UNIVERSITÀ DI SIENA 1240

DMMS
DIPARTIMENTO DI MEDICINA MOLECOLARE E DELLO SVILUPPO

Università degli Studi di Siena
Dottorato di Ricerca in Medicina Molecolare

Scheda Relazione Annuale Dottorando

Pisa, 04/10/2024

Al Collegio Docenti del Dottorato in Medicina Molecolare

Dr.ssa**: Di Marsico Lorenza**
ciclo: XXXIX Tutor: Prof. Massimo Dal Monte

Scientific activity related to the 1° year of the PhD program, Academic Year**:** 2023/24

Introduction
Stress conditions, such as hypoxia, are known to induce catecholaminergic overstimulation, which alters signaling pathways associated with β-adrenoceptors (ARs). In the retina, β-ARs play a crucial role in regulating vascular responses to hypoxia. While the involvement of β1/2-ARs in promoting hypoxia-induced neovascularization (NV) is well established, the role of β3-AR remains unclear. Since β3-AR expression in the retina is oxygen-dependent, the research activity of the first PhD year was to investigate the role of β3-AR in hypoxia-driven NV using a model of oxygen-induced retinopathy (OIR). The role of β3-AR was also studied in two *in vitro* models consisting of human retinal Müller MIO-M1 macroglial cells and human retinal endothelial cells (MIO-M1 and HREC, respectively) cultured under hypoxic conditions.

Methods
- MIO-M1 and hREC cell culture
- Intraperitoneal or subcutaneous injections
- Ocular surgery and retinal dissection
- Electroretinogram (ERG)
- Molecular analyses: Western blot, RT-PCR and immunofluorescence.

Results
In vivo, subcutaneous administration of the β3-AR agonist BRL37344 during the hypoxic phase of OIR restored the balance between pro- and anti-angiogenic factors, such as VEGF and PEDF, effectively counteracting pathological NV. This was demonstrated by a reduction in peripheral vascular tufts and revascularization of the central retinal area. The revascularization likely occurred through the restoration of the astrocyte template, ultimately improving retinal ganglion cell (RGC) function and viability, as demonstrated by pERG recordings and increased RGC density. Molecular analyses revealed increased NOS pathway activity following β3-AR stimulation, leading to nitric oxide production and exerting a protective effect on endothelial cells.
To further explore the functional role of β3-AR in hypoxia-induced VEGF production, we used MIO-M1 and HREC. The β3-AR antagonist SR59230A was found to reduce NOS isoform expression during hypoxia in both cell lines.

- **Congresses:**
  - 74th Congress of the Italian Society of Physiology (SIF), Roma, September 11-13, 2024.
    Poster session: β3-adrenoceptor role in hypoxia-driven retinal neovascularization: insights from the mouse model of oxygen-induced retinopathy
    Authors: Lorenza Di Marsico[1], A. Melecchi[1], A. Canovai[1], R. Amato[1], M. Dal Monte[1], M. Cammalleri[1]
    [1] Department of Biology, University of Pisa, Pisa, Italy

- **Courses attended:**
  - "Basic and Advanced Microscopy course", 12h (12-27/02/2024), Prof. Michela Ori, Dipartimento di Biologia, Pisa
  - Lezioni Dottorato Medicina Molecolare, 24h (February - May 2024)

- **Soft skills courses:**
  - SOFT SKILLS, corso PUBMED, UNIFI, 3h, 15/04/2024 (online)
  - SOFT SKILLS, Disseminazione dei risultati della ricerca: Open Access e archivi istituzionali, UNIPI, 3h, 16/04/ 2024.
  - SOFT SKILLS, La valutazione della ricerca e gli strumenti disponibili per il settore STEM, UNIPI, 3h, 19/04/2024.
  - SOFT SKILLS, La ricerca Statistica, UNIPI, 3h, 6/05/ 2024.
  - SOFT SKILLS, Open Science: principles and practice, UNIPI, 6h, 27, 28, 31/05/2024.
  - SOFT SKILLS, Open Science: principles and practice, UNIPI, 6h, 3, 5, 7 /06/2024.
  - SOFT SKILLS, L'assicurazione della Qualità nell'Ateneo di Pisa e il ruolo dei dottorandi: UNIPI 3h, 17/09/2024

- **Webinar and seminars:**
  - Online workshop on CRISPR Technologies and iPSC-Based Disease Models, November 2023
  - Webinar: How to Become More Sustainable in the Lab – Approaches, MERK, 15/11/2023
  - Giornata studio "Benessere animale e metodi alternativi", Centro 3R UNIPI – 18/12/2023
  - Webinar: "Bioprinting: from the bench to bedside", Prof. G. Fortunato, 17/01/2024
  - Webinar: "An introduction to Microarrays technology", Dr. Paolo Caiella, Technical Sales Specialist of Illumina, 18/03/2024
  - Webinar "La valorizzazione dei risultati delle attività di ricerca: una opportunità di fare leva sulla specializzazione e sulla massa critica del Centro Nazionale di Ricerca", Dott. Baccanti, 05/04/24
  - Webinar: "*Expert tips and tricks for RNA biomarker research",* Qiagen, 25, 26 e 27 June 2024.

- **Scientific publications**: NO
- **Research experience at other laboratories:** NO