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Immuno-laser microdissection coupled to label-free proteomics for the analysis of human brain cells to identify potential stroke biomarkers

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Background: Cerebral infarction or stroke is a leading cause of death and disability worldwide. Our research group firstly described changes in the proteome of human brain after ischemic stroke, which allow the discovery of new blood biomarkers to predict long-term functional disability. Further analysis of the components of the neurovascular unit, such as neurons and brain vessels, could contribute to a better understanding of the pathophysiology of stroke and might show up specific therapeutic targets or biomarkers.

Objective: To identify differences of protein expression in neurons and brain vessels after ischemic stroke.

Methods: Brain slices from infarcted and healthy contralateral areas of 7 patients who died following an ischemic stroke were visualized by immunofluorescence staining with NeuN (Chemicon) for neurons and with Ulex europaea agglutinin-I (UEA-I, Sigma) for vessels. By means of laser microdissection (LMD6000, Leica) we obtained both neurons and vessels from each sample in 60 µL of 0.1% Rapigest SF surfactant (Waters). Cell lysates underwent sonication, trypsin digestion, acidification (for Rapigest removal) and purification on C18 microspin columns. ESI LTQ-OT mass spectrometry was performed on a LTQ Orbitrap Velos (Thermo Electron) equipped with a NanoAcquity LC system from Waters. Gas-phase fractionation (GPF) for data-dependent analysis was performed with 4 injections of each sample with m/z ranges for precursor ions selection of 400-520, 515-690, 685-979 and 974-2000 Thomsons. Protein identification was done using Mascot and quantification and statistical comparison was performed with Progenesis® LC-MS v4.0 software, after automatic alignment of all runs and combination of the four fractions of each sample. PANTHER was used to classify proteins with Gene Ontology terms. Ingenuity Pathway Analysis (IPA) database was searched to find altered pathways after ischemia.

Results: A total of 768 proteins in neurons and 1078 proteins in vessels were identified and quantified from brain sections of ischemic stroke patients. Of these, 45.7% and 48.3% were identified with at least two peptides, respectively. When paired infarcted and contralateral areas were compared, 58 proteins in neurons and 24 proteins in vessels were found to be differentially expressed ($p \leq 0.05$, fold-change ≥ 2, and peptide count ≥ 2). PANTHER analysis revealed that most proteins are related to metabolic or binding processes. In particular, proteins quantified in neurons were involved in membrane trafficking whereas in vessels they were associated with nucleic acid binding. IPA analysis of proteins from neurons showed their involvement in neurological diseases and in cell-to-cell signaling and interaction processes. In contrast proteins from brain vessels were mainly related to cell death and survival.

Conclusions: We have described changes in protein levels of human neurons and vessels after cerebral ischemia, with differential findings regarding the type of cell. If confirmed, these results could contribute to the molecular knowledge of stroke pathology and maybe highlight candidates to be further explored as therapeutic targets or biomarkers for the diagnosis or prognosis of stroke.

Biography

Teresa García-Berrocoso received her M.Sc. in Biology in 2007 and M.Sc. in Biochemistry in 2008 from the Universitat de Barcelona (Spain). In 2011 she held a research stay in the Biomedical Proteomics Research Group at the University of Geneva (Switzerland) under the supervision of Prof. Jean-Charles Sanchez. Recently, she obtained her Ph.D. in Internal Medicine at Universitat Autònoma de Barcelona (Spain) on "Identification and use of prognostic biomarkers in ischemic stroke" directed by Dr. Joan Montaner. Her scientific career is focused on the application of biomarkers to different indications regarding human cerebral infarction. She supervises the website http://stroke-biomarkers.com, has published 17 original articles (4 of them as first author), a patent and some reviews in stroke biomarkers. She has participated in several national and international congresses and collaborates as a reviewer in European Journal of Neurology and Translational Proteomics. She is member of the International Biomarker in Cerebrovascular Diseases study-group, which was created in 2012, and from the Spanish Proteomics Society.

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