INSTITUTO DE TECNOLOGIA QUÍMICA E BIOLÓGICA /UNL

Knowledge Creation

Master Dissertation Project

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Lab/Institution: Mass Spectrometry - ITQB

TITLE: Mitochondrial proteome characterization during neural differentiation

BACKGROUND

Stem cell therapy is a strategy far from being satisfactory and applied in the clinic. The poor survival and differentiation levels of stem cells after either transplantation or neural injury have been one of the major problems. Therefore, a better understanding of the basic molecular mechanisms, and potential therapeutic approaches, responsible for long-term survival and differentiation of stem cells may represent a step in the right direction. Once identified, these molecular mechanisms could be strategically manipulated to improve neural differentiation as an alternative to cell death, bringing stem cells one-step closer to a successful application in neural replacement therapies. Recent evidence suggests that mitochondria affect the proliferative and differentiation potential of neural stem cells (NSC). In addition, it was already demonstrated the involvement of mitochondrial apoptosis events in mouse NSC differentiation, by mechanisms that do not result in cell death. As a general rule, it thus appear that cell-death-relevant proteins, especially those involved in the core of the executing apoptosis machinery, have a dual function in differentiation and cell death. In fact, essential players of apoptotic executioner pathways cannot be totally blocked to assure differentiation efficiency but, at the same time, must be carefully regulated to avoid cell loss. It was also discovered that the endogenous bile acid tauroursodeoxycholic acid (TU) is a strong inhibitor of apoptosis by modulating mitochondrial-signaling pathways. However, the precise mechanisms by which differentiating cells can block pre-activated apoptosis during differentiation are still largely unknown. A differential proteomic approach will help to advance the global knowledge of the molecular targets that could be manipulated to redirect NSCs for differentiation and/or reprogramming processes, as an alternative to cell death.

This project is within a FCT financed project "Driving Mitochondrial Effectors of Apoptosis Toward Neural Differentiation REF: PTDC/BIM-MED/0251/2012".

OBJECTIVES

Characterize the mitochondrial proteome during neural differentiation.

- Establish differentiation-apoptosis differences in terms of mitochondrial protein patterns, by analyzing neural stem cells under differentiation and non-differentiating stages.
- Test whether TU regulates the mitochondrial proteome.

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PROJECT DESCRIPTION

To screen up- and down-regulated proteins during neural differentiation conditions, as well as the role of TU in modulating these evenst, differential proteomics will be performed with mitochondrial extracts from NSC. The mitochondrial protein extracts will be prepared by the FFUL team

Task 1: The protein extracts for each condition (cells under differentiation and non-differentiating stages with or without TU treatment) will be quantified and digested with trypsin.
Task 2: MS and MS/MS data collection: The differentially expressed proteins between the cells under differentiation and non-differentiating stages and for each one of these groups with or without TU treatment will be quantified and identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based proteomics. Label free relative quantification will be performed using the sum of the chromatographic peak intensities determined for the several peptides used in the identification of each protein.
Task 3:Protein identification and quantification data treatment: Profile data from the MS scans are transformed to *m/z* peak lists. Protein identification will be performed using the MS/MS spectra and submitting to a search engine using a general database. For quantification, all reimported peptides of an identified protein are included, and the total cumulative abundance is calculated by summing the abundances of all peptides allocated to the respective protein.
Task 4: Pathway analysis using the proteins identified with expression differences between each pair of sample groups
Task 5: Validation of the differential proteomic data by the LC-SRM analysis.
Task 6: Writing of Master 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										
Task 4										
Task 5										
Thesis										