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Signaling, physiological functions and clinical relevance of the G protein-coupled estrogen receptor GPER

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Abstract

GPR30, now named GPER1 (G protein-coupled estrogen receptor1) or GPER here, was first identified as an orphan 7- transmembrane G protein-coupled receptor by multiple laboratories using either homology cloning or differential expression and subsequently shown to be required for estrogen-mediated signaling in certain cancer cells. The actions of estrogen are extensive in the body and are thought to be mediated predominantly by classical nuclear estrogen receptors that act as transcription factors/regulators. Nevertheless, certain aspects of estrogen function remain incompatible with the generally accepted mechanisms of classical estrogen receptor action. Many recent studies have revealed that GPER contributes to some of the actions of estrogen, including rapid signaling events and rapid transcriptional activation. With the introduction of GPER-selective ligands and GPER knockout mice, the functions of GPER are becoming more clearly defined. In many cases, there appears to be a complex interplay between the two receptor systems, suggesting that estrogen-mediated physiological responses may be mediated by either receptor or a combination of both receptor types, with important medical implications.

Keywords

Estrogen; Estrogen Receptor; GPR30; GPER1; GPER; Cancer; Immune; Vascular; Reproduction

1. Introduction

Estrogen is well recognized for its role in the regulation of gene expression (Edwards, 2005). However, an underappreciated aspect of its function relates to rapid cellular signaling that is independent of transcriptional activity (Moriarty et al., 2006). At present, there are three recognized estrogen receptors. The two classical nuclear estrogen receptors, ER α and ER β , were identified in the early 1970's and in mid 1990's, respectively (Jensen and DeSombre, 1973; Kuiper et al., 1996). These estrogen receptors function traditionally as ligand-activated nuclear transcription factors that bind regulatory response elements in the promoters of genes (Carroll and Brown, 2006). The third estrogen receptor, GPR30 (now officially designated

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GPER1 by the IUPHAR or simply GPER throughout this review) was identified by multiple groups in the late 1990's as an orphan 7-transmembrane G protein-coupled receptor (GPCR) with low homology to existing GPCRs (Carmeci et al., 1997; Kvingedal and Smeland, 1997; O'Dowd et al., 1998; Owman et al., 1996; Takada et al., 1997). GPCRs are traditionally recognized as mediating rapid changes in the levels of second messengers and regulating kinase pathways (Luttrell, 2006). Additional receptors for estrogen, distinct from those described above, have also been postulated, but they remain less well characterized (Toran-Allerand, 2004).

Although the distinction between modes of cell activation (rapid signaling vs. transcription) appear straight-forward, there exists extensive overlap between these artificially defined categories. For example, although classical estrogen receptors are traditionally thought of as regulators of transcription, there is extensive evidence of their ability to mediate rapid signaling events (Moriarty et al., 2006). In addition, rapid signaling events, whether initiated by nuclear steroid receptors, growth factor receptors or GPCRs, result in the modification of transcriptional activity of conventional transcription factors (Ma and Pei, 2007). Thus the cellular effects of estrogen will depend on the specific receptors expressed and the integration of their stimulatory and inhibitory signaling events.

2. Identification of GPER as an estrogen receptor

In 2000, Filardo and colleagues demonstrated that the rapid activation of ERK in breast cancer cells was dependent upon the presence of GPR30/GPER (Filardo et al., 2000). Exogenous expression of GPER in MDA-MB-231 breast cancer cells, which express little to no ER α or GPER, resulted in estrogen-dependent ERK1/2 phosphorylation, similar to the response in MCF-7 cells, which express both ER α and GPER. Estrogen-dependent ERK1/2 phosphorylation was also observed in SKBr3 breast cancer cells, which express GPER but lack ER α . Together, these results indicated that GPER expression was critical for the rapid activation of ERK.

Studies by other groups in the following years demonstrated that GPER was involved in the upregulation of nerve growth factor in macrophages (Kanda and Watanabe, 2003a), and cyclin D2 and Bcl-2 in keratinocytes (Kanda and Watanabe, 2003b; Kanda and Watanabe, 2004). In addition, estrogen-mediated activation of *c-fos* transcription in breast cancer cells was also shown to occur in a GPER-dependent manner (Maggiolini et al., 2004). In these studies the requirement for GPER was established via knockdown of GPER in cells that endogenously expressed the receptor. Such observations indicated that although classical estrogen receptors were traditionally associated with transcriptional regulation, GPER was also capable of mediating estrogen-dependent gene expression.

Despite these correlative demonstrations, the mechanistic link between GPER expression and estrogen signaling remained ambiguous due in large part to a history of similar rapid signaling events being ascribed to ER α (Razandi et al., 2003). In an effort to resolve this controversy, two studies in 2005 described the binding of estrogen to GPER-expressing cells. Thomas *et al.* reported the specific binding of tritiated estrogen to membranes of SKBr3 (ER α - and ER β -negative, GPER-positive) breast cancer cells and to GPER-transfected human embryonic kidney (HEK) cells (Thomas et al., 2005). The reported binding constant of 3 nM was approximately 10-fold poorer than the value typically reported for estrogen binding to the ligand-binding domain of ER α (Kuiper et al., 1997). In addition, estrogen binding was absent in untransfected HEK cells and reduced in SKBr3 cells treated with siRNA targeting GPER.

In contrast to tritiated estrogen binding, Revankar *et al.* visualized the cellular and subcellular binding of estrogen to GPER employing a novel fluorescent estrogen derivative (Revankar et al., 2005). Confocal microscopy demonstrated that binding to GPER occurred in the

endoplasmic reticulum with no detectable binding at the plasma membrane, consistent with antibody staining of the endogenously expressed receptor in numerous cell types. This subcellular pattern of localization has been confirmed in a number of subsequent studies (Brailoiu et al., 2007; Matsuda et al., 2008; Otto et al., 2008b; Sakamoto et al., 2007), as well as through the use of selectively membrane-permeable estrogen derivatives (Revankar et al., 2007), although reports to the contrary also exist (Funakoshi et al., 2006; Thomas et al., 2005). Increasing evidence supports the intracellular localization and function of certain GPCRs, including lipid-activated GPCRs (for prostaglandins, platelet-activating factor and lysophosphatidic acid), and associated signaling molecules including G proteins and ion channels (Zhu et al., 2006).

Flow cytometric analysis of GPER-transfected cells by Revankar *et al.* demonstrated that estrogen binding was proportional to GPER expression, similar to that observed in cells transfected with either ER α or ER β , supporting the conclusion that GPER expression directly generates estrogen-binding sites (Revankar et al., 2005). Competition binding assays revealed a binding constant for estrogen of approximately 6 nM, similar to the value of 3 nM reported by Thomas *et al.* using a completely different approach (Thomas et al., 2005). These two studies strongly implicated GPER as an estrogen-binding protein with low nM affinity, alleviating one of the barriers to the acceptance of GPER as a true estrogen receptor.

3. Ligand specificity of GPER

Given that GPER and the classical ERs share a common ligand, the question arose as to the relative selectivity of each receptor type for estrogenic substances. Estrogen is a small ligand with no conformational flexibility and limited sites of stereospecific recognition. Thus, it might be predicted that two receptors (with no homology in primary or secondary structure) that both bind estrogen, would bind a similar spectrum of structurally related compounds. As described below, a large number of compounds that bind to classical estrogen receptors have also been demonstrated to bind to or activate GPER.

3.1. Estrogens and Steroids

Classical estrogen receptors exhibit a strong preference for the physiological isomer of estrogen, namely 17-beta estradiol compared to 17-alpha estradiol. Competitive binding similarly demonstrated that 17 α -estradiol failed to bind GPER (Thomas et al., 2005). In addition, 17 α -estradiol failed to induce a calcium response at concentrations 1000 times higher than 17 β -estradiol (Revankar et al., 2005). Furthermore, other physiological forms of estrogen, namely estrone and estriol, as well as other steroids including progesterone, cortisol and testosterone, failed to bind GPER (Thomas et al., 2005).

3.2. Anti-estrogens and SERMS

The synthetic non-steroidal anti-estrogen tamoxifen, a substituted triphenylethylene, is the best-known selective estrogen receptor modulator (SERM) (Ariazi et al., 2006). It exhibits tissue-dependent antagonist/partial agonist activities with respect to the known actions of estrogen and has proven effective as adjuvant therapy for estrogen receptor positive breast cancer (Jordan, 2007). The 4-hydroxy metabolite represents the high affinity ligand for estrogen receptors. In contrast, the synthetic 7 α -substituted estrogen derivative ICI182,780, which binds ER α with high affinity, exhibits a pure antiestrogenic profile (Ariazi et al., 2006). Binding studies have demonstrated that tamoxifen and ICI182,780 display significant binding to GPER (Thomas et al., 2005). However, unlike the antagonistic properties these compounds display with respect to the classical estrogen receptors, both compounds serve as GPER agonists (Filardo et al., 2000; Revankar et al., 2005). Although the biological effects of these compounds have been interpreted primarily through genomic responses resulting from

their interactions with the classical nuclear receptors, there is increasing evidence of nongenomic cellular signaling effects in various cell types and tissues. In particular, an increased incidence of endometrial hyperplasia and low grade endometrial cancers exists in breast cancer patients treated with tamoxifen, raising the possible role of GPER in this process (Senkus-Konefka et al., 2004; Smith et al., 2007). The synthetic stilbene estrogen diethylstilbestrol (DES), which exhibits binding affinity for classical estrogen receptors failed to show significant binding to GPER (Thomas et al., 2005).

3.3. Phytoestrogens

A multitude of chemical compounds, including natural phytoestrogens and manmade xenoestrogens (see below) are known to mimic the biological effects of estrogen in human and animals. Among phytoestrogens, the phenolic isoflavone phytoestrogen genistein, found in soy products, displays high binding affinity and selectivity for ER β but also demonstrates significant binding and activity towards GPER (Maggiolini et al., 2004; Thomas and Dong, 2006). Genistein and the related flavinoid quercetin have also been shown to mediate GPER-dependent upregulation of *c-fos*. Furthermore, genistein has been shown to mediate ERK phosphorylation in breast cancer cells (Maggiolini et al., 2004) and proliferation of thyroid cancer cells (Vivacqua et al., 2006a) via GPER.

3.4. Xenoestrogens

Humans are exposed to multiple xenoestrogens that range from synthetic precursors in plastics to pesticides (Singleton and Khan, 2003). For example, the estrogenic activity of the pesticide dichlorodiphenyl-trichloroethane (DDT) has long been recognized (Tiemann, 2008). The halogenated xenoestrogens p,p'-DDT, ortho, para-dichlorodiphenyldichloroethylene (o,p'-DDE) and kepone all displayed low but significant affinities for GPER and acted as agonists (Thomas and Dong, 2006; Thomas et al., 2005). Binding studies also reveal that a number of phenolic xenoestrogens including bisphenol A (BPA) and nonylphenol display affinity for GPER (Thomas and Dong, 2006). The pesticides methoxychor and atrazine have also been shown to exhibit limited binding to GPER (Thomas and Dong, 2006) with atrazine inducing GPER-dependent *c-fos* expression in breast cancer cells and producing proliferative effects in ovarian cancer cells that required both ER α and GPER (Albanito et al., 2008).

3.5. GPER-selective ligands

Given the extensive overlap in specificity apparent between classical estrogen receptors and GPER, our understanding of the biological roles of GPER would be greatly enhanced through the application of agonist and antagonists that demonstrate selectivity for GPER over classical estrogen receptors. In 2006, Bologa *et al.* identified a non-steroidal compound, named G-1, through a combination of virtual and biomolecular screening targeting GPER (Bologa et al., 2006). Binding studies demonstrated a binding affinity of G-1 for GPER of about 11 nM, compared to 6 nM for estrogen. No significant binding of G-1 at concentrations up to 1 μ M could be demonstrated for ER α or ER β . Functional characterization of G-1 demonstrated a GPER-dependent activation of calcium mobilization and PI3-kinase activation with no activation or inhibition of either ER α or ER β . Since its discovery, G-1 has been used in a large number of studies to investigate the role of GPER in numerous systems including the nervous, immune, reproductive and vascular systems as well as cancer (see below). To complement the activities of the GPER agonist G-1, we have recently identified a structurally related antagonist, G15 that has revealed roles for GPER in the reproductive and nervous systems (Dennis et al., 2009).

4. Intracellular signaling pathways initiated by GPER

4.1. Kinase activation

In the original report describing GPER-dependent activation of ERK, Filardo *et al.* demonstrated that this activation occurred via the transactivation of EGFR (Filardo *et al.*, 2000). This pathway involved the receptor-mediated activation of a pertussis-toxin sensitive G protein (presumably $G_{i/o}$), $G_{\beta\gamma}$ -mediated activation of Src and phosphorylation of Shc, resulting in matrix metalloproteinase-mediated release of HB-EGF and transactivation of the EGFR. However, other studies also reported the ER α -mediated transactivation of EGFR (Razandi *et al.*, 2003). To directly compare the signaling capabilities of both ER α and GPER, Revankar *et al.* (Revankar *et al.*, 2005) used COS7 cells transfected with either receptor and evaluated cellular PI3K signaling. Estrogen stimulation of cells expressing either ER α or GPER yielded activation of PI3K, blocked by the PI3K inhibitor, LY294002, revealing that both receptors are capable of mediating this response. Interestingly, stimulation with 4-hydroxytamoxifen resulted in activation of PI3K in GPER-expressing cells but not in ER α -expressing cells. Furthermore, the EGFR inhibitor AG1478 only blocked GPER- but not ER α -mediated PI3K activation demonstrating that estrogen-mediated PI3K activation occurred through two different pathways depending on the receptor being utilized. Revankar *et al.* also demonstrated that estrogen-mediated nuclear activation of PI3K occurred in SKBr3 breast cancer cells that endogenously express only GPER, with knockdown of GPER establishing a requirement for GPER in both estrogen- and tamoxifen-mediated signaling. A role for sphingosine 1 phosphate produced by sphingosine kinase has been proposed as an intermediate in the GPER-mediated transactivation of the EGFR by estrogen (Sukocheva *et al.*, 2006). GPER-mediated MAPK and/or PI3K activation have now been shown to occur upon estrogen treatment in many cells, and in all cases examined to date, to occur through the transactivation of the EGFR.

4.2. cAMP production

The earliest report of estrogen-mediated cAMP elevation was reported in the rat uterus by Szego and Davis in 1967 (Szego and Davis, 1967) with gene regulation in response to rapid increases in cAMP concentrations described later (Aronica *et al.*, 1994). We have also reported rapid increases in cAMP levels following estrogen stimulation in human coronary arteries (Mugge *et al.*, 1993). The estrogen-mediated production of cAMP has more recently been reported for GPER using membrane preparations of SKBr3 breast cancer cells that do not express ER α or ER β as well as in whole cells transfected with GPER (Filardo *et al.*, 2007; Filardo *et al.*, 2002). The generation of cAMP resulted in the attenuation of MAPK activity by estrogen via a GPER-dependent stimulation of adenylyl cyclase that subsequently inactivated Raf-1. The results suggested a pathway in which estrogen first stimulates and then attenuates MAPK activity through a single GPCR, GPER, via two distinct G protein-dependent signaling pathways with opposing effects on the upstream signaling events leading to MAPK activation. Subsequent studies have also shown that numerous phyto- and xenoestrogens can stimulate cAMP production via GPER (Thomas and Dong, 2006; Thomas *et al.*, 2005).

4.3. Calcium mobilization

GPER has also been shown to mediate intracellular calcium mobilization in multiple cell types. Revankar *et al.* first demonstrated this response on GPER transfected COS7 cells (Revankar *et al.*, 2005) and subsequently in SKBr3 cells (Dennis *et al.*, 2009). They subsequently utilized calcium mobilization to demonstrate that only membrane permeable estrogen derivatives could mediate a rapid calcium response (Revankar *et al.*, 2007). Filardo *et al.* have also reported mobilization of intracellular calcium in GPER-transfected HEK293 cells (Filardo *et al.*, 2007). Estrogen and G-1 have been shown to mobilize intracellular calcium in hypothalamic neurons (Brailoiu *et al.*, 2007). However, G-1 was observed not to mobilize calcium in GnRH-

releasing neurons (Romano et al., 2008), although it did reproduce the effect of estrogen in inducing a rapid excitatory effect on primate LHRH neurons (Noel et al., 2009) and calcium accumulation in sensory neurons (Fehrenbacher et al., 2008). Finally, activation of GPER by G-1 in human aortic vascular smooth muscle has also been shown to inhibit subsequent vasoconstrictor-induced changes in intracellular calcium (Haas et al., 2009).

4.4. Transcriptional activation

Although ER α (and to some extent ER β) is almost exclusively associated with transcriptional responses to estrogen, rapid signaling events initiated by GPER have also been shown to regulate gene expression (Prossnitz and Maggiolini, 2009). Upregulation of nerve growth factor via *c-fos* expression in macrophages (Kanda and Watanabe, 2003a) and cyclin D2 and Bcl-2 upregulation in keratinocytes (Kanda and Watanabe, 2003b; Kanda and Watanabe, 2004) are GPER-mediated. Estrogen-mediated Bcl-2 upregulation by GPER has also been shown to attenuate hepatic injury induced by trauma-hemorrhage (Hsieh et al., 2007). Induction of *c-fos* via EGFR transactivation leading to MAPK activation has also been demonstrated in ER-negative SKBr3 breast cancer cells (Maggiolini et al., 2004). Estrogen and tamoxifen induced the expression of *c-fos* through GPER in thyroid and endometrial cancer cells resulting in proliferation (Vivacqua et al., 2006a); (Vivacqua et al., 2006b). In ovarian cancer cells, G-1, like E2, upregulated multiple estrogen-responsive genes including *c-fos*, pS2 and cyclins A, D1 and E. However, G-1 failed to induce PR transcription, which is known to be a primary target of ER α (Albanito et al., 2007). Although *c-fos* induction (and cell proliferation) was entirely GPER-dependent in SKBr3 cells, knock down of either GPER or ER α in ovarian cancer cells that express both receptors revealed a co-dependence between these receptors in the stimulation of *c-fos* by G-1 and E2. E2 and G-1 have also been shown to activate *c-fos* and cyclin D1 expression in mouse spermatogonia GC-1 cells (Sirianni et al., 2008).

To date, only two microarray-based studies have been carried out to examine the contribution of GPER to gene expression on a genome-wide scale. An analysis of MCF-7 breast cancer cells stimulated with a membrane impermeable estrogen dendrimer suggested that approximately 25% of all estrogen-regulated genes are induced independently of the recruitment of ER α to estrogen response elements (Madak-Erdogan et al., 2008). In addition, antiestrogens or ER α knockdown, as well as MAPK and c-Src kinase inhibition, prevented the up-regulation of these genes. Treatment of MCF-7 cells with G-1 for four hours yielded no significant changes in gene expression. The second study utilized ER-negative SKBr3 cells to identify a set of genes that may contribute to the proliferative activities of GPER (Pandey et al., 2009). Stimulation with either estrogen or tamoxifen resulted in the pronounced induction of a number of genes including connective tissue growth factor (CTGF), EGR1, ATF3, FOS, TNF and many more. Estrogen-induced GPER-mediated CTGF secretion contributed to cell proliferation and cell migration and CTGF was also induced by tamoxifen in fibroblasts obtained from breast tumor biopsies. Together, these results suggest possible roles for GPER in the metastasis of breast cancer and resistance to anti-estrogens.

5. Physiological functions of GPER as an estrogen receptor

Although the cellular activities of GPER have been examined in a number of systems, the physiological functions of GPER are just beginning to be investigated. Based on the extensive effects of estrogen in a vast array of physiological systems, the contributions of GPER to estrogen-mediated effects could be substantial. Below, we examine the evidence to date that GPER plays a role in a diverse collection of physiological responses.

5.1. Cancer

GPER is hypothesized to play a potentially important role in cancer with GPER expression reported in a number of cancer cell lines, including MCF-7 and SKBr3 breast cancer cells (Carmeci et al., 1997; Filardo et al., 2000; Revankar et al., 2005; Thomas et al., 2005), Hec1A (Vivacqua et al., 2006b) and Hec50 (Revankar et al., 2005) endometrial cancer cells, JEG choriocarcinoma cells (Revankar et al., 2005), BG-1 ovarian cancer cells (Albanito et al., 2007), and thyroid carcinoma cell lines (Vivacqua et al., 2006a). Furthermore, GPER expressed in primary breast and endometrial cancers (Filardo et al., 2006; Smith et al., 2007). Functionally, GPER has been shown to upregulate genes involved in proliferation (e.g. cyclins) and survival (e.g. Bcl-2), suggesting that its expression and/or activation could play an important role in the process of carcinogenesis.

Filardo and colleagues have carried out immunohistochemistry for ER, PR, and GPER in over 300 breast carcinomas and observed that among the invasive and *in situ* cases, approximately half were GPER positive whereas in 43%, coexpression of ER α and GPER was present. Overexpression of GPER was positively associated with tumor size, the presence of metastases, and HER-2/neu overexpression, leading to the conclusion that GPER may be a predictor of biologically aggressive breast cancer (Filardo et al., 2006). A subsequent study of infiltrating ductal carcinoma of the breast, however, failed to identify similar correlations (Kuo et al., 2007). Interestingly, in models of drug resistance to SERMs and aromatase inhibitors, GPER is overexpressed, suggesting that GPER upregulation may compensate for a deficiency in estrogen-mediated proliferative signaling (Jordan et al., 2007). To investigate the potential involvement of GPER in endometrial cancers, Smith and colleagues performed immunohistochemistry for ER, PR, GPER, EGFR and Ki-67 (a proliferative marker) (Smith et al., 2007). GPER overexpression correlated positively with EGFR and was present more frequently in endometrial carcinomas with deep myometrial invasion, high-grade, biologically aggressive histologic subtypes, and advanced stage. Most importantly, in patients with GPER overexpression, survival was significantly decreased. Recently, a role for GPER was described in the estrogen-mediated recruitment of fibronectin-engaged $\alpha 5 \beta 1$ integrins to fibrillar adhesions and the synthesis of fibronectin fibrils in breast cancer cells (Quinn et al., 2009). Together, these reports indicate that GPER may represent an important marker for high-risk breast and endometrial cancers and, furthermore, may play a mechanistic role in carcinogenesis.

5.2. Nervous system

Estrogen is known to exhibit widespread neurological effects ranging from cognition to emotional status to sensory processing. Immunohistochemical studies have revealed that GPER is expressed in both the central and peripheral nervous system, with expression in the brain of both adult male and non-pregnant female rats (Brailoiu et al., 2007). High GPER expression was present in the Islands of Calleja and striatum. In the hypothalamus, GPER was detected in the paraventricular nucleus and supraoptic nucleus. The anterior and posterior pituitary contained numerous GPER expressing cells and terminal-like endings. Cells in the hippocampal formation as well as the substantia nigra were also positive for GPER. In the brainstem, GPER was detected in the area postrema, nucleus of the solitary tract, and dorsal motor nucleus of the vagus. A cluster of cells in the nucleus ambiguus demonstrated strong expression (Brailoiu et al., 2007). Expression of GPER in pyramidal cells of CA1-3 and granule cells of the dentate gyrus was observed at both the mRNA and protein levels in rats (Matsuda et al., 2008). In the paraventricular nucleus and supraoptic nucleus, GPER was reported in magnocellular oxytocin neurons at both mRNA (Sakamoto et al., 2007) and protein levels (Brailoiu et al., 2007; Sakamoto et al., 2007) but in vasopressin neurons, only one report observed expression (Brailoiu et al., 2007) while the other did not (Sakamoto et al., 2007). Recently GPER stimulation was shown to attenuate serotonin receptor signaling in the PVN

as demonstrated by reduced oxytocin and ACTH responses, suggesting GPER may play a role in mood disorders (Xu et al., 2009).

One of the major feedback targets for estrogen in the brain are the gonadotropin-releasing hormone (GnRH) neurons, which regulate gonadal function and fertility in mammals. G-1 (at 100 nM) displayed no effect on the calcium dynamics of GnRH neurons, where estrogen and ER α -selective agonists exhibited activity (Romano et al., 2008), leading to the suggestion that GPER is not involved in this response. Nevertheless, GPER has been implicated as the mediator of estrogen-mediated LHRH release through modulation of LHRH neuron firing and calcium oscillations in primates (Noel et al., 2009). GPER was observed to be expressed in a subset of LHRH neurons and G-1 mediated changes in calcium oscillations similar to estrogen while knockdown of GPER completely blocked the estrogen-mediated response.

A role for GPER has been suggested in the estrogen-mediated peripheral induction of mechanical hyperalgesia through PKC ϵ activation (Kuhn et al., 2008). G-1 but not classical ER agonists activated PKC ϵ in neurons of dissociated dorsal root ganglia. In addition, ICI 182,780, an ER α antagonist, but a GPER agonist, mimicked the mechanical hyperalgesia of G-1, supporting a role for GPER in this pathway. The ER α agonist PPT and G-1 have also been shown to activate ERK in trigeminal ganglion neurons and to increase allodynia after CFA injection into the masseter of ovariectomized rats, suggesting that both ER α and GPER may play a role in temporomandibular disorder and migraine (Liverman et al., 2009). GPER has also been shown to be expressed in neurons of the dorsal and ventral horn as well as in sensory and autonomic neurons, and to be functional in cultured spinal neurons (Dun et al., 2009). Finally, at the time of editing, a study using G-1 and GPER knockdown, demonstrated that GPER was also responsible for the estrogen-dependence of visceral hypersensitivity in the absence of mucosal inflammation (Lu et al., 2009).

In another study, the contribution of membrane estrogen receptors to the estrogen-mediated modulation of dopamine transporters in nerve growth factor differentiated PC12 pheochromocytoma cells was examined (Alyea et al., 2008). Whereas exposure to estrogen initiated the efflux of preloaded dopamine, knockdown of ER α blocked estrogen-mediated efflux, whereas GPER depletion increased efflux. Although treatment with G-1 alone had no effect on efflux, co-administration of estrogen and G-1 resulted in an inhibition of the estrogen response, suggesting that GPER may serve to antagonize the stimulatory effect of ER α . Based on these numerous studies, it appears that GPER plays a role in multiple aspects of the central and peripheral nervous system.

5.3. Immune system

Estrogen is known to have extensive effects in the modulation of the immune response from regulation of T cell development to autoimmune disease. Using a GPER knockout mouse as well as G-1, GPER has been shown to contribute, along with ER α , to estrogen-induced thymic atrophy (Wang et al., 2008a). Whereas ER α exclusively mediated the early developmental blockage of thymocyte development, GPER was required for thymocyte apoptosis, occurring preferentially in T cell receptor β chain^{-/low} double-positive thymocytes. Other studies, however, using different knockout mouse models have not observed such effects, although one did report lower numbers of CD62L-expressing T cells, consistent with impaired production of T cells in the thymus (Isensee et al., 2009).

Estrogen exerts protective effect in many autoimmune diseases such as multiple sclerosis. In experimental autoimmune encephalomyelitis (EAE), estrogen-mediated protection against EAE was significantly impaired in GPER knockout mice (Wang et al., 2009). Furthermore, treatment of mice with G-1 reproduced the ability of estrogen to protect against clinical and histological manifestations of EAE. G-1 treatment enhanced suppressive activity of

CD4⁺Foxp3⁺ T regulatory cells through GPER-mediated upregulation of programmed death 1-dependent, ligation of which downregulates lymphocyte responses. The regulatory and protective effects of GPER activation on immune responses, if demonstrated in additional systems, may provide a novel target for the clinical application of GPER agonists.

5.4. Reproductive system

The possible functions of GPER in the reproductive system have been examined in numerous species. In Atlantic croaker and zebrafish oocytes, *in vitro* treatment with estrogen as well as G-1 reduced both the spontaneous and progestin-induced maturation of oocytes (Pang et al., 2008). In addition, injection of GPER antisense oligonucleotides in zebrafish oocytes blocked the inhibitory effects of estrogen on oocyte maturation, establishing a role for GPER in the regulation of meiotic arrest. GPER has also been shown to play a role in the estrogen-mediated stimulation of primordial follicle formation in the hamster ovary (Wang et al., 2008b). In the hamster ovary, GPER is expressed in both granulosa and theca cells and its expression is regulated by gonadotropins (Wang et al., 2007).

Recent descriptions of GPER knockout mice failed to reveal obvious defects in reproductive organs (Isensee et al., 2009; Martensson et al., 2008; Otto et al., 2008a; Wang et al., 2008a). *In vivo* experiments with G-1 to examine the effect on mammary glands and the uterus failed to reveal expression of Wnt-4, Frizzled-2, IGF-1 or cyclin E1 (Otto et al., 2008b). In addition, a proliferative response to G-1 was not observed in the endometrium or the mammary gland. Furthermore, G-1 failed to induce ductal growth or endbud formation in the mammary gland or imbibition in the uterus. In contrast to these observations, Dennis et al. observed that, although G-1 did not mediate uterine imbibition, it did increase the number of proliferative cells 3–4 fold over basal levels (Dennis et al., 2009). Although this response was lower than that induced by estrogen, it does suggest that GPER contributes to the proliferative response in the uterus mediated by estrogen. In support of this, a novel GPER-selective antagonist G15 was able to reduce the proliferative index mediated by estrogen by about 50% (Dennis et al., 2009). Thus, although in the uterus there is an absolute requirement for ER α in terms of imbibition, proliferation and expression of ERE-dependent genes, GPER appears to play a supportive or co-dependent role, much as has been described in the proliferation of cancer cell lines that express both ER α and GPER as described above.

5.5. Cardiovascular system

The potential vascular relevance of GPER function was perhaps first suggested by one of the approaches used to clone the cDNA for the receptor, namely the fluid shear stress-induced nature of its expression in human vascular endothelial cells, resulting in one of its designations as Flow-induced Endothelial G protein-coupled receptor gene-1 (Takada et al., 1997). However, GPER was also cloned in cells distinct from vascular cells, suggesting more widespread expression and function (Carmeci et al., 1997; O'Dowd et al., 1998; Owman et al., 1996; Takada et al., 1997). GPER is also expressed in non-endothelial vascular cells, such as smooth muscle cells (Haas et al., 2007; Isensee et al., 2009), being expressed in both human arteries and veins and being regulated in response to estrogen (Haas et al., 2007). Non-genomic vasodilator effects have been described for estrogen (a non-selective estrogen receptor agonist) and phytoestrogens (such as genestein) as well as antagonists of the “classical” estrogen receptors (ICI182,780) and SERMs (such as tamoxifen and raloxifene) (Leung et al., 2007; Meyer et al., 2009; Pinna et al., 2006). For many years, the relaxant effects of the latter remained unexplained mechanistically. Recently, ICI182,780, as well as SERMs, have been identified as potent GPER agonists (Filardo et al., 2000; Revankar et al., 2005), giving rise to the hypothesis that this receptor might regulate vasomotor tone and blood pressure. Using the selective GPER-agonist G-1 and mice deficient in GPER, we recently provided strong evidence that GPER is indeed functional in the cardiovascular system, and that its activation mediates

acute vasodilation and reductions in blood pressure (Haas et al., 2009). Mechanistically, activation of GPER abrogates calcium flux induced by the vasoconstrictor serotonin, suggesting calcium-antagonistic or desensitizing effects. The vasodilator effects of G-1 were more pronounced than those of estrogen, both in murine as well as in human arteries, suggesting that the non-genomic dilator mechanism related to GPER may involve cross-talk with the classical estrogen receptors. Cross-talk in the acute dilator effects related to estrogen receptors alpha and beta has been recently demonstrated (Traupe et al., 2007).

Finally, we found that GPER activation potentially inhibits cell proliferation of human vascular smooth muscle cells that express neither ER α nor ER β (Haas et al., 2009). These findings are consistent with previous studies in rat cardiac myocytes and fibroblasts, in which ICI 182780 and tamoxifen (GPER agonists) potentially inhibited cell growth (Mercier et al., 2003). Studies using LacZ-reporter mice have found that GPER is expressed throughout the vascular system (Isensee et al., 2009); however, these investigators found that GPER was present only in small but not in larger arterial vessels and was found in endothelial as well as vascular smooth muscle cells. We could not confirm these findings, as we found GPER expression also in large conduit arteries such as the aorta and the carotid arteries of wild-type mice (Haas et al., 2009). Mårtensson *et al.* also reported vascular expression of GPER in wild-type mice, with expression particularly high in the well-vascularized kidney (Mårtensson et al., 2008). It is currently unclear whether adipocyte expression of GPER, which can be detected in perivascular fat in rodents (Haas et al., 2009) and also in human adipocytes (Hugo et al., 2008), plays a role in regulation of vascular homeostasis. Taken together, an important role of GPER has been recently established in the vascular system with expression of this receptor throughout the vascular system in animals and humans and results demonstrating that GPER plays a role in maintenance of vascular tone, blood pressure and vascular cells growth (Meyer et al., 2009).

5.6. Metabolism and Obesity

Based on studies in knockout mice, a role for GPER in the regulation of glucose handling and obesity has recently been proposed. Indeed, in mice generated using cre-lox recombination techniques, GPER was shown to mediate in part insulin release in response to stimulation with estrogen (Mårtensson et al., 2008). Moreover, these investigators reported impaired glucose tolerance and increased glucose levels in GPER-deficient mice; however, these effects were seen only in female animals. In GPER knockout animals generated using homologous recombination of embryonic stem cells, glucose tolerance tests were not different from wild-type animals, in both male and female mice (unpublished observation), suggesting that the transgenic technique employed used could have played a role. We found that GPER deficiency is associated with abdominal obesity in both male and female animals (Haas et al., 2009). These data suggest that, in contrast to current concepts, estrogen-dependent signaling through GPER helps to counteract obesity development in a gender-independent fashion. These data are consistent with data from humans, where GPER has been detected in adipocytes (Hugo et al., 2008). Indirect evidence from studies using the agonist raloxifene, unlike estrogen, suggests that GPER could be involved in adipocyte differentiation (Murase et al., 2006). Finally, a role for GPER in bone metabolism has been suggested by Mårtensson et al., who have demonstrated reduced bone growth in female knock-out animals only; however, these findings remain to be confirmed (Mårtensson et al., 2008).

6. Potential roles of GPER in disease and therapy

The experimental data suggest roles for GPER in several physiopathologies and diseases, and it is reasonable to speculate that regulation of GPER expression and/or activity could have therapeutic relevance for clinical medicine. Estrogen-based therapy (through anti-estrogen/SERMs or aromatase inhibitors) is the major neoadjuvant therapeutic approach used for breast cancer treatment. However, tamoxifen treatment leads to an increase incidence of endometrial

cancer, where GPER expression has been correlated with poor survival (Smith et al., 2007). Furthermore, a significant proportion of SERM-responsive breast cancer patients relapse with anti-estrogen-resistant breast cancer, where in model systems, GPER is also overexpressed (Jordan et al., 2007). Combined with studies demonstrating GPER-mediated regulation of cell proliferation (Albanito et al., 2007; Pandey et al., 2009; Sirianni et al., 2008; Teng et al., 2008; Vivacqua et al., 2006b), GPER may therefore represent both a novel and important biomarker for aggressiveness of disease as well as a therapeutic target for multiple cancers.

There is an extensive literature describing the beneficial effects of estrogen in multiple sclerosis, which predominantly affects women (Trenova et al., 2004). Overall, these studies support a role of estrogens as potent anti-inflammatory and neuroprotective agents. Evidence from several studies in humans and animals demonstrate that there is a reduced relapse rate of multiple sclerosis during pregnancy (Abramsky, 1994; Damek and Shuster, 1997; Polanczyk et al., 2003; van Walderveen et al., 1994). These initial observations were subsequently confirmed in the large Pregnancy In Multiple Sclerosis (PRIMS) study, which demonstrated a 70% reduction in relapse rate during late pregnancy (Houtchens, 2007). With the recent demonstration that GPER plays a critical role in this response (Wang et al., 2009), modulation of GPER activity may represent an important target in multiple sclerosis and other autoimmune diseases.

Given that GPER modulates murine immune responses (Wang et al., 2009; Wang et al., 2008a), and improves functional recovery with reduced infarct size in isolated rat hearts following ischemia/reperfusion (Deschamps and Murphy, 2008), its involvement in inflammatory vascular diseases such as atherosclerosis appears likely. The GPER agonist raloxifene inhibits atherosclerosis in animals (Bellosta et al., 2007; Choi et al., 2008) and humans (Colacurci et al., 2007), partly by inhibiting vascular inflammation and partly by increasing plaque stability. In healthy postmenopausal women raloxifene enhances endothelium-dependent vasodilation (Sarrel et al., 2003). A very recent paper suggests that raloxifene may reduce cardiovascular events in younger women at high risk for myocardial infarction (Collins et al., 2009). Indeed, age appears to be important as no effects were seen in older women (Collins et al., 2009; Mack et al., 2007). Also, given that shortly after its discovery of the GPER gene, its locus on human chromosome 7p22 was linked to the genetic susceptibility to hypertension (Lafferty et al., 2000), and that GPER agonism has potent vasodilator effects in human arteries (Haas et al., 2009), GPER agonism could become a new antihypertensive therapeutic principle. Indeed, there is evidence for acute antihypertensive effects (Haas et al., 2009) as well as preliminary data to suggest that G-1 may have chronic antihypertensive effects (Hoffmann-Lindsey and Chappell, 2008). Obesity is an important risk factor for diseases such as hypertension and vascular disease. The findings from knockout animals, demonstrating obesity (Haas et al., 2009) or impairment in insulin release (Martensson et al., 2008), strongly suggest a role of estrogen signaling via GPER in obesity and insulin resistance. This is further supported by a role of GPER in diet-induced obesity (D.J. Clegg, personal communication). Indeed, in obesity there is an increased production of estrogen from aromatase in fat tissue. Interestingly, like GPER, aromatase is localized to the endoplasmic reticulum resulting in proximity of estrogen production and target, making interactions likely. Finally, GPER signaling could be of relevance for diseases related to thyroid function and growth as well as bone metabolism. The latter would be supported by the clinical finding that activators of GPER such as raloxifene have beneficial effects as anti-osteoporotic agents (Goldstein et al., 2008; Sweet et al., 2009).

7. Concluding comments

It has taken more than a decade since the discovery of the first 7-transmembrane G protein-coupled estrogen receptor, now called GPER, to begin to understand the role of this new player

in sex hormone signaling. At present, over 100 publications on GPER have appeared in the literature, with approximately two-thirds of these published within the past two years. These mainly experimental studies have suggested roles for this receptor as a causative agent and/or modulator of physiology and disease; however, the role in human physiology and disease is still unclear. Given that compounds used clinically as “anti-estrogens”, such as certain SERMs, or used as estrogen replacements, such as phytoestrogens, are GPER agonists, it is likely that some of the clinical effects observed with these compounds are at least in part mediated by GPER. With the increasing use of GPER knockout mice and the development of GPER-selective agonists and antagonists, future studies should clarify and deepen our knowledge of the functions and therapeutic roles of this G protein-coupled estrogen.

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Abbreviations

cAMP	cyclic adenosine monophosphate
EGF(R)	epidermal growth factor (receptor)
ER(s)	estrogen receptor(s)
ERE	estrogen response element
GPCR	G protein-coupled receptor
MAPK	mitogen-activated protein kinase
PI3K	phosphatidylinositol 3-kinase
PIP3	phosphatidylinositol (3,4,5)-trisphosphate
SERM	selective estrogen receptor modulator

References

- Abramsky O. Pregnancy and multiple sclerosis. *Ann Neurol* 1994;36(Suppl):S38–41. [PubMed: 7517125]
- Albanito L, Lappano R, Madeo A, Chimento A, Prossnitz ER, Cappello AR, Dolce V, Abonane S, Pezzi V, Maggiolini M. G protein-coupled receptor 30 and estrogen receptor alpha are involved in the proliferative effects induced by atrazine in ovarian cancer cells. *Environ Health Perspect* 2008;116:1648–1655.
- Albanito L, Madeo A, Lappano R, Vivacqua A, Rago V, Carpino A, Oprea TI, Prossnitz ER, Musti AM, Ando S, Maggiolini M. G protein-coupled receptor 30 (GPR30) mediates gene expression changes

and growth response to 17 β -estradiol and selective GPR30 ligand G-1 in ovarian cancer cells. *Cancer Res* 2007;67:1859–1866. [PubMed: 17308128]

Alyea RA, Laurence SE, Kim SH, Katzenellenbogen BS, Katzenellenbogen JA, Watson CS. The roles of membrane estrogen receptor subtypes in modulating dopamine transporters in PC-12 cells. *J Neurochem* 2008;106:1525–1533. [PubMed: 18489713]

Ariazi EA, Ariazi JL, Cordera F, Jordan VC. Estrogen receptors as therapeutic targets in breast cancer. *Curr Top Med Chem* 2006;6:181–202. [PubMed: 16515478]

Aronica SM, Kraus WL, Katzenellenbogen BS. Estrogen action via the cAMP signaling pathway: stimulation of adenylate cyclase and cAMP-regulated gene transcription. *Proc Natl Acad Sci U S A* 1994;91:8517–8521. [PubMed: 8078914]

Bellosta S, Baetta R, Canavesi M, Comparato C, Granata A, Monetti M, Cairoli F, Eberini I, Puglisi L, Corsini A. Raloxifene inhibits matrix metalloproteinases expression and activity in macrophages and smooth muscle cells. *Pharmacol Res* 2007;56:160–167. [PubMed: 17582780]

Bologa CG, Revankar CM, Young SM, Edwards BS, Arterburn JB, Kiselyov AS, Parker MA, Tkachenko SE, Savchuck NP, Sklar LA, Oprea TI, Prossnitz ER. Virtual and biomolecular screening converge on a selective agonist for GPR30. *Nat Chem Biol* 2006;2:207–212. [PubMed: 16520733]

Brailoiu E, Dun SL, Brailoiu GC, Mizuo K, Sklar LA, Oprea TI, Prossnitz ER, Dun NJ. Distribution and characterization of estrogen receptor G protein-coupled receptor 30 in the rat central nervous system. *J Endocrinol* 2007;193:311–321. [PubMed: 17470522]

Carmeci C, Thompson DA, Ring HZ, Francke U, Weigel RJ. Identification of a gene (GPR30) with homology to the G-protein-coupled receptor superfamily associated with estrogen receptor expression in breast cancer. *Genomics* 1997;45:607–617. [PubMed: 9367686]

Carroll JS, Brown M. Estrogen receptor target gene: an evolving concept. *Mol Endocrinol* 2006;20:1707–1714. [PubMed: 16396959]

Choi BG, Vilahur G, Zafar MU, Cardoso L, Yadegar D, Ibanez B, Tunstead J, Viles-Gonzalez JF, Schaffler MB, Fuster V, Badimon JJ. Selective estrogen receptor modulation influences atherosclerotic plaque composition in a rabbit menopause model. *Atherosclerosis*. 2008

Colacurci N, Fornaro F, Cobellis L, De Francis P, Torella M, Sepe E, Arciello A, Cacciapuoti F, Paolisso G, Manzella D. Raloxifene slows down the progression of intima-media thickness in postmenopausal women. *Menopause* 2007;14:879–884. [PubMed: 17667145]

Collins P, Mosca L, Geiger MJ, Grady D, Kornitzer M, Amewou-Atisso MG, Effron MB, Dowsett SA, Barrett-Connor E, Wenger NK. Effects of the selective estrogen receptor modulator raloxifene on coronary outcomes in the Raloxifene Use for The Heart trial: results of subgroup analyses by age and other factors. *Circulation* 2009;119:922–930. [PubMed: 19204301]

Damek DM, Shuster EA. Pregnancy and multiple sclerosis. *Mayo Clin Proc* 1997;72:977–989. [PubMed: 9379704]

Dennis MK, Burai R, Ramesh C, Petrie WK, Alcon SN, Nayak T, Bologa C, Leitao A, Brailoiu E, Deliu E, Dun NJ, Sklar LA, Hathaway HJ, Arterburn JB, Oprea TO, Prossnitz ER. In vivo effects of a GPR30 antagonist. *Nat Chem Biol*. 2009in press

Deschamps AM, Murphy E. Activation of a novel estrogen receptor, GPR30, is cardioprotective in the rat. *Circulation* 2008;118:S357–358.

Dun SL, Brailoiu GC, Gao X, Brailoiu E, Arterburn JB, Prossnitz ER, Oprea TI, Dun NJ. Expression of estrogen receptor GPR30 in the rat spinal cord and in autonomic and sensory ganglia. *J Neurosci Res* 2009;87:1610–1619. [PubMed: 19125412]

Edwards DP. Regulation of signal transduction pathways by estrogen and progesterone. *Annu Rev Physiol* 2005;67:335–376. [PubMed: 15709962]

Fehrenbacher JC, Loverme J, Clarke W, Hargreaves KM, Piomelli D, Taylor BK. Rapid pain modulation with nuclear receptor ligands. *Brain Res Rev*. 2008

Filardo E, Quinn J, Pang Y, Graeber C, Shaw S, Dong J, Thomas P. Activation of the novel estrogen receptor, GPR30, at the plasma membrane. *Endocrinology* 2007;148:3236–3245. [PubMed: 17379646]

Filardo EJ, Graeber CT, Quinn JA, Resnick MB, Giri D, DeLellis RA, Steinhoff MM, Sabo E. Distribution of GPR30, a seven membrane-spanning estrogen receptor, in primary breast cancer and its association

with clinicopathologic determinants of tumor progression. *Clin Cancer Res* 2006;12:6359–6366. [PubMed: 17085646]

Filardo EJ, Quinn JA, Bland KI, Frackelton AR Jr. Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF. *Mol Endocrinol* 2000;14:1649–1660. [PubMed: 11043579]

Filardo EJ, Quinn JA, Frackelton AR Jr, Bland KI. Estrogen action via the G protein-coupled receptor, GPR30: stimulation of adenylyl cyclase and cAMP-mediated attenuation of the epidermal growth factor receptor-to-MAPK signaling axis. *Mol Endocrinol* 2002;16:70–84. [PubMed: 11773440]

Funakoshi T, Yanai A, Shinoda K, Kawano MM, Mizukami Y. G protein-coupled receptor 30 is an estrogen receptor in the plasma membrane. *Biochem Biophys Res Commun* 2006;346:904–910. [PubMed: 16780796]

Goldstein SR, Duvernoy CS, Calaf J, Adachi JD, Mershon JL, Dowsett SA, Agnusdei D, Stuenkel CA. Raloxifene use in clinical practice: efficacy and safety. *Menopause*. 2008

Haas E, Bhattacharya I, Brailoiu E, Damjanovic M, Brailoiu GC, Gao X, Mueller-Guerre L, Marjon NA, Gut A, Minotti R, Meyer MR, Amann K, Ammann E, Perez-Dominguez A, Genoni M, Clegg DJ, Dun NJ, Resta TC, Prossnitz ER, Barton M. Regulatory role of G protein-coupled estrogen receptor for vascular function and obesity. *Circ Res* 2009;104:288–291. [PubMed: 19179659]

Haas E, Meyer MR, Schurr U, Bhattacharya I, Minotti R, Nguyen HH, Heigl A, Lachat M, Genoni M, Barton M. Differential effects of 17beta-estradiol on function and expression of estrogen receptor alpha, estrogen receptor beta, and GPR30 in arteries and veins of patients with atherosclerosis. *Hypertension* 2007;49:1358–1363. [PubMed: 17452498]

Hoffmann-Lindsey S, Chappell MC. GPR30-mediated vasorelaxation is enhanced in female mRen2.Lewis rats and attenuated by high salt. *The FASEB Journal* 2008;22:968.910.

Houtchens MK. Pregnancy and multiple sclerosis. *Semin Neurol* 2007;27:434–441. [PubMed: 17940922]

Hsieh YC, Yu HP, Frink M, Suzuki T, Choudhry MA, Schwacha MG, Chaudry IH. G protein-coupled receptor 30-dependent protein kinase A pathway is critical in nongenomic effects of estrogen in attenuating liver injury after trauma-hemorrhage. *Am J Pathol* 2007;170:1210–1218. [PubMed: 17392161]

Hugo ER, Brandebourg TD, Woo JG, Loftus J, Alexander JW, Ben-Jonathan N. Bisphenol A at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes. *Environ Health Perspect* 2008;116:1642–1647. [PubMed: 19079714]

Isensee J, Meoli L, Zazzu V, Nabzdyk C, Witt H, Soewarto D, Effertz K, Fuchs H, Gailus-Durner V, Busch D, Adler T, de Angelis MH, Irgang M, Otto C, Noppinger PR. Expression pattern of G protein-coupled receptor 30 in LacZ reporter mice. *Endocrinology* 2009;150:1722–1730. [PubMed: 19095739]

Jensen EV, DeSombre ER. Estrogen-receptor interaction. *Science* 1973;182:126–134. [PubMed: 4354173]

Jordan CV, Lewis-Wambi J, Kim H, Cunliffe H, Ariazi E, Sharma CG, Shupp HA, Swaby R. Exploiting the apoptotic actions of oestrogen to reverse antihormonal drug resistance in oestrogen receptor positive breast cancer patients. *Breast Suppl* 2007;2:S105–113.

Jordan VC. SERMs: meeting the promise of multifunctional medicines. *J Natl Cancer Inst* 2007;99:350–356. [PubMed: 17341726]

Kanda N, Watanabe S. 17Beta-estradiol enhances the production of nerve growth factor in THP-1-derived macrophages or peripheral blood monocyte-derived macrophages. *J Invest Dermatol* 2003a;121:771–780. [PubMed: 14632195]

Kanda N, Watanabe S. 17beta-estradiol inhibits oxidative stress-induced apoptosis in keratinocytes by promoting Bcl-2 expression. *J Invest Dermatol* 2003b;121:1500–1509. [PubMed: 14675202]

Kanda N, Watanabe S. 17beta-estradiol stimulates the growth of human keratinocytes by inducing cyclin D2 expression. *J Invest Dermatol* 2004;123:319–328. [PubMed: 15245432]

Kuhn J, Dina OA, Goswami C, Suckow V, Levine JD, Hucho T. GPR30 estrogen receptor agonists induce mechanical hyperalgesia in the rat. *Eur J Neurosci* 2008;27:1700–1709. [PubMed: 18371086]

- Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology* 1997;138:863–870. [PubMed: 9048584]
- Kuiper GG, Enmark E, Peltö-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci U S A* 1996;93:5925–5930. [PubMed: 8650195]
- Kuo WH, Chang LY, Liu DL, Hwa HL, Lin JJ, Lee PH, Chen CN, Lien HC, Yuan RH, Shun CT, Chang KJ, Hsieh FJ. The interactions between GPR30 and the major biomarkers in infiltrating ductal carcinoma of the breast in an Asian population. *Taiwan J Obstet Gynecol* 2007;46:135–145. [PubMed: 17638621]
- Kvingedal AM, Smeland EB. A novel putative G-protein-coupled receptor expressed in lung, heart and lymphoid tissue. *FEBS Lett* 1997;407:59–62. [PubMed: 9141481]
- Lafferty AR, Torpy DJ, Stowasser M, Taymans SE, Lin JP, Huggard P, Gordon RD, Stratakis CA. A novel genetic locus for low renin hypertension: familial hyperaldosteronism type II maps to chromosome 7 (7p22). *J Med Genet* 2000;37:831–835. [PubMed: 11073536]
- Leung HS, Seto SW, Kwan YW, Leung FP, Au AL, Yung LM, Yao X, Huang Y. Endothelium-independent relaxation to raloxifene in porcine coronary artery. *Eur J Pharmacol* 2007;555:178–184. [PubMed: 17113071]
- Liverman CS, Brown JW, Sandhir R, McCarron KE, Berman NE. Role of the oestrogen receptors GPR30 and ERalpha in peripheral sensitization: relevance to trigeminal pain disorders in women. *Cephalalgia*. 2009
- Lu CL, Hsieh JC, Dun NJ, Oprea TI, Wang PS, Luo JC, Lin HC, Chang FY, Lee SD. Estrogen rapidly modulates 5-hydroxytryptophan-induced visceral hypersensitivity via GPR30 in rats. *Gastroenterology*. 2009
- Luttrell LM. Transmembrane signaling by G protein-coupled receptors. *Methods Mol Biol* 2006;332:3–49. [PubMed: 16878684]
- Ma L, Pei G. Beta-arrestin signaling and regulation of transcription. *J Cell Sci* 2007;120:213–218. [PubMed: 17215450]
- Mack WJ, Dhungana B, Dowsett SA, Keech CA, Feng M, Li Y, Hodis HN. Carotid artery intima-media thickness after raloxifene treatment. *J Womens Health (Larchmt)* 2007;16:370–378. [PubMed: 17439382]
- Madak-Erdogan Z, Kieser KJ, Kim SH, Komm B, Katzenellenbogen JA, Katzenellenbogen BS. Nuclear and extranuclear pathway inputs in the regulation of global gene expression by estrogen receptors. *Mol Endocrinol* 2008;22:2116–2127. [PubMed: 18617595]
- Maggiolini M, Vivacqua A, Fasanella G, Recchia AG, Sisci D, Pezzi V, Montanaro D, Musti AM, Picard D, Ando S. The G protein-coupled receptor GPR30 mediates c-fos up-regulation by 17beta-estradiol and phytoestrogens in breast cancer cells. *J Biol Chem* 2004;279:27008–27016. [PubMed: 15090535]
- Martensson UE, Salehi SA, Windahl S, Gomez MF, Sward K, Daszkiewicz-Nilsson J, Wendt A, Andersson N, Hellstrand P, Grande PO, Owman C, Rosen CJ, Adamo ML, Lundquist I, Rorsman P, Nilsson BO, Ohlsson C, Olde B, Leeb-Lundberg LM. Deletion of the G protein-coupled Receptor GPR30 Impairs Glucose Tolerance, Reduces Bone Growth, Increases Blood Pressure, and Eliminates Estradiol-stimulated Insulin Release in Female Mice. *Endocrinology*. 2008
- Matsuda K, Sakamoto H, Hosokawa K, Ito M, Nishi M, Prossnitz ER, Kawata M. Expression and intracellular distribution of the G protein-coupled estrogen receptor, GPR30, in rat hippocampus. *Neurosci Lett* 2008;441:94–99. [PubMed: 18586395]
- Mercier I, Mader S, Calderone A. Tamoxifen and ICI 182,780 negatively influenced cardiac cell growth via an estrogen receptor-independent mechanism. *Cardiovasc Res* 2003;59:883–892. [PubMed: 14553828]
- Meyer, MR.; Haas, E.; Prossnitz, ER.; Barton, M. *Mol Cell Endocrinol*. 2009. Non-genomic regulation of vascular cell function and growth by estrogen. in press
- Moriarty K, Kim KH, Bender JR. Minireview: estrogen receptor-mediated rapid signaling. *Endocrinology* 2006;147:5557–5563. [PubMed: 16946015]
- Mugge A, Riedel M, Barton M, Kuhn M, Lichtlen PR. Endothelium independent relaxation of human coronary arteries by 17 beta-oestradiol in vitro. *Cardiovasc Res* 1993;27:1939–1942. [PubMed: 8287400]

- Murase Y, Kobayashi J, Nohara A, Asano A, Yamaaki N, Suzuki K, Sato H, Mabuchi H. Raloxifene promotes adipocyte differentiation of 3T3-L1 cells. *Eur J Pharmacol* 2006;538:1–4. [PubMed: 16643890]
- Noel SD, Keen KL, Baumann DI, Filardo EJ, Terasawa E. Involvement of G-Protein Coupled Receptor 30 (GPR30) in Rapid Action of Estrogen in Primate LHRH Neurons. *Mol Endocrinol* 2009;23:349–359. [PubMed: 19131510]
- O'Dowd BF, Nguyen T, Marchese A, Cheng R, Lynch KR, Heng HH, Kolakowski LF Jr, George SR. Discovery of three novel G-protein-coupled receptor genes. *Genomics* 1998;47:310–313. [PubMed: 9479505]
- Otto C, Fuchs I, Kauselmann G, Kern H, Zevnik B, Andreassen P, Schwarz G, Altmann H, Klewer M, Schoor M, Vonk R, Fritzemeier KH. GPR30 Does Not Mediate Estrogenic Responses in Reproductive Organs in Mice. *Biol Reprod*. 2008a
- Otto C, Rohde-Schulz B, Schwarz G, Fuchs I, Klewer M, Brittain D, Langer G, Bader B, Prella K, Nubbemeyer R, Fritzemeier KH. GPR30 localizes to the endoplasmic reticulum and is not activated by estradiol. *Endocrinology* 2008b;149:4846–4856. [PubMed: 18566127]
- Owman C, Blay P, Nilsson C, Lolait SJ. Cloning of human cDNA encoding a novel heptahelix receptor expressed in Burkitt's lymphoma and widely distributed in brain and peripheral tissues. *Biochem Biophys Res Commun* 1996;228:285–292. [PubMed: 8920907]
- Pandey DP, Lappano R, Albanito L, Madeo A, Maggiolini M, Picard D. Estrogenic GPR30 signalling induces proliferation and migration of breast cancer cells through CTGF. *EMBO J*. 2009
- Pang Y, Dong J, Thomas P. Estrogen signaling characteristics of Atlantic croaker G protein-coupled receptor 30 (GPR30) and evidence it is involved in maintenance of oocyte meiotic arrest. *Endocrinology* 2008;149:3410–3426. [PubMed: 18420744]
- Pinna C, Bolego C, Sanvito P, Pelosi V, Baetta R, Corsini A, Gaion RM, Cignarella A. Raloxifene elicits combined rapid vasorelaxation and long-term anti-inflammatory actions in rat aorta. *J Pharmacol Exp Ther* 2006;319:1444–1451. [PubMed: 16943257]
- Polanczyk M, Zamora A, Subramanian S, Matejuk A, Hess DL, Blankenhorn EP, Teuscher C, Vandenbark AA, Offner H. The Protective Effect of 17{beta}-Estradiol on Experimental Autoimmune Encephalomyelitis Is Mediated through Estrogen Receptor-{alpha}. *Am J Pathol* 2003;163:1599–1605. [PubMed: 14507666]
- Prossnitz ER, Maggiolini M. Mechanisms of estrogen signaling and gene regulation via GPR30. *Mol Cell Endocrinol*. 2009in press
- Quinn JA, Graeber CT, Frackelton AR Jr, Kim M, Schwarzbauer JE, Filardo EJ. Coordinate regulation of estrogen-mediated fibronectin matrix assembly and epidermal growth factor receptor transactivation by the G-protein-coupled receptor, GPR30. *Mol Endocrinol*. 2009
- Razandi M, Pedram A, Park ST, Levin ER. Proximal events in signaling by plasma membrane estrogen receptors. *J Biol Chem* 2003;278:2701–2712. [PubMed: 12421825]
- Revankar CM, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER. A transmembrane intracellular estrogen receptor mediates rapid cell signaling. *Science* 2005;307:1625–1630. [PubMed: 15705806]
- Revankar CM, Mitchell HD, Field AS, Burai R, Corona C, Ramesh C, Sklar LA, Arterburn JB, Prossnitz ER. Synthetic estrogen derivatives demonstrate the functionality of intracellular GPR30. *ACS Chem Biol* 2007;2:536–544. [PubMed: 17655271]
- Romano N, Lee K, Abraham IM, Jasoni CL, Herbison AE. Non-classical estrogen modulation of presynaptic GABA terminals modulates calcium dynamics in gonadotropin-releasing hormone (GnRH) neurons. *Endocrinology*. 2008
- Sakamoto H, Matsuda K, Hosokawa K, Nishi M, Morris JF, Prossnitz ER, Kawata M. Expression of GPR30, a G protein-coupled membrane estrogen receptor, in oxytocin neurons of the rat paraventricular and suproptic nuclei. *Endocrinology* 2007;148:5842–5850. [PubMed: 17872373]
- Sarrel PM, Nawaz H, Chan W, Fuchs M, Katz DL. Raloxifene and endothelial function in healthy postmenopausal women. *Am J Obstet Gynecol* 2003;188:304–309. [PubMed: 12592230]
- Senkus-Konefka E, Konefka T, Jassem J. The effects of tamoxifen on the female genital tract. *Cancer Treat Rev* 2004;30:291–301. [PubMed: 15059652]
- Singleton DW, Khan SA. Xenoestrogen exposure and mechanisms of endocrine disruption. *Front Biosci* 2003;8:s110–118. [PubMed: 12456297]

- Sirianni R, Chimento A, Ruggiero C, De Luca A, Lappano R, Ando S, Maggiolini M, Pezzi V. The novel estrogen receptor, G protein-coupled receptor 30, mediates the proliferative effects induced by 17 β -estradiol on mouse spermatogonial GC-1 cell line. *Endocrinology* 2008;149:5043–5051. [PubMed: 18566133]
- Smith HO, Leslie KK, Singh M, Qualls CR, Revankar CM, Joste NE, Prossnitz ER. GPR30: a novel indicator of poor survival for endometrial carcinoma. *Am J Obstet Gynecol* 2007;196(386):e381–389.
- Sukocheva O, Wadham C, Holmes A, Albanese N, Verrier E, Feng F, Bernal A, Derian CK, Ullrich A, Vadas MA, Xia P. Estrogen transactivates EGFR via the sphingosine 1-phosphate receptor Edg-3: the role of sphingosine kinase-1. *J Cell Biol* 2006;173:301–310. [PubMed: 16636149]
- Sweet MG, Sweet JM, Jeremiah MP, Galazka SS. Diagnosis and treatment of osteoporosis. *Am Fam Physician* 2009;79:193–200. [PubMed: 19202966]
- Szego CM, Davis JS. Adenosine 3',5'-monophosphate in rat uterus: acute elevation by estrogen. *Proc Natl Acad Sci U S A* 1967;58:1711–1718. [PubMed: 4295833]
- Takada Y, Kato C, Kondo S, Korenaga R, Ando J. Cloning of cDNAs encoding G protein-coupled receptor expressed in human endothelial cells exposed to fluid shear stress. *Biochem Biophys Res Commun* 1997;240:737–741. [PubMed: 9398636]
- Teng J, Wang ZY, Prossnitz ER, Bjorling DE. The G protein-coupled receptor GPR30 inhibits human urothelial cell proliferation. *Endocrinology* 2008;149:4024–4034. [PubMed: 18467434]
- Thomas P, Dong J. Binding and activation of the seven-transmembrane estrogen receptor GPR30 by environmental estrogens: a potential novel mechanism of endocrine disruption. *J Steroid Biochem Mol Biol* 2006;102:175–179. [PubMed: 17088055]
- Thomas P, Pang Y, Filardo EJ, Dong J. Identity of an estrogen membrane receptor coupled to a G-protein in human breast cancer cells. *Endocrinology* 2005;146:624–632. [PubMed: 15539556]
- Tiemann U. In vivo and in vitro effects of the organochlorine pesticides DDT, TCPM, methoxychlor, and lindane on the female reproductive tract of mammals: a review. *Reprod Toxicol* 2008;25:316–326. [PubMed: 18434086]
- Toran-Allerand CD. Minireview: A plethora of estrogen receptors in the brain: where will it end? *Endocrinology* 2004;145:1069–1074. [PubMed: 14670986]
- Traupe T, Stettler CD, Li H, Haas E, Bhattacharya I, Minotti R, Barton M. Distinct roles of estrogen receptors alpha and beta mediating acute vasodilation of epicardial coronary arteries. *Hypertension* 2007;49:1364–1370. [PubMed: 17470727]
- Trenova AG, Manova MM, Zahariev ZI, Vasileva TV. Female sex hormones and multiple sclerosis. *Folia Med (Plovdiv)* 2004;46:11–15. [PubMed: 15962809]
- van Walderveen MA, Tas MW, Barkhof F, Polman CH, Frequin ST, Hommes OR, Valk J. Magnetic resonance evaluation of disease activity during pregnancy in multiple sclerosis. *Neurology* 1994;44:327–329. [PubMed: 8309584]
- Vivacqua A, Bonofiglio D, Albanito L, Madeo A, Rago V, Carpino A, Musti AM, Picard D, Ando S, Maggiolini M. 17 β -estradiol, genistein and 4-hydroxytamoxifen induce the proliferation of thyroid cancer cells through the G protein-coupled receptor GPR30. *Mol Pharmacol* 2006a;70:1414–1423. [PubMed: 16835357]
- Vivacqua A, Bonofiglio D, Recchia AG, Musti AM, Picard D, Ando S, Maggiolini M. The G protein-coupled receptor GPR30 mediates the proliferative effects induced by 17 β -estradiol and hydroxytamoxifen in endometrial cancer cells. *Mol Endocrinol* 2006b;20:631–646. [PubMed: 16239258]
- Wang C, Dehghani B, Li Y, Kaler LJ, Proctor T, Vandenbark AA, Offner H. Membrane estrogen receptor regulates experimental autoimmune encephalomyelitis through up-regulation of programmed death 1. *J Immunol* 2009;182:3294–3303. [PubMed: 19234228]
- Wang C, Dehghani B, Magrisso IJ, Rick EA, Bonhomme E, Cody DB, Elenich LA, Subramanian S, Murphy SJ, Kelly MJ, Rosenbaum JS, Vandenbark AA, Offner H. GPR30 contributes to estrogen-induced thymic atrophy. *Mol Endocrinol* 2008a;22:636–648. [PubMed: 18063692]
- Wang C, Prossnitz ER, Roy SK. Expression of GPR30 in the hamster ovary: differential regulation by gonadotropins and steroid hormones. *Endocrinology* 2007;148:4853–4864. [PubMed: 17640985]

- Wang C, Prossnitz ER, Roy SK. G protein-coupled receptor 30 expression is required for estrogen stimulation of primordial follicle formation in the hamster ovary. *Endocrinology* 2008b;149:4452–4461. [PubMed: 18499747]
- Xu H, Qin S, Carrasco GA, Dai Y, Filardo EJ, Prossnitz ER, Battaglia G, DonCarlos LL, Muma NA. Extra-nuclear estrogen receptor GPR30 regulates serotonin function in rat hypothalamus. *Neuroscience* 2009;158:1599–1607. [PubMed: 19095043]
- Zhu T, Gobeil F, Vazquez-Tello A, Leduc M, Rihakova L, Bossolasco M, Bkaily G, Peri K, Varma DR, Orvoine R, Chemtob S. Intracrine signaling through lipid mediators and their cognate nuclear G-protein-coupled receptors: a paradigm based on PGE2, PAF, and LPA1 receptors. *Can J Physiol Pharmacol* 2006;84:377–391. [PubMed: 16902584]