Cross-coupling between the oestrogen receptor and phosphoinositide 3-kinase

J.K. Liao

Cardiovascular Division, Department of Medicine, Brigham & Women’s Hospital and Harvard Medical School, Boston, MA, U.S.A., and Vascular Medicine Research Unit, Brigham & Women’s Hospital, 65 Landsdowne Street, Room 275, Cambridge, MA 02139, U.S.A.

Abstract
Gender differences in cardiovascular mortality are well documented and oestrogen replacement therapy in post-menopausal women is associated with improved outcomes from cardiovascular events. Indeed, oestrogen therapy has been shown to restore endothelial function in post-menopausal women and reduce the development of atherosclerotic lesions. Despite extensive evidence on the beneficial effect of oestrogen, relatively little is known regarding the molecular signalling mechanism(s) by which oestrogen exerts some of its vascular effects. While the nuclear function of the oestrogen receptor is clearly established, previous studies regarding the membrane and cytoplasmic actions of oestrogen remain inconclusive. Cross-coupling of the oestrogen receptor to phosphoinositide 3-kinase signalling suggests a potentially critical non-nuclear action of the oestrogen receptor and considerably broadens our understanding of the cellular effects of oestrogen.

Introduction
Oestrogen elicits endothelium-dependent vasodilation through the release of endothelium-derived NO. This is, in part, due to the activation of endothelial NO synthase (eNOS) by the oestrogen receptor (ER) via the mitogen-activated protein kinase (MAPK) pathway [1]. However, the cross-coupling of the ER to the phosphoinositide 3-kinase (PI 3-kinase) signalling pathway leads to a much more robust eNOS activity and is the predominant pathway by which oestrogen elicits some of its vascular protective effects [2,3]. Similarly, the phosphorylation of eNOS in response to laminar shear stress occurs via the PI 3-kinase pathway [4], and activators of PI 3-kinase, such as insulin and vascular endothelial growth factor, increase eNOS activity [5,6]. Indeed, Akt, which is a downstream target of PI 3-kinase, can directly phosphorylate and activate eNOS [4,7].

PI 3-kinase is a critical mediator of the cellular effects of many growth factors, such as platelet-derived growth factor, insulin and vascular endothelial growth factor. PI 3-kinase is a heterodimeric phosphoinositide kinase that is composed of an 85 kDa (p85α) adapter/regulatory subunit and a 110 kDa (p110) catalytic subunit. PI 3-kinase phosphorylates the D-3 position of the phosphatidylinositol ring, catalysing the synthesis of PtdIns(3)P, PtdIns(3,4)P2 or PtdIns(3,4,5)P3. These lipid mediators act as second messengers, which activate proteins containing specific PtdIns(3,4,5)P3-binding or pleckstrin homology domains. For example, the increase in intracellular PtdIns(3,4,5)P3 and PtdIns(3,4)P2 leads to the activation of phosphoinositide-dependent protein kinases (PDKs), such as PDK-1 and PDK-2. The PDKs, in turn, selectively phosphorylate and activate the serine/threonine protein kinase Akt. The activation of Akt through its pleckstrin homology domain mediates many of the downstream cellular effects of PI 3-kinase, including activation of p70 S6-kinase and cell survival pathways.

ER structure and function
The ER, like other members of the family of nuclear hormone receptors, is composed of several distinct regions, which participate in binding to ligand, DNA and regulatory proteins that modulate its function [8,9] (Figure 1). Classically, ER regulates gene expression in target tissues in a ligand-dependent manner: the binding of oestradiol (E2) releases ER from an inhibitory complex and allows for receptor homodimerization and translocation into the nucleus. The receptor then binds to a palindromic oestrogen response element (ERE), which is located in the promoter region of target genes. The concerted actions of the ligand-independent activation function domain (AF-1) in the N-terminus and the ligand-dependent activation function domain (AF-2) in the hormone-binding domain lead to the recruitment of tissue-, cell-, and promoter-specific co-regulator complexes to the ERE, resulting in transactivation or transrepression. 17β-Oestradiol, as opposed to anti-oestrogens such as ICI 182,780 or SERM (i.e. tamoxifen or raloxifene), induce distinct conformational changes in ERα [10], thereby exposing surfaces on the receptor that permit it to interact
Figure 1 | Structure and function of ERα

Structural domains include a ligand-independent transactivation function domain (AF-1), a DNA-binding domain, a hormone-binding domain and a ligand-dependent transactivation function domain (AF-2). Putative regions of interaction with other proteins and sites of phosphorylation by various kinases are also shown.

<table>
<thead>
<tr>
<th>A/B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormone-binding</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA-binding (2 zinc fingers)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AF-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AF-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimersation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hsp90-binding</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caiselin-1-binding</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coactivator-binding (LXXLL motif)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site-specific phosphorylation</td>
<td>(ser116)</td>
<td>(ser157)</td>
<td>(ser531)</td>
<td></td>
</tr>
</tbody>
</table>

with distinct transcriptional machinery apparatus [11,12] or specific adapter molecules (i.e., ERAP160) [13]. Indeed, specific peptide antagonists can inhibit the function of ERα by binding the ligand–ERα complex at sites outside the ligand-binding pocket [14].

It is possible that oestrogen may induce conformational changes in ERα, which allows a ligand–receptor complex to interact with the regulatory subunit of PI 3-kinase, p85α (Figure 2). This is consistent with the finding that oestrogen, but not ICI 182,780 or tamoxifen, promotes the direct interaction of ERα with p85α in a cell-free system that is independent of any accessory or adapter molecules [2]. In addition, other steroid hormone receptors, such as the glucocorticoid and vitamin D receptors, have also been linked to the activation of PI 3-kinase [15,16]. These findings, therefore, raise the intriguing possibility that these traditional steroid hormone ‘nuclear receptors’ may mediate some of their biological effects via a similar non-transcriptional mechanism involving cross-coupling to PI 3-kinase signalling. The role, if any, of ERβ, which is synthesized on a separate gene and is structurally and functionally distinct, in such events has not been explored.

Signalling cascades activated by ER

Oestrogenic transcription-dependent effects, such as those, that contribute prominently in organogenesis and function of the reproductive system, become evident hours after stimulation. Non-nuclear (alternatively referred to as ‘non-transcriptional’ or ‘non-genomic’) oestrogenic action peaks...
minutes after stimulation in multiple cell types. Other characteristics include immunity to inhibitors of DNA transcription or protein synthesis (actinomycin D or cycloheximide) and recruitment of membrane or cytosol-localized signalling components. These include the second messengers Ca$^{2+}$ and NO, receptor tyrosine kinases [e.g. epidermal growth factor receptor and insulin-like growth factor (IGF) 1 receptor (IGF-1R)], G-protein-coupled receptors, and protein kinases (e.g. P1 3-kinase, Akt, MAPK family members, the non-receptor tyrosine kinase Src, and protein kinases A and C).

The PI 3-kinase–Akt signalling cascade is one downstream target of non-nuclear oestrogenic signalling [2,3]. In the vasculature, short-term exposure to E2 leads to NO-dependent vasodilation. The secretion of NO by healthy vessels relaxes smooth muscle cells and inhibits platelet activation in a cGMP-dependent mechanism. In cultured endothelial cells, oestrogen enhances NO release within minutes, without altering expression of eNOS [1,3]. E2 activates eNOS activity in a biphasic manner through MAPK and PI 3-kinase–Akt pathways, leading to enhanced NO release [2]. Myocardial protection by high-dose corticosteroids during ischaemia–reperfusion injury also appears to be mediated by PI 3-kinase–Akt [15]. In both cases, ERα and glucocorticoid receptors activate PI 3-kinase by association with the p85α regulatory subunit in a ligand-dependent manner [2]. Furthermore, the 90-kDa heat-shock protein (hsp90) interacts with both eNOS and Akt, and modulates eNOS activity by acting as a scaffold to regulate Akt-dependent phosphorylation of eNOS [17].

MAPK family members are common targets of non-nuclear oestrogenic signalling. Induction of eNOS and inducible NOS expression in cardiac myocytes is blocked by the MAPK inhibitor PD98059 [18]. This may be clinically relevant since NO inhibits the activation of caspases and prevents the development of congestive heart failure [19]. Oestrogen also activates extracellular-signal-regulated protein kinases 1 and 2 (ERK1/2) in cardiomyocytes, colon cancer, breast cancer and bone, and inhibits ERK1/2 in vascular smooth muscle cells and lung myofibroblasts. In the heart, ERα also selectively activates the 38-kDa isoform of MAPK (p38) to modulate the development of pressure-overload hypertrophy [20], which is consistent with recruitment of p38 in other models of cardiac hypertrophy [21]. In endothelial cells, oestrogen prevents disruption of the actin cytoskeleton during ischaemia, prevents cell death and enhances injury-dependent angiogenesis by rapidly and selectively activating the anti-apoptotic β isoform of p38 (p38β) and inhibiting pro-apoptotic p38α, which leads to the increased expression of MAPK-activated protein kinase-2 kinase and phosphorylation of hsp27 [22]. Downstream effects include preservation of stress fibre formation and membrane integrity, prevention of hypoxia-induced apoptosis, and induction of both endothelial cell migration and the formation of primitive capillary tubes [22].

It is possible that ERα might direct the activation of more receptor-proximal signalling complexes located at the plasma membrane. When over-expressed in cells, ligand-bound ERα induces the rapid phosphorylation of IGF-1R and the activation of ERK1/2. Because these receptors co-immunoprecipitate in a ligand-dependent manner, a direct physical interaction between ERα and IGF-1R could conceivably mediate the activation of ERK1/2. In breast cancer cell lines, ligand-bound ERα promotes the rapid phosphorylation of the proteins Src and Shc, resulting in a Src–Grb2–Sos (where Grb2 is growth factor receptor binding protein 2 and Sos is son of sevenless) complex formation [23], leading to downstream activation of Ras, Raf, and MAPK. Similarly, in both breast cancer and prostate cancer cells, E2 treatment induces the association of ERα phospho-Tyr537 with the Src homology 2 domain, leading to activation of the Src–Ras–ERK pathway and cell cycle progression [24]. In addition, in breast cancer cells, Src modulates PI 3-kinase–Akt signalling through a reversible cross-talk mechanism whereby the ligand-bound ER forms a ternary complex composed of ERα, PI 3-kinase and Src [25]. Cross-talk between PI 3-kinase and Src has also been observed in osteoclasts [26] and bone marrow cells [27].

Non-nuclear signalling can also amplify the nuclear, transcriptional activity of ERα; for example, in lactotroph cells, E2 rapidly activates ERK1/2, leading to increased transcription of PRL, the prolactin gene, thus creating an additive effect on PRL expression by complementing the directERE-dependent transcriptional activation of PRL by ERα [28]. Non-nuclear ERα activity can also elicit ERE-independent transcriptional activation. In cardiac myocytes, E2 rapidly increases ERK1/2-dependent expression of the early growth response-1 gene (egr-1) by inducing the recruitment of serum response factor to serum response elements in the egr-1 promoter [29].

Growth factors such as epidermal growth factor and IGF-1 can stimulate the nuclear activity of ERα through a non-nuclear, E2-independent mechanism. Through the cross-talk of molecular networks, mitogenic extracellular signals are translated into cell-cycle progression or, in cancer cells, into hormone-independent proliferation [30]. Epidermal growth factor- and IGF-1-mediated stimulation of MAPKs result in the direct phosphorylation of ERα at Ser118 [31,32], which enhances the binding of p68 RNA helicase [33], and promotes AF-1-dependent transcriptional activity in uterine [34] and ovarian adenocarcinoma cells [35]. Nuclear co-regulator proteins can also be phosphorylated by ERK1/2, leading to increased transcriptional activity. Finally, Src may enhance AF-1 function of ERα through either an Src–Raf–1–MAPK/ERK kinase (MEK)–ERK pathway that leads to phosphorylation of Ser118, or a pathway that includes Src, MEK kinase, Jun N-terminal kinase and Jun N-terminal kinase that regulates AF-1-associated co-activators.

**Mechanisms for ERα activity at the plasma membrane**

Membrane binding sites for E2 were first implicated in 1977 [36], and additional indirect evidence for a
membrane-associated ERα comes from immunohistochemistry [37], overexpressed nuclear receptors [38], or studies with membrane-impermeable ligands [39]. The trafficking of ERα to different cellular compartments may be regulated by the nature of the stimulation. For example, in vascular smooth muscle cells transfected with ERα, MAPK activation mediates the nuclear translocation of ERα from the membrane fraction by both E2-dependent and E2-independent mechanisms [40]. However, because ERα has no intrinsic kinase or phosphatase activity, does not have hydrophobic stretches that could represent transmembrane domains, and lacks myristoylation and palmitoylation sequences that could anchor it to the membrane, membrane localization of the receptor seems unlikely. Alternatively, the receptor may associate with membrane caveolae in fractionated plasma membranes from endothelial cells, ERα is localized to caveolae, and E2 stimulates eNOS in isolated caveolae in an ERα- and calcium-dependent manner [41]. There is evidence that within the caveolae of endothelial cells, hsp90, eNOS, and cave-1 (the coat protein of caveolae) exist in a heterotrimERIC complex that modulates eNOS activity depending on intracellular calcium levels [42,43].

Non-nuclear ERα signalling also involves membrane-associated heterotrimeric G-proteins. In Chinese hamster ovary cells transfected with ERα cDNA, treatment of membrane fractions with oestrogen activated Gαq and Gq, and rapidly stimulated inositol phosphate production and adenylate cyclase activity respectively [38]. G-protein activation also occurs in endothelial cells, where E2 activation of eNOS can be inhibited with the ER antagonist ICI 182,780, regulator of G-protein signalling (RGS)-4 (a regulator of G-protein signalling specific for Gαq and Gq), or pertussis toxin (specific for Gαi) [44]. Interestingly, the activation of PI 3-kinase by oestrogen is associated with a temporal increase in cytoplasmic ERα where it co-localizes and presumably interacts with p85α [2]. Furthermore, oestrogen-stimulated PI 3-kinase activity is temporally associated with the serine/threonine phosphorylation of Akt, and the activation of Akt and eNOS. The PI 3-kinase/Akt pathway has been implicated in the regulation of growth factor-induced cell growth and proliferation, as well as inhibition of cellular apoptosis [45,46]. Akt is known to block apoptosis via the phosphorylation of multiple downstream signalling molecules [47], including inactivation of the BCL-2 family member BAD [48] and inhibition of cell death pathway enzyme caspase-9 [49]. The phosphorylation of these targets by Akt may potentially provide a link between the PI 3-kinase/Akt pathway and the anti-apoptotic effects of oestrogen since oestrogen is known to elicit anti-apoptotic and mitogenic effects in various tissues [50].

**Conclusion**

We are just beginning to appreciate the complexity of ERα signalling. Cross-coupling of ER with PI 3-kinase may serve as an important mechanism for mediating some of the cardiovascular protective effects of oestrogen. Future research efforts will undoubtedly reveal further intricacies of the expression and translocation of endogenous ERα and possibly the identity of a new receptor that binds E2 and activates non-nuclear signalling. In addition, the activity of co-regulators and their role in distinguishing the nuclear and non-nuclear activities of ERα remain to be defined. A full understanding of these highly cell- and promoter-specific mechanisms will allow the development of specific agonists and antagonists that elicit only the beneficial effects of oestrogen.

**References**


Received 9 August 2002