Introduction

Colorectal Cancer (CRC) presents high incidence and mortality due to the ineffectiveness of conventional therapies, especially in patients with advanced stages. In the past years, immunotherapy has evolved as a therapeutic approach in specific CRC clinical settings.

The Major Histocompatibility Complex class I (MHC-I) consists of a group of cell surface proteins with the function of facilitating antigen-presentation to relevant immune cells, and absence or downregulation of MHC-I in tumour cells represents a common tumour immune evasion mechanism, making immunotherapy inefficient.

Cancer stem cells (CSCs) are a subpopulation of tumour cells displaying tumour initiation capacity and are increasingly recognized as a source of treatment resistance. MHC-I negative cells have shown higher tumour initiation capacity and sphere formation than MHC-I positive cells in a sarcoma model.

Aim

The main goal of this project is to evaluate whether MHC-I negative cancer cells show higher tumour initiation capacity than MHC-I positive cells, thus displaying a CSC properties.

Methodology

Four CRC cell lines (SW480, SW620, LoVo and HKe3) were grown in appropriated medium and the MHC-I and Lgr5 expression was analysed by flow cytometry. Then the cell lines were sorted by MHC-I and Lgr5 expression in four groups (All cells, MHC-I negative, MHC-I positive/Lgr5 positive and MHC-I positive/Lgr5 negative) and sphere formation assays were performed. The cells (1000 per well) to generate spheroids were grown for ten days in DMEM/F12-K medium supplemented with 1XB27, 20 ng/ml human recombinant epidermal growth factor and 20 ng/ml basic fibroblast growth fa-

Tumour xenograft experiments in NOD/SCID mice will be done to assess tumour initiation of the different tumour cells subpopulations. Fresh tumour specimens from patients with CRC will be sorted and sphere formation assays and tumour xenograft experiments will also be performed. Formed spheroids were fixed in formalin and will be characterized by flow cytometry after dissociation, immunofluorescence in paraffin after being embedded in agarose and by 3D immunofluorescence imaging. Experiments were done in triplicates. The statistics were performed by GraphPad where p-value is *0.05, **0.01, ***0.001.

Results

We observed presence of MHC-I negative cells in all the four cell lines analysed. The cell line that shows higher percentage is the LoVo cell line (99.95%), followed by the SW480 cell line (92.33%), with the SW620 (0.14%) and the HKe3 (0.03%) being the two with less expression, the last expressing the least.

In the four sorted cells groups (All cells, MHC-I negative, MHC-I positive/Lgr5 positive and MHC-I positive/Lgr5 negative), spheroid formation ability was different amongst the cell lines, being MHC-I positive/Lgr5 negative group the most efficient. The number of spheroids (mean±SD) formed was different within the CRC cell lines, showing distinct morphologies and sizes. The HKe3 cell line generated the biggest spheroids in all groups when compared with the other CRC cell lines.

Conclusion

These studies will provide new insight in stemness and tumour initiation capacities of MHC-I negative CRC cells to ultimately better understand tumour physiology and organization.

References