LUDC-IRC Postdoctoral Program 2020 – Project 7

Remodeling of glucose metabolism in the liver

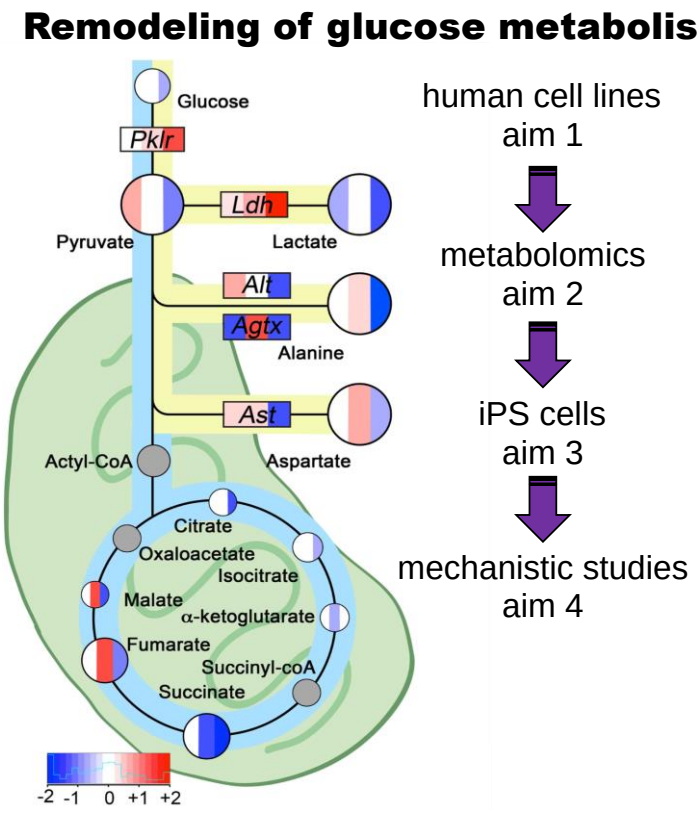
PI: Philipp Kaldis
Co-PIs: Hindrik Mulder and Peter Spégel

Purpose and aims

We have recently shown when hepatocyte division is blocked, the liver remodels its metabolism from using glucose to amino acids [1]. Since this was done in a mouse model, we want to investigate if this is also true in humans, employing human liver cell lines (aim 1) and iPS cells (aim 3). In these cells, we will investigate central glucose metabolism with a focus on how pyruvate is converted into acetylCoA/lactate/ alanine/aspartate (aim 2). The long-term goal is to investigate whether this metabolic remodeling also happens in patients. This project is highly collaborative and involves 4 groups from LUDC: Kaldis, Mulder, Spégel and Franks.

State-of-the-art/background

The liver is the largest solid organ in the mammalian body. It plays essential roles in the gastrointestinal tract, controlling metabolism, detoxification, digestion, and many other essential processes. Due to its functions and the strategic location alongside the gastrointestinal tract, it is constantly exposed to intrinsic and extrinsic damage, which can lead to chronic damage, like non-alcoholic fatty liver disease (NAFLD). NAFLD is the most frequent chronic liver disease in the world. It is characterized by accumulation of lipid droplets in hepatic cells [2], and by dysregulation of metabolic homeostasis and cellular division [3]. In fact, NAFLD, and subsequently non-alcoholic steatatohepatitis (NASH), are emerging as the most damaging complications in Type 2 Diabetes (T2D) and obesity, underlying extensive morbidity in the global population [4]. It has been shown that in NAFLD, hepatocytes display a decreased ability to enter the cell cycle. This is thought to be caused by lipid droplet accumulation in the cells or possible by a "hostile" environment. Nevertheless, we have recently shown that blocking hepatocyte division alone leads to metabolic remodelling even in the absence of exogenous insults to the liver [1]. Therefore, it is possible that the decrease in hepatocyte division is not only a consequence of NAFLD but could contribute to the disease phenotype.



With the development of metabolomics, metabolism has been studied extensively with a strong focus cancer [5]. Although Cancer metabolism is pivotal, metabolism plays important roles in all biological processes both under normal conditions and during disease. Here we focus mostly on metabolism of glucose, the major source of energy and biochemical building blocks. Most glucose molecules are converted to pyruvate, which is a major hub of glucose metabolism. Under aerobic conditions, pyruvate is imported into the mitochondria, where it is converted into

acetylCoA, which drives the TCA cycle and oxidative phosphorylation. In contrast under anaerobic conditions, (lactic acid) fermentation is more favourable with pyruvate in the cytosol converted into lactate by LDH (lactate dehydrogenase), a process which is much less energy efficient compared to oxidative phosphorylation. In cancer cells, Otto Warburg, uncovered that even when oxygen is present pyruvate is converted into lactate, which he termed "aerobic glycolysis". Oxidative phosphorylation and fermentation/aerobic glycolysis are the major branches of glucose metabolism, which have been extensively studied. Nevertheless, pyruvate can also be converted into alanine or, via the TCA-cycle intermediate oxaloacetate, into aspartate, which has garnered much less attention. Levels of alanine aminotransferase (ALT), catalysing the former reaction, are measured in the clinic to determine potential damage to the liver. The functions of alanine and its contribution to whole body metabolism has been recognized but not yet studied in detail. In summary, glucose metabolism is more complicated than just oxidative phosphorylation and fermentation/aerobic glycolysis. In our proposal, we will investigate how cells decide to convert pyruvate into different metabolites as a function of the disease environment. This will help us to understand how cells adapt to hostile environments and how we can take advantage of this information using therapeutic approaches.

Significance and scientific novelty

The liver plays a major role in glucose metabolism. A lot of work has been done to unravel the genomics of diabetes with LUDC being a major driver of this process. Therefore, we have access to rich information about genetic variation associated with T2D. Nevertheless, a large knowledge gap exists in how such risk alleles contribute to the liver disease in T2D. The major focus so far was on islets, and to some extent skeletal muscle, and adipose tissue. Here we focus on the liver, which provides a new dimension. We will be studying the decision making in glucose utilization to determine when and why oxidative phosphorylation, aerobic glycolysis, or amino acid metabolism is activated. This will provide fundamental insight how glucose metabolism is regulated in the liver in the events leading up to T2D. With the obtained data, we will be able identify novel therapeutic targets for the treatment of liver disease in metabolic disorders.

Preliminary and previous results

The Kaldis laboratory has done extensive work on cell cycle regulation and metabolism. In a flagship publication in Developmental Cell (2018), we analyzed the metabolic remodeling of the regenerating liver when hepatocyte division was blocked. All of this work was done in vivo using mouse models. Our approach was to combine RNA sequencing, metabolomics, functional imaging (MRI and intravital imaging), Seahorse analysis, cell biology (primary hepatocytes), with biochemistry (western blots and activity assays). This provided us with a comprehensive picture on how glucose metabolism is remodeled during liver regeneration. Our expectation was that pyruvate would be converted to lactate in order to favor aerobic glycolysis (Warburg effect). To our surprise, this was not the case and regenerating hepatocytes preferred to convert pyruvate into alanine. This was most likely a consequence of impaired mitochondrial function and reduced cellular oxidation. Interestingly, we found that supplementation with alanine increased blood glucose levels suggesting that alanine was supporting whole body metabolism [1]. Notably, gluconeogenic pathways are insufficiently restrained in T2D, contributing to hyperglycemia.

Currently, we are studying the effect on lipid metabolism in this mouse model using lipidomics. It is well known that glucose and lipid metabolism intersect and this is of great importance for liver diseases and T2D. We have observed a decrease in triglycerides, and increase in acylcarnitines, which is probably due to deficient fatty acid oxidation (FAO). As a consequence, the amount of free fatty acids (FFA) increases and over time leads to obesity in these animals. This is also associated with insulin resistance. All these events are reminiscent of what occurs in T2D. Thus, insulin resistance may then drive hepatic steatosis. What we are learning from this project is how tightly connected all these metabolic pathways are. Changes in one of these pathways is likely to affect other pathways too.

In summary, the Kaldis laboratory has extensive experience in RNA sequencing, metabolomics, lipidomics, cell-based assays, and biochemistry. Our strength is to combine all these methods to obtain a comprehensive picture of the biological question. The proposed project builds on our strengths but adds a new dimension by aiming to extend our knowledge to human cells and cells derived from patients and how it relates to the pathogenesis of T2D.

Research plan

Aim 1: Human cell line approach

Our goal here is to translate our findings in mouse models to human cells. In order to achieve this, we will use a widely used liver cell line, HepG2. In the mouse, we deleted the Cdk1 gene to prevent cells from dividing [1, 6] but other approaches would work too (overexpression of the inhibitor p21Cip1/Waf1, cyclin A2KO, MastlKO, or anilinKO just to list a few). In HepG2 cells, we will use multiple approaches including small molecule inhibition, shRNA, and CRISPR to model what can be observed in NAFLD and NASH. The activity of CDK1 can be inhibited by a fairly specific small molecule inhibitor, RO-3306. Alternatively, we can use other CDK1 inhibitors that are commercially available. shRNAs to silence Cdk1 are also available in our lab. Finally, we will use CRISPR to conditionally knockout the Cdk1 gene in HepG2 cells. Yet another approach, perhaps more elegant, is a knock-in of the F80G mutation in the Cdk1 gene, as has been done recently by the Sicinski laboratory [7]. The mutant CDK1F80G, better known as CDK1AS (for analog-sensitive), can be readily inhibited by the analog-sensitive inhibitors 1-NM-PP1 or 3-MB-PP1. This creates a system where CDK1 activity can be specifically inhibited at any time. With these multiple approaches in hand, we will have no problem to recapitulate in human cells what was observed in NAFLD and NASH as well as what we have done in the mouse.

Since there are many reasons to develop NAFLD and a good number of mutations/SNPs have been identified, we will extend our current knowledge by collaborating with Paul Franks (LUDC). His laboratory will identify genetic variants of genes which may play a functional role in or that are associated with T2D and/or metabolic liver diseases. These will be then tested in our cell line system. Bioinformatic analyses will guide us on which variants to test in our system, by comparing the probability of a mutation/SNP to the data from healthy individuals at the gnomAD browser (https://doi.org/10.1101/531210). Using CRISPR, we will introduce and revert SNPs in HepG2 to create isogenic cell lines which will used as disease models and controls, as described in aim 2.

Aim 2: Glucose metabolism

The goal of the second aim is to study central glucose metabolism, i.e. the conversion of pyruvate into acetylCoA, lactate, alanine, or asparagine. This is an extremely important intersection in metabolism, which dictates the metabolic fate of the cell. AcetylCoA drives the TCA cycle and therefore oxidative phosphorylation. Lactate is the product of aerobic glycolysis (Warburg effect). Alanine can lead to gluconeogenesis and support whole body metabolism (see also figure). In the mouse, we had observed that when cell division was blocked, pyruvate was preferentially converted into alanine indicating a switch from glucose to amino acid metabolism [1].

In collaboration with Peter Spégel (LUDC) we will carefully characterize central glucose and amino acid metabolism using mass spectrometry. His laboratory has established methods and extensive experience from studies of metabolism in multiple cell-lines and tissues, including primary hepatocytes. Once the levels of these metabolites are determined, we will evaluate which cell lines (or which treatments) are the best model reflecting the disease. Based on this analysis, we will select 1-2 cell lines or treatments for further analysis in iPS cells.

Aim 3: iPS and organoid approach

Human cell lines represent an excellent tool that is easily manipulated for investigation of biological mechanisms. However, these cell lines rarely reflect the disease in patients because they exhibit a number of abnormalities not observed in primary cells, including the cancer phenotype which is associated with large perturbations in metabolism. In order to get one step closer to investigate liver disease in patients, we will be using iPS cells. This will be done in collaboration with Hindrik Mulder (LUDC). His group is leading an LUDC-sponsored initiative to establish iPS cells as a platform available to our environment. Currently, beta-like cells are being made from patient-derived iPS cells but there is a strong commitment to also develop other cell types, such as hepatocytes. iPS cells are based on primary cells taken from patients, which then are reprogrammed into iPS cells. Such iPS cells will then be differentiated into liver cells using a published protocol [8]. A great benefit of iPS cells is that they harbor the relevant genetic background and can be chosen to carry risk alleles of interest for the disorder to be studied; these SNPs can then be edited by CRISPR, generating isogenic cell lines. In addition, we will grow iPS-derived hepatocytes as liver organoids, which is one step closer to the in vivo situation [9].

With these iPS-derived hepatocytes, we will perform the same analysis as has been described for the HepG2 cells in aim 2, which is done in collaboration with Peter Spégel. These data will allow comparisons with HepG2 cells as well as with our established mouse model. In addition, these data should be informative to determine the state of glucose metabolism in the patient-derived iPS cells. Furthermore, these data will allow us to investigate the mechanisms of glucose metabolism more deeply in aim 4.

Aim 4: mechanistic studies of metabolism

We are confident that we can achieve aims 1-3 in the two-year period. In case there is additional capacity or time, we will embark on mechanistic studies on how patient-derived cells regulate their glucose metabolism. Depending on whether pyruvate is converted into acetylCoA, lactate, alanine, or aspartate, this would give us the direction. The pathways associated (oxidative phosphorylation, fermentation/aerobic glycolysis, or amino acid metabolism) will be further investigated. As a first approach, we will determine the mRNA levels of the genes involved in this pathway using real-time PCR. If we observe up- or downregulated genes, we will further investigate the gene products and their activity using western blots and biochemical assays. In addition, we will consider untargeted metabolomics analyses by mass spectrometry in collaboration with Peter Spégel to determine a larger range of metabolites. The data will help us to get a more global view of glucose metabolism and to determine the reasons why a certain pathway is chosen by these cells. Based on all our data, we hope to get a better understanding of the disease and identify targets for therapeutic intervention.

Time Plan

Aim 1 will take 6 months, aim 2 approximately 12 months, aim 3 will take 10 months (overlapping with aim 2), which leaves approximately 4-6 months for aim 4. We believe this is a realistic plan but one can always encounter difficulties that are not predictable. Therefore, aim 4 is optional and will only be done if we make good progress with aims 1-3.

References

1. Caldez, M.J., et al., Metabolic remodeling during liver regeneration. Dev Cell, 2018. 47(4): p. 425-438.
2. Brunt, E.M., et al., Nonalcoholic fatty liver disease. Nat Rev Dis Primers, 2015. 1: p. 15080.
3. Forbes, S.J. and P.N. Newsome, Liver regeneration - mechanisms and models to clinical application. Nat Rev Gastroenterol Hepatol, 2016. 13(8): p. 473-485.
4. Yki-Jarvinen, H., Diagnosis of non-alcoholic fatty liver disease (NAFLD). Diabetologia, 2016. 59(6): p. 1104-1111.
5. Faubert, B., A. Solmonson, and R.J. DeBerardinis, Metabolic reprogramming and cancer progression. Science, 2020. 368(6487): p. eaaw5473.
6. Diril, M.K., et al., Cyclin-dependent kinase 1 (Cdk1) is essential for cell division and suppression of DNA re-replication but not for liver regeneration. Proc Natl Acad Sci USA, 2012. 109(10): p. 3826-3831.
7. Michowski, W., et al., Cdk1 controls global epigenetic landscape in embryonic stem cells. Mol Cell, 2020: p. in press.
8. Matoba, N., et al., Optimal human iPS cell culture method for efficient hepatic differentiation. Differentiation, 2018. 104: p. 13-21.
9. Ardalani, H., et al., 3-D culture and endothelial cells improve maturity of human pluripotent stem cell-derived hepatocytes. Acta Biomater, 2019. 95: p. 371-381.