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Lab/Institution: Nutraceuticals & Delivery Lab/ IBET&ITQB-UNL; Colon Pathology Study Group/ IPOLFG.
TITLE: Effect of citrus bioactive compound on targeting human colorectal cancer stem cells

BACKGROUND

Cancer is one of the major causes of death worldwide. In particular, colorectal cancer is the second most frequent malignant disease in Europe, causing over 3000 deaths per year in Portugal. Treatments for recurrent and metastatic diseases remain a centre of clinical attention. While continuing efforts have been made for discovering new molecular target based molecules, there is an emerging interest in chemotherapeutic application of natural substances. In particular plant derived bioactive compounds have been demonstrated to reduce tumorgenesis, to revert cancer related epigenetic dysfunctions, to prevent metastasis and to increase chemo and radiotherapy efficacy. More recently, some plant derived bioactive compounds showed to selectively target cancer stem cells (CSC), a minor cell population within the tumor that presents continuous self-renewal and differentiation capability. This CSC population has been considered to be responsible for tumor initiation, relapse and resistance to chemotherapy and thus it is highlighted as a promising target for cancer prevention and therapy.
Epidemiological studies have linked the consumption of fruits and fruit juices with a reduced risk of colorectal cancer. In this field citrus fruits (e.g. orange, lemon) are recognized to be rich sources of natural anticancer compounds. Terpenes and flavonoids (hesperidin, naringenin, tangeretin, sinensetin and nobiletin) are the main bioactive molecules of citrus fruits, juices and peels that already identified to inhibit colorectal tumorogenesis in vitro and in vivo. However, the majority of these studies have been performed mainly on cells grown on monolayers (2D) or animal that present major weakness: compound sensitivity data obtained from 2D systems are often misleading, while animals models are expensive, time consuming and present ethical dilemmas. Additionally, there is no information regarding the activity of citrus compounds on human CSCs. Preliminary data obtained by our group indicated that an orange peel extract containing nobiletin, sinensetin and tangeretin showed anticancer effect in a 3D cell model of colorectal cancer, by inhibiting cell proliferation and inducing cell cycle arrest and apoptosis in HT29 cell aggregates. This cell model was developed in our laboratory and presents similarities with in vivo tumor, including similar phenotype and resistance to chemotherapy agents. Additionally, this cell model presented a higher percentage of CSCs.

OBJECTIVES

The goal of this project is to evaluate the effect of citrus bioactive compounds on CSC population present in aggregates of human colorectal cancer cell line (HT29).
The main objectives proposed are:

- Development and characterization of HT29 cell aggregates enrich in CSCs;
- Evaluation of the effect of citrus bioactive compounds on targeting CSCs: i) decreasing CSC population and ii) modulating CSC self-renewal and stemness pathways.
- Identification of the most effective anticancer compounds and synergetic effects

PROJECT DESCRIPTION

The project work plan is organized into 3 tasks, as described below:

TASK 1- Citrus juices and extracts: phytochemical characterization and cytotoxicity evaluation
In this task, citrus juices and citrus peel extracts derived from orange and lemon that were produced by the host lab will be characterize in terms of phytochemical composition namely, terpenes and flavonoids, using different chromatographic techniques (HPLC-UV-DAD, HPLC-MS, GC). This phytochemical characterization will be also performed to digested samples aiming at identifying and characterizing citrus metabolites obtained after gastrointestinal digestion step. Gastrointestinal cytotoxicity of all citrus samples (juices, extracts and digested samples) and standard compounds will be assessed in human colon and gastric cell lines.

TASK 2- Development of HT29 cell aggregates enrich in CSCs
In this task, 3D cell aggregates of human colorectal immortalized cell line (HT29) will be developed. This cell line will be cultivated as aggregates under stirred condition using computer controlled stirred tank bioreactors where the key environmental conditions for cancer cell culture (e.g. pO2, pH, T) are finely controlled. In this model, the presence of CSCs will be monitored during culture time by measuring specific cell surface and phenotypic markers (e.g. CD44, CD133, CD24 and ALDH activity) using confocal and flow cytometry tools.

TASK 3- Effect of citrus bioactive compounds on targeting CSC population
In this task, the capacity of citrus juices/peel extracts and standard bioactive compounds in targeting CSCs will be tested using the 3D cell model developed in Task 2. In a first approach, the effect of citrus samples and bioactive compounds in decreasing CSC population will be evaluated. Then, the most effective samples will be selected for the evaluation of key signaling pathways related with CSCs self-renewal and stemness capacity (e.g. down regulation self-renewal pathways, anti-apoptotic genes/proteins and EMT related factors; up regulation of pro-apoptotic and tumor suppressor genes/proteins; and induction differentiation and cell cycle arrest) by using several analytical tools, including western blotting analysis, RNAseq, RTPCR, flow cytometry and imunofluorescence microscopy.

TIMELINE (use fill tool for the cells)

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10
Task 1										
Task 2										
Task 3										
Thesis										