Striatin-Dependent Membrane Estrogen Receptor Signaling and Vasoprotection by Estrogens

Running title: Bobik; Membrane Estrogen Receptor Signaling

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It is well known that cardiovascular disease is less frequent in premenopausal women compared with men but rises rapidly in postmenopausal women. Such early observations led to the hypothesis that estrogen therapy will reduce the risk of postmenopausal women developing cardiovascular disease. However, observational studies have led to conflicting results with some studies reporting reductions in cardiovascular disease in postmenopausal women taking estrogens whilst others observed no beneficial effects. Rather increases in the risk of coronary heart disease and stroke have been reported, particularly for women who are older and those with a long hormone-free interval. Such findings have led to the speculation that estrogens have competing cardiovascular effects-beneficial and detrimental and this has intensified efforts to better understand the range of cardiovascular effects mediated by estrogens and their signaling mechanisms, the ultimate aim being to develop new therapies for women that exert the beneficial effects of estrogen whilst minimizing potentially harmful effects. To achieve this aim, new studies to better understand both nuclear and membrane estrogen receptor (ER)-mediated signaling in target tissues such as the heart and blood vessels, immune cells and other target tissues are in progress. In this issue of Circulation, Moens and colleagues provide novel insights on the importance of membrane ER-mediated signaling pathways in blood vessels for vasoprotection and vascular gene regulation.

Estrogen activates multiple signaling pathways in most target cells and tissues including those within the cardiovascular system. Functional ERs, ERα and ERβ are expressed on cardiomyocytes in the heart and by endothelial and smooth muscle cells within blood vessels. Early studies indicated that ERs are ligand activated transcription factors which exist primarily in the nucleus complexed with chaperones. Upon estrogen binding changes in receptor conformation leads to chaperone dissociation and receptor dimerization, followed by DNA
binding at proximal promoter sites, where recruitment of coactivators and/or corepressors leads to gene activation. These nuclear events usually require hours or days for maximal gene activation. ER mediated genomic activation can also be upregulated by growth factor signalling pathways via ER phosphorylation mediated by kinases. ERs also have the ability to signal rapidly, in an apparent non-nuclear manner, by activating specific kinases and their effector molecules. Non nuclear, i.e., membrane ER signaling activates eNOS and promotes endothelial cell growth and migration. In endothelial cells, membrane ERs, which do not possess a transmembrane domain, are mostly located within caveolae/lipid rafts, i.e., specialised cholesterol-rich membrane organelles that compartmentalize signal transduction molecules, with ERs complexed with caveolin-1, Gαi and striatin. Striatin is a member of the WD (tryptophan [W], aspartic acid [D]) repeat protein family containing a caveolin-binding domain as well as a calmodulin binding domain and serves as a molecular anchor and scaffold for assembly of proteins required for rapid, estrogen induced signaling. In the study by Moens and associates in this issue of Circulation, the actions of this receptor complex are defined using a novel transgenic mouse expressing a disrupting peptide consisting of the striatin-binding domain within ERα. This peptide prevents complex formation between ERα and ERβ and striatin and inhibits membrane ER signaling. In this mouse model rapid ERα signaling is lost; specifically, estrogen is unable to initiate phosphorylation of Akt and ERK and elevate eNOS gene transcription. Using aortic tissue they examine the importance of rapid signaling for transcriptional responses of vascular tissue to estrogen. Inhibiting membrane ER signaling resulted in fewer genes being regulated by estrogen with many implicated in vascular development, function and disease. This is consistent with recent findings in MCF-7 breast cancer cells where nuclear and membrane ER signaling contribute to gene expression. About 50% of differentially regulated genes showed opposite
effects in aortas from wild type and the transgenic mice and many estrogen non-regulated or down regulated genes were upregulated when membrane estrogen signaling was inhibited indicating significant cross talk between nuclear and membrane ER signaling processes. Overall, they demonstrate that rapid signaling plays a major role in determining the extent and pattern of estrogens mediated vascular gene responses. Since intact aortic tissue was used it is not possible to assign particular estrogen mediated gene responses to smooth muscle cells, endothelial cells or adventitial fibroblasts but many are likely due to smooth muscle cells, the most abundant cell type in the aorta. It would be of interest to confirm that smooth muscle cells account for most of the observed effects and also determine the extent to which these responses are apparent in vivo, in female ovariectomized mice chronically treated with estrogen. This could provide additional insights into long-term vascular effects of estrogens regulated by membrane ERs, which may be important for assessing the significance of rapid membrane ER signaling in any future hormone replacement strategies. The studies of Moens and associates are a significant advance on earlier studies in cultured endothelial cells which identified PI3K-dependent genes upregulated by estrogen, presumably initiated by membrane ER signaling which included transcription factors, signaling molecules, cytokines and chemokines as well as molecules involved in immune responses. Whilst not specifically studied, the authors propose that changes in transcription factor characteristics induced by kinases activated by rapid membrane ER signaling account for the ability of this signaling system to alter gene expression. Their analysis of differentially expressed genes identifying signaling pathways overrepresented in the microarray array data supports this hypothesis.

The studies of Moens and associates also have important implications for vascular remodelling. They demonstrate that inhibition of platelet-derived growth factor stimulated
vascular smooth muscle cell proliferation and endothelial cell migration are highly dependent on
membrane ER signaling. To determine the in vivo relevance of the in vitro findings the authors
examine the effects of inhibiting membrane estrogen signaling on vessel structure after carotid
artery injury. They demonstrate that inhibiting membrane ER in the injured arteries attenuates
estrogen mediated reductions in smooth muscle cell proliferation. Furthermore, the estrogen
mediated reduction in media thickness was also attenuated. Previously, an estrogen dendrimer
conjugate which activates membrane ER without initiating nuclear ER signaling was shown to
attenuate intima development in injured arteries.\textsuperscript{11}

This study by Moens and associates makes several important advances in our
understanding of rapid membrane ER initiated cardiovascular effects of estrogens. First, the
study definitively establishes that striatin-dependent membrane ER signaling is critical for
vascular estrogen responses and for determining overall ER mediated vascular gene responses,
including those important for vascular function and disease. Second, the study demonstrates the
importance of this signaling system in determining blood vessel responses to injury in vivo.

However, whilst the study demonstrates the importance of striatin-dependent membrane ER
signaling in endothelial cells and vascular smooth muscle in response to vessel injury (see Figure
1), further studies will be required to obtain a more comprehensive picture as to the overall
importance of this signaling pathway in protecting against more complex vascular disorders such
as atherosclerosis, instances where inflammation regulated by immune cells influences disease
outcome. Estrogen regulates macrophage characteristics and activity via ER\textsubscript{α} dependent
signaling, including their phenotype, phagocytic capacity and expression of BAFF (B cell
activating factor belonging to the tumor necrosis family), a cytokine critical for survival of
proatherogenic B2 cells,\textsuperscript{12-14} but the importance of striatin-dependent membrane ER signaling
has not been investigated. This also applies to CD4+ T cells where estrogen enhances responsiveness. As estrogen can also influence thromboembolism, it will also be important to determine whether the striatin-dependent ER signaling pathway influences megakaryocytes and the platelet proteome.

The findings of Moens and associates clearly establish and important role for estrogen mediated rapid striatin-dependent membrane ER signaling in blood vessels, which regulates gene expression and initiates vasoprotective effects in mice. The elegant study is an important step forward in more clearly defining membrane ER dependent mechanisms by which estrogen regulate blood vessel responses in vivo. However, the clinical relevance of the findings remains to be explored and will require additional studies in human blood vessels. This is particularly important given the disparity between the beneficial effects of estrogen therapy in many animal models of vascular disease and humans.

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**References:**


**Figure Legend:**

**Figure 1.** ER signaling in vascular cells. ERs are ligand activated transcription factors that translocate to the nucleus upon binding estrogen (E) to regulate gene expression. They bind to estrogen response elements (ERE), displace corepressors (CoR) and recruiting coactivators (CoA). Estrogen also binds to ERs at the cell membrane, within caveolae where the receptor exists as a complex with other proteins including Gαi, caveolin-1 (Cav-1) and striatin and rapidly activates various kinases including Akt, PI3K and MAPK altering coactivator and corepressor characteristics and transcription factor (TF) function as well as vascular cell function. Moens and associates demonstrate that rapid striatin-dependent membrane ER signaling by estrogen is vasoprotective regulating endothelial and vascular smooth muscle cell function in vivo. They also demonstrate that this pathway influences that extent and pattern of gene activation by nuclear ERs.4 Macrophages, lymphocytes, megakaryocytes and platelets all express ERs and are important participants in vascular pathologies. The significance of rapid striatin-dependent membrane ER signaling in regulating their function remains to be determined.
E-ER

Cell Membrane

caveola

Rapid Effects

Vasoprotective

Endothelial Cells

eNos↑
migration↑
proliferation↑

Vascular Smooth Muscle

proliferation↓

Macrophages and Lymphocytes?

Megakaryocytes and Platelets?

Akt, PI3K

MAPK

GoI

Striatin

Cav-1

Akt, PI3K

MAPK

ER

TFs

ERE

CoA

CoR

Nucleus

ERE

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