Regulation of chromatin accessibility in the hypoxic tumour microenvironment of glioblastoma

The precious genetic material that is hidden in every cell of our body is neatly wrapped around special protein units called histones, that together are assembled into chromatin. It is very important how tightly the chromatin thread is packed with histones, as this decides which genes are accessible to be switched on, and consequently, how our cells will behave. The more condensed are the histone complexes on the chromatin – the less accessible the gene will be. It turns out that in cancer cells, the accessibility of certain fragments of chromatin is changed when compared to the healthy cells, and by learning about these differences we could help to understand the behaviour of cancer, and potentially, to find some new cures. The recent technological advances give the possibility to record the changes in the accessibility of chromatin in every measured cell, and provide a huge resolution in the understanding of the diseases.

In this project, we are focusing on the chromatin changes in glioblastoma, which is the malignant and the most common primary brain tumour. Unfortunately, despite many decades of research, we are still missing the effective cure against glioblastoma, and after diagnosis, the average expectancy of a patient to survive is approximately just over a year. There are several reasons why we fail in curing glioblastoma. Firstly, this tumour is very invasive, which means that it spreads into the surrounding brain, preventing its complete removal during the surgery, and leading to the inevitable tumour regrowth. Secondly, glioblastoma recruits normal cells that support the development of the disease. For example, the microglia are the innate immune cells that reside in the brain and mediate the responses to different stresses or inflammation. However, once being attracted to the tumour tissue, microglia can constitute up to 30% of the tumour mass and secrete certain molecules that support the tumour growth. Also, the physical conditions that develop within tumours, for example, a loss of oxygen (hypoxia), can additionally promote the cross-talk between the cancer cells and microglia and further support the expansion of the tumour. It is very important to learn about the biology of the response to hypoxia because the clinical data show that the more hypoxic is the tumour, the smaller are the chances for the patient's survival.

The aim of this project is to understand the changes in the chromatin accessibility in microglia and tumour cells that were exposed to hypoxia. We will, therefore, learn about the activity of particular genes under hypoxic stress and how hypoxia may stimulate the cross-communication between the microglia and glioblastoma cells within the tumour microenvironment.

In our work, we will use the glioma tumours grown in mouse brains, to most closely recapitulate the natural environment of glioblastoma with all the infiltrating immune cells. We will use the state-of-the-art technology called Pi-ATAC, which simultaneously allows to measure the markers of specific cell types present within the tumour (for example microglia and tumour cells) and the chromatin accessibility in each labelled cell. Also, we will use the markers to detect hypoxia in these cells, and therefore, we will be able to distinguish the chromatin changes in microglia and cancer cells that were exposed to hypoxia. Once we learn about the hypoxia-induced chromatin changes *in vivo*, we will then model the hypoxic conditions for the microglia and tumour cell co-culture in the hypoxic chamber. The Pi-ATAC method was recently shown to detect similar chromatin profiles in the breast cancer cells exposed to hypoxia in the tumour *in vivo* and in the laboratory growth conditions, and so we are expecting to be able to model the hypoxia-induced chromatin changes in glioblastoma as well. Once we develop our laboratory model, we will further explore the properties of chromatin in microglia/glioma cell co-cultures by studying the properties of histones, e.g. which particular histone types are being incorporated into chromatin in response to hypoxia or how these are modified in hypoxia, which could affect the chromatin accessibility. We will also measure the gene expression in these co-culture models in hypoxia, which we will compare with the changes in the chromatin accessibility. Once we identify some potential genes and chromatin regions that are changed specifically in microglia or glioma cells in response to hypoxia, we will aim to validate these in the mouse and human tumour tissues using immuno-labelling methods.

Overall, our project will characterise the chromatin changes in cancer and microglia cells under hypoxic stress, and potentially will identify some new markers that could be used to detect the activity of these cells under the natural hypoxic microenvironment within the glioblastoma. As a result, we may discover novel hypoxia-specific directions to target the chromatin changes in glioblastoma and to develop some new therapeutic approaches against this deadly disease.