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P02.07.B FIBROBLAST ACTIVATION PROTEIN EXPRESSING MESENCHYMAL CELLS INFLUENCE T CELL ABUNDANCE AND FUNCTION IN GLIOBLASTOMA
N. Ternerova[1], M. Houdova Megova[1], T. Buna[1], T. Svablova[1], B. Vymolova[1], E. Balaziova[1], Z. Vanickova[1], E. Krepela[1], P. Hrabal[2], R. Tomas[3], D. Netuka[4], P. Busek[1], A. Sedo[1]; [1]Laboratory of Cancer Cell Biology, Institute of Biochemistry and Experimental Oncology, First Faculty

of Medicine Charles University, Prague, Czech Republic, [2]Department of Pathology, Military University Hospital, Prague, Czech Republic, [3]Department of Neurosurgery, Na Homolce Hospital, Prague, Czech Republic, [4]Department of Neurosurgery and Neurooncology, First Faculty of Medicine, Charles University and Military University Hospital, Prague, Czech Republic.

BACKGROUND: Glioblastomas (GBMs) are aggressive brain tumors with strong immunosuppressive properties. In epithelial cancers, mesenchymal cells expressing fibroblast activation protein (FAP) play an important role in modulating the T cell response. We have recently shown that FAP$^+$ mesenchymal cells are present in human GBMs. The aim of this study was to determine their effect on T cell abundance and function. MATERIAL AND METHODS: GBM tumors (16 samples) were mechanically and enzymatically dissociated and analyzed for immune cell subpopulations by flow cytometry. Expression of T cell related genes was analyzed by qRT-PCR in GBMs selected based on FAP protein concentration determined by ELISA (14 FAP high [upper tercile], 16 FAP low [lower tercile]). Immunohistochemistry (IHC) was used to compare the number of CD3$^+$, CD4$^+$, and CD8$^+$ T cells in GBMs with high (upper tercile, n=21) and low abundance of FAP+ stroma (lower tercile, n=20). Co-localization of FAP+ mesenchymal cells and CD3, PD-L1 and PD-L2 was evaluated by immunofluorescence in frozen sections. 8 FAP$^+$ mesenchymal and 3 glioblastoma stem-like cell cultures were derived from human GBMs and PD-L1 and PD-L2 expression was determined using ELISA. Peripheral blood mononuclear cells were isolated from healthy donors´ buffy coats. Proliferation of stimulated T cells in conditioned media from FAP$^+$ mesenchymal cells was analyzed by flow cytometry. RESULTS: The percentage of T cells in CD45$^+$ cells from GBMs positively correlated with FAP expression. Expression of the T cell inhibitory molecule PD-L2 was higher in FAP high GBMs. Immunohistochemisty revealed that the numbers of CD3$^+$ and CD8$^+$ T cells were higher in GMBs with a high abundance of FAP$^+$ stroma and T cells were frequently in close proximity to FAP$^+$ mesenchymal cells, which were often PD-L2 positive. *In vitro*, FAP$^+$ mesenchymal cells expressed more PD-L2 than glioblastoma stem-like cells. T cell proliferation was decreased after exposure to conditioned media from FAP$^+$ mesenchymal cells. CONCLUSION: FAP$^+$ mesenchymal cells influence T cell abundance in GBMs and may affect T cell functions by limiting their proliferation via soluble factors or cell-cell contact. Acknowledgment: This work was supported by the National Institute for Cancer Research (LX22NPO5102), the Center for Tumor Ecology (CZ.02.1.01/0.0/0.0/16_019/0000785), Charles University project GAUK 365022 and Cooperatio Program Oncology and Haematology“.