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TITLE: Elucidating the mechanisms of the chemotherapeutic effect of natural compounds using cell models of colorectal cancer - Correlation of molecular structure with functionality .

BACKGROUND

Recently there is a growing interest in cancer treatment through the use of natural compounds. In particular, phytochemicals (e.g. phenolic compounds, monoterpenes, isothiocyanates) found in fruits and vegetables are very attractive in the prevention and chemotherapy of various types of cancer. The mechanisms by which these compounds inhibit tumorigenesis includes i) antioxidant, antiproliferative and anti-inflammatory effects, ii) induction of cell cycle arrest and apoptosis, and iii) inhibition angiogenesis and metastasis.

The successful design of novel natural anticancer agents requires the use of predictable and robust cancer models that better mimic the in vivo tumors. Until now, research testing the efficacy of novel anticancer drugs has been performed mainly on cells grown on monolayers (2D) or animal model. However, these two systems present major weakness: drug sensitivity data obtained from 2D systems are often misleading, while animals models are expensive, time consuming and present ethical dilemmas. Recent research has demonstrated that 3D culture systems are promising candidates. By providing a cellular context closer to actually occur in in vivo cancer microenvironments, these strategies would provide a higher degree of predictability and improved robustness.

OBJECTIVES

This master workplan is a part of a project aiming at correlating the structure of natural anticancer biomolecules present in different natural matrices with their chemoterapeutic effect towards colorectal cancer. The main objectives proposed are:

- Evaluation of the anticancer properties of several phytochemical-rich extracts and standard compounds using 2D and 3D cell models of colorectal cancer;
- Establishment of structure-activity relationship.

PROJECT DESCRIPTION

The project work plan is organized into 4 tasks, as described below:

TASK 1- Selection of phytochemical-rich extracts

Natural extracts derived from several sources (e.g. fruits, vegetables and plants) previously developed and characterized by the host lab, will be selected taking into account their phytochemical composition.

TASK 2- Gastrointestinal cytotoxic and antiproliferative effect in 2D cell models of colorectal cancer

The antiproliferative activity of phytochemical-rich extracts selected in Task 1 and standard compounds will be evaluated on HT29 cell monolayers. For each sample, different incubation times and several concentrations will be tested in order to determine the effective dose values (ED50). Cell viability will be evaluated using two assays namely MTS, CyQuant. Additionally, BrdU method will be used to quantify actively dividing cells and thus better characterizing the antiproliferative effect of extracts. The impact of phytochemical-rich extracts/standard compounds on inducing cell cycle arrest will be assessed by flow cytometry. Cell apoptosis will be evaluated by caspase 3 activity detection. Gastrointestinal toxicity of natural extracts/standard compounds will be evaluated in human colon (Caco2) cell lines. The assay will be performed using Cyto-tox-Glow kit to measure cell dead and viability. The IC50 (index of cytotoxicity) values will be determined for each sample.

TASK 3- Anticancer effect in 3D cell models of colorectal cancer

In this task, the anticancer effect the most promising extracts and standard compounds (selected from Task 2) will be evaluated in a 3D cell model of colorectal cancer already developed by the host lab (aggregates of HT29 cell line). Several hallmarks of cancer will be studied including antiproliferative effect, induction of cell cycle arrest, induction of apoptosis, inhibition angiogenesis and metastasis. Additionally, the effect of samples on the phenotypical characteristics of cell aggregates will be also explored by analyzing stem and epithelial/mesenchymal character of cells.

TASK 4- Structure – activity relationship

In this task, the results obtained in TASK 2 and TASK 3 will be combined with the structure of the molecules for the quantitative structure activity relationship analysis –QSAR studies- using chemometric methods. Correlation studies between phytochemical composition of natural extracts with bioactivity results will be performed aiming at identifying synergistic/antagonistic effects between natural compounds of the samples.

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										
Task 4										
Thesis										