The role of SRC-3 in estrogen-dependent vasoprotection during vascular wall remodeling postinjury

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Estrogen receptors are hormone-inducible transcription factors requiring coactivators such as members of the SRC/p160 family to modulate the transcription of their target genes. This perspective will examine the interplay between estrogen receptors and their coactivators in vasoprotection during vascular wall remodeling.

Received April 26th, 2003; Accepted June 1st, 2003; Published June 15th, 2003 | **Abbreviations:** **ECs:** Endothelial cells; **HERS:** Heart Estrogen-progestin replacement study; **VSMCs:** Vascular smooth muscle cells

Cite this article: Nuclear Receptor Signaling (2003) **1**, e002

Introduction

The vasoprotective effects of estrogen are well recognized. The incidence of cardiovascular disease among premenopausal is significantly less than that among age-matched men and postmenopausal women [Mendelsohn and Karas, 1999]. Estrogen replacement therapy for postmenopausal women significantly reduces the incidence of atherosclerotic diseases [Mendelsohn and Karas, 1999]. Additionally, estrogen increases vasodilatation and inhibits the response to vascular injury [Mendelsohn and Karas, 1999]. The vasoprotective effects of estrogen are attributed to both systemic effects, such as decreased serum total cholesterol and alteration of serum lipoproteins, and direct effects on blood vessels through rapid non-genomic and longer-term genomic pathways [Mendelsohn and Karas, 1999]. Both the non-genomic and the genomic cardiovascular effects of estrogen are mediated by estrogen receptor α (ER α) and ER β [Bakir et al., 2000; Chen et al., 1999].

ERs are hormone-inducible transcription factors requiring coactivators to modulate the transcription of their target genes [Xu and O'Malley, 2002]. The steroid receptor coactivator-3 (SRC-3/p/CIP/RAC3/ AIB1/ACTR/TRAM-1) belongs to the p160 SRC family that also contains the other two homologous coactivators, SRC-1 and SRC-2 (TIF2/GRIP-1) [Xu and O'Malley, 2002]. These coactivators interact with ERs and other nuclear receptors (NRs) in a ligand-dependent manner and strongly coactivate the transcription of cellular target genes by NRs.

SRC-3 was recently shown to be associated with ER α bound to the estrogen responsive promoters of target genes after estrogen treatment [Shang et al., 2000]. The levels of SRC-3 are also related to the estrogen-dependent growth of certain breast cancer cells [Planas-Silva et al., 2001]. These findings suggest that SRC-3 may play an important role in mediating ER function *in vivo*.

Coexpression of SRC-3 with ER α and ER β in vascular smooth muscle cells (VSMCs) and endothelial cells (ECs)

Previous studies have shown that both ER α and ER β are expressed in VSMCs and ECs [Karas et al., 1994; Venkov et al., 1996]. As an initial step to study the role of SRC-3 in cardiovascular system, we investigated its expression pattern by using the phenotypically normal SRC-3$^{+/-}$ mice harboring a knock-in *LacZ* reporter that was regulated by the endogenous SRC-3 promoter [Xu et al., 2000]. Our results demonstrated that SRC-3 is highly expressed in both VSMCs and ECs throughout the entire blood vessel system, but not expressed in the myocardial cells [Yuan et al., 2002]. These findings indicate that SRC-3 is coexpressed with ERs in both VSMCs and ECs, suggesting that SRC-3 and ERs may have a functional partnership in these two cell types.

Faster Neointima Growth in Intact SRC-3-/- Mice

To investigate the role of SRC-3 in estrogen-facilitated vascular remodeling after vessel injury, we used a carotid artery ligation model whereby neointimal formation is consistently induced after cessation of blood flow [Kumar and Lindner, 1997]. The vasoprotective effect of estrogen in this model appears to protect the vascular wall from thickening through inhibiting neointimal formation during the injury-induced vessel wall remodeling. The basic experimental steps are illustrated in Figure 1 A. In WT mice, significant growth of a stenotic neointima was observed 4 weeks after ligation. Much higher levels of arterial neointima formation were observed in SRC-3$^{-/-}$ mice compared to WT mice (Figure 1 B, a & b). Quantitative measurements revealed that the average intimal area in SRC-3$^{-/-}$ mice was 2 fold larger than that in the age-matched WT mice. No differences in medial wall growth were observed between WT and SRC-3$^{-/-}$ mice [Yuan et al., 2002]. These observations demonstrate that disruption of the *SRC-3* gene facilitates

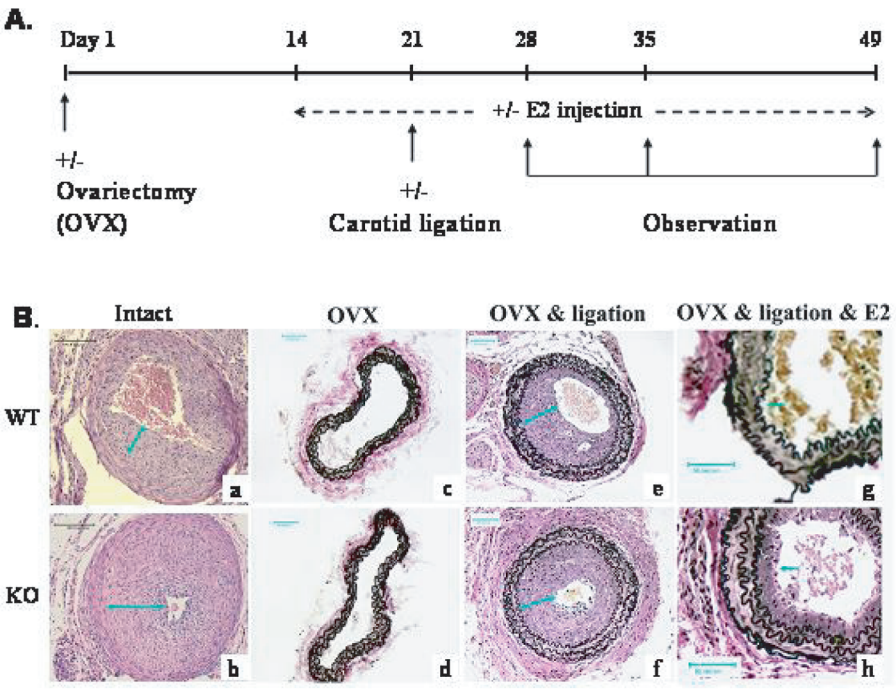

Figure 1 . Cardiovascular phenotype of SRC-3 knockout mice (A), experimental schedule for animal treatment. (B). (a) and (b), representative photomicrographs of neointima formation 4 weeks after carotid ligation in intact WT and SRC-3 knockout (KO) mice. (c) – (h), morphology of right control (c & d) and left ligated (e-h) carotid arteries of ovariectomized WT and SRC-3-/- mice treated with vehicle (c-f) or E2 (g & h) 2 weeks after ligation.

neointimal growth in the process of injury-induced vascular remodeling.

The faster growth of neointima in SRC-3$^{-/-}$ mice was not due to alteration of ER α or ER β levels since no changes of their protein levels in the vascular wall could be detected [Yuan et al., 2002]. Other contributing factors might involve estrogen and ER functional levels in SRC-3$^{-/-}$ mice. Actually, lower serum estrogen levels have been identified in female SRC-3$^{-/-}$ mice [Xu et al., 2000]. Therefore, analyses of neointimal formation were repeated in ovariectomized WT and SRC-3$^{-/-}$ mice, in which estrogen and estrogen-dependent ER function were largely eliminated. Indeed, the neointima was formed equally in both ovariectomized WT and SRC-3 $^{-/-}$ mice (Figure 1 B, e & f) [Yuan et al., 2002]. These results suggest that the neointimal overgrowth after vascular injury in the intact SRC-3$^{-/-}$ mice is likely accredited to the reduction in estrogen levels and/or ER functions.

Attenuated Inhibition of Neointima Formation in SRC-3-/- Mice by Estrogen

Next, we compared the degrees of estrogen (E2)-dependent inhibition of neointimal growth between ovariectomized and E2-treated WT and SRC-3$^{-/-}$ mice. E2 treatment almost completely inhibited the neointima formation after the carotid ligation in ovariectomized WT mice (Figure 1 B, g). In contrast, the parallel treatment with equal amounts of E2 only partially inhibited the neointima formation in the age-matched and ovariectomized SRC-3$^{-/-}$ mice (Figure 1 B, h). Statistics revealed a highly significant difference ($p < 0.01$) between the average intimal areas of WT and SRC-3$^{-/-}$ mice treated with E2. In addition, minimal changes in medial wall area were observed in vehicle- or E2-treated WT and SRC-3$^{-/-}$ mice. Correlated with the insensitive neointimal inhibition by E2, the neointima in SRC-3$^{-/-}$ mice also exhibited a higher degree of cell proliferation [Yuan et al., 2002]. In conclusion, the loss of SRC-3 in VSMCs and/or ECs results in a loss of sensitivity to estrogen-induced inhibition of neointima formation, probably due to an impaired suppression of intimal cell proliferation during vascular remodeling through a direct or indirect estrogen-regulated pathway.

Conclusion

Our study demonstrates that SRC-3, an ER coactivator, is expressed in VSMCs and ECs and required for the estrogen-dependent vasoprotective effect mediated by ERs. It is clear that removal of SRC-3 only partially desensitizes the inhibition of neointima formation by estrogen. This likely is due to the compensation of other ER coactivators such as SRC-1 and SRC-2 [Xu and O'Malley, 2002]. Former studies have shown that both ER α and ER β are responsible for the inhibition of the

vascular injury response by estrogen [Karas et al., 2001] . ER α is required for estrogen-accelerated reendothelialization [Brouchet et al., 2001], while ER β is required for regulation of vasoconstriction and blood pressure [Zhu et al., 2002]. Since a poor reendothelialization and a higher vasoconstriction may contribute to the neointima formation, SRC-3 may serve as a coactivator for both ERs in the inhibition of intima formation by estrogen. The clinical trial Heart Estrogen-Progestin Replacement Study (HERS) was unable to prove therapeutic effects of estrogen on pre-existing coronary artery diseases [Tolbert and Oparil, 2001]. Probably, an active estrogen-signaling pathway is required in the early stages of vascular injury to inhibit neointima formation as demonstrated in animal models [Mori et al., 2000]. In addition to the lack of estrogen and the reduction in ER levels as seen in diseased human coronary arteries [Losordo et al., 1994], the loss of ER coactivator functions in the diseased arteries will also result in the loss of estrogen-induced vasoprotection.

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