Al collegio docenti del Dottorato in Medicina Molecolare

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Introduction

Müller cells are the main retinal macroglia [1] and play major supporting roles. [2] They express glutamine synthetase (GS), an enzyme crucial to maintain the glutamate-glutamine cycle. [3,4] Moreover, they exert an angiostatic effect, secreting both pro- and antiangiogenic factors. [5–7] In hypoxic conditions, Müller cells become the main producer of vascular endothelial grow factor (VEGF). [7]

Retinopathy of prematurity (ROP) is a blinding disease affecting preterm newborns driven by excessive retinal production of VEGF. [8] Studies in a mouse model of ROP demonstrate that β-adrenoreceptors (BARs) plays a role in ROP pathogenesis. [9] In particular, it has been shown that retinal noradrenaline levels increase in hypoxia, and that BAR2 has a pivotal role in VEGF regulation and retinal neovascularization. [9-13] Indeed, lack of BAR2 resulted in protection from the hypoxic damage [14] and blocking BAR2 with selective antagonists reduced VEGF levels to those of control mice. [10]

BAR2 is expressed by mouse and rat Müller cells. [9,15]
Our aim is to verify the presence of BAR2 in human Müller cells and whether their level is modulated by hypoxia.
Demonstration of their presence and their possible modulation by hypoxia, could pave the way to further studies acting specifically at them to regulate VEGF levels.

Methods

We used the human Müller cell line MIO-M1.

Immunocytochemistry (ICC) was performed using anti-BAR2 and anti-GS as antibodies.

A solution of BAR2 siRNA was incubated with the cells. RNA was extracted and qPCR was performed on cDNA with primers hybridizing to BAR2 and actin.

Subsequently to starvation, MIOM1 were exposed to hypoxia.

Western Blot (WB) was performed, using anti-BAR2 and anti-actin antibodies.

Results

ICC shows the presence of GS and BAR2 in MIOM1. The staining of slides with only the secondary antibody is unspecific, suggesting the specificity of the primary antibodies.

To further validate BAR2 antibody specificity, silencing of *BAR2* was performed, and verified by qPCR. Silenced MIOMI show a significant decrease in BAR2 signal in both WB and ICC. These outcomes definitely validated BAR2 antibody specificity and, thus, data reliability.

Hypoxia significantly increases the protein level of BAR2 in MIOM1.
Additional analysis is needed to confirm this result. The possible link between this increase and an increase in VEGF levels remains to be evaluated.

References

[1] Bringmann *et al., Ophthalmol.,* 2012,227:1–19
[2] Bringmann *et al., Prog. Retin. Eye Res.,* 2006,25:397–424
[3] Pow *et al., Neurosci.,* 1996,70:295–302
[4] Hurley *et al., J. Neurosci. Res.,* 2015,93:1079–1092
[5] Reichenbach *et al., Glia,* 2013,61:651–678
[6] Eichler *et al., Exp. Cell Res.,* 2004,299:68–78
[7] Li *et al., Transl. Res.,* 2019,207:96–106
[8] Chen *et al., Angiogenesis,* 2007,10:133–140
[9] Casini *et al., Prog. Retin. Eye Res.,* 2014,42:103–129
[10] Ristori *et al., Invest. Ophthalmol. Vis. Sci.,* 2011,52:155–170
[11] Dal Monte *et al., Invest. Ophthalmol. Vis. Sci.,* 2012,53:2181–2192
[12] Martini *et al., J. Neurochem.,* 2011,119:1317–1329
[13] Dal Monte *et al., Exp. Eye Res.,* 2013,111:27–35
[14] Dal Monte *et al., Invest. Ophthalmol. Vis. Sci.,* 2015,56:59–73
[15] Walker *et al., Invest. Ophthalmol. Vis. Sci.,* 2007,48:5276–5281

Partecipazione a congressi e corsi:

- Responsabile Dott.ssa Antonella Pochini, “Protezione degli animali impiegati nella ricerca: aspetti scientifici, etici e applicativi” (8,15,22/10/2019)
- Luis Puelles, “Mouse brain develpment and structure” (23,24,25/10/2019)
- George M. Malacinski “Will DNA Science Change Everything, Including The Answer to The Question: What Does It Mean To Be Human?” (13/11/2019)
- George M. Malacinski “After earning a graduate degree, what comes next?” (19/11/2019)
- Marina Mione “Uncovering mechanisms of cancer development in Zebrafish” (18/12/2019)
- Filippo Del Bene “Reelin and meteorins: and old friend and new players in neural circuit wiring in Zebrafish” (18/12/2019)

- Gaetano Vattemi “Caratteristiche biochimiche e binding partners del SERCA1 – verso una migliore comprensione della miopatia di Brody” (22/01/2020)
- Stefano Lagravinese, Carla Polimeni “Formazione nel Farmaceutico” (14/04/2020-24/05/2020, 48 ore totali)
- “Covid19: come si insegue e sconfigge un virus” (28-29/05/2020)
- Paolo Glisenti, Marta Antonelli, Paolo Bonaretti, Paola De Bernardi, Rosanna Zari, Francesco Mastrandrea, Cristiana Smurra, Angelo Riccaboni “Scenari e prospettive delle imprese agroalimentari fra sostenibilità e innovazione” (22/07/2020)
- Gerald Crabtree “Chromatin regulation: Insights from New Methods and the Genomics of Human Disease” (26/08/2020)