ImmunoTools *special* Award 2022

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The role of hypoxia in the immune tumour microenvironment of glioblastoma

Chromatin structure is often dysregulated in cancers, including glioblastoma (GBM), the most aggressive type of primary brain tumor. GBM has the poorest prognosis with no efficient cure to date due to diffusive growth into the brain, resistance to treatments and the immunosuppressive tumor microenvironment (TME). The growth and invasiveness of GBM is supported by the heterogeneous TME including local microglia and bone-marrow-derived macrophages (collectively known as glioma-associated microglia and macrophages, GAMs), but also by physical aspects of tumor microenvironment such as shortage of oxygen (hypoxia). Hypoxia can globally and rapidly alter gene expression, induce cancer cell invasiveness, stemness and lead to therapy resistance. Hypoxia can influence the pro-tumorigenic function of GAMs by inducing the expression of cytokines and cell surface receptors. Recently, it has been shown that hypoxia can also impose global changes on the chromatin properties, increase the heterochromatin content and ultimately impact multiple downstream signalling pathways. In our current work, we are investigating hypoxia-imposed chromatin changes in GAMs and glioma cells, which can in turn impact the interaction between these cell populations. We have adapted a recently published single-cell chromatin accessibility method called Pi-ATAC-seq (Protein-indexed Assay of Transposase Accessible Chromatin with sequencing, doi: 10.1038/s41467-018-07771-0.). Pi-ATAC-seq is ideal for studying the effects of hypoxia within the TME of GBM as it allows fixation of the tissue prior its dissociation and further analysis by flow cytometry and ATAC-seq. Therefore we can avoid cellular reoxygenation that could mask

the effects of hypoxia induced in tumors *in situ*. While Pi-ATAC-seq allows a simultaneous staining of proteins (both cell surface proteins and intracellular markers) by flow cytometry and analysis of chromatin accessibility by ATAC-seq– it is a great tool to study epigenetic changes in single cells populations defined by expression of specific markers.

To date we have optimised Pi-ATAC-seq to analyse $CD11b^+/CD45^+$ cell populations that have been exposed to hypoxia within the tumor (hypoxic marker Glut1 or pimonidazole) and we are currently analysing chromatin accessibility changes in these cell populations in a murine orthotopic GL261 glioma model.

With the ImmunoTools special Award we would like to expand our current project and screen for additional cell populations that might be recruited to hypoxic areas.

In particular, we would like to further confirm the balance between microglia and bone marrow-derived infiltrating macrophages (CD49d in addition to already used CD45 staining combined with CD11b) recruited to hypoxic areas, antigen presenting cells (CD80), markers of lymphocytes and monocytes (CD48), NK cells, $CD8^+$ and $CD4^+$ cells or cancer stem cell markers (CD90 or CD44). This study will allow us to better understand the balance of particular cellular populations recruited to hypoxic areas of the tumors and support future investigation of dependencies of these cells in the tumor-promoting hypoxic microenvironment.

Since GL261 glioma cells in our model are stably expressing GFP protein, we would like to test potential infiltrating populations in question with antibodies labelled with a PE fluorophore.

ImmunoTools *special* AWARD for **Katarzyna Leszczyńska** includes 10 reagents

PE - conjugated anti-mouse CD49d, CD48, CD80, isotype control IgG2b, NK-cells, CD4, CD8a, CD11a, CD90 and CD44

DETAILS more AWARDS