Summary of the work on the project "Identification of immunosuppressive mechanisms in glioblastoma and their evaluation as therapeutic targets"

The goal of the research projekt was to investigate the mechanisms of immune escape in glioblastomas and to identify novel targets for counteracting immunosuppression. Three specific aims had been defined: 1. Identification of genes that are up- or downregulated in tumor cells or host cells and are responsible for the manifestation of the immunosuppressive micromileu in glioblastomas. 2. Evaluation of the therapeutic efficacy of inhibiting arginase as monotherapy or as combination treatment. 3. Investigation of the extracellular vesicle (EV)-mediated interaction between glioblastoma cells and immune cells.

To address the first aim, we compared the growth of orthotopic syngeneic gliomas in the brains of immunocompetent C57BL6/Wildtyp (WT) mice versus immunodeficient $Pfp^{-/-}$ /$Rag2^{-/-}$ knockout mice. Immunocompetent mice survived significantly longer and tumors in immunodeficient mice grew strikingly more invasive. Experiments in which glioma cells were marked with a lentiviral red-green-blue (RGB)-labeling system revealed that the clonal heterogeneity was significantly reduced in tumors in WT mice so that obviously the selection pressure of the immune system allows fewer clones to survive than in $Pfp^{-/-}$ /$Rag2^{-/-}$ mice. This result was validated by using the opical barcoding (OBC) method which confirmed that clonal heterogeneity was highest in $Pfp^{-/-}$ /$Rag2^{-/-}$ mice while tumors in WT mice as well as in $PD1^{-/-}$ knockout mice were far less polyclonal. Repeat experiments showed that always the same clones became dominant in immunocompetent mice so that these appear to be distinguished by specific escape mechanisms allowing them to escape immune control. In order to identify mechanisms by which the tumor cells manage to escape immune control, we performed gene expression analyses of tumor cells as well as tumor-infiltrating host cells, which are mainly comprised of macrophages/microglia. These analyses showed that the vast part of the immunosuppressive signature in these tumors originates from the stromal cells. The actual tumor cells in immunocompetent mice mainly upregulated interferon-induced genes, including both immune-activating as well as immunosuppressive genes. In summary, our results demonstrate that gliomas cells adapt to the infiltration by interferon-producing T cells with upregulation of both immunostimulatory as well as immune evasion-associated genes, and in conjunction with the stromal cells which overexpress even higher levels of immunosuppressive molecules a microenvironment results that favors the immune escape of the tumor.

The second aim was based on our observation that ARG1 - the gene encoding the enzyme arginase - is among the highest upregulated genes in both macrophages/microglia as well as the tumor cells themselves in immunocompetent mice. Further, it is known that arginase acts as an immunosuppressor by cleaving and depleting L-arginine which is necessary for the activation and proliferation of cytotoxic T cells. In order to inhibit arginase, we used a small molecule inhibitor that we infused continuously intratumorally into gliomas in mice, and we first performed toxicity and pharmacokinetic tests. Subsequently, we treated glioma-bearing mice over several weeks with the inhibitor, either as monotherapy or in combination with an anti-PD1 antibody. Treatment with the antibody prolonged the survival of the animals significantly, however, combination with the arginase inhibitor did now confer any additional beneficial effect. Morover, monotherapy with the inhibitor did not prolong survival compared to vehicle-treated mice. In summary, these findings do not support our hypothesis that arginase could be a promising target for counteracting immunosuppression in malignant gliomas in order to support the effect of immunotherapy.

In the third part of the project we investigated the effect of activated T cells on the composition of EVs secreted by human and murine glioma cells. To this end, we first established and refined the Imaging Flow Cytometry (IFCM) technique for characterizing EVs, which resulted in a publication. Subsequently, we confronted glioma cells with activated T cells, PBMCs or with interferon-γ and performed proteomic analyses of tumor cell-secreted EVs. We discovered that in particular FASN (fatty acid synthase) is upregulated in tumor cell EVs that were confronted with activated lymphocytes or interferon-γ. Further experiments showed that FASN is highly expressed in glioblastoma tissue and that FASN-positive EVs are elevated in the blood of glioblastoma patients. In summary, we identified FASN as a potential marker for circulating glioblastoma EVs which is upregulated upon immune confrontation.