Cytokine profiling of drug-disrupted tumor cell/fibroblast crosstalk provides insights to understand the protective role of the stroma.

Background: The knowledge of which factors mediate the crosstalk between tumor cells and fibroblasts (most abundant stromal cell type) and how this cytokine repertoire is modulated by cancer therapy might provide insights to fight the disease. Results of our group (poster 2396) determine that IL1β and TGFβ1 secreted by tumor cells triggers the activation of normal colonic fibroblasts (NCF) to become CAFs. The role of IL1β is not as well known as TGFβ1 in a cancer context. We used cytokine arrays in order to determine: i) cytokine profiling of IL1β-treated NCF and ii) cytokine profiling of tumor cell-NCF cocultures in the presence of inhibitors of IL1β and TGFβ1 signaling, main triggers of NCF activation.

Methods: Conditioned media (CM) from normal colonic fibroblasts (NCF), IL1β-treated NCF (10ng/ml) and IL1β-treated NCF + neutralizing IL1β antibody (10μg/ml) was obtained after 24 hours in culture in FBS-free conditions. With this CM we also performed migration assays and cytotoxicity assays (WST-1) in the presence of Oxaliplatin (L-OHP) and 5FU to involve IL1β-responsive soluble factors in cancer progression and chemoprotection against these conventional drugs. Additionally, we evaluated the composition of CM obtained from cocultures between tumor cells and NCF in conditions where the crosstalk is disrupted with IL1β blocking antibody (2μg/ml) and an TGFBR1 inhibitor (0.1nmM). In both experiments we interrogated 174 cytokines/growth factors in glass-slide based arrays using fluorescent signal readout (RayBiotech Inc.).

Transwell coculture DLD1/NCF

Growth factors

IL1β

TGFβ1

IL1β + TGFβ1

IL1β + TGFβ1 + inhibitor

IL1β + TGFβ1 + CM

We evaluated the cytokine profile of the crosstalk between NCF and tumor cells considering the blockade of the two main triggers of fibroblast activation: cytokine, cytokine + IL1β blocking antibody, cytokine + TGFβ1 inhibitor or cytokine + IL1β blocking antibody + TGFβ1 inhibitor. 23 out of 174 factors were altered showing decreased concentration from cytokine alone to cytokine with the two blocking agents. Some of these factors are MMP1, EGF, KITLG or CXCL10. But surprisingly, 34 factors were progressively upregulated. Some of these factors were HGF, IL6, IL11, IL17, CCL5 or IL2RG.

Conclusions: Here we show an example of the usefulness of cytokine profiling as a complementary approach for microenvironment studies in assessing reciprocal activation of tumor cells and stroma, mediators of such interplay, treatment effectiveness and new target interventions.