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Effects of Cancer Associated Fibroblasts in resistance to oxaliplatin, 5-fluorouracil and cetuximab in Colorectal Cancer

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Introduction

Colorectal cancer is one of the most frequent with high mortality rates wideworld. Conventional chemotherapy (i.e oxaliplatin and 5-fluorouracil) and the use of target agents like anti-EGFR monoclonal antibody cetuximab, are the treatments for this tumor (Van Cutsem, E. et al, New England Journal of Medicine, 2009). However, not all patients respond to treatment, and some resistance mechanisms are still unknown.

Cancer-Associated Fibroblasts (CAFs) are a heterogeneous population of activated fibroblasts present in the tumor microenvironment that promote tumor growth and progression (Herrera et al, Clinical and Translational Oncology, 2016). It has been observed that the secretion of several soluble factors by CAFs or the mechanisms of the tumor cells adhesion to the extracellular matrix (ECM) are determinant in the chemoresistance processes (Kalluri, R., Nat Rev Cancer, 2016).

Under this scenario, we propose to investigate the involvement of CAF-derived ECM and paracrine CAFs signals on the resistance to oxaliplatin, 5-FU and cetuximab of the tumor colon cells.

Objectives

We generated ECM derived from immortalized fibroblasts cell lines as BJhTERT which were stimulated with PDGF-BB or TGF-b to analyse possible differences in ECM composition and fibers organisation. Secondly, chemoresistance of colon cancer cells Difi and SW480-ADH seeded in generated matrices was evaluated.

In parallel, ECM derived from human primary established CAFs were developed to identify and validate possible chemoresistance mediators by in vitro experiments. Ongoing experiment will include primary CAFs classification based on resistance conferred to oxaliplatin and cetuximab in colon cancer cells.

Methodology

Fresh colon tissue samples were obtained from patients operated at Hospital Ramón y Cajal. Normal tissue and tumor samples were cultured and normal fibroblasts (NFs) and CAFs were obtained as previously described by Herrera et al (Bioprotocol, 2016) and stored at -80ºC to further use.

ECM were generated with immortalized fibroblasts BJhTERT, NFs and CAFs (Herrera et al, Oncogenesis, 2018). SW480-ADH and DIFI tumor cell chemoresistance was tested, by fluorescence, in previously dye labelled cells, seeded on generated ECM after 24 or 48 h

cultures. IC50 doses were previously obtained for oxaliplatin, 5-FU and cetuximab using a panel of colon cancer cells.

Informed written consent was obtained from all participants. Colon cancer-derived samples were obtained immediately after surgery, and processed within 24 hours.

Results

Preliminary results show that PDGF-activated fibroblasts stimulate extracellular matrix fiber remodelling and deposition and increase resistance to oxaliplatin in SW480-ADH cells and to cetuximab in DIFI cells after 24 h culture. Similarly, TGF-b-stimulated fibroblasts generate ECM which confer more resistance to oxaliplatin and 5-FU in SW480-ADH after 24 h culture.

Moreover, ECM generated from different human primary established primary CAFs showed significant differences in ECM-derived chemoresistance effects on cancer cells.

Conclusions

Fibroblasts PDGF or TGF-b stimulation induce tumor cells chemoresistance mediated by derived changes in ECM generation. Moreover, matrices derived from human primary established CAFs confer different levels of resistance to oxaliplatin and/or cetuximab of colon cancer cells. Matrices derived by CAFs promote higher survival rates to colon cancer cells to oxaliplatin or cetuximab treatment than NFs´ matrices.

Future experiments will include identification of possible mediators and pathways involved in these mechanism by RNA sequencing and in vitro or in vivo experiments.

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