Effect of H. pylori and P. mirabilis ureases in the protein expression of cell lines and their relation with neurodegenerative diseases

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Neurodegenerative diseases, particularly Alzheimer's Disease (AD) and Parkinson's Disease (PD), have been afflicting an increasing proportion of the population, especially in developed countries, where the senior population is larger. Both diseases are characterized by complex and multifaceted etiologies, and so far have eluded any demonstrably effective means of prevention or treatment. Epidemiologic studies indicate a correlation between chronic infection by *Helicobacter pylori* and the incidence of both AD and PD. There are also clinical data showing an increased prevalence of *Proteus mirabilis* in the disbiosios associated with PD. Literature reports that rats treated with a *H. pylori* cell culture filtrate have developed biochemical brain alterations compatible with those of AD. There are also reports of mice colonized by *P. mirabilis* presenting motor deficit and biochemical alterations, both in the brain and colon, compatible with PD. Both bacteriae are huge producers of urease, present as microvesicles released into the intracellular medium, and capable of penetrating the blood-brain barrier.

Studies by our group have shown evidence that many ureases from different sources possess neurotoxic and neuroinflammatory activity, both *in vitro* and *in vivo*, amongst those the ureases of *H. pylori* (HPU) and *P. mirabilis* (PMU). In order to investigate a potential contribution of HPU and PMU towards pathogenesis or progression of AD and PD we have, in this work, treated *in vitro* cell cultures (SH-SY5Y, human neuroblastoma; BV-2, murine microglia) with these ureases, in order to investigate, through proteomic analysis, changes in protein expression that could be associated with these and other neurodegenerative diseases

After their incubation with urease, cellular cultures were subjected to protein extraction and analysis by mass spectrometry (nLC-MS/MS in a LTQ Orbitrap spectrometer), the resulting spectra were analyzed in the FragPipe e Perseus software. The DAVID, Reactome e GSEA software were uses to identify the ontologies and metabolic routes affected by the urease treatment. The Malacards database was used to identify genes related to AD, PD and other neurodegenerative pathologies that had their protein levels altered by the treatments.

Experiments aiming to directly identify the molecular targets of the ureases were performed through *in vivo* treatment of the cell cultures with ureases conjugated with the photoactivable extremity cross-linker SDAD, followed by an immunoprecipitation step utilizing anti-urease antibodies in order to isolate the urease, along with any protein attached to the by the cross-linker, which could then be identified by mass spectrometry.

The results obtained indicated significant changes in the protein expression of urease-treated cells, particularly in proteins related to the energy metabolism, RNA transcription and stress response routes. Of note, the glutathione-S-transferase, a protein from the eicosanoid route strongly related to neuroinflammatory processes, was found to be affected. Several proteins were also identified as possible molecular targets of the urease, such as superoxide dismutase and the Parkinson Disease Protein 7.

These results confirm previous cytological data, and reinforce the hypothesis of a pro-inflammatory and neurotoxic role for the HPU and PMU, as well as their possible contribution to neurodegenerative diseases associated with urease-positive pathogens.

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