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Reversal of Fortune: Estrogen Receptor-Beta in Endometriosis

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Abstract

Enhanced inflammation and reduced apoptosis sustain the growth of endometriotic lesions. Alterations in the expression of estrogen receptor (ER) α and β accompany the conversion of resident endometrial cells within the normal uterine environment to ectopic lesions located in extra-uterine sites. Recent studies highlighted in this focused review linked ER β to dysregulation of apoptotic and inflammatory networks involving novel interacting partners in endometriosis. The elucidation of these non-genomic actions of ER β using human cells and mouse models is an important step in understanding key regulatory pathways that are disrupted leading to disease establishment and progression.

Keywords

endometriosis; estrogen receptor-beta; inflammation; non-genomic; apoptosome

Estrogens are key mediators of endometrial homeostasis, hence, any dysregulation in their synthesis, metabolism, and/or activities irrefutably leads to a broad range of endometrial pathologies. Two major estrogen receptor proteins, estrogen receptor-alpha (ER α) and estrogen receptor-beta (ER β) which are encoded by distinct genes (White *et al.*, 1987; Kuiper *et al.*, 1996), mediate the actions of estrogens in target cells. The significant homologies in DNA (94%) and ligand (59%)-binding domains between these ERs enable both proteins to bind estrogens with equal affinity and to transcriptionally regulate common subsets of ER-responsive genes. ER α and ER β display distinct spatial, temporal and physiological expression. Genetic deletions support most roles for ER α in the uterus and for ER β to prevail in the ovary (Hamilton *et al.*, 2014). While ligand-bound ER α is requisite for mitogenesis of uterine cells, ER β is considered to inhibit ER α -dependent cell proliferation in part, through its ability to form ER α /ER β heterodimers with different ligand specificity, interacting partners, and transcriptional targets than ER α homodimers (Pace *et al.*, 1997).

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Declaration of interest

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Endometriosis is an estrogen-dependent gynecological disorder, defined as the growth of endometrial stroma and glands in extra-uterine sites such as in the peritoneum and the ovary (Burney & Giudice, 2012). The disease affects 1 in 10 reproductive age women and can lead to debilitating pelvic pain, dysmenorrhea, and reduced fertility. While benign, endometriosis is a chronic condition that requires long-term treatment throughout a woman's reproductive years. The goals of treatment for endometriosis include hormonal suppression of active endometriosis or surgical excision/ablation of visible lesions (Vercellini *et al.*, 2014). However, medical therapies are not without side effects and surgical removal of the ovaries and uterus, while considered a definitive management for the condition, is not without associated morbidity.

In recent years, ER β has emerged as an important player in the pathogenesis of endometriosis. Human endometriotic lesions, whether of ovarian or peritoneal locations, display higher ER β expression when compared to normal human endometrial cells; this was not shown for ER α (Bulun *et al.*, 2012). The reversal in ER β to ER α ratio in lesions ($\gg 1$), relative to normal endometrial cells ($\ll 1$) has been similarly demonstrated in many animal models of endometriosis (Fazleabas *et al.*, 2003; Greaves *et al.*, 2014; Heard *et al.*, 2014) and raises the intriguing question of how the conventionally anti-proliferative ER β in the endometrium, in the context of endogenous estrogens, dismantles its customary role and orchestrates a pro-proliferative/anti-apoptotic/pro-inflammatory response to drive disease pathogenesis. In a recent issue of *Cell* (Han *et al.*, 2015), an elegant study by O'Malley and co-workers unequivocally demonstrated that ER β is required for the progression of endometriosis in mice and defined the apoptosome and inflammasome as endogenous targets of non-genomic ER β action. Katzenellenbogen and colleagues in a related study published in *Science Translational Medicine* (Zhao *et al.*, 2015) described two ER antagonists, one specific for ER α (chloroindazole, CLI) and the other specific for ER β (oxabicycloheptane sulfonate, OBHS), that individually function to inhibit the estrogen-inflammatory axis to suppress endometriosis in mice. These two studies collectively define a novel role for ER β in the upper echelon of the inflammatory regulatory hierarchy. Importantly, as inflammation is well-considered as one of the major contributors to the development and progression of endometriosis, the study findings offer promise for novel therapeutic strategies that may be relevant to endometriosis and other reproductive and non-reproductive diseases associated with chronic inflammation.

To uncover the underlying mechanism(s) by which ER β promotes endometriosis, O'Malley and his group used ovariectomized+E₂-pelleted immunocompetent mouse models in which endometriosis was surgically-induced through auto transplantation of ER β over-expressing (gain-of-ER β -function) and ER β null (loss-of-ER β -function) endometrial tissues in the peritoneal cavity. Ectopic lesions generated from ER β over-expressing (ER β -OE) endometrium had larger volumes while those from ER β null endometrium had smaller volumes, than did wildtype (WT; control) ectopic lesions. Consistent with these observations, the use of the ER β selective antagonist PHTTP (a pyrazolol [1.5- α] pyrimidine-based ligand) suppressed ectopic lesion growth relative to vehicle alone. The inhibitory effect of PHTTP on lesion growth was accompanied by the loss of recruitment of CD163-positive monocyte/macrophage cells that normally infiltrate lesions. Conversely, the ER β specific agonist ERB-041 enhanced the growth of mouse ectopic lesions compared with

vehicle alone. Because these noted changes in lesion growth occurred in the absence of perturbations in ER α , enhanced ER β expression and activity appear sufficient to promote endometriosis progression. In an earlier study (Harris *et al.*, 2005), athymic nude mice surgically implanted with human endometriotic lesions showed lesion regression when administered ERB-041, a response clearly contrasting with that obtained with the immunocompetent mouse model in the *Cell* study. Intriguingly, ectopic lesions formed in control and ERB-041-treated nude mice did not express ER β , a major departure from lesions of women and those generated in other animal models of endometriosis, in which ER β is the predominant ER isoform. While the molecular basis underlying the differential responses of the two mouse models to ERB-401 remains unknown, these results provide support for the complex interactions between the immune system and ER-mediated signaling in the development and progression of endometriosis. Additionally, these results highlight innate limitations of the animal models utilized for many endometriosis studies, including those described in the two highlighted papers in this review (e.g. the need to implant estrogen pellets, which most likely does not reflect/only approximates the situation in humans) and underscore the continuing need for the development of more relevant models to fully understand the human disease.

How might ER β alter lesion biology distinct from its role in non-diseased endometrial cells? The authors analyzed by mass spectroscopy, flag-tagged ER β -containing protein complexes that were immunoprecipitated from eutopic endometria of endometrial ER β -overexpressing mice. Further confirmation by Western blotting revealed that a majority of proteins interacting with ER β are involved in inflammation and apoptosis signaling. Apoptosis plays an important role in inflammation and in the resolution of inflammatory reactions, and the two are irrefutably linked since cell death signaling initiated by tumor necrosis factor-alpha (TNF- α) activates inflammasomes to initiate IL-1 β driven inflammation (Vince & Silke, 2016). An attractive candidate identified in the study as an ER β -interacting protein is apoptosis signal-regulating kinase-1 (ASK-1). ASK-1 is a component of TNF- α -induced apoptosis complex 1, whose formation is required for TNF- α -induced apoptosis. Serine/threonine kinase receptor-associated protein (STRAP) and 14-3-3 protein were also identified to interact with ER β in the same screen. Interestingly, STRAP and 14-3-3 proteins have been previously demonstrated to bind ASK-1 in a tripartite complex (Jung *et al.*, 2010). The formation of this complex interferes with the functional association between ASK-1 and the TNF receptor-associated factor 2 in response to TNF- α signaling, resulting in the inhibition of TNF- α -induced apoptosis. To validate this model, the authors demonstrated: 1) lower status of phosphorylated ASK-1 (phospho-Thr845 ASK-1), without accompanying changes in total ASK-1 protein levels, indicating loss of ASK-1 activation and hence, function, in ER β -OE ectopic lesions compared with WT ectopic lesions; 2) conversely, increased levels of phosphorylated ASK-1 in ER β -null ectopic lesions compared with WT ectopic lesions; and 3) lower mitochondrial cytochrome c levels in ER β -OE ectopic lesions, consistent with the disruption of TNF- α -induced ASK-1 activation that normally leads to caspase 9 activation. The link between ER β and caspase 9 was further illuminated by demonstrating that ER β -OE ectopic lesions had lower caspase 9 levels and lacked detectable interactions between caspase 9 and apoptotic peptidase-interacting factor; by contrast, the latter interaction was easily detected in ER β -null ectopic lesions. Taken together, these novel

findings identify TNF- α -induced apoptosis as a key regulatory pathway disrupted by cytoplasmic-based ER β to promote lesion survival. The cytoplasmic-localized ER β anti-apoptotic action likely occurs in conjunction with nuclear-localized ER β transcriptional activation of gene targets such as the serum and glucocorticoid-regulated kinase, which by phosphorylating the pro-apoptotic FOXO3, inhibits apoptosis (Monsivais *et al.*, 2016). However, the relative contribution of nuclear vs. cytoplasmic actions of ER β to lesion survival is unknown.

The intriguing idea that ER β multitasks outside of the nucleus was further demonstrated in other experiments from the same study. The authors identified caspase 1 and the NLR family pyrin-domain-containing 3 as additional ER β -interacting proteins. These findings are significant in the context of the inflammatory process, given the requisite participation of caspase 1 and NLR in the processing of pro-IL-1 β to the mature bioactive IL-1 β , a key regulator of adhesion and proliferation in endometrial cells (Kao *et al.*, 2011). In this regard, ER β -OE lesions had higher IL-1 β and cleaved caspase 1 levels than did control ectopic lesions and conversely, ER β -null lesions had lower levels of both components. Given that primary human endometriotic stromal cells also showed elevated levels of IL-1 β and increased anti-apoptosis signaling when treated with TNF- α , the results suggest that the key ER β -mediated events elucidated in these mouse models of endometriosis are relevant to the human disease.

Interestingly, the authors found, using immortalized human endometrial epithelial cells expressing Myc-tagged human ER α genes that TNF- α treatment of these cells did not elicit comparable anti-apoptotic responses and an increase in IL-1 β expression noted for the same human cells expressing ER β . More intriguingly, co-transfection of ER α and ER β in these cells resulted in ER α inhibition of ER β -mediated IL-1 β production, suggesting that ER α may assume a negative regulatory role in ER β -mediated promotion of inflammation. Whether this effect of ER α reflects its competitive displacement of ER β from its interacting proteins, or its sequestering of ER β through formation of ER α /ER β heterodimers is unknown. This result, while baffling, sheds new light on the contrasting activities of ER β and ER α in endometriosis and must be reconciled with previous findings that ER α is an equally active player in the pathology of this disease in mice (Burns *et al.*, 2012). In the study by Katzenellenbogen and colleagues (Zhao *et al.*, 2015), specific ER α (CLI) and ER β (OBHS) antagonists exhibited ER-dependent anti-inflammatory activities in a mouse model of endometriosis and in human endometriotic stromal cells. In these models, CLI and OBHS equally inhibited estrogen-dependent processes including cell proliferation, cyst formation, vascularization, cytokine production, macrophage infiltration and lesion growth. A previous study has reported the presence of both ER α and ER β in peritoneal fluid macrophages and shown that both ERs display higher expression in peritoneal fluid macrophages of women with endometriosis when compared to women without endometriosis (Montagna *et al.* 2008). Interestingly, this same study showed that only ER α expression was positively correlated with increased pro-inflammatory cytokine levels in peritoneal fluids of women with endometriosis. ER β levels while higher in peritoneal fluid macrophages of women with endometriosis, were correlated with expression of proinflammatory cytokines, irrespective of disease status, suggesting a role for macrophage ER β in basal pro-inflammatory cytokine production and for macrophage ER α as a likely

‘driver’ of increased inflammation seen in endometriosis. Since mouse ectopic lesions, similar to human lesions display higher levels of ER β than ER α , one intriguing question raised by the Katzenellenbogen study pertains to the unexpected comparable levels of inhibition of estrogen-dependent responses with disruption of ER α signaling to that mediated by the more highly-expressed ER β . Perhaps the significant contribution of recruited macrophages expressing higher ER α and hence, equally higher production of pro-inflammatory cytokines may underlie the favorable response of lesions to ER α antagonists. It is also tempting to speculate that the cellular locations of the respective actions of ER α and ER β may be at play, given that in O’Malley’s study, ER β disruption of inflammasome and apoptosome functions takes place outside of the nucleus, invoking non-genomic actions and involving novel interactions with proteins not previously identified to interact with ER β . If the latter is true, then a follow-up question is whether ER α interacts (or not) with some of the same proteins that were identified for ER β . Yet another question is whether ER β preferentially acts outside of the nucleus in endometriotic lesions and if this may underlie the reversal of its (generally good) fortune of being the better half to ER α in controlling mitogenesis. The answer is not likely going to be straightforward given other nuclear actions attributed to ER β in ectopic lesions, one of which is its transcriptional regulation of ER α in endometriotic stromal cells (Trukhacheva *et al.*, 2009). Time (and more in-depth scrutiny) will tell.

The molecular details of complex mechanisms have gained much ground from careful dissection of context-dependent cross-talk among seemingly unrelated molecules. For an enigmatic condition such as endometriosis, the identification of novel partners elucidated here for ER β begs the question of whether progesterone receptors (whose expression and function are equally compromised in endometriosis) and other steroid receptor co-activators or co-repressors may similarly assume new extra-nuclear (cytoplasmic) roles to sustain ectopic growth. A tipoff to this possibility comes from the recent discovery of a new 70 kDa (truncated) Steroid Receptor Co-Activator 1 (SRC-1) isoform, which similar to ER β , was found to have little expression in normal endometrium, but displayed significant expression in endometriotic lesions (Han *et al.*, 2012). Moreover, the truncated SRC-1 protein, similar to ER β , was demonstrated to be essential in the initial stages of endometriosis establishment. In the current *Cell* paper, SRC-1 isoform was shown to form a complex with ER β and caspase 8, inhibiting the latter from interacting with its usual partner Fas-associated via death domain protein to generate apoptosis complex II that augments TNF- α -induced apoptosis. Whether the truncated SRC-1 only partners with ER β or exhibits a more expansive repertoire of interacting proteins to promote endometriosis remains to be explored.

The present studies provide fundamental insights into the adaptive functions of ER β in inflammation and apoptosis (Figure 1). Fine-tuning our awareness of the different networks orchestrated by steroid hormone receptors and their changing partners in normal and endometriotic cells may offer the much-needed therapeutic opportunities to address the development and progression of endometriosis.

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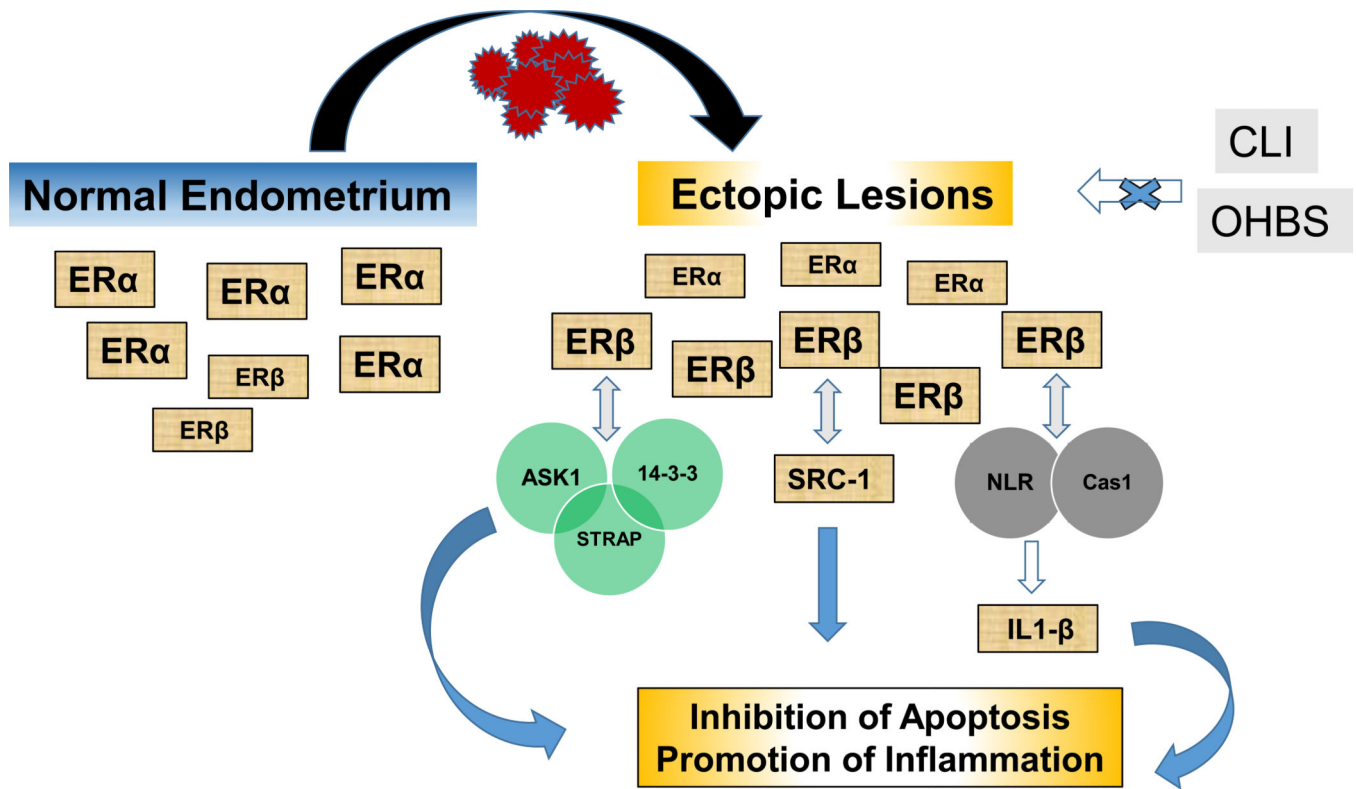


Figure 1. Proposed model for Estrogen receptor-β regulation of inflammation and apoptosis in endometriotic lesions. Normal endometrium and ectopic lesions display distinct levels of estrogen receptor (ER)-α and -β. The direct interactions of ERβ with proteins associated with inflammation and apoptosis in ectopic lesions (Han *et al.*, 2015) provide novel, non-genomic mechanisms for ERβ-mediated pathogenesis of endometriosis. CLI and OHBS are respectively, specific ERα and ERβ antagonists shown to inhibit ER-mediated promotion of inflammation underlying endometriosis progression (Zhao *et al.*, 2015). ASK-1, STRAP, 14-3-3, NLR, Cas1, and IL-1β are defined in the text.