

Looking for 'X' genetic factor(s) controlling levels of BMP6, a master regulator of the key iron hormone hepcidin

Iron is a microelement that plays a crucial role in various vital functions (e.g. it is indispensable for haemoglobin synthesis in red blood cells). Due to its chemical properties, however, excessive iron may be harmful, and thus can cause organ damage, ultimately leading to disease. Therefore, both in cells and at the level of the organism iron availability must be tightly controlled.

It has been known for decades that disorders associated with inappropriately high or low body iron status belong to the most common diseases worldwide. Surprisingly, only in last two decades the molecular mechanisms that control iron levels in the human body have been revealed. A small hormone produced by liver hepatocytes called hepcidin has been discovered as a key regulator of systemic iron homeostasis. Interestingly, it was shown to act in a very similar manner as another critical metabolic regulator, insulin. Analogously as insulin is produced in response to elevated blood sugar levels to subsequently lower the amount of circulating glucose, hepcidin decreases plasma iron concentration under conditions of high iron supplies.

Over last few years scientists identified another secreted protein, a cytokine called bone morphogenetic protein (BMP) 6, which acts as a key upstream hepcidin regulator. Like hepcidin, BMP6 is indispensable to maintain iron homeostasis, and the lack of this factor leads to severe iron overload disease. It is well-established that BMP6 acts as a sensor of systemic iron status, and modulates hepcidin levels according to the body iron needs. Interestingly, very recent data showed that BMP6 synthesis occurs not in hepatocytes, but in less abundant liver cells: endothelial cells that line hepatic blood vessels and Kupffer cells, specialized liver macrophages. Strikingly, the molecular mechanisms which are responsible for BMP6 regulation by iron supplies are completely unknown. With this project I would like to explore this intriguing topic and search of an 'X' genetic factor(s) that links body iron signals with BMP6 responses.

To this aim I will employ an innovative genetic engineering system - CRISPR/Cas9. Over only last 2-3 years, this technology has been adapted from Bacteria defence mechanisms and became a breakthrough and versatile technology to modify mammalian genomes. It is based on an enzyme called Cas9 that is directed by pre-designed small RNA molecules to specific DNA sequences. Cas9 cleaves the DNA, which may either lead to gene disruption, or may allow for a precise insertion of a pre-designed DNA fragment at a desired location.

Using CRISPR/Cas9 genome editing, I will first generate a suitable cell model, where the levels of the BMP6 protein could be monitored using a fluorescent molecular probe. Next, I will employ CRISPRs to inactivate approximately 90 candidate genes in parallel in these engineered reporter cells and search for those, that are required for BMP6 synthesis upon iron supplementation. I will further characterize the most promising hits from this screen and ultimately assess if they are required to maintain appropriate iron balance *in vivo* in mice.

Classical pathologies of iron homeostasis, such as iron-restriction anaemias or iron overload disorder hemochromatosis are very frequent in our population. Furthermore, recent advances in the field clearly demonstrate that iron plays also an important role in other common disorders, such as metabolic syndrome, cancer, atherosclerosis, osteoporosis, neurodegeneration or inflammatory diseases. 'To understand' is always a prerequisite to 'treat'. Our knowledge about the mechanisms that control mammalian iron homeostasis has progressed enormously over the last decade. This has brought major medical implications, with several 'bench to bedside' approaches currently being developed to treat iron-related diseases. In frame of this project, another important gene involved in regulation of body iron levels may be identified. In a broader perspective, this may reveal still unknown molecular pathways triggered by iron signals and may lead to new pharmacological options for correcting inappropriate iron levels in human patients.

Taken together, with this project I aim to address one of the most exciting open question in the field of iron biology, which may be also of high clinical importance. To reach my goals, I would like to apply a breakthrough CRISPR/Cas9 genetic editing system, which is currently expected to revolutionize not only basic science, but also medicine and biotechnology.