«Estrogen receptor beta as direct regulator of mitochondrial function»

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ABSTRACT

Estrogens are crucial regulators of the nervous system physiology, exerting anti-oxidant, anti-apoptotic and anti-inflammatory actions. They act via binding to their cognate nuclear, mitochondrial and membrane estrogen receptors (ERs), but also in an ER-independent manner. Recently, the neuroprotective effect of mitochondrial ERs, especially mitochondrial ERβ (mtERβ), has been documented. The exact mechanism of mtERβ action in this organelle has not been fully elucidated and is the subject of study by various research groups. Considering the significant role of mitochondria in the maintenance of neurons survival and function, the characterization of the role and the biochemical mechanisms of the actions of mtERβ in neural cells is of great importance. In this context, the aim of this PhD thesis was the characterization of the direct action of mtERβ on several mitochondrial processes, such as mitochondrial transcription, energy production, anti-oxidant defense, mitochondria-associated apoptosis, as well as the elucidation of mtERβ effects on carcinogenesis, cell differentiation and defense against neurotoxic agents. To this aim, a stable mouse neuroblastoma cell line Neuro-2a (N2A) overexpressing a mitochondrial-targeted ERβ (mtERβ) fused with the green fluorescent protein (N2AmtGFPERβ), and a control N2A cell line stably overexpressing a mitochondrial-targeted GFP (N2AmtGFP), was generated. The produced stable cell lines along with the human neuroblastoma cell line SH-SY5Y, that exhibits considerable endogenous ERβ protein levels with mitochondrial ERβ localization as well, were cultured in the presence or absence of a specific inhibitor of nuclear transcription or differentiation- or neurotoxicity-inducing factors, in order to study the role of mtERβ in mitochondrial function, by applying real-time PCR, fluorescence microscopy, immunocytochemistry and Western blotting, measurement of ATP levels or / and MTT assays. Moreover,

stable cell lines were used for the development of a xenograft mouse model, for in vivo tumorigenesis studies.

The results of this PhD thesis revealed the direct involvement of mtERβ in the activation of mitochondrial transcription and ATP production, even in the presence of a specific inhibitor of nuclear polymerase and in the presence or absence of estradiol (Estradiol, E2), in both the SH-SY5Y and N2AmtGFPERβ cells. Furthermore, investigation of the role of mtERβ in the regulation of neural cell differentiation uncovered the involvement of mtERβ in neuronal differentiation as indicated by the statistically significant increase in the neuronal length and Tuj-1 protein levels in mtERβ-overexpressing cells, compared to control cells. Moreover, the increased anti-apoptotic and anti-oxidant defense of mtERβ-overexpressing N2A cells, in the presence of apoptosis- and oxidative stress- inducing factors compared to the control cells, demonstrate the direct involvement of mtERβ in the regulation of apoptosis and oxidative stress, verifying the neuroprotective effect of mtERβ.

The actions and effects of the mtERβ actions were confirmed and further investigated by applying a xenograft mouse model of mtERβ-overexpressing N2A cells. In tumorigenesis studies, the direct action of mtERβ in the regulation of mitochondrial transcription was also verified. Thus, in mtERβ expressing tumors the increased synthesis of OXPHOS subunits was observed that possible lead to the enhancement of energy production through the respiratory chain - OXPHOS. Our findings indicate that these mtERβ-induced actions contribute to the inhibition of carcinogenesis, through direct or indirect regulation of other biochemical mechanisms such as, cellular energy metabolism, mitophagy and cell cycle, thereby reversing the trends for metabolic reprogramming observed in Warburg effect.

Finally, the study of both the potential estrogenic action of aluminum (Aluminium, Al) and the involvement of this action in Al-induced neurotoxicity, highlighted the Al-induced reduction in ERβ protein levels. This action may be linked to the observed involvement of Al in the phosphorylation at S118 residue of ERα, which cause the inhibition of ERα proteolytic degradation and the increase of ERα protein levels. Increased ERα levels, as we know from the literature, induce the reduction in ERβ protein levels and consequently ERβ-induced neuroprotective action. In addition, the

neurotoxic effect of Al could be attributed to the observed Al-induced increase in mitochondrial localization of ERβ, possibly triggering mitochondrial metabolism and increasing production of ROS and induction of apoptosis.

In summary, the results of the present PhD thesis support the direct action of mtERβ in the regulation of mitochondrial function in both normal and pathological conditions, rendering mtERβ a potential pharmaceutical target for mitochondrial related diseases, such as cancer and neurodegenerative diseases.