Not Lost in Translation: Emerging Clinical Importance of the G Protein-Coupled Estrogen Receptor GPER

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Summary

It has been 20 years that the G protein-coupled estrogen receptor (GPER) was cloned as the orphan receptor GPR30 from multiple cellular sources, including vascular endothelial cells. Here, I will provide an overview on the historic development of rapid vascular estrogen signaling and review the recent advances in the understanding of the mechanisms underlying GPER function, its role in physiology and disease, some of the currently available GPER-targeting drugs approved for clinical use such as SERMs (selective estrogen receptor modulators) and SERDs (selective estrogen receptor downregulators). Many of currently used drugs such as tamoxifen, raloxifene, or faslodexTM/fulvestrant were discovered targeting GPER many years after they had been introduced to the clinics for entirely different purposes. This has important implications for the clinical use of these drugs and their modes of action, which I have termed 'reverse translational medicine'. Similarly, environmental pollutants known as 'endocrine disruptors' have been found to bind to GPER. This article will discuss recent evidence in these areas and also opportunities in translational clinical medicine and GPER research, including medical genetics, personalized medicine, prevention, and its theranostic use.

Abbreviations used

ER, estrogen receptor; GPER, G protein-coupled estrogen receptor; GPR30, G protein-coupled receptor 30; SERD, selective estrogen receptor downregulator; SERM, selective estrogen receptor modulator; DDT, dichlorodiphenyltrichloroethane

Key words

Estrogen biology; faslodexTM, fulvestrant; clinical medicine; GPER-1; GPR30, tamoxifen, ICI 182,780; theranostics; cancer, atherosclerosis; coronary artery disease; multiple sclerosis; inflammation; autoimmune disease; patients; niacin; dyslipidemia; blood pressure; hypertension

Contents

1. Clinical importance of estrogen signaling.....	4
2. Estrogen receptors: cellular mediators and therapeutic targets.....	4
3. A G protein-coupled receptor that mediates estrogen action.....	5
4. Drugs targeting GPER: Opportunities for ‘reverse translational medicine’....	7
5. Targeting GPER in cardiovascular disease, obesity, and diabetes.....	8
6. Targeting GPER in immune disorders, infectious diseases, and cancer.....	10
7. Endocrine disruptors and xenoestrogens as activators of GPER	11
8. Summary and Conclusion.....	12
Acknowledgments.....	13
References.....	15

1. Clinical importance of estrogen signaling

Estrogens have been known only for about 80 years after German biochemist Adolf Butenandt (1903-1995) while at the University of Danzig (today Gdansk, Poland) and still in his twenties identified estrogen and other female sex hormones [1, 2] in collaboration with Schering AG, Berlin, isolating a mere 15 mg of crystallized estrogen from 25'000 liters of urine. Butenandt's discovery was awarded the 1939 Nobel Prize in Chemistry, which he shared with the Croatian biochemist Leopold Ružička (1887-1976) who had succeeded in isolating testosterone in Zürich [3, 4]. Today, we know that in premenopausal women, endogenous estrogen production is mainly derived from the ovaries [5-7]. However in males and females estrogen is also produced in the arterial wall (from the aromatase-mediated conversion of testosterone [8, 9]) as well as other tissues, including adipose [10]. Estrogens are regulators of cell proliferation, bone growth, mammary gland proliferation, uterine growth, vascular tone, blood pressure, myocardial contractility, sympathetic activation, glucose metabolism, inflammation, adipogenesis and lipid metabolism [7, 9, 11-13], and consequently, atherogenesis [13]. In women, aberrant estrogen signaling or decreases/impairment of estrogen function (clinically well evident in patients diagnosed with polycystic ovary syndrome (PCOS) who are prone to develop arterial hypertension, diabetes, and obesity [14, 15]), promotes the development of disease such as cancer, atherosclerotic vascular disease, osteoporosis, hypertension and diabetes [7, 16]. Though estrogens are known as prototypical "female sex hormones", they also play a role on male physiology [6, 7, 17].

Given their involvement in disease processes, the understanding of how estrogens mediate cellular signaling, the function of estrogen receptors and their tissue specificity, and their agonism and antagonism has become increasingly important not only for estrogen-dependent diseases but particularly for therapeutic approaches targeting estrogen receptors. Estrogen-based therapies (utilizing anti-estrogens/SERMs or aromatase inhibitors) have become the major neoadjuvant therapeutic approach used for the treatment of breast cancer [18, 19].

2. Estrogen receptors: cellular mediators and therapeutic targets

The so-called “classical” estrogen receptors, ER α and ER β [5, 20, 21], were cloned in 1985 [22-26] and 1996, respectively [26, 27], and nuclear as well as membrane-bound subpopulations of both receptors have been identified that are derived from the same transcript as the nuclear receptors [28, 29]. Today, particularly in clinical medicine, estrogens are still mainly known for mediating their cellular effects through “genomic” signaling regulating gene transcription, which involves the formation of receptor homo- or heterodimers, and binding to estrogen response elements (ERE) in the promoter and regulatory regions of target genes [20, 30]. Nuclear ERs (even in the absence of ligand binding or direct binding to DNA) also interact with other classes of transcription factors and DNA modifying proteins through complex protein–protein interactions, thus further modifying cellular responses and functions [31].

By contrast, rapid, i.e. “non-genomic” vascular effects mediated by estrogen noted already at the beginning of the Second World War when in November 1939 Reynolds and Foster published rapid peripheral vasodilator effects of estrogen in healthy men that occurred within 3 to 15 minutes after infusion [32]. Half a century ago at the University of California Los Angeles cell biologist Clara M. Szego (1916 -) observed in cell experiments that 17 β -estradiol rapidly stimulates cAMP in uterine tissue within seconds after application [33-35], and it has been 40 years since Venter and Buonassisi [36] and Pietras and Szego [37] detected binding sites for estrogen on human endothelial cell and endometrial cell membranes, respectively; these experiments were subsequently confirmed in plasma membrane fractions of normal and tumor mammary human tissues [38]. The role of rapid estrogen signaling, however, has become increasingly appreciated only in the past two decades, and knowledge about the contribution of the receptors mediating rapid estrogen action to physiology and disease has only begun to evolve.

3. A G protein-coupled receptor that mediates estrogen action

In the early 1990s, I came across the early studies by Szego and coworkers [33-35, 37] and set up similar experiments to study acute effects of 17 β -estradiol on cAMP production in human coronary arteries [39, 40]. Following exposure to 17 β -estradiol rapid increases of vascular cyclic AMP content (and also cyclic GMP content) could be

observed within 30 minutes of application [39]. Within the same time frame a rapid and potent vasodilation of precontracted coronary artery rings in response to 17β -estradiol occurred, in both intact arteries from women and men [40, 41]. Both observations could not be explained by genomic mechanisms [39]. At that time no membrane receptor mediating rapid responses to estrogen was known [41]. In the following year, Benita Katzenellenbogen and associates confirmed these findings also demonstrating rapid increases in cyclic AMP content following exposure 17β -estradiol within 30 minutes utilizing intact uterine tissue that was associated with activation of adenyl cyclase [42].

Shortly after these studies demonstrating rapid increases in cellular cAMP in response to 17β -estradiol had been published, a 7-transmembrane spanning G protein-coupled receptor was identified at Lund University in Sweden in 1996 [43]. Since at the time no known ligand existed this receptor was received the orphan receptor designation GPR30 [10]. The receptor was subsequently cloned from multiple other cell types, including inflammatory white blood cells [43, 44], breast cancer cells [45], endothelial cells [46]. The receptor was also identified following a database mining [47], degenerate oligonucleotide screening of genomic DNA [48], and as its rat homologue, GPR41 [49-51]. High expression of GPR30 was detected in vasculature, heart, renal tubules, lung, placenta, and the reproductive system, among others tissues and organs [10].

GPR30 was 'de-orphanized' as an estrogen receptor in 2000 in seminal studies by Filardo and associates, who demonstrated that abundance of GPER was required for the rapid estrogen-mediated ERK1/2 activation [52] and increasing cyclic AMP content [53]. At the same time, Levin and coworkers reported membrane subpopulations of $ER\alpha$ and $ER\beta$ [29] subsequently showing that they function as receptor dimers [54] while Nadal et al. reported rapid actions of estrogen and xenoestrogens that were mediated through a then unknown membrane receptor unrelated to $ER\alpha$ and $ER\beta$ [55]. Prossitz et al. and Thomas, Filardo and coworkers subsequently reported that GPR30 mediates rapid effects of estrogen [56, 57].

In 2007, GPR30 received the official new designation as « G protein-coupled estrogen receptor-1 » (GPER-1) from the *International Union of Basic and Clinical Pharmacology* (IUPHAR) [58, 59]. The data currently available provide evidence that

GPER not only plays a role in physiology of the reproductive system, vasculature, kidney, brain, in obesity and endocrine metabolism, but that it is also involved in estrogen-dependent diseases including cancers of the reproductive system, male fertility [60], obesity, osteoporosis, arterial hypertension, atherosclerosis, and autoimmune diseases including multiple sclerosis, as well as aging (reviewed in [10, 61, 62]). It is important to remember that GPER, like most other GPCRs, not only mediates “non-genomic” rapid effects, but also exerts tonic (constitutive/basal) activity that contributes to cell function and growth, as demonstrated by numerous studies in GPER-deficient mice [63-70]. A recent study found that GPER is responsible for the abnormal estrogen-dependent vasoconstriction and elevated blood pressure in female mice deficient in ephrin B3, and that these effects can be abrogated by ovariectomy [71], suggesting cross-talk between estrogen-dependent GPER function and ephrin pathways.

4. Drugs targeting GPER: Opportunities for ‘reverse translational medicine’

It is noteworthy that selective estrogen receptor modulators, now known to activate GPER[10, 59, 61], were not initially intended for their current use. In fact, tamoxifen (ICI 46,474) was developed as a contraceptive pill but failed [72]. Tamoxifen had been patented by as a hypocholesterolemia drug in the early 1960’s and related compounds also affected cholesterol metabolism and biosynthesis [72]. Of note, more than 50 years later activation of the tamoxifen target GPER has been implicated in beneficial effects on cholesterol metabolism [62, 68, 73]. The number molecules targeting GPER with agonistic or antagonistic activity continues to grow and those currently known been recently summarized [10, 62]. Among others, these substances include natural steroid hormones such 17 β -estradiol (a non-selective ER agonist), its metabolites (2-hydroxy-estradiol [74], a catechol estrogen [75]), selective estrogen receptor modulators (SERMs, including tamoxifen, raloxifene) [59], selective estrogen receptor downregulators (SERDs, ICI 182, 780, faslodexTM/fulvestrant [59]), vitamin B₃/niacin [76], phytoestrogens such as genistein [59], daidzein [59], the daidzien metabolite equol [77], resveratrol, green tea catechins [78], synthetic compounds non-selectively (PPT) [59, 79] or selectively targeting GPER (G-1, G15, G36 [59]), as well as endocrine

disruptors/xenoestrogens such as bisphenol A and DDT [10, 59] (see Figure 1 for an overview of GPER activators and inhibitors).

GPER may not only have direct effects upon ligand activation, but its function critically involves receptor interactions, i.e. cross-talk with nuclear steroid receptors, including ER α [80], ER β [81], mineralocorticoid receptor (MR), vitamin D receptor (VDR), and the glucocorticoid receptor (GR) (reviewed in [17, 61]). Moreover, the recent availability of drugs that selectively activate ER β [82-84] or GPER [59] and given that substances such as propyl pyrazole triol (PPT, commonly used as ER α -selective agonist) can also activate ER β [85] and GPER [59, 79], have added to the complexity of estrogen action and understanding the roles and interplay between individual estrogen receptors [11, 86]. Finally, under certain pathological conditions activation of individual estrogen receptors may exert comparable beneficial effects [12, 87]. In contrast to data indicating functional crosstalk between MR and GPER [17, 88], there is no evidence to support the notion that GPER functions as a receptor for aldosterone [88, 89] as has been proposed by some [90, 91] since no binding of aldosterone to GPER could be detected [92, 93].

Over the past decade, numerous molecules were identified to either activate or inhibit GPER [10, 59, 61]. Several of these drugs were discovered to target GPER many years after they had been already introduced to the clinics for entirely different purposes, e.g. as neoadjuvant cancer therapeutics [10, 61]. These drugs particularly include SERMs such as tamoxifen, raloxifene, and SERDs like fulvestrant (ICI 182, 780) [17]. These newly discovered modes of action of clinically approved drugs require us to rethink and extend our therapeutic rationale that we apply when administering these drugs to patients [10]. In fact, we need to go ‘from bench to bedside and back’ (as proposed 20 years ago by Marie Gerhard and Peter Ganz [94]) to understand how estrogen receptor-targeting drugs work and may benefit patients, an approach which I have termed ‘reverse translational medicine’. In the following two paragraphs, I will give some examples and discuss how this may impact and change our understanding of clinical treatments of disease.

5. Targeting GPER in cardiovascular disease, obesity, and diabetes

The protective effects of endogenous estrogen on cardiovascular disease, arterial hypertension, obesity, dyslipidemia, and diabetes in women become evident after menopause [7, 9]. These effects are only partly mediated by the “classical” ER α [95, 96], and also involve GPER-dependent mechanism [62, 68]. Notably, many of the salutary effects of estrogen are absent in GPER-deficient mice [65, 66, 97]) and can be mimicked by GPER-activating compounds, which exert beneficial effects on arterial hypertension [9, 98] and the associated hypertensive renal injury [99], insulin resistance and diabetes [12, 70, 87], dyslipidemia [68, 73], and also reduces adipogenesis [97, 100] and thus obesity – which conversely is increased in animals lacking GPER [65, 66, 97]. The latter suggests an important role of GPER as a receptor with “constitutive”, i.e. ligand-independent basal activity.

Many compounds currently in clinical use exhibit activity towards GPER; in view of the recent discovery that SERMs and SERDs - which not only block the “classical” estrogen receptors ER α and ER β (which would not explain the beneficial effects) – but like the soy phytoestrogens genistein [59] and daidzein [59], green tea catechins [78] or niacin [76] also exhibiting agonistic activity towards GPER, the clinical data currently available needs to be reassessed. As phytoestrogens also improve lipid and glucose metabolism [101-105] as well as neointima formation after balloon angioplasty [106], the above drugs and substances are considered for primary or secondary prevention of cardiovascular and metabolic diseases [13, 62].

The GPER gene maps to chromosome 7p22.3, a region linked to arterial hypertension in humans [65, 107]. Moreover, recent clinical studies have found an association between *Gper* gene function and arterial blood pressure [108]. In addition to numerous preclinical investigations [109-115], clinical studies have shown that SERMs or SERDs improve vasodilation [116-118] and arterial stiffness [119], lower blood pressure [120, 121], improve the lipid and glucose profile [122-126], reduce circulating fibrinogen levels [127], and educe cardiovascular events in postmenopausal women [128, 129] – all effects that are compatible with GPER activation while blocking ER α and/or ER β would be expected to yield the opposite effects [9]. Finally, a common side effect in breast cancer patients receiving the SERD fulvestrant frequently report

hypotension as an unwanted side effect [121], which so far could not been explained mechanistically. We [109] and others [130] have demonstrated that the GPER agonist fulvestrant causes rapid vasodilation which might explain the frequent occurrence of hypotension observed in patients receiving this drug.

Niacin (vitamin B₃) has used for more than half a century as an effective treatment for dyslipidemia in patients at risk for or diagnosed with coronary, cerebrovascular, or peripheral artery disease [131]. Its mode of action has been recently been attributed to two G protein-coupled receptors of high and low affinity, respectively, GPR109A (MH74A) and GPR109B (HM74) [131-133]. Recent evidence however suggests that niacin is also capable of activating GPER [76] which would be compatible with the lipid-lowering effect seen in patients treated with SERMs and SERDs [122, 123]. A role for GPER as a modulator of lipid metabolism is also supported by clinical studies demonstrating influences of its gene function on the lipid profile [73]. Of note, selective GPER activation has been shown to inhibit angiogenesis [61, 62], which may confer additional therapeutic benefits in cancer patients. A recent study found that nicotinamide, the amide form of niacin, inhibits vasculogenic mimicry in vitro [134], a pathway that has implicated in the pathogenesis of highly aggressive melanoma. An inhibitory effect on vasculogenesis would be compatible with GPER activation [10]. Also, a dramatic beneficial effect of niacin on UV-induced DNA damage in patients with autosomal recessive xeroderma pigmentosum (Cockayne syndrome) has been recently reported, which possibly involves GPER activation.

Taken together, clinically used SERMS, dyslipidemia therapeutics as well as phytoestrogens show therapeutic benefits on cardiovascular and metabolic profiles in patients that are consistent with a GPER-activating function rather than with blocking the “classical” estrogen receptors. Further research is required to delineate of whether and how much these effects involve GPER.

6. Targeting GPER in immune disorders, infectious diseases, and cancer

Estrogens play an important role in modulating immune responses, thereby contributing to autoimmune diseases, infection, and diseases with a strong inflammatory component such as vascular and renal disease, obesity, and diabetes [10, 61]. GPER

was cloned from white blood cells [43], among other cellular sources, and its initial suggested role was that of a cytokine receptor [10]. It is not surprising that an important immunomodulatory role of GPER could be demonstrated [10].

GPER is expressed and functional in human monocytes [135], macrophages [135], eosinophiles [136], lymphocytes [43, 137-139], and the fish equivalent of human neutrophils [140]. There is now preclinical evidence that activation of GPER reduces inflammation in endothelial cells [141, 142], in atherosclerotic plaque [68] and myocardium of failing hearts [143]. Moreover, GPER is involved in protective, estrogen-mediated effects of autoimmune diseases in models of multiple sclerosis [144-146], as well as chronic infections caused by hepatitis C virus (HCV) [147]. Moreover, roles of GPER in different forms of cancer of the reproductive and non-reproductive cancers have been demonstrated (reviewed in [10]). Recent clinical studies suggest that SERMs may indeed provide therapeutic efficacy as adjuvant therapy in non-gynecological cancers such as metastatic prostate cancer [148] or as sensitizers of cytostatic drugs in patients with pancreatic cancer [149]. Moreover, the use of SERMs for the treatment of *Candida albicans* or *Staphylococcus aureus* infections [150] or infections caused by Ebola virus [151] has been proposed.

As with the studies demonstrating beneficial effects of SERMs, SERDs, phytoestrogens, and niacin/vitamin B₃ in cardiovascular and metabolic diseases, similar results using these compounds were obtained regard to immune-mediated diseases and cancer ([152, 153] and reviewed in [59]). Raloxifene has been shown to inhibit inflammatory activation and cell growth [154, 155], as has been demonstrated for GPER-activating phytoestrogens [156-159] and niacin [160]. The profound anti-inflammatory effects of these substances have also been observed clinical studies studying diseases with inflammatory activation [161-163]. As discussed above, currently it is unclear whether and to what extent the beneficial effects of these compounds can be ascribed to GPER. Future preclinical and clinical studies should be directed toward addressing this question.

7. Endocrine disruptors and xenoestrogens as activators of GPER

Xenoestrogens are synthetic or natural substances of high stability that exert toxicity by mimicking the effects of estrogens [164]. These stable chemicals are products of modern technology used in manufacturing consumer products including detergents, surfactants, resins, lubricants, plasticizers, fire retardants, and pesticides [59] and they accumulate in the environment, including in ground water, fruits and vegetables [165], and can even be found in bottled mineral water [166]. Because of their endocrine activity they have been designated ‘endocrine disruptors’, also known as ‘environmental pollutants’ [10]. Numerous synthetic compounds (e.g. pesticides and plastic precursors) that act as endocrine disruptors with metabolic and reproductive effects have been found to activate GPER (reviewed in [59]). These chemicals include atrazine, bisphenol A, nonylphenol, kepone, chlorinated hydrocarbons and their metabolites and degradation products such as *p,p*-DDT, *o,p*-DDE and 2,2,5,- PCB-4-OH (reviewed in [10, 59, 61, 62]), as well as the mycotoxin zearaloxone [167]. At this point it is not known to what degree endocrine disruptors mediate their deleterious effects in humans through GPER, and to which other mechanisms are involved. Research also concentrates on chemical classes such as alkyl phenols and esters of parahydroxybenzoic acid, phthalates, polybrominated diphenyl ethers, organophosphates, chlorinated hydrocarbons, and biphenyls [59]. Bisphenol A (BPA) is produced in great quantities globally, primarily for use in polycarbonate plastics and epoxy resins, with the safety of human exposures to BPA being subject of intensive scientific assessment and regulatory efforts [59, 168, 169]. Since endocrine disruptors have implicated in a number of health concerns, including embryonic and early life development [164, 170], progenitor cell function [171], female and male reproduction and development [164, 172], infertility, cancer [172], autoimmune diseases [164], obesity and metabolic disorders [173, 174], epigenetic programming in adolescents [164, 175], arterial hypertension [176], polycystic ovary syndrome (PCOS) [177-179] as well as deleterious effects of prenatal exposure to xenoestrogens [164, 180]. Given that many endocrine disruptors also activate GPER future clinical research should be directed to explore which of the known estrogen receptors mediates their deleterious effects, and/or whether other mechanisms also play a role.

8. Summary and Outlook

As discussed, the obvious and potent beneficial effects of clinically used drugs such as SERMs, SERDs, and niacin (which can also be observed for certain phytoestrogens and green tea constituents [181]) cannot be explained by blocking the “classical” estrogen receptors and based on recent preclinical finding likely involve an activating effect on GPER. Applying “reverse translational medicine” we should rethink our therapeutic approaches when using these drugs which also suggest possible new therapeutic indications such as for the treatment of bleeding in hereditary hemorrhagic telangiectasia [182] and possibilities for future drug development. Medical genetics studies have found that genetic polymorphisms of GPER are associated with changes in cell function [183] or disease susceptibility for dyslipidemia [73], arterial hypertension [108], seminoma [184], gastric cancer [185], breast cancer [186], leiomyoma [187] and gynecomastia in adolescents [188] providing us with new opportunities for personalized medicine, theranostics, and thereby improving treatment and prevention. Continued clinical research is required to increase our understanding of estrogen receptors and the clinically approved drugs targeting them and how these drugs mediate therapeutic benefits and unwanted side effects in patients, which ultimately will improve patient safety and survival.

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Figure Legend

Activators (left, green) and inhibitors (right, red) of G protein-coupled estrogen receptor (GPER) signaling. GPER is activated by natural estrogens (endogenous estrogen such as 17 β -estradiol (E₂) or 2-methoxyestradiol) as well as by phytoestrogens (such quercetin, genistein, epicatechin, resveratrol), highly stable environmental pollutants (“endocrine disruptors”) also known as xenoestrogens [including atrazine, bisphenol A, nonylphenol, and p,p0-dichlorodiphenyltrichloroethane (DDT)]. Although zearalonone is plant (fungus)-derived and thus by definition a phytoestrogen as shown in the figure, due to its toxicity as a environmental mycotoxin is also considered a xenoestrogen. Similarly, daidzein - which like genistein is a soy-derived phytoestrogen - is also considered an environmental xenoestrogen REF. Equol is a metabolite of daidzein and thus must be considered of phytoestrogen-derivative rather than of plant origin REF. Importantly, GPER is also activated by niacin (vitamin B₃), selective estrogen receptor (ER) modulators (SERMs), and selective ER downregulators (SERDs). The latter two exhibit inhibitory activity on the “classical” estrogen receptors, ER α and ER β . GPER is also activated by selective synthetic, non-steroidal, small molecule compounds, such as G-1. Inhibition of GPER can be achieved utilizing synthetic GPER-selective small molecule compounds G15 and G36, and the non ER-selective compound ethyl 3-[5-(2-ethoxycarbonyl-1-methylvinyl)-1-methyl-1H-indol-3-yl]but-2-enoate (MIBE). The catechol estrogen metabolite 2-hydroxy-17 β -estradiol (2-OH-E₂) and the 16-OH-DHEAS metabolite estriol (E₃) also act as GPER antagonists. Figure reproduced from *Trends Endocrinol Metabol* 2015; 26: 185-192, with the permission of Cell Press.

Figure 1

Graphical Abstract