play a crucial role in the regulation of cell adhesion, proliferation, differentiation and migration. We hypothesize that activation or inhibition of these pathways will also lead to changes in cellular determination in vitro. A detailed analysis of these changes would help us to better understand molecular mechanisms and cellular dysfunctions observed during gut diseases such as cancer or inflammatory bowel diseases.

Experimental plan: (i) existing protocols will be applied in order to establish intestinal stem cell cultures of mouse wild-type gut in vitro. Methods: Primary cell culture, cell viability tests. (ii) selected recombinant proteins will be applied in optimized concentrations on intestinal stem cells and cell growth will be monitored after short and long-time treatment. Methods: time-lapse imaging. (iii) detailed analysis of epithelial proliferation, differentiation and apoptosis. Methods: BrdU assay, TUNEL assay, immunocytochemistry, RT-PCR.

**e-mail: [marco.metzger@uni-wuerzburg.de](mailto:marco.metzger@uni-wuerzburg.de)**

**phone: 0049-937-3186686**

### **3. Dr. Joachim Nickel**

**Project Title: Role of shRNAs targeting components in “epithelial-mesenchymal-transition”**

#### **Project description:**

Bone morphogenetic proteins (BMPs) belong to the TGF- $\beta$  superfamily of secreted growth factors. Over 20 members of BMPs have been identified in a wide variety of organisms.<sup>1</sup> As the name implicates, BMPs were originally discovered by their ability to induce endochondral bone formation in vivo. But, after two decades of extensive research they can be considered as components of a highly conserved signaling pathway that also controls cell growth, differentiation, apoptosis, motility, angiogenesis, and matrix synthesis not only during embryogenesis but also in adult life.<sup>2,3</sup> Given the diversity of responses to BMPs and the complexity of morphogenic events, their activities are delicately regulated by secretory antagonists (such as Noggin, Chordin, Follistatin, and others), signaling inhibitors and co- and pseudoreceptors.<sup>4</sup> The discovery that perturbations in BMP pathways are genetically responsible for certain hereditary cancer syndromes (such as familial juvenile polyposis and a subset of Cowden syndrome<sup>5,6</sup>) has prompted the delineation of their significance in carcinogenesis. Evidence now indicates that various sporadic human cancers also exhibit aberrations in BMP signaling, contributing to tumor development and progression.<sup>7-11</sup>

The aim of the project is to apply shRNAs targeting components of the BMP signaling pathway in order to clarify the question whether this might results in “epithelial - mesenchymal-transition”, a process being essential for a primary tumor to metastasize.

1. Balemans W, Van Hul W. *Dev Biol.* 2002;250:231–250.
2. Whitman M. *Genes Dev.* 1998;12:2445–2462.
3. Botchkarev VA. *Bone J Invest Dermatol.* 2003;120:36–47.
4. Hsu MY, Rovinsky S, Penmatcha S, et al. *Cancer Metastasis Rev.* 2005;24:251–263.
5. Howe JR, Sayed MG, Ahmed AF, et al. *J Med Genet.* 2004;41:484–491.
6. Waite KA, Eng C. *Nat Rev Genet.* 2003;4:763–773.
7. Langenfeld EM, Calvano SE, Abou-Nukta F, et al. *Carcinogenesis.* 2003;24:1445–1454.
8. Kim IY, Lee DH, Lee DK, et al. *Cancer Res.* 2004;64:7355–7360.
9. Horvath LG, Henshall SM, Kench JG, et al. *Loss of BMP2, Prostate.* 2004;59:234–242.
10. Wen XZ, Miyake S, Akiyama Y, et al. *Biochem Biophys Res Commun.* 2004;316:100–106.
11. Helms MW, Packeisen J, August C, et al. *J Pathol.* 2005;206:366–376.

**e-mail:** [joachim.nickel@uni-wuerzburg.de](mailto:joachim.nickel@uni-wuerzburg.de)

**Phone number:** not available

#### **4. Dr. Sarah Nietzer**

**Project Title: Steps in the Development of a human Colonic 3D Tumor Model as well as a 3D lung tumor model.**

##### **Project description:**

In our group we work on the establishment of a human 3D tumor model for colonic carcinoma which is the second common kind of cancer with 235000 deads in Germany each year (DKFZ, cancer information service). We cultivate two different adenocarcinoma cell lines from colorectal cancer patients, Caco-2 (Fogh and Trempe 1975) and SW480 (Giard, Aaronson et al. 1973), on a 3D collagen matrix deriving from porcine jejunum segments, the so-called BioVaSc (Schanz, Pusch et al. 2010). This model is extraordinary because it enables human cells to grow in a 3D environment which resembles the in vivo conditions. The advantage of these 3D test systems over conventionally used 2D and animal models is the more complex histology of tumors and should lead to better reliability and transferability of drug tests to patients. Caco-2 cells differentiate in this system to enterocytes with characteristics of epithelial cells of the small intestine (Artursson and Karlsson 1991; Le Ferrec, Chesne et al. 2001), in contrast SW480 exhibit malignant invasive growth on the 3D Matrix. In co-cultivation together with fibroblasts, the induction of more tumor-like morphology can be observed. Further models we built up by using lung cancer cell lines like A549 and HCC827 with specific mutations in for lung adenocarcinoma important pathways

In order to characterize the tumor models we built up in a static system until now, various tests are needed: e.g. immunohistochemical characterizations, western blotting analyses, qRT-PCR and Transmission electron microscopy (TEM) analyses. Additionally, these static experiments have to be transferred to the dynamic cultivation system by using a bioreactor. All these analyses can be done in our lab and in the lab of the anatomy (TEM-analyses).

##### Literature:

- Artursson, P. and J. Karlsson (1991). *Biochem Biophys Res Commun* 175(3): 880-885.  
Fogh, J. and G. Trempe (1975). *Human Tumor Cells In Vitro Plenum*: 115-141.  
Giard, D. J., S. A. Aaronson, et al. (1973). *J Natl Cancer Inst* 51(5): 1417-1423.  
Le Ferrec, E., C. Chesne, et al. (2001). *Altern Lab Anim* 29(6): 649-668.  
Schanz, J., J. Pusch, et al. (2010). *J Biotechnol* 148(1): 56-63

**e-mail:** [sarah.nietzer@uni-wuerzburg.de](mailto:sarah.nietzer@uni-wuerzburg.de)

**Phone number:** not available