Al collegio docenti del Dottorato in Medicina Molecolare

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Ciclo XXXV Tutor Massimo Dal Monte

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Introduction

Retinopathy of prematurity (ROP) is a blinding disease affecting preterm newborns characterized by a first phase of hyperoxia and a second one of hypoxia, driven by vascular endothelial growth factor (VEGF) increase.[1]. This latter causes leakages in the blood-retinal barrier (BRB)[2]. Studies in an oxygen-induced retinopathy (OIR) mouse model of ROP demonstrate that β-adrenoreceptors (BARs) play a role in ROP pathogenesis [3–8].
In mouse OIR retina, BAR3 is the only BAR subtype to be upregulated by hypoxia, in association with noradrenalin increase [6]. While BAR3 responsiveness to hypoxia has been clearly elucidated *in vivo,* as well as its contribution to VEGF production in such condition *ex vivo* [9], its putative sensitivity to hyperoxia is still to be elucidated.
Thus, we decided to investigate the role of oxygen tension as a possible BAR3 modulator in two different kinds of retinal cells. We analysed for the first time the effects of 72 hours (h) hyperoxia or hypoxia on the expression of BAR3 in two different human retinal components: Müller glial cells (MIO-M1), the major retinal glia [10], and retinal endothelial cells (HREC), essential components of the BRB [11]. In addition, we examined the response of BAR1 and BAR2 to such treatments. We also evaluated cell viability.

Methods

Subsequently to 24h starvation, both HREC and MIO-M1 cells were exposed to hyperoxia (75% oxygen) or hypoxia (1% oxygen).
Western Blot (WB) was performed, using anti-BARs and anti-actin antibodies.
Cell viability was evaluated by Trypan Blue.

Results

We demonstrated for the first time that oxygen abundance significantly reduced BAR3 protein level in both cell lines, while BAR1/2 were not affected by hyperoxia.
Consistently with previous *in vivo* results [6], hypoxia strongly upregulated BAR3 in both cell lines. Surprisingly, BAR1 expression increased too, showing for the first time a hypoxia-dependent regulation in these two cell lines. BAR2 was not influenced by oxygen deprivation.
Cell viability was not influenced by the treatments.

We are actively investigated mRNA expression of HREC and MIO-M1 in these experimental conditions, as well as the effects of shorter (24, 48h) exposure times on both protein and transcript levels. The possible correlation between BARs oxygen-dependent modulation in ROP and VEGF levels remains to be evaluated.

References

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2. Kaur *et al*., *Prog Retin Eye Res*. 2008,27:622-647
3. Dal Monte *et al*., *Invest Ophthalmol Vis Sci*. 2012,53:2181-2192
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5. Dal Monte *et al*., *Investig Ophthalmol Vis Sci*. 2015,56:59-73
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- Partecipazione al 71° Congresso Nazionale SIF (Società Italiana di Fisiologia)

Autori della presentazione: Martina Lucchesi, Silvia Marracci, Luca Filippi, Massimo Dal Monte

Titolo della presentazione: Oxygen dependent expression of β adrenoceptors and members of the HIF 1/VEGF axis in human Müller and retinal endothelial cells

Abstract:
Retinopathy of prematurity (ROP) is a disease characterized by a first phase of hyperoxia and a second one of hypoxia. Hypoxia leads to neovascularization through an increase in the levels of hypoxia-inducible factor (HIF) 1 and its target vascular endothelial growth factor (VEGF). In hypoxic conditions, VEGF, mainly produced by Müller cells, stimulates endothelial cell proliferation acting at its receptor VEGFR2. An involvement of β-adrenoceptors (BARs) in VEGF production and retinal neovascularization has been assessed. Here, using human Müller and retinal endothelial cells (MIO-M1 and HREC, respectively) we evaluated for the first time the effects of hyperoxia (75% oxygen) and hypoxia (1% oxygen) on the expression of BARs, HIF-1, VEGF and VEGFR2.
Hyperoxia did not affect BAR1 and BAR2, whereas decreased BAR3 in both MIO-M1 and HREC. Hypoxia, instead, upregulated both BAR1 and BAR3 in MIO-M1 and HREC, having no effect on BAR2. In both cell lines, HIF-1 and VEGF were increased by hypoxia. In HREC, VEGFR2 was decreased by hyperoxia while it was not influenced by hypoxia; in MIO-M1, VEGFR2 was upregulated by hyperoxia and downregulated by hypoxia.
Although a correlation between BARs levels and the activity of the HIF-1/VEGF axis under hyperoxia/hypoxia is still to be demonstrated, the present results lay the ground for assessing whether targeting BARs during the different phases of ROP may be a strategy to avoid the development of neovascularization.

Date del Congresso: 7,8,9/09/21

- Partecipazione ai seguenti corsi e seminari:

    - 21.09.20 “Immunoistochimica. Colorazione singola, dualplex e multiplex: una panoramica degli step critici per l’ottimizzazione” Euroclone
    - 22.10 – 4.12.20: “Protezione degli animali impiegati nella ricerca: aspetti scientifici, etici e applicativi” Responsabile Dott.ssa Antonella Pochini
    - 11-13.11.20 “I-Gene Training School” Vittoria Raffa, Chiara Gabellini, Andrea Cioni, Michele Lai, Giulia Freer, Dariusz Witt, Douwe Geuzebroek, Arnoud Everhardt, Francesco De Angelis, Martín Ricardo Abraham, Marta d’Amora and Francesco Tantussi.
    - 23.11.20 “Vaccini e monoclonali per liberarci dal covid19” Rino Rappuoli (Accademia dei Fisiocritici, Università di Siena)
    - 26.11.20 “Life science: amazing professors at Meiji University in Tokyo” Hiroshi Nagashima, Kentaro Yano, Sara Philips
    - 17.12.21 “SARS-Cov2: origine, trasmissione e patogenesi” Maria Grazia Cusi (Accademia dei Fisiocritici, Università di Siena)
    - 14.01.21 “Control of Ca2+ in the heart: free and beyond” David Eisner
    - 19.01.21 “Connexin43 hemichannels and intracellular calcium: An axis of dysfunction in sudden cardiac death” Henrique Girao, Mario Delmar, Tania Marques, Rachel Padget
    - 25.01.21 “Ricerca Biomedica tra diritto alla salute e valori costituzionali” Paola Binetti, Elena Cattaneo, Lorenzo Chieffi, Paolo De Angelis, Giuliano Grignaschi
    - 10.02.2021 “Phenocell-hiPSC based Dry AMD in vitro model”
    - 24.02.2021 “Perché il COVID-19 colpisce in modi diversi?” Alessandra Renieri
    - 04.03.2021 "Nano and the environmen” Fabio Pulizzi
    - 18.03.2021 “Minisimposio su sperimentazione animale in biomedicina” Luca Bonini, Marco Tamietto, Nicola Simola
    - 23.03.2021 “Aspetti medico legali della pandemia da COVID-19” Mario Gabbrielli (Accademia dei Fisiocritici, Università di Siena)
    - 24.03.2021 “Webinar N° 1 Sharing CYTOScience: Mieloma Multiplo” EuroClone
    - 12.04.2021 “Nervous System Development and Plasticity in Model Organisms II” Diego Garcia-Gonzalez, Matthias Carl
    - 19-23.04.2021: Western Blot University - Courses designed to make you a western blotting expert (Paul Pease, Gary F. Ross, Omar Qazi, Mauhamad Baarine, Brad VanderWielen, Kenneth Oh)
    - 27.04.2021 “L’esitanza vaccinale pandemica in Italia - 2nd Vaccine Hesitancy Forum Covid-19” Cinzia Caporale, Andrea Grignolio Corsini (Centro Interdipartimentale per l'Etica e l'Integrità nella Ricerca)
    - 04.05.2021 “Nervous System Development and Plasticity in Model Organisms III” Nathalie Jurisch-Yaksi, Christine Charvet
    - 09.06.2021 “Monitoraggio molecolare del trascritto BCR-ABL1 tramite Droplet Digital-PCR (ddPCR): pronti per una sua applicazione nel management clinico dei paziente con Leucemia Mieloide Cronica?” Carmen Fava, Jessica Petiti, Enrico Gottardi
    - 14.06.2021 “Cells Webinar | Nervous System Development and Plasticity in Model Organisms IV” Alessandra Pierani, Stephane Nedelec

- 22.06.2021 "COVID-19 e vaccini: diritto alla salute, giustizia distributiva e solidarietà tra bioetica e diritto internazionale" Cinzia Caporale, Gilberto Corbellini, Corrado Spinella, Andrea Corsini, Francesca Moccia, Isabella Mori, Stefania Negri, Raffaele Cadin, Richard Pavone, Valentina Zambrano

- Pubblicazioni:

  - Martina Lucchesi, Silvia Marracci, In vitro models of retinal diseases, Annals of Eye Science, submitted

  - Rosario Amato, Francesco Pisani, Emiliano Laudadio, Martina Lucchesi, Silvia Marracci, Maurizio Cammalleri, Luca Filippi, Roberta Galeazzi, Maria Svelto, Massimo Dal Monte, Paola Bagnoli, Novel insights into beta3 adrenoceptor regulation by oxygen levels in the retina: evidence from the oxygen-induced retinopathy model, Journal of Cellular and Molecular Medicine, submitted