ER α on the cell membrane helps the heart

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17 β -Estradiol is a lipophilic steroid hormone that can pass through the cell's phospholipid membranes and bind to two different classes of estrogen receptors (ERs): the nuclear steroid hormone receptors ER α and ER β [1] and the G protein-coupled receptor (GPER) also known as the G protein-coupled receptor 30 (GPR30) [2, 3]. Hormone binding triggers distinct responses in different tissues through genomic, non-genomic, and combined pathways.

The classical ERs are ligand-activated transcription factors. Hormone binding triggers their dimerization, migration to the nucleus, and binding to hormone-response elements on DNA. A separate gene, *ESR1* and *ESR2*, encodes each receptor. ER α and ER β exhibit significant overall sequence homology, and both are composed of five domains. Since ER α and ER β are co-expressed in many cell types, the receptors may form ER α ($\alpha\alpha$) or ER β ($\beta\beta$) homodimers or ER $\alpha\beta$ ($\alpha\beta$) heterodimers. However, there is increasing evidence that ERs are also present on the endothelial cell surface where they can signal in a non-genomic fashion. ERs may associate with cell membranes by attachment to caveolin-1 and form complexes with G proteins, striatin, and receptor tyrosine kinases such as epidermal growth factor (EGFR), insulin-like growth factor (IGF-1), and non-receptor tyrosine kinases such as Src. By interacting with striatin, ERs may influence cytosolic Ca²⁺ and nitric oxide. As mentioned, estrogen also binds to GPER resulting in intracellular calcium mobilization and synthesis of phosphatidylinositol (3,4,5)-triphosphate [3].

Endogenous estrogens contribute to the low prevalence of atherosclerotic vascular disease in premenopausal women with intact ovarian function, and cessation of estrogen production following menopause increases cardiovascular risk [4]. Myocardial remodeling, defined as the molecular and cellular events after an injury to the myocardium such as

myocardial infarction, is more favorable in women than in men, and estrogen is believed to be responsible [5]. Work presented in this issue of J Mol Med may bring us a little closer to the mechanisms involved [6]. Lee et al. present an evidence that membrane ER α attenuates myocardial fibrosis via Ras homolog member A (RhoA) and Rho-associated, coiled-coil containing protein kinase 1 (ROCK)-mediated actin remodeling [6, 7].

The authors ovariectomized (OVX) female Wistar rats. Two weeks later, the left-anterior descending coronary artery was ligated, a standard myocardial infarction model. The rats were then assigned to placebo or the implantation of a 60-day release pellet of 17 β -estradiol. Four weeks were allowed to pass to permit remodeling to be completed in intact female rats that have endogenous estrogen, OVX rats that have no estrogen, or OVX rats receiving replacement 17 β -estradiol. The collagen area fraction was twice as large in the estrogen-deficient OVX group than in the two groups with estrogen, a finding that was verified by measuring the proline content. RhoA was more phosphorylated in the OVX controls than in the two groups with estrogen, while cofilin was more phosphorylated in the estrogen-deficient OVX group. Actin-binding proteins, such as cofilin, regulate assembly and disassembly of actin filaments. The data suggest that 17 β -estradiol treatment was associated with RhoA/Rho kinase inhibition as evidenced by increased phosphorylation of RhoA and decreased phosphorylation of downstream factors including cofilin.

The authors then moved to an in vitro model. They isolated infarcted hearts from female OVX rats and subjected these hearts to vehicle, 17 β -estradiol, 17 β -estradiol plus an inhibitor of GPER, ICI 182,780, which is also an ER α agonist, and 17 β -estradiol conjugated with bovine serum albumin. This configuration provides a membrane-impermeable conjugate of estrogen. The authors then included the ER α agonist, PPT, or a specific ER β agonist, DPN. The hearts were perfused with a non-circulating modified Tyrode solution in a Langendorff preparation. After perfusion, the hearts were harvested for western blotting. 17 β -estradiol administration led to decreased cofilin phosphorylation. The 17 β -estradiol

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Fig. 1 RhoA is a small GTPase. The RhoA is active when bound to GTP and inactive when bound to GDP. GTPase-activating proteins (GAPs) accelerate GTP hydrolysis, while guanine nucleotide exchange factors (GEFs) catalyze GTP exchange. GTP-RhoA acts on ROCK, which in turn phosphorylates LIM that phosphorylates cofilin, thereby regulating actin filaments. Lim et al. argue that cofilin phosphorylation is diminished through the action of 17 β -estradiol

plus GPER antagonist, ICI, led to increased cofilin phosphorylation back to control levels. The cell-membrane impermeable 17 β -estradiol inhibited cofilin phosphorylation to the same degree as 17 β -estradiol, implying that cellular entry of 17 β -estradiol was not necessary for reducing cofilin phosphorylation. PPT resulted in attenuated cofilin phosphorylation similar to 17 β -estradiol, while DPN did not influence cofilin phosphorylation, which remained at control levels. This experiment suggested that ER β is not involved directly in regulating cofilin.

The authors then performed additional *in vitro* studies to address the role of ROCK in regulating cofilin phosphorylation. They used a ROCK inhibitor, Y-27632, and relied on the perfused heart model described above. Compared to controls, cofilin was inhibited to the same degree by the ROCK inhibitor as it was by 17 β -estradiol. The authors thus concluded that the inhibition of cofilin phosphorylation relies on the ROCK-signaling pathway. Putting these components together suggests that 17 β -estradiol activates surface GPER and membrane-docked ER α . Both act to inhibit RhoA/ROCK/cofilin pathway. Transcriptional machinery is not necessary for this function of 17 β -estradiol signaling.

The role of RhoA/ROCK as a key regulator of actin organization and thus regulator of cell migration deserves additional attention. ROCK belongs to the PKA/PKG/PKC family of serine–threonine kinases and is pivotal in regulating the shape and movement of cells by acting on the cytoskeleton. RhoA is a small GTPase. When RhoA-GTP is not present, an auto-inhibitory intramolecular fold reduces the kinase activity of ROCK and vice versa (Fig. 1) [8]. ROCK can phosphorylate various substrates including LIM kinase, myosin light chain (MLC), and MLC phosphatase [9]. When these substrates are phosphorylated, actin filament organization and contractility are affected. For instance, ROCK inhibits the depolymerization of actin filaments. ROCK phosphorylates and activates LIM kinase, which in turn phosphorylates

cofilin, which inactivates its actin-depolymerization activity. This state of affairs stabilizes the actin filaments and increases their numbers. The increased stable actin filaments and loss of actin monomers reduce cell migration.

How does decreasing the phosphorylation of cofilin result in less fibrosis after myocardial infarction? According to our schema (Fig. 1), phosphorylated cofilin leads to actin filament stabilization, while inactive cofilin favors actin depolymerization. Cofilin functions with other actin-binding proteins, integrin–talin complexes, formins, etc. [10]. Cofilin is a central component of actin dynamics in migrating cells by catalyzing either actin polymerization or depolymerization through its severing activity. All of this activity allows the locomotory protrusions of migrating cells. Cofilin inhibition promotes protrusion defects and decreases chemotaxis. Presumably, the production of fibrosis is an active process of myofibroblasts. In any event, the ROCK inhibitor, fasudil, has been shown to reduce myocardial fibrosis [11]. Are there therapeutic lessons here? Possibly, but the disappointing results of hormone-replacement therapy suggest that much more basic work needs to be done.

Respectfully,
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