M.Sc. Project

Title: Cerebrospinal fluid metabolome: an instructive niche for CNS metastasis

Supervisors: Dr. Luís G. Gonçalves/ Prof. Helena Santos/ Dr. Tânia Carvalho

Host Laboratory: Cell Physiology & NMR, ITQB-UNL, Oeiras

Duration: 1 year

It is estimated that 10% to 30% of patients with solid tumors including breast, lung, melanoma and colorectal cancer, are diagnosed with central nervous system (CNS) metastasis. Within the CNS, tumor cells are frequently in direct contact with the cerebrospinal fluid (CSF), often spatially distant from blood vessels, and although vascular sources of secreted proliferative and migratory signals in cancer are well characterized, the same is not true for the CSF.

Metabolism has recently emerged as a “rediscovered” research area, with increasing number of Labs interested in finding causal links between metabolic traits (adaptation or selection) of tumor cells and phenotypical features granting tumor cells advantage over their normal counterparts. One of the biochemical parameters routinely used for diagnosing CNS metastasis is the low CSF glucose levels, which is due to consumption of this metabolite by tumor cells, but very little is known about the metabolic switches on CNS. We propose to study the CSF of cancer patients with/without CNS metastasis using a metabolome-based approach to define a “Metabolic signature” of CNS compromise/invasion by tumor cells.

The CSF samples will be analyzed by ^{1}H-NMR, at high field (800 MHz Bruker Avance IV instrument). From our preliminary screening of CSF samples, the use of the first transient of tnnoesy-presaturation pulse sequence (noesypr1d) provides good signal ratio and suitable water suppression. Identification of compounds will be performed by Chenomx NMR Suite program and Human Metabolome database (http://www.hmdb.ca). Classification of the different types of samples will be performed by Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA), and Orthogonal Projections to Latent Structures (OPLS-DA) methods which are well established in metabolomic studies. This part of the workflow will be implemented in the R language (http://www.r-project.org), specifically using the package 'Metabonomic' and its dependencies, available at The Comprehensive R Archive Network (http://cran.r-project.org).

In a second part of the work, in collaboration of Dr. Tânia Carvalho (IMM), the cells present in the CSF will be recovered and used for ex-vivo cell culture assays. The

glycolytic activity of tumor cell will be assessed by culturing CSF tumor cells retrieved by centrifugation in artificial CSF supplemented with [^{13}C] labeled glucose, glutamine or oleic acid, 24 h in 24-well plates; metabolites derived from the labeled substrates will be analyzed by ^{13}C-NMR both in cell extracts and supernatants. Tumor matched cell lines and, whenever possible, tumor cells from the same patients' primary tumor will be used as control.

Methodologies

- NMR spectroscopy;
- Metabolite profiling;
- Statistical analysis;
- Cell culturing

Contacts

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