BLOCKING TUMOR SUPPORT FROM CANCER-ASSOCIATED FIBROBLASTS IN TUMOR MICROENVIRONMENT

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Introduction

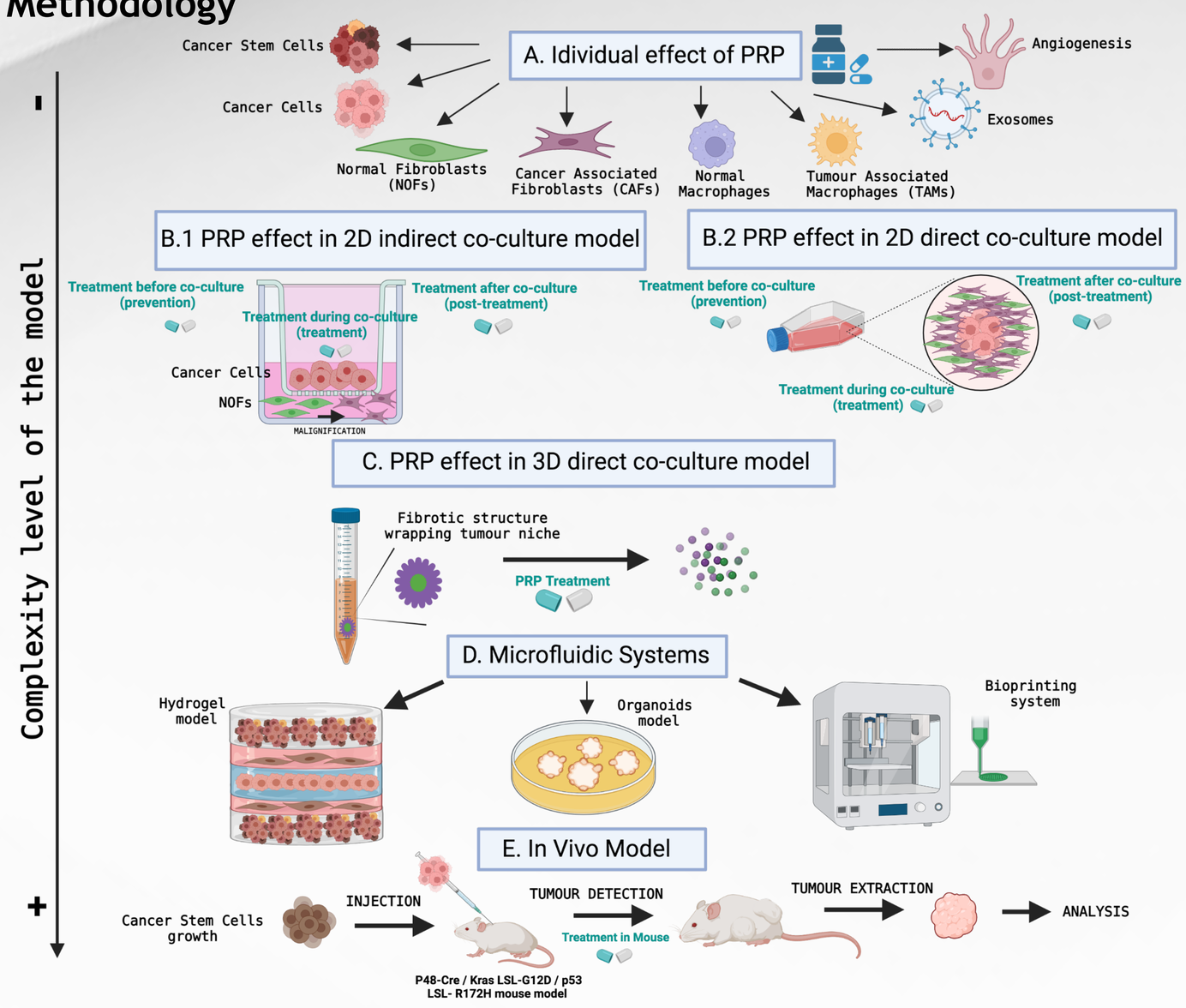
Despite the cancer research budget continuing to grow, together with our knowledge of the complex interaction between the tumour and the host organism, standard treatments are still inefficient, leading to extremely serious side effects and the development of tumour relapses with difficult treatment due to acquired chemoresistance. Cancer Associated Fibroblasts (CAFs), as the main cell population of the tumour microenvironment (TME), play a determinant role in all stages of tumorigenesis, from the tumour initiation, to the induction of the pre-metastatic niche settlement, thus it seems evident that novel therapeutic approaches should hamper CAFs support to tumour cells.

We have proven in vitro, in vivo and in patients with advanced solid tumours, the efficacy of a protein-based treatment. Here, we present the effect of the same formulation on CAFs modulation.

Objectives

To develop a 3D TME capable of representing a simplified model of in vivo organisms and reflecting the heterogeneity of cells and their behavior in a more physiological environment, thus overcoming the spatial and physiological limitations established by 2D cell culture systems and studying the influence of cell-cell contact on tumor differentiation and functionality, in order to subsequently develop a pre-clinical protocol of anti-tumor efficacy using a protein-based treatment, in order to study its possible negative effect on the TME, thus reducing the appearance and progression of tumors, metastasis and tumor recurrence.

Methodology



Results

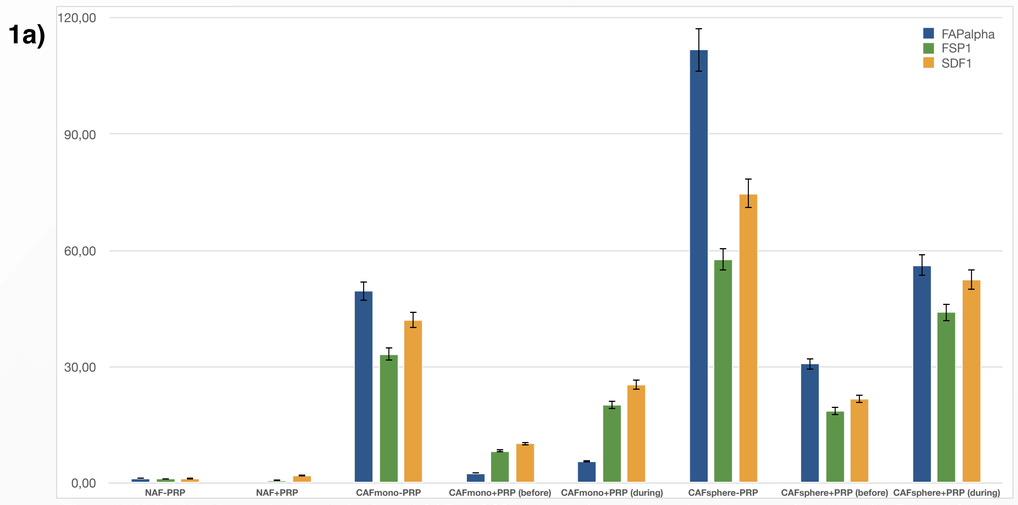
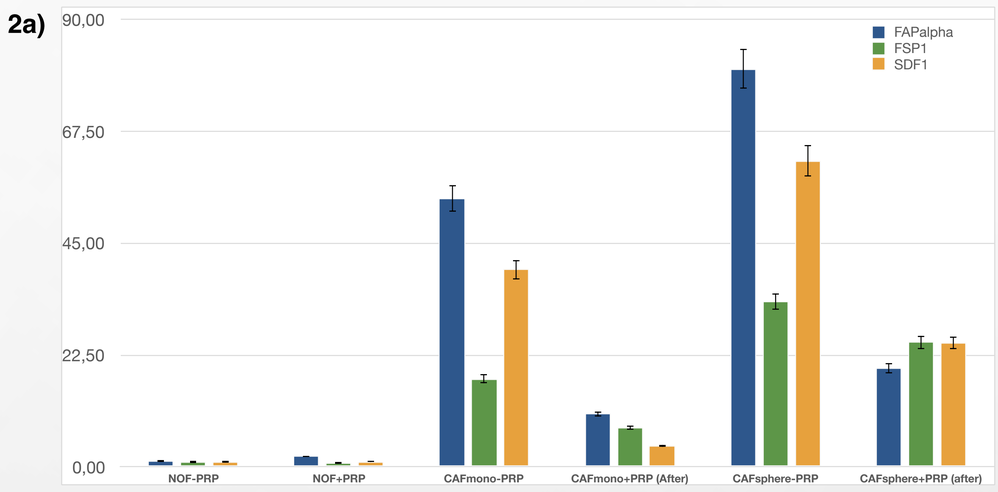


Figure 1. Malignification and characterization of CAFs. qPCR analysis of tumor biomarkers in normal fibroblasts (NAFs) and Cancer associated fibroblasts (CAFs) with and without protein-based treatment during and before malignification (1a). Immunofluorescent detection of αSMA (green) and FAPα (red) in indirect (1b) and direct (1c) coculture between fibroblasts and tumor cells with/without treatment.



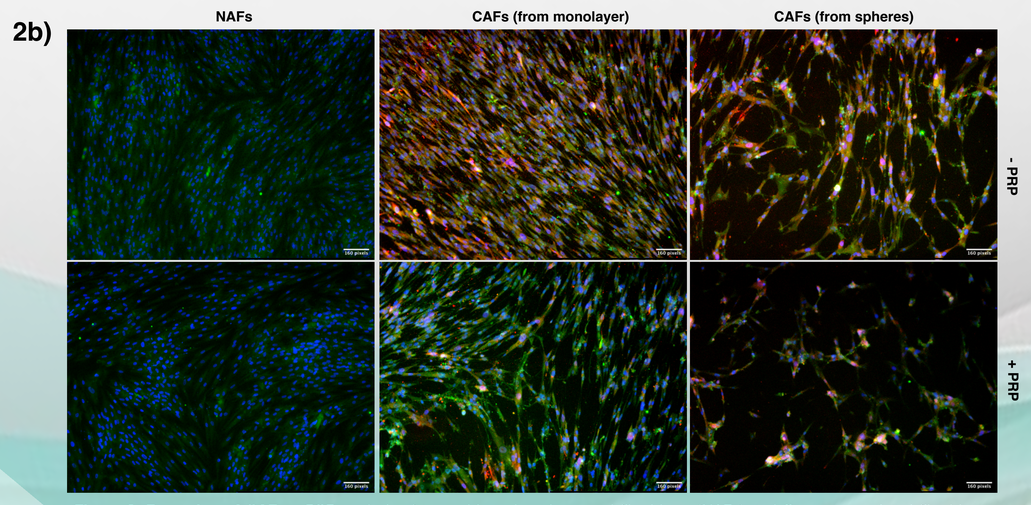


Figure 2. Reversion of CAFs. qPCR analysis of tumor biomarkers in normal fibroblasts (NAFs) and Cancer associated fibroblasts (CAFs) with and without protein-based treatment after malignification (2a). Immunofluorescent detection of αSMA (green) and FAPα (red) in indirect and direct coculture between fibroblasts and tumor cells with/without treatment after coculture (2b).

Figure 3. Functional capacity of CAFs. Wound healing assay to determine cell migration of pancreatic cancer cells seeded in mediums from normal and cancer fibroblasts with and without treatment. Representative phase-contrast microscope images showing the area covered by the cells at 72h after wounding (3a,3b). Clonogenity assay to determine proliferation and viability of pancreatic cancer cells seeded in mediums from normal and cancer fibroblasts with and without treatment (3c,3d).

Conclusions and Acknowledgments

Fibroblasts malignification was proved by morphological, phenotypical and protein expression changes, and in fact, induced CAFs showed an increased expression of αSMA, FAPα and SDF1 when compared with non-tumour fibroblasts (NAFs). In addition, CAFs promoted tumour cells proliferation, viability and migration rate and interestingly, those tumour supporter parameters were decreased after protein-based treatment. CAFs "re-education" instead of CAFs eradication could be a novel therapeutic strategy to decrease TME influence in drug uptake, immune evasion, tumour progression and further tumour dispersion.

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