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ew clinical studies have impacted clinical care more dramatically than those addressing the role of hormone replacement therapy (HRT) as a strategy to reduce cardiovascular risk. Based on large observational and sound biological studies addressing cardiovascular risk, and on the efficacy of HRT in reducing perimenopausal symptoms, HRT was the standard-of-care for postmenopausal women for many years. Then, as a result of landmark prospectively randomized clinical trials published within the last few years,2,3 the recommendations changed, such that HRT is no longer routinely prescribed for postmenopausal women.4

How could observational studies and biology have been so wrong? The present study by Smith et al5 may be a beginning in understanding subtleties implicit in the use of HRT. It is postulated that estrogen has pleiomorphic cardiovascular effects including direct effects on the vessel wall and on extracellular matrix proteins and components, complex regulation of lipid metabolism7 and nitric oxide synthesis,8 and effects on the balance between thrombosis and fibrinolysis9—all areas of critical importance in atherogenesis. Many of these effects are mediated via interactions of estrogen with two receptors: ERα and ERβ.10 Important downstream events modulated by ERα and ERβ include regulation of the expression of a number of genes, one of which is plasminogen activator inhibitor-1 (PAI-1), arguably among the most important of the regulators of fibrinolytic balance.

At first glance, one might ask why the present study, which identifies a new estrogen response element (ERE) in the PAI-1 promoter that responds to ERα activation, would merit editorial comment. After all, many EREs have been identified in the regulatory regions of countless genes that might be important in atherogenesis.11 When considered in light of other recent reports, however, the significance of the findings of Smith et al5 is worthy of additional consideration. In brief, the authors demonstrate that after treatment of bovine aortic endothelial cells (BAECs) with estrogen, ERα interacts with at least one estrogen response element in the PAI-1 promoter, resulting in activation of PAI-1 expression. In contrast, ERβ fails to induce, and possibly suppresses, PAI-1 promoter activity in an estrogen-dependent manner. Based on mutational analysis of the PAI-1 promoter, it is clear that these interactions likely involve not only two AP1-like elements (P-box and D-box) but likely other EREs. The precise molecular mechanisms involved—whether because of changes in the confirmation of receptor subtypes and dimerization on agonist stimulation, direct effects through binding to the PAI-1 promoter, effects of coregulators (coactivators or corepressors), or intracellular signals related to estrogen effects on membrane-linked receptors—remain to be defined. The role of coregulators warrants consideration because coregulators have been shown to be important in determining not only the promoter specificity of ER but also the cell specificity. Recently, it has been proposed that coregulators may be the basis for differential transcriptional activity of ER-ligand complexes in different tissues and cells—uterus versus aorta, vascular smooth muscle cells versus endothelial cells (ECs)—and may explain differences in cultured ECs in contact (or not) with macrophages.10

The role of coregulators may well explain the observations of Smith et al5 that tamoxifen, a selective estrogen receptor modulator (SERM), differentially regulates ERs. Tamoxifen-suppressed ERα-induced activation of PAI-1 promoter but had no effect on ERβ-induced suppression of the gene. However, a statistically significant activation of PAI-1 promoter was observed in cells transfected with equimolar concentrations of ERα+ERβ in the presence of tamoxifen. The most likely explanation for these findings is that promoter-specific ER-coregulator interactions occur in tamoxifen-treated cells and explain the differential expression of ERs in various tissues. The known effects of raloxifene, another SERM that demonstrates estrogenic activity in bone and cardiovascular system while it inhibits estrogenic action in uterus and breast, further support this hypothesis.12

The clear implication of these findings is that if estrogen effects on PAI-1 expression are dependent on the balance between ERα and ERβ, then an entirely new understanding of the variable effects of estrogen can begin to be formulated. ERα and ERβ are both expressed in the vasculature, and are differentially regulated.11 ERβ expression is upregulated by vascular injury13 and an inverse relationship between ERβ and vasoconstriction in response to estrogen has been defined in mouse studies14 and may also be the case in humans, based on studies of an ERβ polymorphism and hypertension in postmenopausal women.15 Although regulation of vascular tone depends on a variety of physiological effects (many of which involve nitric oxide), differential effects of a single mediator (estrogen) via its action on two receptors (ERα and ERβ) support the likelihood that gene-environment interactions may play a critical role in understanding the effects of estrogen. For instance, one might imagine environmental factors that differentially regulate the
Differential regulation of PAI-1 gene activation by estrogen receptors in endothelial cells. Estrogen (E) binding to ERα induces conformational changes of the ERα, which lead to its dimerization. After binding of a coactivator to the receptor dimer, the dimer can directly interact with estrogen response element(s) (ERE) on the promoter of PAI-1 gene and initiate transcription. ERα may suppress the ERα-induced transcription of PAI-1 gene in two different ways. First, ERβ may bind to other response element(s) on the PAI-1 promoter and, together with a corepressor, inhibit gene transcription. Second, ERβ may form heterodimers with ERα and negatively regulate transcription of PAI-1. It is also possible that unique response elements exist within the promoter of the PAI-1 gene that interact preferentially with ERα/ERβ heterodimers. ERβ may also exert an indirect effect on PAI-1 gene transcription through a membrane-linked receptor system (E-GPCR?). Another indirect mechanism for ER-induced gene regulation is via activation of intracellular signaling pathways. Arrows indicate activation and inhibitory mechanisms.

expression of ERα and ERβ and, hence, the effect of estrogen on PAI-1 expression, and likely its effect on the expression of other important proteins. Or, polymorphisms may exist in the promoter region of PAI-1, or of other important estrogen-regulated genes, that result in effects that reflect more ERα than ERβ effects, or vice versa.

As illustrated in the Figure, these findings provide a paradigm for studying estrogen effects in cultured cells. The finding of differential regulation by ERα and ERβ in regard to PAI-1 gene expression is of particular note. PAI-1 is regulated by many factors thought to be important in vascular dysfunction, including not only estrogen, but glucose and insulin, and prominently by components of the renin-angiotensin system. PAI-1 plays distinct roles in regulation of fibrinolytic balance, but also in cell migration and in atherosclerotic plaque stability. For understanding the complex role of estrogen in the regulation of cardiovascular disease, it would be difficult to choose a more important target than PAI-1. To be sure, the present study is limited by the fact that the only studies reported are those in cultured BAECs, a reasonable model, but a model nonetheless. An added level of complexity would involve analysis of a number of estrogen-responsive genes in a like manner. Or, as more sophisticated approaches to analysis of promoter response elements in mouse models become available, the hypothesis proposed by Smith et al. may be able to be tested in vivo. Likewise, additional genetic studies examining cardiovascular parame-

ters in individuals with polymorphisms in this region of the promoter would be of interest. The principle point is that in demonstrating differential effects of ERα and ERβ on PAI-1 promoter activity, Smith et al. have outlined a paradigm that may be useful in better understanding whether there will ever be a general role for estrogen in the prevention of cardiovascular disease, and also a potential system for testing various hormones for their effect in this important arena.

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In an article by Madamanchi et al (Circ Res. 2004;95:228–229), the name of the second author was misspelled. The name is Xi-Lin Niu, not Xilin Liu. The authors apologize for this error.