Poster 19: Modulating Colorectal Cancer-Associated Fibroblast Subtypes Through In Vitro Culture Optimization: Implications for Therapeutic Advancements

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The tumor microenvironment (TME) is a complex ecosystem comprising tumor cells and non-cancerous cells, including cancer-associated fibroblasts (CAFs), endothelial cells, and pericytes. CAFs are key components of the TME, influencing tumor progression through mechanisms like immune evasion, metastasis, and extracellular matrix remodeling. Recent research in colorectal cancer (CRC) identifies various CAF subsets, including contractile CAFs, known for enriched genes involved in the regulation of cell contraction; ECM-remodeling CAFs, known to highly express ECM proteins and to be strongly associated with fibrotic matrix; and secretory CAFs, which secrete various growth factors, signal molecules, complements, and chemokines. However, these subsets have not yet been completely characterized, and further investigation is needed to understand their function and relevance in disease modeling, disease progression, and drug discovery.

This study investigates the phenotype of colorectal CAFs cultured in different commercially available media. We analyzed a diverse cohort of CRC-CAFs, focusing on proliferation, transcriptomic profiles, and cytokine secretion patterns across various culture media conditions. As a result, we demonstrated differences in the growth and activation of signaling pathways among media conditions. Further comparative analyses with publicly available CRC single-cell RNA sequencing datasets validated the relevance of these phenotypes *in vivo* and revealed two culture media conditions that significantly altered CAF toward two phenotypes present in CRC: contractile CAFs with immunosuppressive features and secretory CAFs with pro-inflammatory and cancer survival features.

Our findings highlight the importance of culture conditions in shaping CAF phenotype and their functional implications in CRC. Future research will focus on integrating these findings into co-culture systems to explore the maintenance of specific CAF subtypes in complex models and their interactions with immune and epithelial cells. By refining *in vitro* models, this work contributes to a better understanding of CAFs heterogeneity, tumor-stroma interactions, and their potential as therapeutic targets in colorectal cancer.