ImmunoTools *special* Award 2018

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Development of new therapeutic strategies increasing drug permeability by modulating the cytokine microenvironment of the blood-brain barrier/glioblastoma cells interface

Glioblastoma Multiforme (GB) is a central nervous system tumor with high aggressiveness and chemoresistance due to the presence of cancer stem cells (SCs). The presence of tight junctions (TJs) and the high levels of drug efflux pump like P-glycoprotein (Pgp/ABCB1) on the blood–brain barrier (BBB) further limits the success of chemotherapy in GB, preventing the drug delivery and antitumoral efficacy.

Since the BBB is disrupted in the presence of GB[1] and GB SCs are more chemoresistant than GB differentiated cells (adherent cells, AC)[2], the grade of differentiation and/or stemness of GB cells may influence drug permeability across BBB.

Using human primary GB cells co-cultured with primary BBB cells we found that GB cells increased the permeability to doxorubicin and dextran-70, indexes of Pgp and TJs functions, compared to BBB alone. This effect was stronger with GB AC than with GB SCs and was due to changes in the expression of Pgp and TJ proteins. Also conditioned medium derived from AC increased doxorubicin and dextran-70 permeability more than conditoned medium of SC, suggesting that AC cells release soluble factors that may mediate these changes in BBB permeability. The nature of these factors is still unknown, but by screening cytokines and chemokines by PCR Arrays we showed that IL-1b, IL-8, IL-10, SDF1α, TNFα, INF-gamma are significantly more produced by AC than by SC. To verify the role of these cytokines in the BBB breakdown, I will validate by ELISA human IL-1b, IL-8, SDF1α, and TNFα release in medium of GB cells alone and in the presence of BBB. If the release of these cytokines is increased in GB/BBB co-cultures I will, on one hand, incubate BBB cells alone or with AC/SC-conditioned medium with human IL-8, IL-1b, IL-10, TNFα, SDF1α/CXCL12α and IFN-gamma, as positive stimulation, and on the other hand, downregulate expression with blocking-antibodies or specific receptor inhibitors. Then I will check BBB permeability and changes in the ABC/TJs expression.

The disruption and increased permeability of BBB by tumor also contribute to the interaction between the central nervous system and immune system[3]. In order to investigate this aspect, I will incubate a co-coltures of BBB and GB cells with T lymphocyte obtained from peripheral blood of GB patients and analyse the effects of

this interaction on GB cells. T cells will be sorted by flow cytometry according to the expression of $CD3^+$, $CD4/CD8^+$, $CD45RO^+$ (FITC), IFN-gamma(PE), CD107a. GB cells, growing under BBB, will be tested by flow cytometry for HLA-DR (PE) and TNFα (PE). Annexin (FITC) assay will be used as index of apoptotic death.

These experiments will give us more information about the mechanisms related to the immune-environment underlying GB-induced disruption of the BBB. Modulating this immune-environment could be a good strategy to improve the drug delivery across BBB and the consequent killing of tumor itself.

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