Striatin assembles a membrane signaling complex necessary for rapid, nongenomic activation of endothelial NO synthase by estrogen receptor α

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Steroid hormone receptors (SHRs) are ligand-activated transcription factors that regulate gene expression. SHRs also mediate rapid, nongenomic cellular activation by steroids. In vascular endothelial cells, the SHR for estrogen, estrogen receptor (ER) α , is targeted by unknown mechanisms to a functional signaling module in membrane caveolae that enables estrogen to rapidly activate the mitogen-activated protein kinase and phosphatidylinositol 3-Akt kinase pathways, and endothelial NO synthase (eNOS). Here we identify the 110-kDa caveolin-binding protein striatin as the molecular anchor that localizes ER α to the membrane and organizes the ER α -eNOS membrane signaling complex. Striatin directly binds to amino acids 183–253 of ER α , targets ER α to the cell membrane, and serves as a scaffold for the formation of an ER α -Gai complex. Disruption of complex formation between ER α and striatin blocks estrogen-induced rapid activation mitogen-activated protein kinase, Akt kinase, and eNOS, but has no effect on ER-dependent regulation of an estrogen response element-driven reporter plasmid. These findings identify striatin as a molecular scaffold required for rapid, nongenomic estrogen-mediated activation of downstream signaling pathways. Furthermore, by demonstrating independent regulation of nongenomic vs. genomic ER-dependent signaling, these findings provide conceptual support for the potential development of “pathway-specific” selective ER modulators.

Estrogen, acting via its two receptors, ER α and ER β , exerts diverse effects on a variety of tissues (reviewed in ref. 1). Estrogen receptors (ERs) regulate cellular function via two signaling pathways referred to as “genomic” and “nongenomic.” Genomic effects of estrogen are mediated by nuclear ERs that act as ligand-activated transcription factors. Nongenomic effects of estrogen are also mediated by ERs, but they occur relatively rapidly and do not involve alterations in gene expression. We and others (2–9) have shown that vascular cells express ERs capable of mediating both genomic and nongenomic effects. Nongenomic effects of estrogen in the vasculature have been best studied in vascular endothelial cells. In these cells, estrogen rapidly and sequentially activates the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase–Akt kinase pathways, which increases the activity of endothelial NO synthase (eNOS) (4, 10–13). This pathway contributes to the rapid, estrogen-induced arterial vasodilation observed in clinical studies (14, 15). ER α is required for rapid activation of eNOS by estrogen (4, 10–12), and recent reports demonstrate that these nongenomic effects are transduced by a subpopulation of ER α localized to caveolae (3), cell membrane microdomains important for many signaling pathways. However, the molecular mechanisms by which ER α localizes to the caveolae and the details by which ER α activates membrane-associated eNOS remain obscure.

We now report the identification of striatin as an ER α -binding protein. Striatin is a 780-aa protein first isolated from rat brain synaptosomes (16, 17). Striatin is a calmodulin-binding member of the WD-repeat family of proteins and contains at least four

protein–protein interaction domains, including caveolin-binding (amino acids 55–63), coiled-coil (amino acids 70–116), Ca²⁺-calmodulin-binding (amino acids 149–166), and a series of eight WD repeat (amino acids 419–780) motifs (16) (Fig. 1A). WD repeats are found in a wide variety of proteins, including the signal-transducing G protein β -subunit, as well as other proteins that organize and regulate signal transduction, cytoskeletal organization, and vesicular fusion (18). Striatin also forms a complex with protein phosphatase 2A (19), which we have shown recently also binds directly to and regulates the function of ER α (20). We now show further that striatin targets ER α to the cell membrane and serves as a scaffold for the assembly of proteins required for rapid, estrogen-induced activation of eNOS.

Methods

Cell Culture, Transfection, and Luciferase Assay. Rad91 cells are a spontaneously immortalized vascular smooth muscle cell line derived from a human radial artery. EAhy926 cells, a human aortic endothelial cell hybridoma, were the kind gift of C.-J. Edgell (University of North Carolina, Chapel Hill). GH3B6 cells are a rat pituitary tumor cell line; Cos1 cells and MCF7 human breast cancer cells were obtained from American Type Culture Collection. Where indicated, cells were treated with 10^{−8} M 17 β -estradiol (E2) or an identical amount of vehicle for 20 min. In coinubation experiments, cells were preincubated with either the ER antagonist ICI 182,780 (10^{−7} M) for 30 min or pertussis toxin (PTX) (100 ng/ml) for 120 min before the E2.

For the protein expression studies, cells were transfected by electroporation as described (6, 21). For luciferase assays, cells were transfected with PolyFect (Qiagen, Valencia, CA). Cos1 cells were plated in 12-well plates and cultured in DMEM without phenol red with 10% estrogen-deficient FBS overnight. After transfection (20 h), the cells were washed and treated with either 10^{−8} M E2 or vehicle for 9 h. Cells were lysed in reporter lysis buffer (Promega), and luciferase assays (luciferase assay system, Promega) and β -galactosidase assays (Tropix, Bedford, MA) were performed according to the manufacturer’s guidelines.

Plasmids. The bait plasmid PGBT9-ER α was constructed by inserting the 1.8-kb full-length cDNA of human ER α into the *EcoRI* site of the Gal4 DNA-binding domain vector pGBT9 (Clontech). The pGAD10-ER α and pGAD10-ER β plasmids were cloned by insert-

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Abbreviations: ER, estrogen receptor; MAPK, mitogen-activated protein kinase; eNOS, endothelial NO synthase; E2, 17 β -estradiol; IGFR, insulin-like growth factor receptor; EGFR, epidermal growth factor receptor; PTX, pertussis toxin.

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