Research

Cell Division and Cancer Research

In vivo studies of proliferative diseases

Human diseases are often context dependent and therefore reductionist approaches do not always work. Cell cycle progression and division is essential for organ development, tumor growth and metastasis. We study proliferative and developmental diseases using genetically modified mouse models. This allows us to study cells within tissues, where several cell types interact with each other. Our main aim is to understand how cell division and metabolism regulate each other during tissue repair, regeneration, and development. In our laboratory we focus mainly on liver and testis.

We analyze genetically modified mice to study human diseases using a combination of genetic, transcriptomic, proteomic, metabolomic, lipidomic, bioinformatics, microscopy, cell biology, and biochemical tools. The main projects in the laboratory are:

We have generated a number of knockout mouse lines and are working on three different projects:

1. Liver development, regeneration, metabolism and cancer

2. Male sterility (testis, male germ cell development)

3. Regulation of biogenesis by cyclin-dependent kinases

Liver development, regeneration, metabolism and cancer

The incidence of the metabolic syndrome (MS) is increasing alarmingly worldwide. Non-alcoholic fatty liver disease (NAFLD) is one example of a metabolic syndrome. NAFLD develops as a chronic disorder, with constant damage to the hepatic parenchyma inducing fibrosis, cirrhosis and potentially leading to hepatocellular carcinoma (HCC), the most frequent type of liver cancer. For a long time, South East Asian countries have been suffering from hepatitis B virus infection (HBV), which was until recently one of the main prognostic and causing factors for the incidence of HCC in the region. However, in recent years the incidence of NAFLD has been increasing in the local population. Unfortunately, from most of the patients with liver disease, liver resection and transplantation is the most common therapeutic strategy offered as first line therapy. However, it has been observed that NAFLD patients that undergo liver surgery suffer from liver failure, due to reduced hepatic proliferation. In healthy individuals, liver regeneration is mostly driven by division of differentiated cells (hepatocytes) rather than stem cells. Therefore, understanding the metabolic requirements for hepatic division under physiological and pathological conditions is essential.

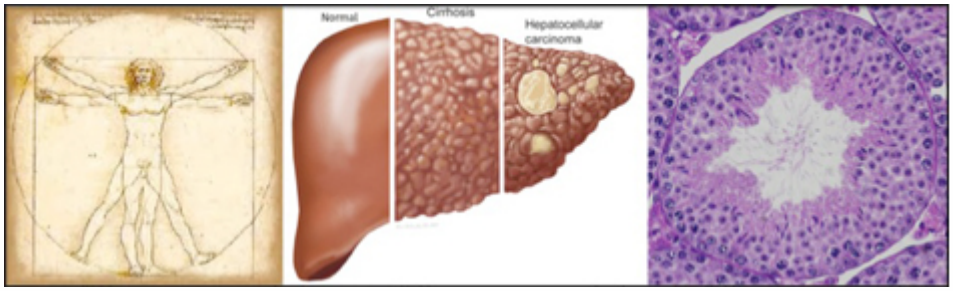

Figure 1: Human disease, liver associated disease, and a section through testis

Contribution of proliferation and metabolism to liver regeneration

During liver regeneration, hepatocytes exit from quiescence and have to re-enter the cell cycle in order to undergo one to two rounds of cell division. In order to achieve entry into the cell cycle, DNA replication and cell division, cells have to generate the required building blocks including nucleotides, amino acids/proteins, lipids, etc. To achieve this, the cell has to simultaneously coordinate cell cycle progression and metabolism but how the cells achieve this is unclear. We aim to decipher the mechanisms behind this coordination.

A hallmark of liver disease is the decreased capacity of hepatocytes to proliferate. In order to compare wild type liver, where hepatocytes divide upon injury, with diseased liver where hepatocytes cannot divide, we are taking advantage of our Cdk1flox/flox Albumin-Cre (Cdk1Liv-/-) mice (PNAS109, 3826-3831) as well as other mouse models. We have shown that liver regeneration in these mice happens with a similar kinetics as in wild type mice and did not affect viability. Although the division of hepatocytes in Cdk1Liv-/- knockout mice was blocked, the liver regenerates by compensatory cellular hypertrophy. Nevertheless, the molecular pathways by which metabolism supports this regeneration process was not known. To understand the molecular mechanism and identify the implicated pathways supporting this regeneration process, we used RNAseq, metabolomics and lipidomic analyses (by mass spectrometry) and combined it with intravital imaging, functional MRI (13C-pyruvate), and biochemical experiments. These experiments generated large datasets that we are mining with the help of bioinformatics experts. Our results indicate that when the liver regenerates by compensatory cellular hypertrophy, there is a profound rewiring of the metabolic pathways. In collaboration with clinicians, our data will help to identify biomarkers that could be helpful to stratify liver resection patients in the clinic.

Collaborators: Mikael Björklund (Dundee), Uwe Sauer (Zürich), Markus Wenk (NUS), Hanry Yu (A*STAR/NUS), Philip Lee (A*STAR), Hyungwon Choi (IMCB/NUS)

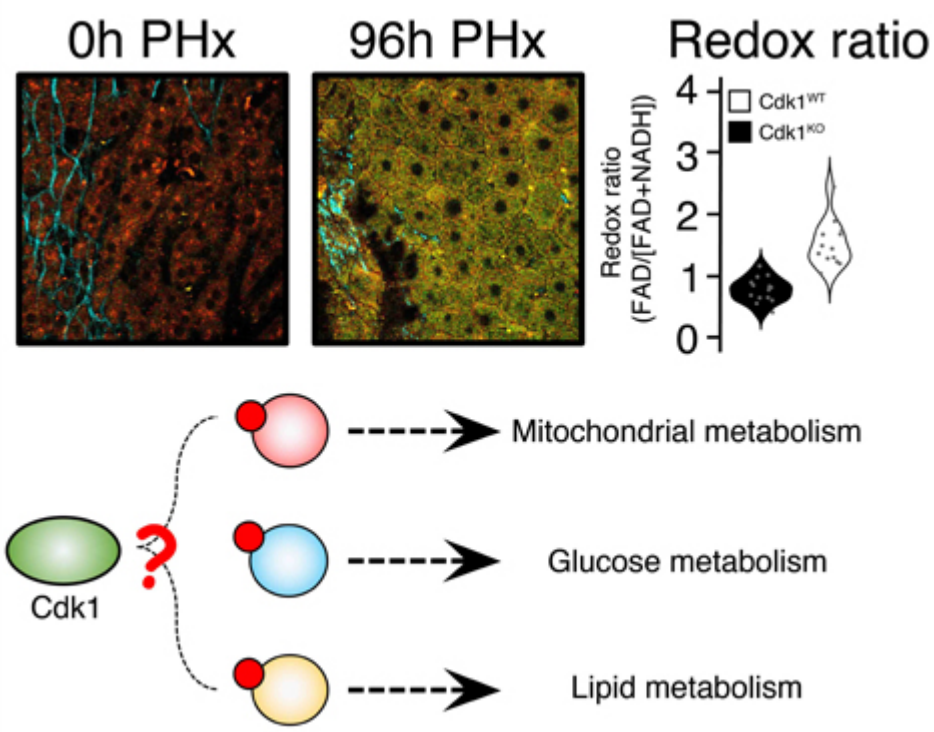

Figure 2: The coordination of cell division and metabolism during liver regeneration

Liver regeneration when hepatocyte division is impaired

The liver consists of approximately 80% hepatocytes and under normal conditions liver regeneration is driven by hepatocyte proliferation. Nevertheless, in diseased livers when hepatocyte proliferation is impaired, liver regeneration may be supported by other cell types that eventually differentiate into hepatocytes. Among the possible cell types are liver stem/progenitor cells, and transdifferentiating biliary cells. We have developed systems where we can impair the division of specific cell lineages to investigate how the liver develops under these conditions. The results will tell us how the different cell types work together during liver development and regeneration. In addition, we hope to learn how the different lineages can be stimulated to proliferate, which could be useful for liver disease patients in the clinic.

Collaborators: Hyungwon Choi (IMCB/NUS), Walter Hunziker (IMCB).

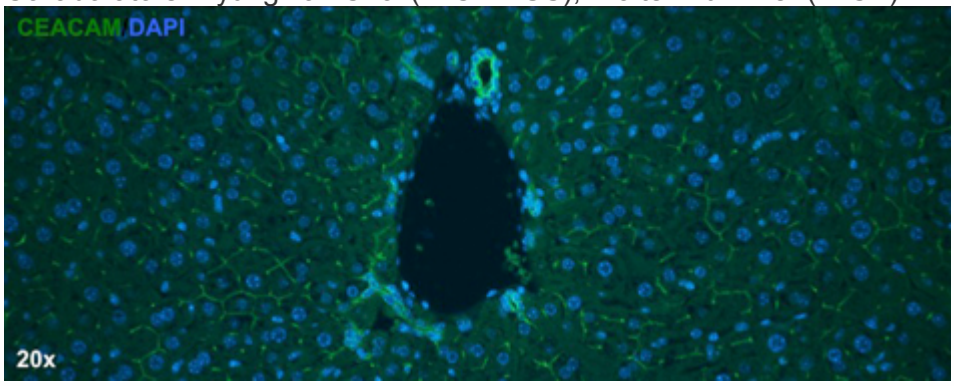

Figure 3: A liver section that was stained with DAPI and antibodies against CEACAM

Male sterility (testis, male germ cell development)

Fertility is an essential component of human life and any disturbance can lead to severe consequences personally as well as for the society. A recent report indicated that the average sperm count in males has dropped by 50-60% from 1973 to 2011. If this trend continues, it could lead to the extinction of the human race in the future.

Male germ cell development in the testis is an intricately controlled process that includes meiosis. As a difference to mitosis, where the genome is duplicated and then divided in two daughter cells to maintain the amount of genetic information constant, meiosis leads to a reduction of genetic material in order that gametes contain only 1N DNA content. Therefore, the genome needs to be divided twice in each cycle. In addition, during meiosis the genome is rearranged by recombination in order to increase genetic diversity. These characteristics of meiosis require changes to the wiring of the cell cycle machinery compared to mitosis.

Cyclin-dependent kinases (Cdks) not only regulate mitosis but are also known for regulating meiosis. Even more interesting is that some Cdks are not essential for mitosis but are indispensable for meiosis. We are investigating how the functions of Cdks differs in meiosis compared to mitosis since this will teach us new tricks that may be useful down the road for the treatment of infertility and other disease where the functions of Cdks are deregulated.

Some of our work focuses on Cdk2, Speedy A, and Emi2 but we are also interested how the entire network of Cdks and cyclins cooperates to regulate meiosis. Furthermore, we are studying how Cdks regulate transcription and the epigenome in this context.

Our work help us to understand some of the reasons for sterility in males and may also provide novel targets either to improve male infertility or to interfere with fertility to develop novel contraceptive agents.

Collaborators: Ernesto Guccione (IMCB), Diana Low (IMCB), Daniel Messerschmidt (IMCB), Kui Liu (Gothenburg)

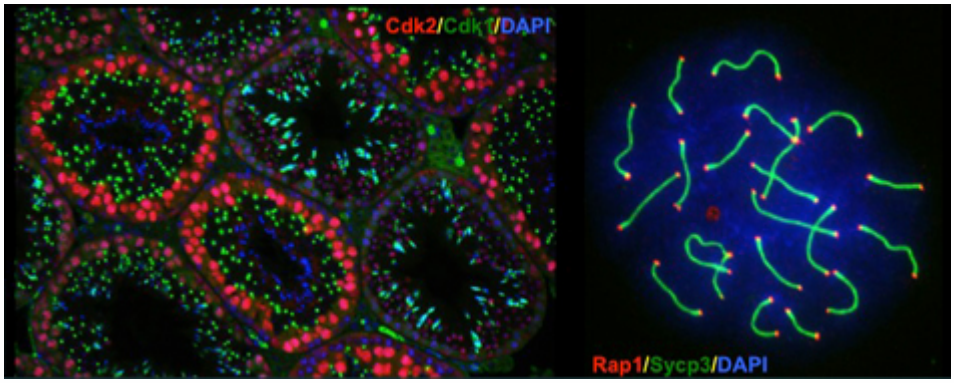

Figure 4: Testis section and chromosome spread stained with antibodies

Regulation of biogenesis by cyclin-dependent kinases (Cdk1/Cdk2)

In order that cells can multiply, cell proliferation and biogenesis must be coordinated. Although the molecular mechanisms remain unclear, there is convincing evidence that biogenesis processes are controlled as cells progress through the cell cycle. Our hypothesis is that cyclin-dependent kinases (Cdks) could provide the required signal to coordinate with biosynthesis pathways. To study this process, we have generated Cdk2 and Cdk1 knockout mice and cells but found that they compensated for the loss of the other Cdk in the G1-S-G2 phase of the cell cycle. Therefore, the identification of specific in vivo substrates for each Cdk is almost impossible since every candidate substrate was phosphorylated by the Cdk that was not knocked out (Cdk1 in the case of Cdk2KO and Cdk2 in the case of Cdk1KO). To circumvent this problem and to identify Cdk1- and Cdk2-specific substrates we knocked out Cdk2 and Cdk1 at the same time; therefore we generated four different MEF lines (1) wild type, (2) Cdk2KO, (3) Cdk1KO, and (4) Cdk2Cdk1DKO. Each MEFs line was released synchronously in the cell cycle, samples were collected at different time points, samples were phospho-enriched, and labeled with isobaric TMTs before being analyzed by mass spectrometry. Using this approach we are able to evaluate the total level of each protein as well as their phosphorylation status at each time point corresponding to the different phases of the cell cycle. The large amount of generated data requires state-of-the-art bioinformatics to normalize and assess significant modifications occurring during cell cycle progression but affected by the loss of Cdk1/Cdk2. This project confirms numerous known substrates but also has unveiled unexpected new substrates in biogenesis pathways. The function of the phosphorylated substrates and their impact in normal and pathological conditions will require combining cell biology, biochemistry, and microscopy techniques.

Collaborators: Chris Soon Heng Tan, Radoslaw Sobota, Pär Nordlund (IMCB/NTU)

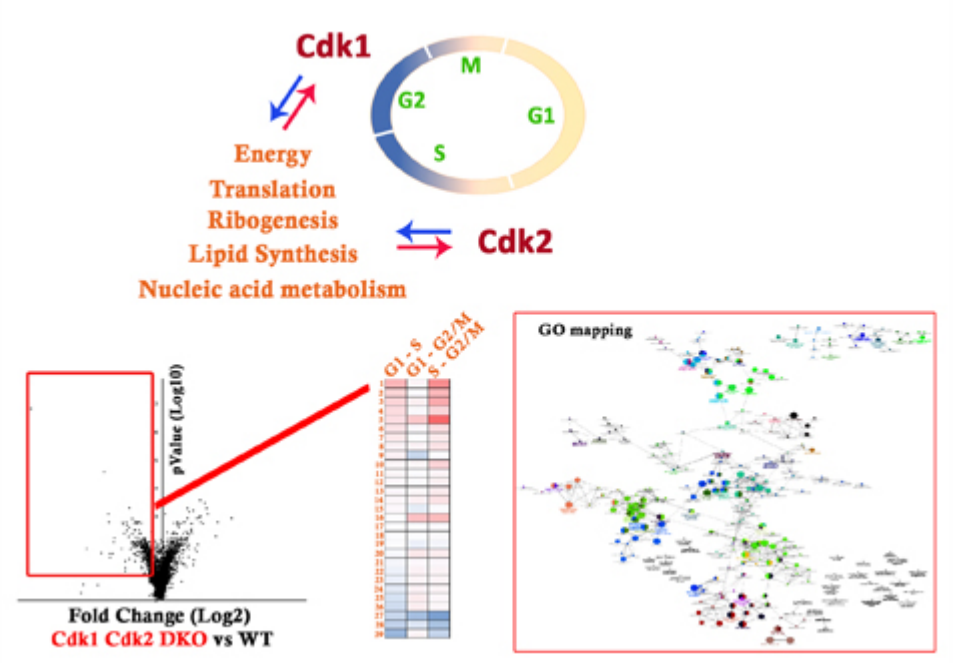

Figure 5: Cyclin-dependent kinases regulate biosynthesis pathways