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XVII SIMPOSIUM

Acquired spinal cord and brain injuries



## **PROTECTION FROM STROKE-INDUCED NEURONAL INJURY BY INHIBITION OF AUTOPHAGY. ROLE OF BMF AND NOXA**

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## 1. Project summary

Ischaemic stroke is a leading cause of death and disability. Apart from early thrombolysis, there is no pharmacological approach for the treatment of acute stroke. In the ischaemic core, neurons and astrocytes die rapidly. In the ischaemic 'penumbra', neuronal injury progresses over time. This injury is associated with the activation of the energy sensor AMPK. In this project, we explored the role of AMPK-induced autophagy and cell death activation, and its direct relevance for the pathophysiology and future treatment of ischaemic stroke. Based on preliminary work by the applicants, we hypothesised that AMPK and autophagy inhibition may exert protective effects.

### **Methodology**

We aimed to explore the therapeutic effects of inhibition of autophagy and AMPK signalling in primary cortical neuron cultures and differentiated human neural stem cells subjected to oxygen/glucose deprivation, and in an in vivo model of transient middle cerebral artery occlusion in mice using both pharmacological and genetic approaches. We also explored the molecular mechanisms that AMPK and autophagy employ to mediate these effects. In particular we focused on the role that the Bcl-2 family proteins Noxa and Bmf, which are activated by AMPK, play in mediating cell death signalling.

## 2. Results

- Neurons subjected to ischaemia/reperfusion-like conditions in vitro were protected from cell death when Noxa and Bmf are reduced. We confirmed in vivo that deficiency of Bmf prevented cell death and reduced ischaemic damage.
- We identified that inhibition of autophagy is not suitable to prevent damage by ischaemia as it increases cell death in ischaemia/reperfusion-like conditions.
- Neuronal cell death upon ischaemia/reperfusion showed features of necroptosis and was prevented by chemical necroptosis inhibitors when cells are preincubated with these drugs.

- We also identified miRNA that were activated by the protein kinase AMPK in cerebral ischaemia and regulated cell death after ischaemia/reperfusion-like conditions.

### **3. Relevance and possible clinical implications**

This study provides significant evidence for a role of Bmf as a BH3-only protein contributing to hypoxic/ischaemic neuronal injury, but also demonstrated that the early induction of noxa did not influence neuronal survival or ischaemic injury, suggesting functional redundancy among BH3-only proteins for ischaemia-induced neuronal death, or functions of noxa independent of cell death signalling. These findings are important for future target selection strategies such as neuroprotective interventions to combat ischaemic brain injury.

Additionally, our results indicate that some inhibitors of necroptosis could be useful to ameliorate ischaemic symptoms during stroke when provided as a preventive treatment.

Identification of endogenous microRNAs (miRNA) as potent regulators of gene function elevated following ischaemia, with crucial roles as regulators of signalling pathways involved in ischaemia-reperfusion injury, represent novel and clinically useful diagnostic and prognostic indicators for outcome in patients after ischaemic stroke, and neuroprotective agents aimed at rescuing ischaemic neurons from irreversible injury, improving neurological outcome and facilitating brain recovery. The roles played by microRNAs and their dysregulation in disease, their ability to regulate multiple genes in similar pathways, combined with remarkable stability leave them uniquely poised as ideal therapeutic targets, entering many clinical trials. Data from this grant has provided the basis for progression to in vivo trials of one microRNA as a novel therapeutic target and prognostic blood biomarker in clinical intervention for neuroprotection in ischaemia-reperfusion injury.

This research has significantly increased our understanding of the pathophysiology of stroke, but also helped to define new therapeutic approaches for the treatment of ischaemic stroke.

## 4. Publications

### Published articles

“Glucose-starved cells do not engage in prosurvival autophagy.”

Ramírez-Peinado S, León-Annicchiarico CL, Galindo-Moreno J, Iurlaro R, Caro-Maldonado A, Prehn JH, Ryan KM, Muñoz-Pinedo C.

J Biol Chem. 2013 Oct 18;288(42):30387-98

Open access:

<http://www.jbc.org/content/early/2013/09/06/jbc.M113.490581.full.pdf+html>

“Analysis of BH3-Only Proteins Upregulated in Response to Oxygen/Glucose Deprivation in Cortical Neurons Identifies Bmf but not Noxa as Potential Mediator of Neuronal Injury.”

Shona Pfeiffer, Gang Chen, Silvia Ramírez-Peinado, Ujval Anilkumar, Orla Watters, Cristina Muñoz-Pinedo, and Jochen H. M. Prehn.

Cell Death Dis. 2014 Oct 9;5:e1456

Open access: <http://www.nature.com/cddis/journal/v5/n10/full/cddis2014426a.html>

### Conference presentations

Protection from oxygen glucose/deprivation-induced neuronal injury by deletion of noxa and bmf

Shona Pfeiffer, Silvia Ramírez-Peinado, Gang Chen, Cristina Munoz-Pinedo, Jochen H.M. Prehn

Cold Spring Harbor Laboratories Cell Death meeting 2013

Identification of miRNA regulated by activation of AMP-activated protein kinase during ischemic neuronal injury

Shona Pfeiffer, Gang Chen and Jochen H.M. Prehn

Society for Neuroscience Meeting, Chicago, IL, USA (2015).

### Article in preparation

“Neurons under glucose/oxygen deprivation die by necroptotic cell death”

J. Galindo-Moreno, D. Domínguez-Villanueva, J.H.M. Prehn and Cristina Muñoz-Pinedo.

## **PhD thesis**

Papel de proteínas de la familia Bcl-2 en el estrés metabólico. Implicación en cáncer y en daño neuronal por isquemia.

Javier Galindo Moreno. Universidad de Barcelona, 2015.

## **Articles related to this subject in which general funding to the lab from La Marató is acknowledged**

Oxidative stress modulates mitochondrial failure and cyclophilin D function in X-linked adrenoleukodystrophy.

López-Erauskin J, Galino J, Bianchi P, Fourcade S, Andreu AL, Ferrer I, Muñoz-Pinedo C, Pujol A.

*Brain*. 2012 Dec;135(Pt 12):3584-98.

Emerging concepts: linking hypoxic signaling and cancer metabolism

C A Lyssiotis, M G Vander-Heiden, C Muñoz-Pinedo and B M Emerling

*Cell Death and Disease* (2012) **3**, e303; doi:10.1038/cddis.2012.41

Autosis: a new addition to the cell death tower of babel

C Muñoz-Pinedo and S J Martin

*Cell Death and Disease* (2014) **5**, e1319