EFFECT OF FIBROBLASTS-DERIVED EXTRACELLULAR MATRIX TREATED WITH PGE_2 ON EPITHELIAL-MESENCHYMAL TRANSITION OF COLORECTAL CANCER CELLS

Renata Machado Brandão Costa, Jose Andrés Morgado-Díaz
Grupo de Biologia Estrutural, Programa de Biologia Celular, Centro de Pesquisas, INCA

INTRODUCTION

Tumor development involves a set of abilities, including sustained inflammatory response and metastasis. The inflammatory environment is a characteristic of colorectal cancer (CRC), which is the third most common type of cancer among men and women. In these tumors, increased expression of Cyclooxygenase 2 (COX-2) is often associated with a poor prognosis. Prostaglandin E2 (PGE_2) is the main product generated by the action of COX-2 on the conversion of arachidonic acid, and has been described as inducing the epithelial-mesenchymal transition (EMT) of tumor cells. In addition to the inflammatory component, tissue context also provides crucial information for tumor development. Extracellular matrix (ECM) confer physical, molecular and biomechanical signals that are essential for the regulation of cellular behavior. In the tumor inflammatory environment, tumor cells and fibroblasts produce an altered ECM, corroborating for tumor progression through the activation of integrin-mediated intracellular signaling pathways, which are the main receptors that connect the cells to the ECM. However, the effect of PGE_2 on fibroblast activation and synthesis of ECM proteins, contributing to the progression of CCR, is still scarce in the literature. Our study aims to elucidate the effect of ECM derived from fibroblasts treated with PGE_2 on the behavior of CCR.

RESULTS

Effect of PGE_2 treatment on the induction of the CAF phenotype in fibroblasts

One-day treatment of FGH cells with different doses of PGE_2

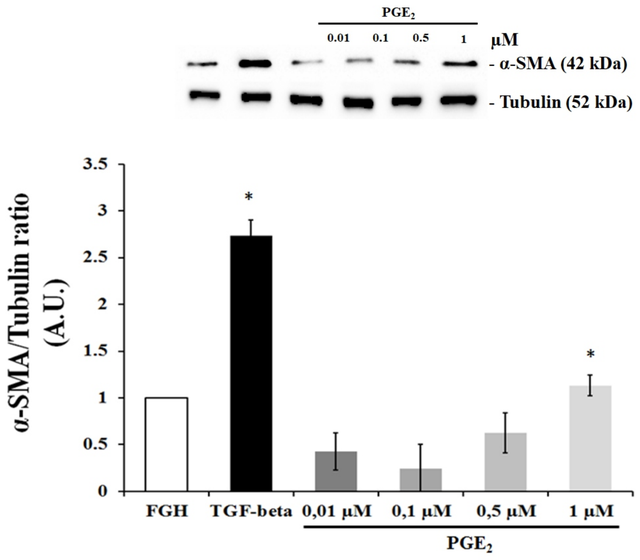


Figure 1: FGH cells were cultured in standard conditions for 72 h and treat on the first day with or without 0.01, 0.1, 0.5 and 1 µM of PGE_2. Lysates of FGH cells were immunoblotted with anti-α-SMA antibody. α-Tubulin was used as load. The results are shown as the mean ± SD fold increase relative to the control (FGH), calculated from 3 individual experiments (*p<0.05).

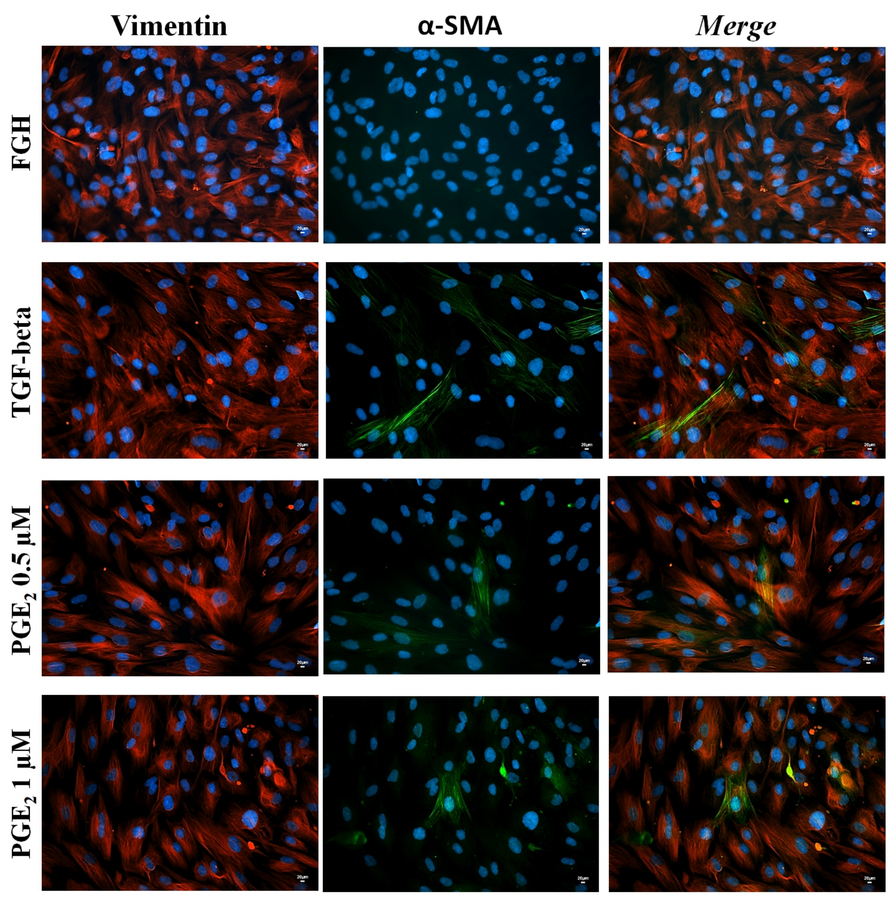
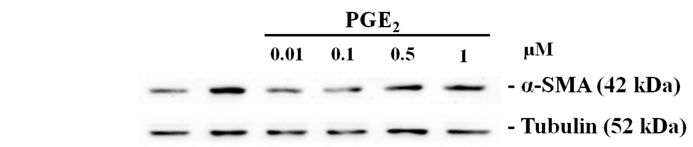


Figure 2: FGH cells were cultured in standard conditions on coverslips for 72 h and treat on the first day with or without 0.01, 0.1, 0.5 and 1 µM of PGE_2. Cells were fixed, washed and labelled. For double staining of α-SMA (green) and vimentin (red), cells were marked with α-SMA antibody and vimentin marker. Nuclei were stained with DAPI. Representative images were captured on fluorescence microscopy at 40x magnification.

Three-day treatment of FGH cells with different doses of PGE_2



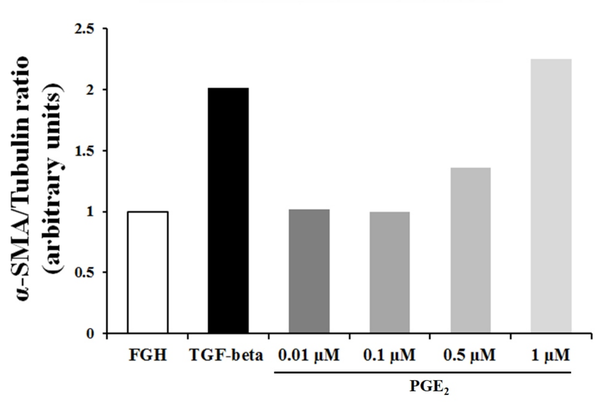


Figure 3: FGH cells were cultured in standard conditions for 72 h and treat each day with or without 0.01, 0.1, 0.5 and 1 µM of PGE_2. Lysates of FGH cells were immunoblotted with anti-α-SMA antibody. α-Tubulin was used as load. The graph is representative of two experiments and shows the fold increase relative to the control (FGH).

Three-day treatment of fibroblasts NF-33 obtained from patients with different doses of PGE_2

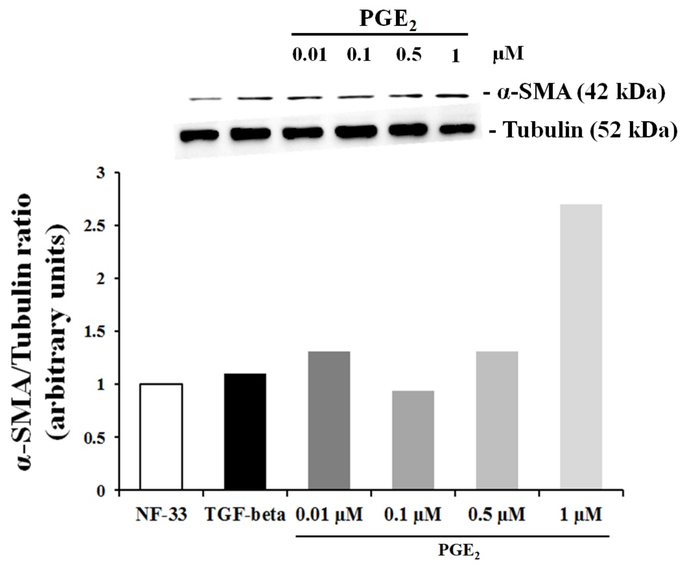
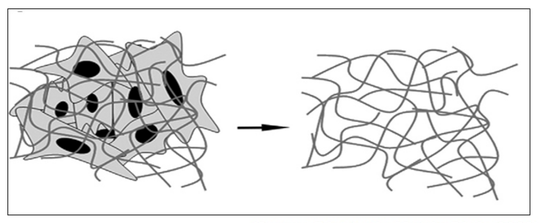


Figure 4: Fibroblasts NF-33 extracted from normal tissue (5-10 cm away from the tumor) of patients diagnosed with colorectal cancer and indicated for surgery of resection of colonic or rectal tissue were cultured in standard conditions for 72 h and treat each day with or without 0.01, 0.1, 0.5 and 1 µM of PGE_2. Lysates of NF-33 cells were immunoblotted with anti-α-SMA antibody. α-Tubulin was used as load. The graph is representative of one experiment and shows the fold increase relative to the control (NF-33).

PROSPECTS

Characterize the ECM produced by FGH stimulated with TGF-beta and PGE_2

3D Model of the matrix extraction from MDAMB-231 and MCF-7: MDAMB-231 and MCF-7 cells were seeded at confluence on 96-well plates at 37ºC and 5% of CO_2. Celular and nuclear materials were extracted by incubation with 0.1% Triton X-100 and 100 mM NH4OH until the cells were floating to obtain only the matrix derived from MCF-7 and MDAMB-231 cells. Matrix was blocked with BSA 0,1% for 30 min and used immediatelly.



(Soucy PA et. al., Matrix Biol., 2009)

Investigate the effect of fibroblast-derived ECM on colorectal cancer cells EMT.

- Epithelial markers
- Mesenchymal markers
- Invasive and migratory potential

Study the signaling pathways involved, such as signaling dependent activation of integrins responsible for cell-MEC adhesion.

CONCLUSION

Our preliminary data show that PGE_2 is able to activate fibroblasts. Activated fibroblasts can modify the production of ECM proteins, contributing to the remodeling of the ECM in the tumor microenvironment, being able to lead EMT and promote the progression of colorectal cancer

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA