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Review

A role for G-protein coupled estrogen receptor (GPER) in estrogen-induced carcinogenesis: Dysregulated glandular homeostasis, survival and metastasis

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ABSTRACT

Mechanisms of carcinogenesis by estrogen center on its mitogenic and genotoxic potential on tumor target cells. These models suggest that estrogen receptor (ER) signaling promotes expansion of the transformed population and that subsequent accumulation of somatic mutations that drive cancer progression occur via metabolic activation of cathecol estrogens or by epigenetic mechanisms. Recent findings that GPER is linked to obesity, vascular pathology and immunosuppression, key events in the development of metabolic syndrome and intratissular estrogen synthesis, provides an alternate view of estrogen-induced carcinogenesis. Consistent with this concept, GPER is directly associated with clinicopathological indices that predict cancer progression and poor survival in breast and gynecological cancers. Moreover, GPER manifests cell biological responses and a microenvironment conducive for tumor development and cancer progression, regulating cellular responses associated with glandular homeostasis and survival, invading surrounding tissue and attracting a vascular supply. Thus, the cellular actions attributed to GPER fit well with the known molecular mechanisms of G-protein coupled receptors, GPCRs, namely, their ability to transactivate integrins and EGF receptors and alter the interaction between glandular epithelia and their extracellular environment, affecting epithelial-to-mesenchymal transition (EMT) and allowing for tumor cell survival and dissemination. This perspective reviews the molecular and cellular responses manifested by GPER and evaluates its contribution to female reproductive cancers as diseases that progress as a result of dysregulated glandular homeostasis resulting in chronic inflammation and metastasis.

This review is organized in sections as follows: I) a brief synopsis of the current state of knowledge regarding estrogen-induced carcinogenesis, II) a review of evidence from clinical and animal-based studies that support a role for GPER in cancer progression, and III) a mechanistic framework describing how GPER-mediated estrogen action may influence the tumor and its microenvironment.

1. Estrogen-induced carcinogenesis

A clear case can be made for the role of estrogen in the genesis and progression of breast and gynecological cancers. Key observations buttressing this argument are: i) prolonged, uninterrupted exposure to endogenous estrogen is a risk factor for the development of breast, ovarian and endometrial cancer [1–3], ii) administration of exogenous estrogen to postmenopausal women is associated with increased risk of developing breast, ovarian and endometrial cancer [4–6], iii) oophorectomy in premenopausal women reduces the risk for developing hereditary breast and endometrial cancer [7–9], and iv) pharmacological agents which block estrogen biosynthesis or estrogen receptor (ER) action are effective measures for the treatment of early breast cancer [10]. However, inconsistencies exist in arguments supporting estrogen-induced carcinogenesis and are cause to reconsider the underlying mechanisms commonly attributed to estrogen action. Principal among these observations is the fact that based on cancer SEER statistics, the majority of breast and gynecological cancers occur in postmenopausal women, who demonstrate reduced serum estrogen concentrations. Secondly, while estrogen-targeted therapies are highly effective for the treatment of early breast cancer, these intervention strategies are not effective for advanced disease and yield mixed results in gynecological cancers [11,12]. Thirdly, while breast and ovarian cancer are more aggressive diseases in premenopausal women, this is not the case for endometrial cancer [12]. Finally, an ER-dependent mechanism of estrogen-induced proliferation in breast cancer does not readily explain why tumors of premenopausal women with intact ovarian function are more commonly ER-negative relative to tumors derived from postmenopausal women [13].

Models explaining the role of estrogen-induced carcinogenesis have focused on the capacity of estrogen to promote cellular proliferation, and consequently increase the chance for somatic mutations to occur, and accumulate with increasing exposure to estrogen [14]. The mitogenic effects of estrogen are presumed to be largely manifested via the

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ER since pharmacological agents that block ERα, or its more recently defined homologue, ERβ, inhibit estrogen-mediated cellular proliferation [15,16], although the precise mechanism by which ERs triggers cellular proliferation remains unclear. Regardless of the molecular details of ER-dependent proliferation, estrogen exerts strong mitogenic effects in breast [17] and endometrial [18] tissue, and induces strong proliferative effects on isolated breast and endometrial cancer cell lines. Estrogens also have long been implicated as etiologic agents for ovarian cancer [19,3], although their capacity to promote cellular proliferation in ovarian surface epithelia, the most common target cell type for cancers that arise from this tissue, or in isolated ovarian carcinoma cells [20], is less clear. By the same vantage point, further confusion regarding the role of estrogen in the carcinogenesis of female reproductive cancer comes from the disconnect between menopausal status and proliferative index, as measured by Ki-67 labeling in tumor biopsy tissue. High mitotic indices are observed in breast tumors from patients with intact ovaries, while postmenopausal women with ER-positive breast cancer receive estrogen-targeted therapy regardless of Ki-67 index [21]. Thus, the addition of chemotherapeutic agents, which target rapidly proliferating cells to estrogen-targeted therapy, is a mainstay for treating aggressive estrogen-dependent cancers [10]. The most recent example of this is observed in results from the PALOMA-III trials which have shown that addition of palbociclib, which targets cyclin-dependent kinases, CDK4 and CDK6, to ER-targeted therapy (fulvestrant) provides increased overall survival for patients with advanced ER-positive breast cancer [22]. Early results achieved with palbociclib in metastatic breast cancer are encouraging, yet they do not resolve whether palbociclib selectively targets proliferation in fulvestrant-resistant, ER-positive breast cancer cells, or whether its actions directly impact cellular responses associated with tumor cell metastasis and disease progression.

While naturally occurring and synthetic estrogens are unequivocally labeled as carcinogens [23], the molecular mechanism that promotes disease progression remains unclear. Evidence has been provided to support the influence of either genetic − or epigenetic- based mechanisms for promoting estrogen-induced carcinogenesis. Each model has implications with regards to the involvement of ERs in cancer progression. Metabolic activation of catechol estrogens that induce oxidative stress [24] and genotoxicity [25] occurs independently of ER action [26,27], while epigenetic mechanisms that drive estrogen-induced carcinogenesis have been attributed largely to global changes in gene expression initiated by ER-mediated gene transcription. Neither model alone provides a satisfying explanation with regards to cancer progression of estrogen-sensitive target cells. The epigenetic model suffers from the observation that proliferating cells of normal mammary epithelia do not express either ERα or ERβ [28], an observation consistent with the description of breast cancer as a heterogenous disease categorized by both luminal (ER-positive) and basal-like (ER-negative) target cells [29]. On the other hand, the genotoxic model provides a mechanism describing the evolution of ER-independent cancers. Yet concerns regarding its involvement in the progression of estrogen-induced cancers have been raised. Primary among these is the observation that while catechol estrogen metabolites have been linked to direct DNA damage that occurs in target cells [30,31], they do not elicit mutagenic activity as measured in standard bacterial or mammalian cell activity assays [32]. Still, it is important to consider that not all carcinogens report in cell-based assays, and thus *in vivo* genotoxicity assays remain as the most critical measure in determining mutagenicity and carcinogenicity [33]. Based on this idea, catechol estrogen metabolites, including 2- and 4-hydroxyestradiol, and quinolones derived thereof, remains as a popular means to describe estrogen-induced, ER-independent carcinogenesis [34]. This mechanism of estrogen-induced carcinogenesis has been championed in breast cancer and also used to explain the role of estrogen in gynecological cancers, whose biology is not easily explained by ER-dependence. This is particularly true for ovarian carcinogenesis since ovarian tissue estrogen levels are estimated to be at least 100-fold higher than circulating levels of serum estrogen, and the follicular fluid of ovarian follicles is still higher [35]; [36]. In fact, indirect mechanisms of estrogen action have been proposed to play a role in ovarian carcinogenesis [37]. Likewise, genotoxic mechanisms of endometrial carcinogenesis have been employed to describe the genesis of type II endometrial carcinomas [38], which generally fail to express ER [39]. Moreover, mitogenic and genotoxic mechanisms of tamoxifen action have been suggested as a means to drive tamoxifen-induced uterine hyperplasia leading to subsequent carcinogenesis. In the former case, mitogenicity of this popular ER antagonist is suggested to be the result of proliferative responses mediated by tissue specific cofactors that associate with ER and cause tamoxifen to function as an ER agonist [40]. In addition, evidence of receptor-independent action of tamoxifen and its metabolites in the uterus has been linked to adduct formation in endometrial cancer [41].

The recent acceptance by the International Union of Clinical Pharmacologists (IUPHAR) of GPER as a unique estrogen receptor [42] expands our perspective of estrogen responsiveness, which also affects our view of estrogen-induced carcinogenesis. Simple multistage models of carcinogenesis suggest that cancer arises as the result of cellular, genetic and epigenetic changes that transform normal cells into cancer cells [43,44]. These alterations are initiated by the loss of mechanisms that regulate normal growth and are promoted by events that enable the transformed cell to spread and grow at distant sites. This description fits with classic models of chemical carcinogenesis that categorize carcinogens as either tumor initiators or promoters [reviewed in [45]]. The observations listed below suggest that GPER manifests biological activities associated with tumor promotion and progression that provide a plausible explanation for ER-dependent and receptor-independent mechanisms of estrogen-induced carcinogenesis. The fact that GPER is linked to the development of pathophysiological events defined as the metabolic syndrome that drives cancer progression is further reason to consider its cellular and molecular mechanism of action in estrogen-induced cancers. The purpose of this review is to describe recent progress with regards to our understanding of the biological role of GPER, and to suggest a perspective of GPER as an estrogen receptor that mediates physiological functions usurped by malignant cancer cells to facilitate estrogen-mediated cancer cell survival and dissemination.

2. GPER and estrogen-induced carcinogenesis

2.1. GPER acts independently of ER and is a unique measure of estrogen responsiveness

Three receptors have been suggested to manifest estrogen action: ERα, ERβ, and GPER. In order to discuss GPER and its impact on estrogen-induced carcinogenesis, it is necessary to establish that GPER functions autonomously from the ER homologues that possess structural and functional homology to the nuclear steroid hormone receptor superfamily [46]. The ERs are primarily considered to function as hormone-activated transcription factors and are described as promoting the *genomic* actions of estrogen. While each ER imparts distinct molecular and biological activity, here they are considered synonymously as they are more closely aligned by structure and function than they are to GPER. Evidence has been provided to suggest that ERs, and derivatives thereof, also trigger *pregenomic* signaling from the plasma membrane [47], although the molecular mechanisms that determine their physical association with the plasma membrane or recruitment of signaling effectors that link to second messenger or protein kinase signaling has yet-to-be clearly defined. In contrast, GPER exhibits structural and functional characteristics of GPCR superfamily members. GPER functions as a Gs-coupled heptahelical receptor that promotes second messenger signaling as well as Gβγ-dependent coordinated activation of plasma membrane-associated matrix metalloproteinases, integrin α5β1 and epidermal growth factor receptors [42,48]. In this regard, GPER