Fibroblast plasticity and heterogeneity in skin cancer

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The tumor microenvironment plays a crucial role during cancer development by providing tumor promoting as well as inhibiting signals, which show an impact on tumor origination, growth, invasion and metastatic behavior. Fibroblasts are the most common cell type in the tumor microenvironment (TME), responsible for a wide variety of processes like extracellular matrix remodeling and angiogenesis, thus modulating tumor progression. However, research on fibroblast function in the TME is challenging because of the heterogeneity as well as high plasticity of this cell type. Our work focuses on the phenotypic characterization of fibroblast subpopulations in skin cancer including their spatial distribution, as well as on how the tumor-stoma interaction induces this functional diversity.

Multiplex staining with diverse fibroblast markers of skin cancer tissue (Merkel cell carcinoma and Melanoma) confirmed fibroblast heterogeneity by the presence of different stromal cell subpopulations located in a defined manner around and in the tumor area. The spatial distribution of fibroblast subpopulations reveals that the subpopulations may also have different functionality. Fibroblasts in proximity to the tumor cells express Caveolin-1 (Cav-1) and S100A4, which is a characteristic of metastasis-associated fibroblasts.

Since tumor cells are able to activate resting fibroblasts in normal tissue to become cancer-associated fibroblasts (CAFs), we wanted to reproduce this activation in vitro to allow functional analysis. Therefore we generated primary fibroblast cultures (n=16) from healthy human skin. Surprisingly, primary fibroblasts display high plasticity and in some cases are activated even by default culturing conditions. Culture on polystyrene surface could induce CAF-related features (i.e. expression of α-SMA, Cav-1 and S100A4) and senescent behavior. These phenotypic changes were omitted by culturing the cells on a collagen matrix. In co-culture with Squamous (SCL-1) and Merkel cell carcinoma (WaGa) cell lines in hanging drops, presence of fibroblasts allowed more solid spheres and enhanced growth of the tumor cells. In this model, fibroblasts also displayed an activated phenotype based on their α-SMA expression, thus verifying the bi-directional crosstalk between tumor cells and fibroblasts. In the chicken chorioallantoic membrane (CAM) model we observed that fibroblasts induced strong angiogenesis, resulting in bleeding into the tumor, which was not observed in the tumors without fibroblasts.

In summary our data demonstrates that fibroblast polarization in skin cancer depends on their spatial distribution. This observation could be reproduced in vitro and in preclinical in vivo models, which will enable us to scrutinize the respective functional consequences.