INSTITUTO DE TECNOLOGIA QUÍMICA E BIOLÓGICA /UNL
Knowledge Creation

MSc Research Project

Supervisor(s): Luís Gafeira Gonçalves
Supervisor(s) email address: lgafeira@itqb.unl.pt
Lab/Institution: Cell Physiology & NMR, ITQB
TITLE: A metabolomics approach to the study of Ovarian Cancer

BACKGROUND

Ovarian cancer (OC) is the most lethal of all common gynecologic malignancies, with more than 204,000 new cases and 125,000 deaths each year, accounting for 4% of all cancer cases and 4.2% of all cancer deaths in women around the world. In Portugal, according to the National Oncologic Registry in 2001, the incidence of ovarian cancer was 8.3 per 100,000 people.

Early ovarian cancer is not associated with symptoms therefore detection is often by chance. Currently, there are no acceptable screening techniques available. Though, early detection strategies to identify ovarian cancer precursor lesions or early-stage carcinomas should theoretically have a major impact on mortality and survival in patients. New insights and approaches should be considered, leading to the development of ground-breaking detection techniques and therapeutic interventions.

Tumour cell metabolism was recently raised as an emerging hallmark in cancer. Data accumulated in recent years suggest that tumour microenvironment plays a key role in tumour development and metastases, with impact on the response to therapy. Therefore, metabolic profiling of tumour microenvironment presents exciting opportunities for the development of new therapeutic approaches. The very broad range of NMR techniques and the non invasive character makes NMR a very powerful tool to study cell metabolism.

OBJECTIVES

Characterize the metabolic profiles of ascitic fluid and serum from ovarian cancer patients.

To define the metabolic profile of different histological types of ovarian cancer, using peripheral blood (PB), in order to identify metabolites that can be used in the future as biomarkers for ovarian cancer development.

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PROJECT DESCRIPTION

Task 1: Metabolomic characterization of ascitic fluid and peripheral blood (PB) of ovarian cancer patients.

Experimental approach:

Biological samples: Ascities fluid and PB serum samples will be centrifuged for cell separation and immediately stored at -80 °C. Sample processing will involve deproteinization.

NMR-based quantitative metabolomics: ^{1}H NMR spectra will be acquired on a high-field spectrometer (800 MHz Bruker Avance II) equipped with liquid sample probes hosted in CERMAX facility at ITQB. The analyses of metabolites will be performed by using 1D ^{1}H spectra. Metabolite identification will make use of ^{1}H-^{1}H total correlation (TOCSY) NMR spectra, 2D ^{13}C-^{1}H Heteronuclear Single Quantum Coherence (HSQC) and with the aid of the publicly available Human Metabolome database (http://www.hmdb.ca) and CHENOMX software. Metabolite concentrations will be measured from the peak areas when referenced internally to compounds of known concentration or using external a calibration method.

Chemometrics: The NMR data sets will be analysed using multivariate analysis methods including Principal Components Analysis (PCA), Partial Least Squares Regression Discriminant Analysis (PLS-DA) or Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA). These methods will allow the establishment of predictive models of OC and the identification of upregulated/downregulated metabolites.

Task 2: Metabolic analsysis of ex vivo and in vitro cell cultures of ovarian cancer

Biological samples: The metabolic activity of tumour cell will be assessed by culturing ovarian carcinoma cells (primary and immortalised) in culture medium supplemented with [^{13}C] glucose, [^{13}C] glutamine or [^{13}C] oleic acid, 24 h in 24-well plates; metabolites derived from the labeled substrates will be analysed by ^{13}C-NMR both in cell extracts and supernatants**.** Lipidic and water-soluble cell/tissue fractions will be extracted using a mixture of methanol/chloroform/water.

NMR spectroscopy: Besides the NMR experiments described in the task 1, it will also acquired ^{13}C spectra and ^{13}C-^{13}C correlation spectroscopy (COSY) NMR.

Task 3: Writing of the thesis

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										