ImmunoTools *special* Award 2021

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Decoding novel therapeutic targets against rectal cancer iron metabolism and immune microenvironment

Background

Rectal carcinoma accounts for more than 30% of all colorectal cancer cases, being the seventh most incident cancer worldwide. However, rectal cancer is usually referred to as “Colorectal”, comprising both the rectum as well as the colon cancer. Nevertheless, these two identities are distinct in terms of molecular, physiology and therapy response, and awareness of studying these anatomic locations separately is mandatory. Neoadjuvant radiotherapy corresponds to the standard treatment for this cancer, once 50% of all cases present an advanced disease at diagnosis. Nevertheless, the response to ionizing radiation differs widely, ranging from individuals with complete remission response or to patients whose tumour is resistant to therapy.

Aims of the study

Considering that 1) most of the studies only focused on the colon section, 2) most research is performed on tumour cells alone, ignoring the intrinsic interactions of the tumour with its microenvironment, and 3) the scarcity of knowledge on the impact of ionizing radiation on bridging rectal cancer microenvironment and its iron metabolism, we propose to uncover altered molecular pathways from the iron metabolism and immune profile of rectal cancer microenvironment, paving the way for relevant scientific questions. Furthermore, we will scrutinize the iron metabolism and immune-related alterations in the irradiated rectal tumour milieu, a novel approach for the discovery of new therapeutic targets for rectal cancer, and unveil new combinatory therapies to radiotherapy-treated tumours.

Methodology

Task 1 – Development of a biomimetic 3D rectal cancer immunospheroids (RCIS)
Within this task, we aim to develop an innovative radiosensitive (rs) and radioresistant (rr) RCIS culture composed of rectal tumour cells, primary macrophages, and T lymphocytes. RCIS will be characterized for the respective tumour features and acquired immune profile by flow cytometry, namely by CD14, CD86, CD80 and CD163, to characterize pro-inflammatory and anti-inflammatory macrophage phenotype lineage, respectively (ImmunoTools). CD3, CD8, CD4, CD25 and Foxp3 to assess T lymphocyte lineage, namely, cytotoxic T, T helper and Tregs (ImmunoTools). Thereafter, radiation mimicking one week of short scheme standard radiotherapy treatment of rectal cancer patients (5 Grays (Gy) /5 days) will be employed, followed by the validation of the radiation action, and subsequent characterization of the immune profile changes of

the irradiated RCIS, namely the expression of CD14, CD86, CD80, CD163, CD3, CD8, CD4, CD25 and Foxp3 markers (ImmunoTools).

Task 2 - Identification of novel iron metabolism and immune targets on irradiated RCIS
Molecular targets altered upon radiotherapy treatment will be scrutinized in irradiated and non-irradiated rsRCIS and rrRCIS. This unprecedented analysis will be performed not only using a transcriptomic analysis of a 579-multiplex panel of immunology- and iron-related genes but also by mass spectrometry (Proteomics) and inductively coupled plasma-mass spectrometry (ICP-MS) analysis.

Task 3 - Clinical validation of the identified targets in a retrospective rectal cancer patient cohort submitted to neoadjuvant radiotherapy
The identified key players will be validated in RCIS cultures and in a retrospective rectal cancer cohort of 150 patients (biopsy samples at diagnosis and surgical resections upon neoadjuvant radiotherapy). The results will also be cross-analyzed with clinical-pathological data.

Task 4 - Modulation of the identified targets in an orthotopic mouse model of rectal cancer
Within this task, we aim to develop an orthotopic animal model of rectal cancer. This model will be critical to assess the effect on cancer progression and metastasis following the modulation of the most promising identified targets using pharmacological inhibitors coupled with the conventional radiotherapy schemes.

The projects' results with the generous help of *ImmunoTools* will allow the identification of new biomarkers and improved therapeutic options that, in combination with radiotherapy, could decrease radioresistance and improve overall survival and quality of life in rectal cancer patients.

Key references

The Royal College of Radiologists. Radiotherapy dose fractionation, third edition. Clin. Oncol. 17 (2019).
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Peirsman, A.et al. MISpheroID: A Knowledgebase and Transparency Tool for Minimum Information in Spheroid Identity. 1–26 2021.

ImmunoTools *special* AWARD for **Diogo Estêvão** includes 9 reagents

FITC - conjugated anti-human CD14, CD47, CD53

PE - conjugated anti-human CD3, CD71, CD80

APC - conjugated anti-human CD8, CD14, CD86

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