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"Establishing a 3-D multicellular Tumor Model to assess the impact of fatty acids activated immunosuppressor cells on Breast Cancer spheroid growth"

Background and Hypothesis: Studies have shown that post-menopausal women who are obese have a 20-60% risk of developing breast cancer compared to women with a healthy weight (body mass index < 25). Obesity is characterized by metabolic dysfunction, including excess of free fatty acids (FFAs) in circulation and low-grade chronic inflammation. Myeloid-derived suppressor cells, or MDSC, are immature myeloid cells that exhibit unusual behavior of immunosuppression associated with tumor immune evasion, promotion of angiogenesis and metastasis in cancer. It has been shown that the immunosuppressive function of MDSC is enhanced by the metabolism of lipids such as FFAs; however, it has not yet been explored the effect of FFAs on MDSC in facilitating tumor growth independently of their immunosuppressive features. We **hypothesized** that the FFAs Oleic (OA), Palmitic (PA) and Linoleic (LA) increase the expression of S100A8 and MMPs on MDSC and are associated with increasing the spheroid size of the mouse Luminal B cell line EO711.

Methods: To test this hypothesis, we induced mouse bone marrow-derived MDSC (mBM-MDSC) from bone marrow cells and cultured in presence of recombinant cytokines IL-6, GM-CSF, and G-CSF for 4 days. Exposure to the different FFAs, or bovine serum albumin (BSA) as the FFAs carrier control, was performed after 24h of initial induction of MDSC during the last 72h. After culture, the cells were collected and used for cell extract and for the coculture with 3D EO711 spheroids. Western blot was performed for MMP9, S100A8, and Arginase 1. To perform the multicellular 3D spheroid assay, EO711 cells expressing green fluorescent protein (GFP) were cultured in a 96-spheroid microplate. After spheroid formation for 3 days, the mBM-MDSC were added. Spheroid growth was followed for a 10-day period.

Results: Arginase 1 (Arg1), a marker of MDSC activation, was significantly affected by the FFAs. While OA and LA significantly increased the Arg1 protein expression, PA downregulated its expression. Contrary to Arg1, the expression of MMP9 and S100A8 were not dramatically influenced by the FFA treatment. Interestingly, coculture of EO711 with mBM-MDSC treated with PA, but not with OA or LA, did increase the size of EO711 spheroid. From the Spheroid Assay model, we learned that adding fresh media in the middle of the culture time disturbed the spheroids. Also, wells at the border of the plate should not be used for spheroids, instead for adding media to protect evaporation from following wells containing spheroids.

Conclusions: OA, LA, and PA have a different effect on MDSC features. PA decreases the expression of Arg1, a marker of immunosuppression, but enhances the ability of MDSC to increase tumor growth by undefined mechanisms. S100 proteins and MMP9 should be evaluated in the supernatant since no changes were observed at intracellular level of MDSC. Other factors derived from MDSC such as cytokines should also be analyzed. Additional optimization of the 3D system is needed including lower number of seeded cells (from 10,000 to 5,000) to avoid disturbances in coculture by adding media during the culture time.

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