SOCIETÀ ITALIANA DI FARMACOLOGIA

MODELLO PER INVIO RELAZIONE DI METÀ E FINE PERIODO

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TIPOLOGIA DI BORSA RICEVUTA: Borsa di Ricerca SIF per brevi periodi all'estero

TIPOLOGIA DI RELAZIONE (es.: metà periodo o finale): Relazione finale

TITOLO DELLA RELAZIONE: Role of the β-Amyloid Precursor Protein (APP) in endothelial cells functionality and in regulating the VEGF/VEGFR2 signaling

RELAZIONE:

Background:

The β-Amyloid Precursor Protein (APP) is a ubiquitous type-1 integral membrane protein, abundantly expressed in vascular endothelium (Ott & Bullock, 2001), mainly known for its cleavage product Aβ and its detrimental role in the pathogenesis of neurodegenerative disease such as Cerebral Amyloid Angiopathy (CAA) and Alzheimer's disease (AD).

Although accumulation of neuron-derived Aβ peptides is considered the primary influence driving AD and CAA pathogenesis, recent studies highlighted the importance of the physiological role of its precursor APP in cell homeostasis, suggesting a potential role of this protein in maintaining vascular endothelium homeostasis (Ristori et al., 2020a). Moreover, increasing evidence supports the hypothesis that vascular dysfunction plays a major role in CAA and AD and several studies raise the possibility that vascular dysfunction could be an early step in these diseases and could even precede significant Aβ deposition (de la Torre, 2018), emphasizing the importance of a better understanding of APP role in vascular endothelium.

Under physiological conditions, APP is cleaved by different secretases through two main proteolytic pathways: the non-amyloidogenic and amyloidogenic processing. The APP processing leads to the release of several intracellular and soluble metabolites, including Aβ. Despite the role of the Aβ peptides in the development of AD and CAA pathogenesis, the full-length APP and its cleavage products, including low concentrations of soluble Aβ, exert several beneficial physiological roles in endothelial cells (Cantara et al.,

2004; Cameron et al., 2012; d'Uscio et al., 2017; Ristori et al., 2020a). Indeed, in vivo studies show a strong correlation between APP loss-of-function models and vascular dysfunction supporting the importance of this protein and its metabolites in vascular homeostasis (Luna et al., 2013; d'Uscio et al., 2018). Moreover, evidences suggest that APP mediates endothelial cells' response to angiogenic growth factors and modulates angiogenesis (Cantara et al., 2004; Cameron et al., 2012).

Aim:

To date, most of the research effort focused in the role of this protein on neuronal tissue and only few studies investigate the function of APP in the vascular endothelium. Thus, the aim of my study was to understand the role of APP in endothelial cells functionality and in the regulating vascular endothelial growth factor (VEGF) signalling.

Methods and Results:

To investigate the physiological role of APP full-length in endothelial cells, I generated an in vitro APP-knockdown model using human umbilical vein endothelial cells (HUVECs). I used a proteomic approach to identify the major cellular targets and the biological pathways affected by APP down-regulation, and I validated the proteomic results with functional and molecular assays.

Before leaving for Yale University, I showed that the loss of APP resulted in altered cellular morphology and reduction of cell migration and proliferation.

Using a proteomic approach I was able to identify the biological pathways affected by APP silencing. In particular, I observed a significant reduced expression of actin cytoskeleton-interacting proteins, mainly involved in actin organization, cell adhesion, cell–cell contact and also an altered expression of protein involved in VEGF-mediated angiogenesis

I spent 12 months in the laboratory of Professor Michael Simons (Yale University), where I was able to validate the proteomic results and to deepen my knowledge in angiogenic growth factors signaling.

I first showed that loss of APP inhibits endothelial cells adhesion on different substrates (fibronectin and collagen I); moreover, loss of APP reduces the expression levels of focal adhesion proteins such as Integrins, kindlin, vinculin and paxillin. Therefore APP regulates endothelial cell-extracellular matrix interacting proteins.

Moreover, APP-silenced endothelial cells lost barrier function due to altered expression of tight junctions proteins, including ZO-1 and claudin-5, indicating that APP is also involved in endothelial cell-cell interactions.

Finally, I investigated the effect of loss of APP on VEGF signalling. APP silencing significantly inhibited VEGF-mediated cell migration, cell proliferation and on the ability of endothelial cells to form angiogenic tubes, suggesting an implication of APP in the response of endothelial cells to VEGF. I also observed a significant inhibition of VEGF receptor (VEGFR2) activation. Indeed APP-silenced endothelial cells showed VEGFR2 reduced phosphorylation levels following VEGF stimulation at its major phosphorylation sites, located, respectively, in the kinase insert domain (Y951), in catalytic domain (Y1054/1059), as well as in the carboxyl-terminal domain (Y1175), suggesting that APP controls ECs response to VEGF by modulating VEGFR2 activation.

Finally, I observed that APP silencing also affected the activation of VEGF–VEGFR2 downstream signalling. In particular I observed a reduced activation of p-ERK, essential for VEGF-mediated endothelial cells proliferation and survival and p-SRC and p-FAK, involved in endothelial cells migration and angiogenesis. These results indicate that APP is involved in VEGF/VEGFR2 signalling.

Conclusion:

In conclusion, I was able to show that APP interacts with cell membrane components modulating the expression and the stability of cytoskeleton interacting proteins and that APP is necessary to mediate endothelial response to pro-angiogenic stimuli such as VEGF.

Loss of APP leads to endothelial cells dysfunction, thus, the intact expression and processing of APP is required for normal endothelial function. Part of my research work has been published (Ristori et al., 2020b).
Further research is needed to investigate the specific role of APP in different vascular beds. I believe that a deep understanding of APP function in maintaining vascular homeostasis might shed light on new therapeutic targets and provide a new perspective on treatment options of neurodegenerative diseases.

References

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