The role of secondary modification of S100B in protein aggregation and its influence on Alzheimer's disease pathology.

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Objectives: In this project, we want to investigate the interrelationship between S100B/oxidative modifications of S100B, S100B aggregation, and trace metal homeostasis in a physiological context, but also in the background of Alzheimer's disease (AD) with focus on effects on Amyloid beta (Aβ) protein aggregation. These interactions will be modelled in vitro, where cells will be exposed to S100B/modified S100B in different concentrations with and without presence of Aβ aggregates, and where trace metal levels as well as aggregation status and kinetics of both S100B and Aβ aggregation will be monitored. In addition, interactions will be modelled in vivo after injection of S100B/oxidative modifications of S100B into wild type (WT) and 5xFAD mice, a model for AD. There, S100B aggregation will be determined in WT vs. the AD mouse in an age dependent, treatment time dependent, and dose dependent manner, and effects on trace metal levels and Aβ aggregation quantified. Based on the obtained data, using a bioinformatics based approach, the interrelationship of the aforementioned factors will be modelled and used for proteomics studies to identify common pathways affected by the dysregulation of one or multiple of these factors, which may act as promising targets for drug development in the future.

Methodology: S100B proteins and especially oxidative modifications of S100 proteins may be important functional regulators of Aβ aggregation and metal-binding/regulation of Aβ. However, the relationship between S100B protein modification, trace metals levels and Aβ aggregation are not yet determined. To establish a model for these interactions, we propose several in vitro and in vivo studies:

In vitro: Primary neuronal cells from mouse cortex will be used and exposed to different concentrations of S100B/modifications of S100B and/or trace metals in presence and absence of Aβ. S100B and modified S100B protein will be produced and characterized in Dr. Gomes' lab. Using immunocytochemistry and high resolution fluorescent microscopy, protein aggregates and trace metals will be visualized and quantified in Dr. Grabrucker's lab. Cell culturing methods are established in his lab and the lab was recently awarded 620.000€ to complement their confocal microscope with an ImageXpress-Micro High-Content Imaging System and a real-time xCELLigence CardioECR. Using these devices, cell treatments and fluorescence detection will be performed using a high content imaging system with automated robotic liquid handling for on-board compound addition, controlled temperature, CO2, and humidity for live and fixed samples in a 96 well format. Further, protein aggregation will be analysed using advanced biophysics (Circular dichroism, fluorescence and ATR-FTIR) and biochemical approaches in Dr. Gomes' and Dr. Grabrucker's lab. Based on the obtained data, the interrelationship between the aforementioned factors will be modelled using a computational approach.

In vivo: WT and AD mice will be exposed to different concentrations of S100B/ modified S100B at different developmental stages and brains analysed. We will use immunohistochemistry, fluorescence micropscopy and protein biochemistry to investigate the relationship between S100B modification, trace metal levels and AD pathology. We will further use a RT-QuIC assay (Dr. Gomes' lab). This assay allows to determine if a tissue can seed the aggregation of a given protein. We will use brain extracts from AD and different S100B injected AD mice to probe (systematically using different regions of the brains) if they contain seeds that can be detected/amplified in these RT-QuIC assays. We further analyse protein expression by Proteomics methods such as mass spectrometry (MS). MS will be carried out in University of Limerick in conjunction with the new Science Foundation Ireland funded BioPoint

structural project, a state of the art MS facility. The brain tissue for analysis will be examined using quantitative label free MS to generate expression-based datasets. These datasets will be internally verified and downstream proteomic analysis will be used to detect key markers and pathways for further evaluation. We will identify of a core subset of proteins significantly regulated across all experimental groups or only within one experimental group. This core subset will be employed to create a training set for downstream machine learning techniques. In this way, we can identify biomarkers for specific conditions (AD, high S100B levels, trace metal interactions) and verify them in our cell culture system. This data will be integrated in the computational model.

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