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Review article

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The G protein-coupled oestrogen receptor GPER in health and disease: an update

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Abstract

Oestrogens and their receptors contribute broadly to physiology and diseases. In premenopausal women, endogenous oestrogens protect against cardiovascular, metabolic and neurological diseases and are involved in hormone-sensitive cancers such as breast cancer. Oestrogens and oestrogen mimetics mediate their effects via the cytosolic and nuclear receptors oestrogen receptor-α (ERα) and oestrogen receptor-β (ERβ) and membrane subpopulations as well as the 7-transmembrane G protein-coupled oestrogen receptor (GPER). GPER, which dates back more than 450 million years in evolution, mediates both rapid signalling and transcriptional regulation. Oestrogen mimetics (such as phyto-oestrogens and xenooestrogens including endocrine disruptors) and licensed drugs such as selective oestrogen receptor modulators (SERMs) and downregulators (SERDs) also modulate oestrogen receptor activity in both health and disease. Following up on our previous Review of 2011, we herein summarize the progress made in the field of GPER research over the past decade. We will review molecular, cellular and pharmacological aspects of GPER signalling and function, its contribution to physiology, health and disease, and the potential of GPER to serve as a therapeutic target and prognostic indicator of numerous diseases. We also discuss the first clinical trial evaluating a GPER-selective drug and the opportunity of repurposing licensed drugs for the targeting of GPER in clinical medicine.

Sections

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Key points

- Oestrogens exert multiple activities in physiology, including reproduction, immunity, cardiovascular and endocrine functions, and ageing, as well as in diseases such as hormone-sensitive cancers, arterial hypertension, atherosclerosis and osteoporosis.
- Oestrogen signalling mediates both acute (non-genomic) and chronic (transcriptional) effects through cytosolic or nuclear oestrogen receptors ERα and ERβ and membrane subpopulations and the G protein-coupled oestrogen receptor (GPER), which is a 7-transmembrane protein.
- Molecules that activate oestrogen receptors include natural oestrogens, phytooestrogens, mycooestrogens and synthetic compounds, such as selective oestrogen receptor modulators and downregulators and xenooestrogens (also known as endocrine disruptors), activate oestrogen receptors and/or GPER.
- Research using *Gper*-deficient animals, GPER-selective agonists and antagonists, and non-selective compounds has revealed multiple roles of GPER in physiology and disease, including as a constitutive activator of the reactive oxygen species-producing enzyme NOX1.
- GPER holds potential to become a diagnostic, prognostic and therapeutic target in clinical medicine, including the repurposing of licensed drugs targeting GPER and the ongoing first-in-human clinical trial of the GPER-selective agonist G-1.

Introduction

Although actions of sex steroid hormones were described more than 2,000 years ago[1], the concept of a 'hormone' was first introduced in 1910 by Starling[2]. It has been a hundred years since the chemical structures of oestrogens (and other steroids) were determined[3,4] (Box 1). Identification and characterization of oestrogen receptors began in the 1950s by Jensen, Szego and others[5–7], leading to the cloning of oestrogen receptor-α (ERα) by Chambon and associates in 1985 (ref. 8) (Box 1). In 1996, Kuiper et al.[9] and Mosselman et al.[10] cloned and identified oestrogen receptor-β (ERβ) contemporaneously with several reports describing the cloning of the orphan G protein-coupled receptor GPR30 (reviewed in refs. 7,11) (Box 1). GPR30 is a protein that predates the evolutionary divergence of fish and tetrapods more than 450 million years ago[12]. The discoveries that oestrogen binds to and activates cell signalling via GPR30 (refs. 13–15), establishing it as a transmembrane oestrogen receptor, resulted in its designation as the G protein-coupled oestrogen receptor (GPER) by the International Union of Basic and Clinical Pharmacology in 2008 (refs. 11,16). Following up on our previous article in *Nature Reviews Endocrinology*[11], we now provide an update on the field of GPER research over the past decade. We will discuss advances made in cell signalling, molecular biology, pharmacology and genetics related to GPER. Special emphasis is given to the roles of GPER in pathophysiology and human disease and as a potential diagnostic, prognostic and therapeutic target in numerous and diverse areas of clinical medicine.

Molecular signalling mediated by GPER

G protein-coupled receptors (GPCRs) are 7-transmembrane spanning proteins that conventionally reside at the plasma membrane and signal to heterotrimeric G proteins, among other proteins, upon binding of ligands to their extracellular surface or within their transmembrane helices. GPER is predominantly expressed on intracellular membranes (the endoplasmic reticulum and Golgi apparatus), with little detected at the plasma membrane in many cell types[14]. While most investigations support this localization, limited expression in the plasma membrane in certain cell types (for example, uterine and renal epithelium), with constitutive internalization, has been reported[17]. Nuclear localization of GPER has also been observed and was suggested to be required for the GPER-mediated induction of transcription and cell migration[18].

GPER signals through multiple G proteins, including $G\alpha_s$[15,19] and $G\alpha_i$[14,20] proteins, as well as via $G_{\beta\gamma}$ signalling[13], and possibly $G\alpha_{q/11}$ protein[21] (Fig. 1). GPER signalling involves, or possibly requires, epidermal growth factor (EGF) receptor transactivation[13], a mechanism that, at the time this study was published in 2000, had only recently been discovered[22]. In addition to adenylyl cyclase[19] and ERK1/2, GPER also activates PI3K–Akt signalling, which has been implicated in tumour cell survival[23], activation of endothelial nitric oxide synthase (NOS3, also known as eNOS), nitric oxide (NO) formation and, thus, in cGMP-dependent vasodilation[24,25] (Fig. 1). GPER also regulates ion channels, including those for calcium[26], sodium[27] and potassium[28], and has been implicated in mTOR signalling and autophagy[29].

Transcriptional regulation is often a consequence of rapid signalling, yielding sustained genomic effects (Fig. 1). Rapid signalling pathways initiated by GPER that lead to transcriptional regulation include adenylyl cyclase-generated cAMP-dependent phosphorylation of CREB[30] and MITF[31] by PKA. GPER inactivates the FOXO3 transcription factor via Akt, promoting cell survival[23]. GPER-mediated ERK1 and ERK2 activation leads to Elk1-mediated transcription, which upregulates *FOS* and subsequently CTGF, FGF2 and CYP1B1 production[32,33]. GPER can either activate or inhibit NF-κB transcriptional activity, depending on the cellular context[34,35]; GPER also γ-secretase-dependent activation of Notch, resulting in expression of HES1 and SNAIL[36]. GPER stimulation can activate YAP and TAZ, two homologous transcription coactivators and key effectors of the Hippo tumour suppressor pathway, via $G\alpha_{q/11}$, PLCβ–PKC, ERK1/2 and the Rho–ROCK signalling pathways[37] (Fig. 1). GPER expression, and therefore function, is also regulated by multiple microRNAs[38]. Finally, basal expression and activity of GPER constitutively regulate expression and activity of the NADPH oxidase NOX1 (ref. 39) (Fig. 1), a reactive oxygen species (ROS)-producing enzyme implicated in many non-communicable diseases[40].

Natural and synthetic ligands of GPER

Oestrogen receptors are activated by a wide range of chemical entities derived from diverse sources, including endogenous oestrogens, phytooestrogens (plant-derived oestrogens), mycooestrogens (fungus-derived oestrogens) and xenooestrogens (synthetic molecules also known as 'endocrine disruptors') (Fig. 2). The identification and characterization of oestrogen receptors facilitated the development of targeted drugs, including selective oestrogen receptor modulators (SERMs) and selective oestrogen receptor downregulators (or degraders) (SERDs), some of which were, in fact, already available in the 1960s[41] (Box 1). In the following section, we will discuss GPER-targeting steroidal ligands, xenooestrogens, plant-derived and fungus-derived molecules, and synthetic receptor-selective ligands and their activities with respect to GPER (Fig. 2).

Steroid hormones

GPER, at the time still known as the orphan receptor GPR30, was first linked to oestrogen-mediated signalling, in 2000, through the activation of ERK via transactivation of the EGF receptor[13] (Box 1).

Box 1

Timeline of key discoveries in oestrogen and oestrogen receptor research

This timeline shows the important milestones in the discovery and study of oestrogen. These include oestrogen chemistry, its receptors, mechanisms of action and pharmacology, with particular emphasis on the recent advances related to the study of GPER functions in health, disease and drug discovery.

1920s

- **1920s:** Isolation and purification of oestrogens[3,4]
- **1928:** Progynon (a 16α-oestriol glucuronide extract) commercially produced and prescribed to treat amenorrhoea[4]
- **1929:** Acute vasodilatation in response to oestrogen of tissue transplanted into the eye[277]

1930s

- **1930:** Ovarian extracts containing oestrogens acutely lower capillary pressure[278]
- **1930:** Emmenin (16α-oestriol glucuronide extract) commercially produced and prescribed as oestrogen replacement[279]
- **1938:** Diethylstilbestrol (DES) discovered[280]
- **1939:** Acute vasodilation by oestrogens shown in humans[281]

1940s

- **1941:** Urine extract from pregnant mares (Premarin) marketed by Pfizer as an oestrogen replacement[282]
- **1941:** FDA approves DES for atrophic vaginitis, menopausal symptoms and lactation suppression[283]

1950s

- **1950s:** Contraceptive pill developed[284]
- **1958–1960:** First non-steroidal anti-oestrogen ethamoxytiphetol discovered[285]
- **1958–1960:** Radioactive tracers concentrate in reproductive tissues; the binding sites are called 'oestrogen receptors'[5]

1960s

- **1960s:** ICI-46,474 (later named Tamoxifen) developed for use as a contraceptive[286]
- **1966–1968:** Oestrogen binding characterized in rat uterus[287,288]
- **1967–1975:** Rapid oestrogen effects on cAMP and intracellular calcium release discovered[289,290]
- **1969:** Purification of an oestrogen receptor from rat uterus; anti-receptor immunoglobulin abolishes 17β-oestradiol binding[6]

1970s

- **1972:** Tamoxifen repurposed for breast cancer treatment[291]
- **1979:** Plasma membrane oestrogen receptors identified[292]

1980s

- **1985–1986:** Cloning of oestrogen receptor-α (ERα)[8]

1990s

- **1996:** Cloning of oestrogen receptor-β (ERβ)[9,10]
- **1996–1998:** Cloning of GPR30 (refs. 293–299)

2000s

- **2000–2002:** The role of GPR30 in mediating rapid 17β-oestradiol signalling discovered[13,19]
- **2005:** 17β-Oestradiol binding to GPR30 demonstrated[14,15]
- **2006–2009:** GPR30 activation dilates human arteries and lowers blood pressure; 17β-oestradiol regulates human arterial GPR30 expression; GPR30 expression prevents obesity[91,121]
- **2006–2011:** First GPR30-selective agonist (G-1) and antagonists (G15 and G36) developed[67–69]
- **2008:** International Union of Basic and Clinical Pharmacology designates GPR30 as G protein-coupled oestrogen receptor (GPER)[16]
- **2009:** Role of GPER and efficacy of G-1 treatment in multiple sclerosis shown[119,249]

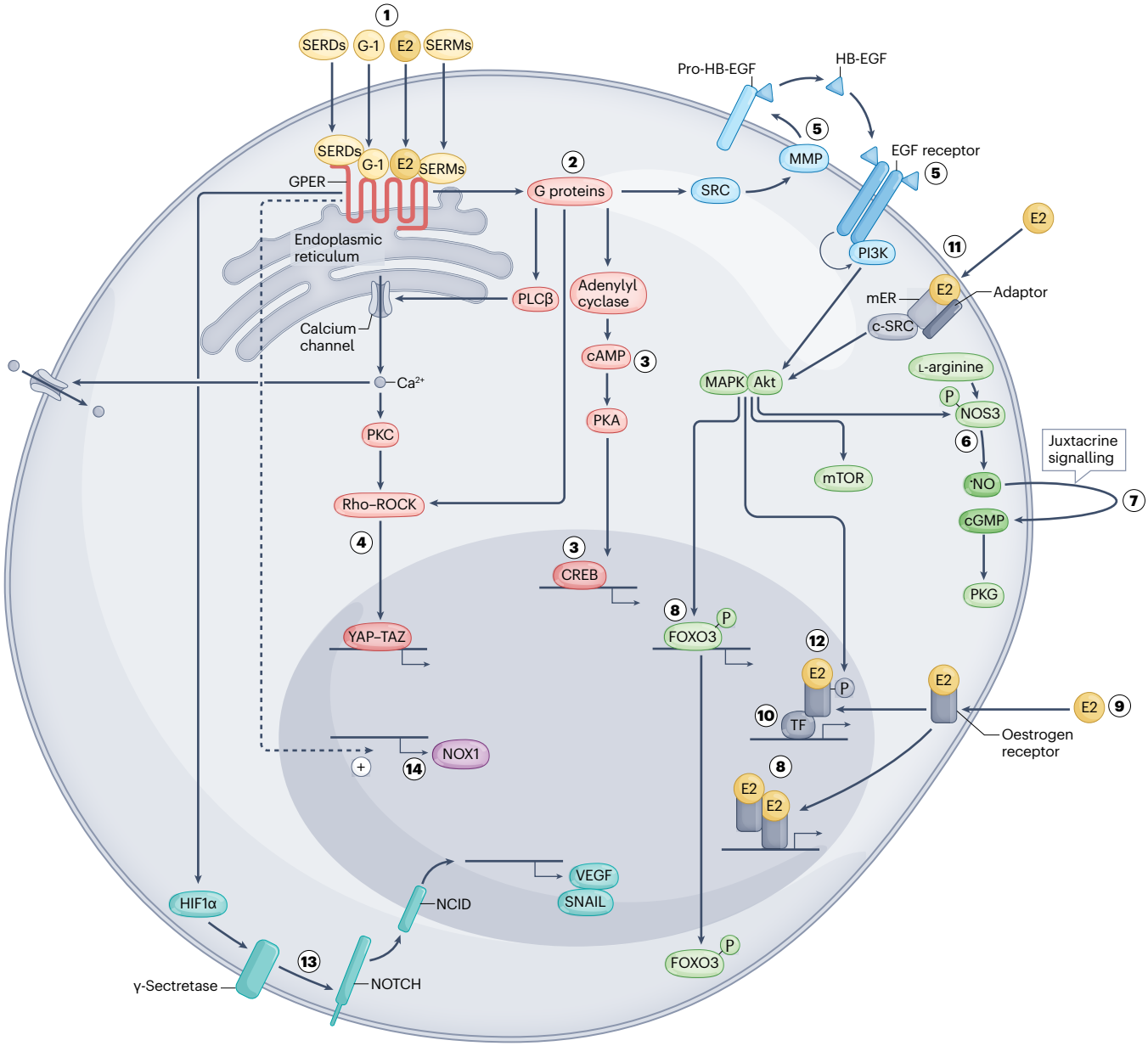
2010s

- **2010:** Protective effects of GPER in myocardial reperfusion injury shown[127]
- **2011:** GPER mediates 17β-oestradiol-stimulated pancreatic β-cell insulin secretion[26]
- **2016:** GPER regulates NOX1; G36 identified as NOX1 downregulator[39,157]
- **2016:** Roles of GPER in melanin production and therapeutic effects of G-1 in malignant melanoma shown[207,228]
- **2019:** Phase I clinical trial of G-1 (LNS8801) for cancer[78–80]
- **2019:** First ERα-selective and ERβ-selective agonist AB-1 developed[77]

2020s

- **2020:** Efficacy of G-1 in obesity and diabetes mellitus treatment shown[168]

High-affinity, competitive binding of 17β-oestradiol to GPER was first demonstrated in 2005 (refs. 14,15). In contrast to 17β-oestradiol, oestrogens, such as oestrone and oestriol, exhibit poor binding to GPER[15]. GPER shows no binding to other steroids, such as testosterone, progesterone, aldosterone and cortisol[15,42–44], although aldosterone has been shown to be involved in crosstalk between the mineralocorticoid receptor and GPER and between the EGF receptor and GPER[43]. The catecholoestrogen 2-methoxy-oestradiol[45] and the glucuronic acid metabolite 17β-oestradiol-17-D-glucuronide[46] act as GPER agonists, whereas another catecholoestrogen, 2-hydroxy-oestradiol, is reported to act as an antagonist[47]. Dehydroepiandrosterone shows agonistic behaviour towards GPER[48,49], whereas its metabolite 7β-hydroxy-epiandrosterone antagonizes GPER-mediated oestrogenic responses[50]. Most recently, 27-hydroxycholesterol, a cholesterol metabolite



implicated in oestrogen receptor-negative breast cancer, was reported to bind and activate GPER, although with relatively low affinity compared with its most important physiological ligand, 17β-oestradiol[51].

Xenooestrogens and natural oestrogenic molecules

Xenooestrogens are a large family of chemically stable synthetic molecules with oestrogenic activities often referred to as environmental oestrogens or endocrine-disrupting chemicals (EDCs). They are found in a wide range of consumer products and plastics, and most of them are toxic[52]. Endocrine-disrupting chemicals can be found in detergents, surfactants, resins, lubricants, plasticizers, fire retardants and pesticides[52]. Xenooestrogens that bind and/or regulate the activity of GPER (typically acting as agonists) include bisphenol A (BPA), polychlorinated biphenyls (PCBs), diethylstilbestrol (DES), nonylphenol, dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyltrichloroethylene isomers, kepone, methoxychlor and atrazine (Fig. 2).

Many molecules present in soy or green tea plants also target oestrogen receptors. Such naturally occurring phytooestrogens include flavonoids, isoflavonoids, chalcones, coumestans, stilbenes, lignans, ginsenosides and tetrahydrofurandiols[53]. Phytooestrogens that bind and/or activate GPER include genistein[54], daidzein[55], equol[56], quercetin[57], resveratrol[58], oleuropein[59], icariin[60] and the green tea polyphenol (-)-epicatechin[61]. The mycooestrogen zearalenone also shows agonism towards GPER[54,62].

Discovery of GPER-selective ligands

Owing to the highly conserved nature of the binding sites in ERα and ERβ, the typical affinity difference for oestrogen receptor subtype-specific

Fig. 1 | Cellular signalling pathways activated by ERα, ERβ and GPER. Non-genomic and genomic signalling pathways are activated by oestrogen and oestrogenic ligands (in yellow) through binding to the three known oestrogen receptors, oestrogen receptor-α (ERα), oestrogen receptor-β (ERβ) and the G protein-coupled oestrogen receptor (GPER). 17β-Oestradiol (E2), selective agonists such as G-1, or selective oestrogen receptor modulators (SERMs) and selective oestrogen receptor downregulators and/or degraders (SERDs) activate GPER (1), which is localized predominantly intracellularly at the endoplasmic reticulum. GPER activates several heterotrimeric G proteins (2), leading to multiple downstream cascades, including cAMP production (3) and activation of PKA (3) and CREB (3). G protein activation also leads to calcium (Ca^{2+}) mobilization from intracellular stores, which activates PKC and leads to activation of plasma membrane calcium channels. GPER activation can also lead to regulation of gene expression via activation of the YAP–TAZ transcription factors via Rho–ROCK signalling (4). Activation of SRC via G proteins can also lead to activation of matrix metalloproteinases (MMPs) (5) that cleave pro-heparin-binding epidermal growth factor (HB-EGF) (5), releasing free HB-EGF. HB-EGF then transactivates the EGF receptor (5), which in turn activates MAPK (ERK1/2), Akt and other pathways. These induce additional, rapid (non-genomic) effects such as activation of the L-arginine–endothelial nitric oxide synthase (NOS3)–NO–cGMP pathway (in combination with mobilization of calcium stores). Akt causes phosphorylation of endothelial NOS3 (6), which releases nitric oxide (NO) and leads to juxtacrine signalling from endothelial to vascular smooth muscle cells (7), and activation of PKG. Activation of MAPK and Akt signalling also causes genomic effects regulating gene transcription such as FOXO3 phosphorylation and degradation (8). In the classic, genomic oestrogen receptor pathway, 17β-oestradiol binds cytosolic and nuclear oestrogen receptors (9), inducing receptor dimerization and binding to the promoters of target genes. Alternatively, activated oestrogen receptors modulate the function of other classes of transcription factors (TF) through protein–protein interactions (10). Subpopulations of membrane-bound oestrogen receptors (mER) are present at the plasma membrane (11). Once activated, these oestrogen receptors interact with adaptor proteins (adaptor) and signalling molecules, such as SRC, which mediate rapid signalling events (for example, PI3K–Akt and MAPK signalling) (11). Oestrogen receptor ERα, potentially following transactivation of EGFR by GPER, is regulated by phosphorylation through kinases (such as MAPK and Akt), resulting in the regulation of gene expression (12). HIF1α, following GPER activation, induces γ-secretase-dependent activation of NOTCH (13) and VEGF signalling (13). Basal expression and/or activity of GPER constitutively induces expression of the NADPH oxidase NOX1 (14).

compounds ranges from ~30-fold to 300-fold[63]. Oestrogen receptor subtype-biased ligands, such as propylpyrazoletriol (PPT, an ERα-selective agonist) and diarylpropionitrile (DPN, an ERβ-selective agonist) (Fig. 2), have been developed and are widely used[64,65]. PPT, however, also acts as a GPER agonist[66], complicating the interpretation of its use.

The discovery and development of highly GPER-selective ligands were essential to facilitating research into the physiology and pathophysiology related to this receptor. In 2006, compound library screening led to the identification of G-1 (1-(4-(6-bromobenzo[1,3]dioxol-5-yl)-3a,4,5,9b-tetrahydro-3*H*-cyclopenta[c]quinolin-8-yl)-ethanone), a small molecule that acts as a selective agonist of GPER[67] (Fig. 2). The discovery of GPER-selective antagonists G15 and G36 complemented the use of G-1 as an agonist in understanding the roles of GPER in cell biology and physiology[68,69]. Some reports suggest that the activity of these compounds can vary depending on the system employed[70,71]. Other reported GPER-selective ligands include the agonists GPERL1 and GPERL2 (ref. 72), a series of indole-thiazole derivates that act as GPER agonists[73], the antagonist CIMBA (an acyclic analogue of G36)[74], as well as the pan-oestrogen receptor and GPER antagonist MIBE[75] (Fig. 2). Proteolysis-targeting chimaeras (PROTACs), which are molecules that induce degradation of specific proteins (via selective recruitment of E3 ubiquitin ligases and target ubiquitination followed by degradation in proteosomes), were developed to target ERα as early as 2005 (ref. 76), with a pair of 17β-oestradiol–proteolysis-targeting chimaeras shown to degrade GPER in addition to ERα in a study published in 2021 (ref. 44). The 2019 discovery of AB-1, an agonist of ERα and ERβ that lacks affinity for GPER, should allow further dissection of the functions of ERα and/or ERβ compared with GPER in cells that express multiple oestrogen receptors[77]. Of these GPER-targeting ligands, only G-1 has so far entered clinical trials, specifically for use in combination therapy with immune checkpoint inhibitors (ICIs) in cancer. G-1 exhibits a favourable safety profile in these trials, either alone or in combination with pembrolizumab, with encouraging initial antitumour activity observed to date (NCT04130516)[78–80].

Roles of GPER in physiology and disease

In the following sections, we will review advances in understanding the functions of GPER in cardiovascular and kidney disease, endocrinology and metabolism, gastrointestinal and liver diseases, immunity and immunology, neurology, and the physiological ageing process. Findings are frequently based on effects due to phenotypes of *Gper*-deficient mice or the effects of GPER-selective ligands (Fig. 3). Reported phenotypes of multiple differently derived *Gper*-deficient mice are not entirely consistent, probably due to differences in genetic background and other factors, including age[81]. The available evidence points to multiple roles of GPER in oestrogen-dependent and oestrogen-independent functions and pathologies, allowing the development of possible diagnostic and therapeutic approaches with regard to GPER.

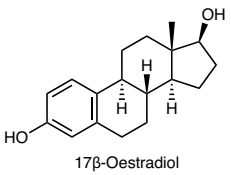
Clinical genetics

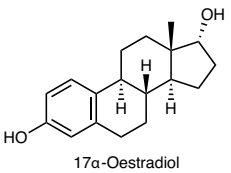
Sex chromosomes, sex steroids and sex steroid receptors contribute to and determine disease risk and efficacy of pharmacological therapy[82,83]. In humans, the *GPER* gene maps to chromosome 7p22.3, a region associated with arterial hypertension in genetic linkage studies[84]. The *GPER* single-nucleotide polymorphism rs11544331, which results in a Pro16Leu alteration in the receptor (amino acid substitution of proline 16 to leucine), produces a hypofunctional variant of GPER. The Leu variant is associated with slightly higher blood pressure than the Pro variant in women but not in men, and its allele frequency is two-fold higher in women with hypertension compared with age-matched men[85]. The inhibitory effect of GPER on pro-inflammatory gene expression in induced pluripotent stem cell-derived endothelial cells is reduced in the GPER Leu variant compared with the Pro variant[86]. Moreover, GPER activation induces LDL receptor expression, in part by downregulating proprotein convertase subtilisin–kexin type 9 (PCSK9) resulting in increased plasma levels of LDL cholesterol in Pro16Leu variant carriers[87]. Finally, expression of the Pro16Leu variant of GPER in cancer-associated fibroblasts increases secretion of paracrine factors promoting migration of breast cancer cells[88]. Together, these genetic observations support potentially important roles for GPER for human diseases.

Cardiovascular and kidney diseases

Endogenous oestrogens in premenopausal women protect against cardiovascular diseases in general, and particularly against arterial hypertension, coronary heart disease (including myocardial infarction) and heart failure[11,89,90]. GPER is widely expressed in the cardiovascular system in mammals, including the arterial wall and the heart[11]. In the cardiovascular system, physiological functions of GPER include

Steroids


17β-Oestradiol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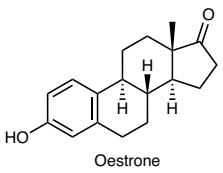
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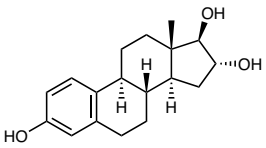
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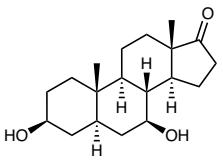
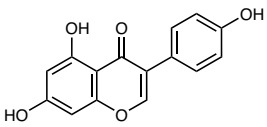

7β-Hydroxy-epiandrosterone

Phytooestrogens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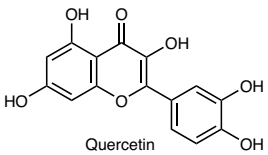
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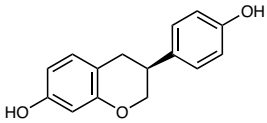
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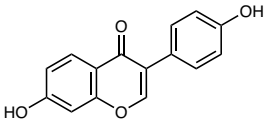
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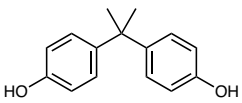
Synthetic xenooestrogens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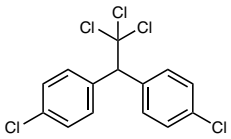
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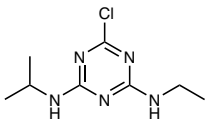
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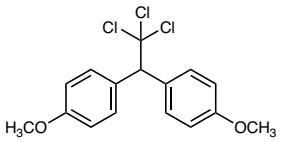
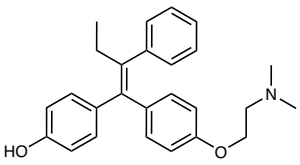
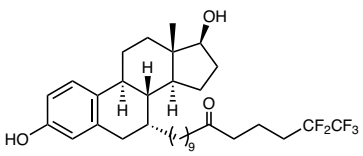
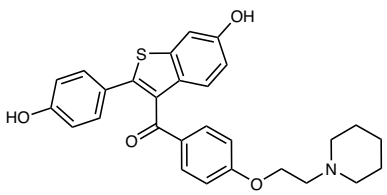

Methoxychlor

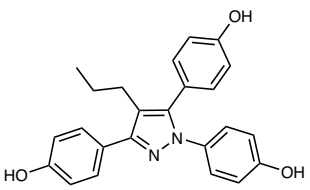
Therapeutic agents


4-Hydroxytamoxifen


Fulvestrant


Raloxifene

Oestrogen receptor-targeted and/or GPER-targeted compounds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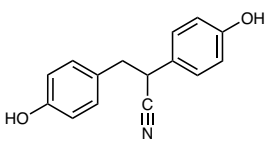
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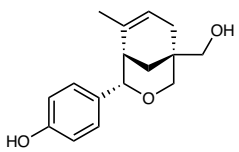
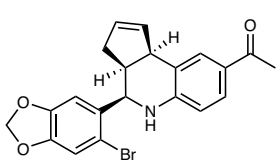
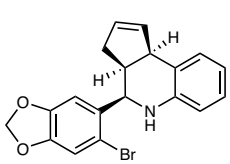

AB-1


G-1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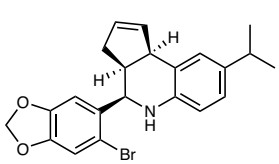
G36

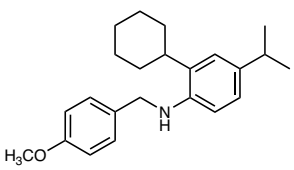
CIMBA

Fig. 2 | Chemical structures of compounds that act as ligands for ERα, ERβ and/or GPER. Shown are examples of natural steroids, phytooestrogens, xenooestrogens/endocrine disrupting chemicals (EDCs), therapeutic agents and experimental compounds that display varying activities towards oestrogen receptor-α (ERα), oestrogen receptor-β (ERβ) and the G protein-coupled oestrogen receptor (GPER) but are generally non-selective. Also shown are synthetic experimental compounds that exhibit selectivity for ERα and/or ERβ, such as propylpyrazoletriol (PPT), diarylpropionitrile (DPN) and AB-1, or for GPER, such as G-1, G15, G36 and CIMBA. p,p′-DDT, p,p′-dichlorodiphenyltrichloroethane.

the regulation of arterial blood pressure, angiogenesis, myocardial contractility and suppression of inflammation[11]. Activation of GPER results in acute vasodilatation of human, pig, rat and mouse arteries[91–93]. The underlying mechanisms include direct effects on vascular smooth muscle[91,92,94] and activation of the endothelial L-arginine–NOS3–NO–cGMP pathway[24,95,96] (Fig. 1). GPER-mediated vasodilatation also involves cAMP-dependent[97] and Rho kinase-dependent mechanisms[98] as well as inhibiting contractile factors such as endothelial vasoconstrictor prostanoids[99] and endothelin-1 (refs. 92,100). GPER-dependent vasodilation is augmented during pregnancy[101] and is reduced by ageing[39,102,103]. Systemic deletion of *Gper* prevents age-dependent, endothelium-dependent dysfunction, probably due to a reduction in NOX1 abundance[39,103]; the main effects are summarized in Fig. 3.

Blood pressure and arterial hypertension. Endothelium-derived contracting factors, such as cyclooxygenase-derived vasoconstrictor prostanoids and endothelin 1, are involved in the pathogenesis of arterial hypertension[89]; their activity is suppressed by constitutive GPER activity and augmented by systemic deletion of *Gper*[99]. Similarly, acute (seconds to minutes)[91] and chronic treatment (hours to days) with the GPER agonist G-1, via its nitric oxide (NO)-liberating and antioxidant effects[24,95], induces vasodilation and lowers blood pressure. Interestingly, deletion of *Gper* prevents angiotensin II-induced elevations of blood pressure, which are also markedly lowered by the GPER antagonist and NOX1 downregulator G36 (refs. 39,40). These data suggest that either agonist-dependent activation (through increased NO bioactivity) or chronic antagonism of GPER (via NOX1 downregulation) could be suitable for the treatment of different forms of arterial hypertension and related diseases such as atherosclerosis, stroke and chronic kidney disease (CKD).

The GPER agonist G-1 prevents hypertension in intrauterine growth-restricted female rat offspring later in life, suggesting a potential role in embryonic priming of adult hypertension[104]. Arterial blood pressure in *Gper*-deficient animals is normal[91,105] or slightly reduced compared with animals expressing GPER[106]. Crosstalk between GPER and endothelin receptors has been described, resulting in natriuretic effects[107]. Aldosterone, which also has natriuretic effects, has been implicated in the actions of GPER, yet there is no evidence of aldosterone binding to GPER[42–44,108,109]. Consistent with this, deletion of *Gper* has no effect on the hypertensive effects induced by aldosterone[110]; however, GPER does regulate autocrine aldosterone synthesis in the renal medulla[111]. In addition, crosstalk between the mineralocorticoid receptor and GPER has been reported[43]. Correspondingly, mineralocorticoid receptor antagonists downregulate the expression of GPER[112]. Moreover, aldosterone triggers both direct interactions between the mineralocorticoid receptor and GPER involving the EGF receptor, which is abrogated by *GPER* gene silencing in endothelial and SkBr3 breast cancer cells in vitro[43]. Such interactions between the mineralocorticoid receptor and GPER might also contribute to aldosterone-mediated regulation of the sodium–chloride cotransporter, which is reduced in male mice lacking *Gper*[113].

Atherosclerosis and coronary artery disease. Atherosclerosis is a chronic systemic inflammatory vascular disease[89] and the underlying cause of coronary artery disease (CAD), peripheral artery disease and stroke. The main complications of CAD are myocardial infarction, fatal ventricular arrhythmias following reperfusion injury after infarction, and heart failure[89]. Natural or surgical menopause accelerates CAD progression and can be alleviated by oestrogen therapy, which activates all three oestrogen receptors[89]. In mice of both sexes fed either a regular diet or a high-calorie diet rich in fat and sugars, deletion of *Gper* results in moderate dyslipidaemia[114,115]. In endothelial cells, oestrogen-mediated activation of GPER attenuates transcytosis of LDL cholesterol into endothelial cells, compatible with an indirect vasculoprotective effect[116]. G-1 also reduces cardiac lipid accumulation and PPARα expression in surgically postmenopausal rats with type 2 diabetes mellitus (T2DM)[117]. In human monocytes, which contribute to the earliest stages of atherogenesis[118], the anti-inflammatory effects of oestrogen might involve both direct effects via GPER[119] as well as crosstalk between ERα and GPER[120].

In the arteries of patients with coronary artery disease, GPER expression is sensitive to 17β-oestradiol regulation[121]. Activation of GPER by G-1 or green tea polyphenols inhibits the growth of coronary vascular smooth muscle cells[61,91,118,122–124], a crucial step during atherogenesis. Deletion of *Gper* increases both perivascular adipose tissue growth and the production of cyclooxygenase-dependent adipose-derived contracting factor (ADCF), suggesting that endogenous GPER activity negatively regulates these processes[125]. In ovariectomized, that is, surgically postmenopausal, *ApoE*-deficient mice or in surgically postmenopausal C57BL/6J mice fed a cholate-containing atherogenic diet, G-1 reduces inflammation and atherosclerosis[126]. G-1 also reduces steady-state mRNA levels of the angiotensin AT_1 receptor in *ApoE*-deficient mice[123], a receptor protein that mediates angiotensin II-dependent vasoconstriction, vascular cell growth, inflammation and oxidative stress.

Myocardial disease and heart failure. GPER activation attenuates reperfusion injury following myocardial infarction through pathways involving GSK3β, mitophagy and mechanisms regulating mitochondrial permeability[127–129]. Arterial hypertension, T2DM and the resulting coronary artery disease and loss of myocardial tissue from myocardial infarction are the most frequent causes of heart failure. While heart failure with reduced ejection fraction (HFrEF) is often due to the loss of contractile tissue following myocardial infarction, heart failure with preserved ejection fraction (HFpEF) is a consequence of diabetes mellitus, arterial hypertension and ageing, all resulting in myocardial fibrosis and stiffening[89,90]. Patients with HFpEF are primarily perimenopausal or early postmenopausal women, suggesting that the cessation of endogenous oestrogen production contributes to the pathogenesis of HFpEF.

In experimental models of HFrEF, oestrogen therapy can reverse heart failure-induced myocardial fibrosis[130]. ERα and ERβ, as well as GPER, are all involved in the inhibitory effects of oestrogen on cardiomyocyte proliferation[131,132]. Interestingly, SERMs and SERDs, which are also GPER agonists, also inhibit cardiomyocyte proliferation[133]. Hypoxia and/or hypoxaemia, which occur during myocardial ischaemia and heart failure, upregulate GPER[134]. GPER controls myocardial contractility involving crosstalk between GPER and β1 adrenoceptors[135].

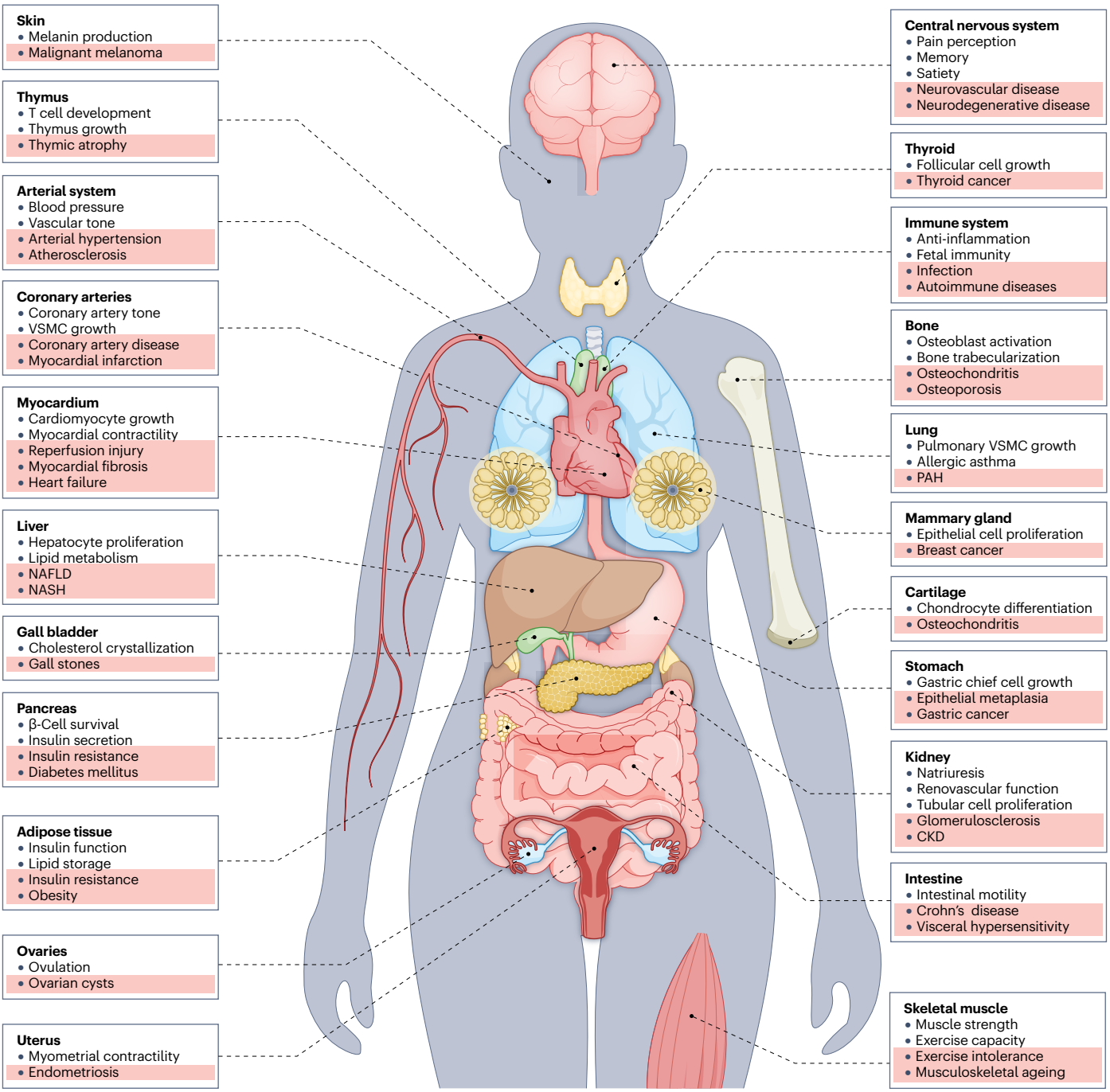

Fig. 3 | GPER in health and disease. The G protein-coupled oestrogen receptor (GPER) regulates many physiological functions (white background) and is involved in multiple pathologies and diseases (pink background) CKD, chronic kidney disease; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PAH, pulmonary arterial hypertension; VSMC, vascular smooth muscle cell.

In a model of ageing-associated HFpEF, systemic deletion of *Gper* in male mice prevents the development of heart failure and myocardial fibrosis, an effect that is related to downregulation of NOX1 protein expression and associated reduction of NOX1 function[39]. In vitro studies using *Nox1*-knock-in experiments in aortic vascular smooth muscle cells from *Gper*-deficient mice further underscored that constitutive NOX1 expression and activity require GPER expression, which, probably through ligand-independent or basal activity, enables ROS formation, inflammation and myocardial fibrosis[39]. By contrast, in young female mice, cardiomyocyte-specific deletion of *Gper* worsens cardiomyocyte

function compared with wild-type mice both in vitro and in vivo, which can be partly rescued by inhibiting cardiac NLRP3 inflammatory pathways[136]. G-1 reduces diastolic dysfunction in experimental HFpEF[137] and in rats with hypertensive cardiomyopathy[137,138]; G-1 treatment also improves cardiac function and reduces cardiac fibrosis in surgically postmenopausal rats[139]. Taken together, either reducing constitutive NOX1-dependent production of ROS by blocking GPER or increasing NO bioactivity by activating GPER, holds potential for pharmacological intervention in heart failure, possibly in a sex-dependent manner.

Renal physiology and disease. Loss of functional kidney tissue, particularly due to CKD, facilitates the development of arterial hypertension and cardiovascular disease. Similar to cardiovascular diseases, CKD displays sex differences with premenopausal women being largely protected from CKD development compared with age-matched men, implicating a role for oestrogens and oestrogen receptors[140]. GPER regulates renal artery and intrarenal vascular tone[103,141], and its activation increases Ca^{2+} flux and H^+-ATPase activity in renal tubular cells[142]; GPER also regulates natriuresis[107] via crosstalk with endothelin ET_A and ET_B receptors[143]. Deletion of *Gper* counteracts the development of focal segmental glomerulosclerosis (FSGS) and the resulting proteinuria[144] and tubulo-interstitial injury caused by inflammation and oxidative stress, by reducing NOX1 upregulation[145]. Activation of GPER also reduces glomerular mesangial cell proliferation induced by hyperglycaemia in vitro (which is associated with oxidative stress)[60], and *Gper* silencing in these cells markedly reduces NOX1 abundance[144]. The GPER antagonist and NOX1 downregulator G36 reduces mRNA expression of podocyte injury markers *NPHS1* (coding for nephrin), *COL4A1* (collagen IV) and *WT1* (Wilms-tumour 1) in human podocytes in vitro[144]. Protective effects of GPER signalling on podocytes have also been demonstrated for treatment with GPER agonists, probably via activation of the L-arginine–NOS–nitric oxide pathway[146]. In a model of hypertensive nephropathy, GPER activation reduces proteinuria as well as tubular injury but not glomerular injury via pressure-independent mechanisms[147,148]. Possibly, the stimulating effect of G-1 on tubular epithelial cell proliferation could contribute to this effect[149]. Protective effects of GPER signalling have also been reported for methotrexate-induced human renal epithelial cell injury in vitro[150] and for acute renal endothelial cell injury following renal ischaemia in female mice[151].

Pulmonary diseases

Pulmonary arterial hypertension (PAH) is a chronic fibroproliferative disorder of the pulmonary vasculature, ultimately leading to right-heart failure. Four out of five patients are women, suggesting a role for sex chromosomes, sex steroids, or sex steroid receptors. In experimental rat models of PAH, ovariectomy increases mortality[152], while 17β-oestradiol (a non-selective oestrogen receptor and GPER agonist)[153] or the GPER agonists G-1 (ref. 154) or 2-ME[152] partially reduce or even reverse established cardiopulmonary injury. G-1 also improves skeletal muscle function and exercise intolerance in rats with PAH, possibly through normalization of SERCA2a and phospholamban expression[154,155]. Finally, in experimental hypoxia-induced PAH in rats, blocking GPER using G36 improves cardiac function by lowering right ventricular pressure, probably involving the downregulation of NOX1 (refs. 156,157). Thus, both agonists and antagonists of GPER might aid in the treatment of PAH.

Endocrinology and metabolism

Metabolic homeostasis is differentially regulated in men and women, with the metabolic actions of oestrogens mediated through both ERα[158–160] and GPER; discussed later in this section. Premenopausal women exhibit lower incidences of obesity and T2DM compared with age-matched men; these protective effects are lost following menopause, with similar effects seen in rodents. Oestrogen therapy can alleviate weight gain and its associated adverse metabolic effects present in postmenopausal women and in surgically postmenopausal mice[161–163].

Obesity and diabetes mellitus. Since the first reports demonstrating roles of endogenous GPER in the regulation of body weight, adipose tissue growth, obesity and insulin function in 2009 (refs. 91,164), studies in mice lacking *Gper* have found that these mice develop dyslipidaemia and show reduced energy expenditure compared with wild-type mice. These effects are probably responsible for the observed increases in visceral and subcutaneous adipose tissue depots, given that food intake and locomotor activity remain unaffected in *Gper*-deficient mice[114,115,165]. Compared with males, female ovary-intact *Gper*-deficient mice exhibit a lower sensitivity to acute leptin-stimulated food intake and short-term cholecystokinin-stimulated satiety signals[165]. The expression of thermogenic genes, such as those encoding uncoupling protein 1 (*Ucp1*) and the β_3-adrenergic receptor, is reduced in brown adipose tissue of *Gper*-deficient mice consistent with the decreased energy expenditure.

17β-Oestradiol treatment protects β-cells from apoptosis and prevents diabetes mellitus in mice[166]. The severity of diabetes mellitus in mice lacking both ERα and ERβ worsens following surgical menopause[167]. 17β-Oestradiol supplementation improves glucose homeostasis in these mice, suggesting alternative mechanisms of oestrogen action other than signalling through ERα or ERβ, for example, through GPER[167]. Indeed, in mice lacking *Gper*, plasma levels of glucose are increased and these animals exhibit glucose intolerance, defective glucose-stimulated and oestrogen-stimulated insulin secretion, and insulin resistance[114,164,165]. Insulin secretion in response to both 17β-oestradiol and G-1 in healthy islets is reduced by pharmacological GPER inhibition and is absent in mouse islets lacking *Gper*[26]. In a mouse model of streptozotocin-induced diabetes mellitus, deletion of *Gper* results in greater loss of pancreatic β-cells, reduced pancreatic insulin content and, consequently, abnormally increased plasma levels of glucose compared with wild-type mice[167].

GPER as a therapeutic target in obesity and diabetes mellitus. Therapeutic targeting of GPER in glucose homeostasis and lipid metabolism has been studied in models of Western diet-induced obesity in male mice and in models of surgical menopause in female mice, both of which result in obesity and metabolic dysfunction. G-1 treatment over a period of 6–8 weeks reduced overall body weight, adiposity and circulating levels of lipids compared with vehicle-treated mice, without affecting lean mass or bone density, via increased basal energy expenditure[168]. No changes in either daily food consumption or locomotion were observed in this study although, in surgically postmenopausal obese rats, G-1 treatment acutely and transiently decreased food intake[169]. G-1 treatment in surgically postmenopausal mice increased the expression of genes involved in mitochondrial biogenesis and fatty acid oxidation in brown and white adipose tissue and in skeletal muscle, while reducing the expression of genes involved in inflammation, hypoxia and angiogenesis[168].

In line with previous results[69,126], G-1 treatment of surgically postmenopausal obese mice was devoid of the feminizing effects of 17β-oestradiol as indicated by the absence of uterine imbibition[168]. In addition to weight loss and improved lipid profiles, G-1 also improved glucose homeostasis at the level of glucose and insulin tolerance tests,

and reduced fasting blood levels of glucose and insulin[168]. In postmenopausal rats with streptozotocin-induced diabetes G-1 treatment reduced disease-induced weight loss to a comparable degree as did 17β-oestradiol treatment, and similarly improved glucose homeostasis and lipid profiles compared with vehicle-treated diabetic rats[170]. While surgically postmenopausal obese mice show improved glucose homeostasis in response to acute or chronic 17β-oestradiol treatment, deletion of *Gper* abrogates this response, indicating a key role of GPER in 17β-oestradiol-mediated glucose homeostasis in vivo[164,165]. Moreover, G-1 amplifies glucose-stimulated insulin secretion ex vivo in pancreatic islets obtained from patients with T2DM, while also suppressing glucagon and somatostatin secretion[171,172]. Thus, selective GPER agonists hold potential for the treatment of obesity and associated diseases such as diabetes mellitus.

Gastrointestinal and liver diseases

Oestrogens modulate multiple gastrointestinal and hepatic functions via their receptors[173], including via GPER[173]. GPER is a cell-specific marker of gastric epithelium chief cells[174] and also controls lower oesophageal sphincter tone[175], colonic motility and severity of visceral pain[176,177]. In human Crohn's disease[178], ulcerative colitis[179] and irritable bowel syndrome (IBS)[180–182], the majority of studies found intestinal GPER expression to be increased compared with healthy individuals. GPER activation reduces inflammation, tissue injury and mortality in a mouse model of Crohn's disease[178] and G-1 reduces colonic crypt cell injury related to reperfusion injury following intestinal ischaemia[183]. Finally, intestinal inflammation in a mouse model of acute colitis induced by dextran sulfate sodium is reduced by GPER activation, improving intestinal mucosal barrier function[184].

GPER regulates liver in zebrafish[185] and contributes to oestrogen-dependent proliferation and lipid metabolism in human hepatocytes[185,186]. In addition, both GPER or ERα protect hepatocytes from fatty degeneration, a predisposing factor propagating non-alcoholic fatty liver disease and steatohepatitis[187].

Obesity in premenopausal women is associated with an increased risk of developing gallstones, which are formed via GPER-dependent mechanisms[188]. Oestrogen-dependent cholesterol crystallization pathways differ markedly between those involving ERα or GPER[189], yet deletion of *Gper*[190] or its pharmacological inhibition[74] completely prevents gallstone formation in female mice.

Cancer biology and oncology

GPER is expressed in tumours and tumour cells of cancer patients, including the mammary gland[191–195], endometrium[66,196], ovaries[197], prostate[198], pancreas[199], thyroid[200], colon[201] and lung[202]. Increased GPER expression correlates with a worse outcome in breast[191–193], endometrial[196] and ovarian[197] cancer. Although pharmacological activation of GPER can increase proliferation and associated signalling in breast[203], endometrial[204], thyroid[200] and ovarian[205] cancer cells, inhibition of proliferation due to GPER signalling has also been reported in breast[206], pancreatic[199] and melanoma[207] cancer cells. With these — sometimes — opposing results in different cell lines, the role of GPER in cancer in vivo appears to be more complex than anticipated. Indeed, in certain forms of cancer, endogenous GPER activity might be protective, possibly through anti-inflammatory effects[208].

Breast cancer. Much has been published regarding GPER and breast cancer due to obvious questions arising from the well-documented importance of presence or absence of ER for the efficacy of anti-oestrogen therapies in cancer treatment[209]. The fact that SERMs, such as tamoxifen[14] and raloxifene[66], as well as SERDs, such as fulvestrant[13], act as GPER agonists to activate growth and survival pathways has led to the suggestion that GPER expression and/or activity could contribute to breast cancer recurrence[194]. This complex pharmacology has also led to a search for ERα-selective compounds that do not cross-react with GPER[77].

Supporting roles for GPER in breast cancer recurrence and metastasis, GPER expression is elevated in metastases of patients with breast cancer compared with matched primary tumours[210,211]. However, this elevated GPER expression, where assessed, is only observed in women originally treated with tamoxifen[211]. Aromatase inhibitors are more effective than tamoxifen at inhibiting tumour growth in primary breast tumours that are both ERα-positive and GPER-positive, with this difference in treatment efficacy being absent in primary ERα-positive and GPER-negative breast tumours[192]. Moreover, aromatase inhibition resulted in better disease-free progression for patients with breast cancer compared with a tamoxifen-based therapy, consistent with a role for GPER in recurrence and metastasis[193]. Using a genetic mouse model of mammary gland tumorigenesis, systemic *Gper* deficiency resulted in reduced tumour size and metastasis compared with wild-type mice, consistent with a pro-tumorigenic role for GPER in vivo[212].

In vitro, tamoxifen induces proliferation of tamoxifen-resistant MCF-7 cells through a GPER-dependent pathway[210,213]. This proliferation can be blocked by *GPER* knockdown or co-treatment with the GPER-selective antagonist G15 (refs. 69,210) as tamoxifen binds to and cross-activates GPER[15,66,214]. Breast cancer cell survival in the presence of tamoxifen might be mediated by Akt-induced inactivation of the pro-apoptotic transcription factor FOXO3, suggesting a mechanism to enhance eventual tamoxifen resistance[23]. Tamoxifen-mediated cross-activation of GPER also induces breast cancer cell migration[215], potentially via the YAP–TAZ pathway[37] (Fig. 1), and increases aromatase expression in tamoxifen-resistant (ERα-positive) cells[216]. In vivo, GPER also contributes to tamoxifen resistance in MCF-7 cells, with tamoxifen-resistant xenografts derived from MCF-7 cells regaining sensitivity to tamoxifen in female mice upon treatment with a combination of tamoxifen and G15, where neither alone had an effect[210]. GPER downregulation and G15 treatment also sensitize breast cancer cells to doxorubicin by inhibiting epithelial-to-mesenchymal transition[217]. Lastly, G-1 (as well as tamoxifen and fulvestrant) increases natural killer cell-mediated growth inhibition of both ERα-negative and ERα-positive breast cancer cells, suggesting a novel role for GPER in cancer therapy[218].

Cancer-associated fibroblasts (CAFs) express GPER, with most studies to date employing breast CAFs, which have previously described roles supporting breast tumour progression[18,219,220]. In breast CAFs, GPER mediates expression of HIF1α and VEGF[195] and has been implicated in promoting tumour progression by increasing migration and invasion[221–223]. Tamoxifen and G-1 induce increased aromatase expression in breast CAFs, resulting in increased oestrogen production[219], potentially leading to tamoxifen resistance[216].

The tumour microenvironment also contains adipocytes, particularly in adipose-rich tissues such as the breast. Obesity has been clinically established as an important contributor to multiple cancers[224]. Adipocytes not only express aromatase, resulting in intracrine oestrogen synthesis, but also adipokines and other (pro-inflammatory) cytokines and hormones that can promote tumorigenesis. The actions of GPER in reducing obesity and mitigating metabolic dysfunction[168], inflammation[194] and chemotherapy-associated cardiotoxicity[225] could,

in part, reduce the incidence of and improve outcomes in breast cancer and other cancers.

Malignant melanoma. Female patients with malignant melanoma have a better clinical outcome than male patients[226], although ICIs, an effective treatment for melanoma, show better therapeutic efficacy in men than in women[227]. A role for GPER activity in melanoma was first suggested by the observation that GPER (but not ERα) mediates oestrogen-induced melanogenesis (melanocyte differentiation and melanin production)[31,228]. Treatment of mouse melanoma cells with G-1 or tamoxifen, interestingly, inhibits proliferation in vitro[229]. Combining ICIs (specifically an anti-PD1 antibody) with G-1 not only reduces tumour growth but also improves survival of melanoma-bearing female mice, far more than either anti-PD1 antibodies or G-1 treatment alone. Combination therapy utilizing immune checkpoint inhibition and G-1 can result in long-term clearance of tumours, indicating immunological memory[207], with similar results in pancreatic cancer mouse xenograft models[199]. This effect is potentially mediated through lowering Myc levels, which results in decreased expression of PDL1 and increased expression of HLA class I in melanoma tumour cells, which together could lead to improved immune recognition of melanoma tumour cells[207]. In 2019, these results led to the initiation of the first Phase 1 clinical trial of G-1 for the treatment of malignant melanoma (NCT04130516)[78].

Other forms of cancer. The type of cancer might determine whether GPER activity promotes or inhibits carcinogenesis and/or metastasis. Pharmacological activation of GPER reduces liver tumorigenesis, at least in part, through inhibiting inflammation and fibrosis[208]. In mouse models of non-small-cell lung cancer (urethane-induced adenocarcinoma), tumour burden increases following treatment with 17β-oestradiol or G-1, and decreases upon treatment with G15 (ref. 202), possibly with the involvement of NOTCH-dependent pathways[230]. GPER expression is increased in castration-resistant prostate cancer[231], and its activation is associated with sustained cytotoxic ERK activation[198]. In a prostate cancer mouse xenograft model, chronic treatment with G-1 for several weeks inhibits cancer progression but only following cancer recurrence after castration[231], suggesting the potential for GPER-targeted therapies in castration-resistant prostate cancer.

GPER expression and function have also been implicated in gastric epithelial metaplasia and gastric cancer[173,174,232,233] as well as in colon cancer[173,234]. In mouse syngeneic pancreatic cancer xenograft models, G-1, alone or in combination with ICIs improves survival compared with vehicle only or ICIs alone, respectively, resulting in a substantial cure rate[199]. In line with the beneficial effects of G-1 on pancreatic cancer, tamoxifen, also acting as a GPER agonist, inhibits the recruitment and polarization of tumour-associated macrophages and interferes with myofibroblastic differentiation of pancreatic stellate cells in the tumour microenvironment[235]. This reduces the cells' ability to remodel the extracellular matrix and to promote cancer cell invasion[235]. GPER is highly overexpressed in Waldenström macroglobulinaemia, yet G-1 treatment, both in vitro and in vivo, induces apoptosis of tumour cells, even in the protective bone marrow milieu[236]. In this study, G-1 treatment improved survival in a murine xenograft model but had no effect on B cells transplanted from healthy donors[236].

Immune system and immunology

Regulation of fish granulocyte functions by oestrogens through GPER predates the evolutionary divergence of fish and tetrapods more than 450 million years ago, which indicates that oestrogens are modulators of the immune response and that GPER have played a pivotal role in immunity throughout evolution[12]. Sex plays an important role in immune responses with oestrogens frequently exerting anti-inflammatory effects, traditionally through ERα and, to a lesser extent, through ERβ[237]. However, 17β-oestradiol also mediates part of its anti-inflammatory effects through GPER, which is widely expressed in white blood cells, (including neutrophils, eosinophils, monocytes and lymphocytes) as well as in macrophages[238].

Regulation of immune cells by GPER. GPER regulates apoptosis in eosinophils[239], suggesting a role for GPER in allergic immune responses. Indeed, in a model of allergic pulmonary inflammation, G-1 attenuates airway hyper-responsiveness, reducing bronchoalveolar levels of inflammatory cells and the T helper 2 (T_H2) cell cytokines IL-5 and IL-13, while increasing the frequency of splenic regulatory T cells (which produce the anti-inflammatory cytokine IL-10), thus establishing cross-talk between GPER and IL-10 (ref. 240). Moreover, G-1 treatment also promotes the formation of IL-10 in pro-inflammatory T_H17 cells[241,242]. In macrophages, G-1 inhibits the production of lipopolysaccharide-induced cytokines, such as TNF and IL-6 (ref. 119), through the inhibition of NF-κB[120], while also downregulating TLR4 expression[243]. Neutrophils show complex responses to G-1 in vitro, with G-1 treatment causing activation of human neutrophils[244] and increased cell death-associated neutrophil extracellular trap formation[245]. In fish granulocytes, G-1 has multiple effects[245], including suppression of ROS production[12].

Regulation of inflammation by GPER. Deletion of *Gper* in mice increases circulating levels of pro-inflammatory cytokines, with a concomitant decrease in adiponectin levels compared with the wild type[114,165]. In a mouse model of diethylnitrosamine-induced liver cancer, deletion of *Gper* increases inflammation, fibrosis and tumorigenesis[208]. Consistent with this, GPER activation reduces expression of fibrosis markers in hepatic stellate cells in vitro, suggesting a possible role for GPER in counteracting liver inflammation and liver cancer[208]. In a mouse model of atherosclerosis, G-1 treatment reduces the increased number of $CD68^+$ macrophages but not of $CD3^+$ T cells, whereas deletion of *Gper* has the opposite effect[126].

Modulation of GPER activity in immunity, inflammation and infection. In surgically postmenopausal mice with diet-induced obesity, chronic treatment with G-1 reduces levels of TNF, MCP1 and IL-6 as well as the expression of inflammatory genes in multiple metabolic tissues[168]. GPER may also play a role in inflammatory bowel diseases; in a model of Crohn's disease, G-1 treatment reduces mortality, improves macroscopic and microscopic injury scores, and lowers C-reactive protein levels[173,178]. In a mouse model of *Staphylococcus aureus* skin and soft tissue infection, G-1 reduces dermonecrosis and increases bacterial clearance, indicating a role of GPER for the innate immune system[246,247]. These effects are more pronounced in females, suggesting a sex-specific response, and are absent in *Gper*-deficient mice, confirming the selectivity of G-1 for its target GPER[247].

Clinical data suggest a sex bias in COVID-19 severity following SARS-CoV-2 infection, with men exhibiting increased hospitalization and mortality compared with women. A role for GPER in this sex bias is suggested based on experimental models of both overexpression of GPER and treatment with G-1, each of which (similar to 17β-oestradiol treatment) leads to reduced SARS-CoV-2 viral load in infected bronchial cells in vitro compared with uninfected cells. These reductions in viral load caused by 17β-oestradiol and G-1 treatment are reversed by treatment with G15 (ref. 248). GPER activation also results

in anti-inflammatory immune responses in numerous neurological diseases[249–251]. Lastly, in a genome-wide CRISPR–Cas9 screen, GPER was identified as a downregulator of type I interferon[252]. GPER expression during pregnancy is both necessary and sufficient to suppress IFNγ signalling, which is elevated in reproductive and fetal tissues in influenza A virus-infected female mice. During virus-induced maternal inflammation, blocking GPER with G15 delays fetal development and promotes fetal demise compared with vehicle-treated mice[252]. Thus, GPER expression and activity are required to protect the fetus during maternal infection. Taken together, pharmacological activation of GPER holds promise for the treatment of diseases and conditions that are associated with activation of inflammation (due to infectious pathogens such as bacteria or viruses) and of conditions associated with an abnormal immune response.

Ageing and neurological diseases

Cardiovascular and renal ageing. Physiological ageing is an unmodifiable risk factor for arterial hypertension, myocardial disease and atherosclerotic vascular disease. In addition, vascular ageing is further accelerated by modifiable risk factors, including obesity (which is often associated with hypertension and diabetes) and smoking[89]. Endogenous *Gper* expression is associated with suppresion of the age-dependent increases in endothelin ET_B receptors, and endothelin-converting enzyme-2 in the heart[253]. Moreover, *Gper* deficiency abrogates age-dependent impairment of vasodilatation by interfering with NOX1-dependent ROS formation, specifically by reducing NOX1 expression, which is induced by GPER[39,103]. Accordingly, *Gper* deficiency prevents ageing-induced myocardial fibrosis and the associated development of diastolic heart failure (HFpEF) and for the most part prevents angiotensin-induced hypertension[39]. In addition, *Gper* deficiency is associated with a supression of development of age-dependent CKD due to FSGS[144]. The effect of *Gper* deficiency could be partly recapitulated pharmacologically by reducing NOX1 abundance and the associated production of ROS with G36, the first NOX1 downregulator[39]. Thus, blocking the GPER–NOX1 axis holds therapeutic opportunities for ageing-associated non-communicable diseases, including arterial hypertension.

Neurological diseases. In premenopausal women, endogenous oestrogens protect against stroke and dementia[254]. GPER, like ERα and ERβ, regulates arterial tone of the cerebral vasculature[255]. Antisense oligonucleotide knockdown of *Gper* in vivo largely abrogates the protective effects of oestrogen on cerebral ischaemia[256], whereas activation of GPER with G-1 reduces reperfusion injury following cerebral ischaemia in both male and female mice[257,258]. This involves inhibition of both apoptosis[259] and inflammatory pathways, such as TLR4 (ref. 258), with concomitant activation of anti-inflammatory pathways[260]. GPER-dependent protective effects have been demonstrated in rodent models of ischaemic[261] and haemorrhagic stroke[262]. G-1-dependent protection from ischaemic stroke is completely abrogated by systemic deletion of *Gper*, while only partial protection was observed in animals with astrocyte- or neuronal cell-specific *Gper* deletion[261]. Activation of GPER by G-1 also attenuates blood–brain barrier injury[263] and improves immunoprotection following stroke[264]. GPER also might play a role in psychiatric disorders such as anxiety[265], depression[266] and addiction[267]. Systemic deletion of *Gper* increases anxiety in rats[265]; accordingly, activation of GPER by G-1 has anxiolytic and also antidepressant effects in rodents[69,266]. Finally, deletion of *Gper* or GPER antagonism enhances morphine analgesia and reduces pain involving μ-type opioid receptors, suggesting the potential of GPER blockade for the treatment of pain, substance addiction, and opioid tolerance[268].

Ageing is the main risk factor for Parkinson disease and Alzheimer disease as well as for vascular dementia. Studies in neurotoxic mouse models of Parkinson disease have shown that 17β-oestradiol-dependent, ERα-mediated protective effects on dopaminergic neurons require crosstalk with GPER and that GPER also has independent protective effects against Parkinson disease[267]. In a mouse model of Parkinson disease, G-1 treatment reduces the release of pro-inflammatory cytokines[251] and also mediates part of the neuroprotective effects of IGF1 on dopaminergic neuronal injury[269]. G-1 treatment also reduces microglial activation and decreases pro-inflammatory cytokine production[251]. GPER is important for maintaining long-term memory, and G-1 enhances object recognition and long-term memory in male mice[270]. Accordingly, in a mouse model of Alzheimer disease and after traumatic brain injury in rats, improvements in neuropsychological functions are observed upon G-1 treatment[271–273]. GPER also mediates the anti-inflammatory effects of genistein in microglia[250].

Elevated levels of 17β-oestradiol present in pregnant women are associated with reduced severity of multiple sclerosis[274], and 17β-oestradiol supplementation reduces symptom severity and immune infiltration in a mouse model of MS (experimental autoimmune encephalomyelitis) in mice of both sexes[275]. In this model, female *Gper*-deficient mice exhibit reduced 17β-oestradiol-mediated protection against multiple sclerosis disease severity and reduced protective effects of 17β-oestradiol on white matter damage compared with wild-type mice[119,249,276]. Conversely, GPER activation by G-1 reduces multiple sclerosis severity, an effect absent in female *Gper*-deficient mice. Mechanistically, G-1 reduced inflammatory cytokine production in macrophages and upregulated PD1 to enhance the activity of T regulatory cells[249].

Conclusions

Progress made in the past decade in the field of GPER has broadened our understanding of the multiple functions of this receptor at the cell, tissue and organismal level, including in humans. Widely expressed, GPER mediates both rapid and genomic effects in all main organs, being involved in multiple aspects of health and disease (Fig. 3). In addition to oestrogens, many natural and synthetic molecules target GPER, either as selective or combined oestrogen receptor agonists or antagonists. Importantly, clinically approved ERα antagonists, such as the SERMS tamoxifen and raloxifene or the SERD fulvestrant, licensed for the treatment of breast cancer[209], show agonistic activity towards GPER[13,14,66]. Diverse molecules present in plants (such as genistein, daidzein and green tea polyphenols) and EDCs also activate GPER; further study is required to determine how their effects on health or disease involve GPER. Utilizing GPER expression as a diagnostic marker in tissues or in circulating cells provides new opportunities to further characterize pathological conditions at different stages during disease progression or even before diseases develop. Targeting GPER pharmacologically could provide new opportunities to treat diseases for which no or only a few effective therapies exist (such as malignant melanoma and other cancers), including inhibition of the constitutive inducing effect of GPER on NOX1 activity. Clinical studies that should also consider sex, genetics and hormonal status are needed to determine whether utilizing or targeting GPER could improve diagnosis, prognosis, therapy and the clinical course of human diseases and thus overall health[82].

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Author contributions

Both authors contributed equally to all aspects of this manuscript.

Competing interests

M.B. and E.R.P. are inventors on U.S. patent Nos. 10,251,870, 10,682,341 and 10,980,785, and E.R.P. is an inventor on U.S. Patent Nos. 10,471,047 and 10,561,648, all for the therapeutic use of compounds targeting GPER ("Method for treating obesity, diabetes, cardiovascular and kidney diseases by regulating GPR30/GPER"). E.R.P. is an inventor on U.S. Patent Nos. 7,875,721 and 8,487,100 for GPER-selective ligands and imaging agents ("Compounds for binding to ERα/β and GPR30, methods of treating disease states and conditions mediated through these receptors and identification thereof"). M.B. has served or serves as a consultant to Abbott, Inc., Abbvie, Inc., Travere, Inc. and Pharmazz, Inc.

Additional information

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Review criteria Abstracts of all articles published on GPR30 or GPER, published between January 1996 and February 2023, were retrieved from the U.S. National Library of Medicine (PubMed.gov). Articles were assessed for relevance, importance and scientific rigour, with a focus on publication in the past 10 years. The authors apologize to their colleagues whose work could not be included due to space and reference restrictions.