In Vivo Functions of GPR30/GPER-1, a Membrane Receptor for Estrogen: From Discovery to Functions In Vivo

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Summary. G protein-coupled receptor 30/G protein-coupled estrogen receptor-1 (GPR30/GPER-1) was reported as a novel membrane receptor for estrogen in 2005. However, the research on GPR30 has produced conflicting reports with regard to its intracellular localization, the tissue distribution of its expression, and some of its functions. Recently, in addition to the finding of G-1, a GPR30 agonist, GPR30 KO mice have been produced in laboratories, and this has significantly increased the confidence in the data. In this review, the intrinsic appearance of GPR30 is approached based mainly on data obtained in vivo.

Key words: GPR30, Membrane, GPCR, Estrogen, In vivo

Many receptors of steroid hormones including estrogen (Estradiol, E2) associate with nuclear receptors, and function as transcription factors. Since the hydrophobic steroid structure of E2 makes it easy to pass through the cell membrane, it is thought to be unlikely to act on the cell membrane through association with cell surface receptors. However, it is known that various events, such as calcium influx and the production of nitric monoxide (NO) elicited by E2, could be observed within a few minutes after its stimulation. When this time course is taken into consideration, it appears that these events are induced without nuclear receptors, i.e., transcription factors. Therefore, the idea that a novel receptor for E2 exists on the cell surface has long been supported despite the high hydrophobicity of E2.

Research on this proposed cell surface E2 receptor was addressed aggressively, and a classical estrogen receptor (ER)α, or its S-palmitoylated ER variant, was found to be localized in the cell membrane with caveolin, and to form complexes with trimeric G protein [1-3]. Some ER-deficient breast cancer cells proliferate in response to E2, suggesting that a novel estrogen receptor other than ER may also exist on the cell surface. In 2002, the ER-X and SIX-sensitive receptors, E2-sensitive seven transmembrane receptors, were reported as membrane receptors for E2, although their molecular structures have yet to be clarified [4, 5]. In neuronal cells, it was reported that a glutamate receptor is sensitive to E2 [6]. Furthermore, it has been reported that a novel cell surface receptor of E2 may exist in sex organs, but its amino acid sequence has not been determined.

We previously reported that GPR30 (also designated rat GPR41) is rapidly induced in the cardiomyocyte ischemia model, and that its induction is associated with apoptotic cell death during ischemia and reperfusion [7]. Another group has cloned GPR30 as a gene expressed

Abbreviations used: NO, nitric monoxide; E2, estradiol; ER, estrogen receptor; GPR30, G protein-coupled receptor 30; GPER-1, G protein-coupled estrogen receptor-1; EGF, epidermal growth factor; PDZ, PSD-95/Disc large/Zonula occludens-1; GPCR, G protein-coupled receptors; MMP, matrix metalloproteinase; HB-EGF, heparin-binding EGF-like growth factor; CTGF, connective tissue growth factor; GLUT2, glucose transporter 2; EAE, Experimental autoimmune encephalomyelitis; oVX, ovariectomy; Ang II, Angiotensin II; 5-HTP, 5-hydroxytryptophan.
in blood vessels using a hypertensive model [8].

We thought that GPR30 might be a receptor for a cytokine or a receptor working in the cardiovascular system with a peptide ligand because GPR30 shows weak homology with a cytokine receptor [9]. However, several groups initially identified E2 as a ligand candidate for GPR30 because GPR30 was cloned based on the difference in gene expression in response to E2 in breast cancer cells [10]. Thomas et al. reported that E2 activates GPR30 in MCF-7 cells [11]. Finally, Revankar et al. showed that an orphan receptor, GPR30, is a receptor for E2 in the endoplasmic reticulum in 2005 [12]. After this discovery, it was shown by several groups, including ours, that GPR30 in the plasma membrane is activated by E2 [13, 14].

A. Basic structure of GPR30

Human GPR30 is located in chromosome 7p22.3, and is composed of three exons. Exon3 coincides with the amino acid coding region of GPR30. Based on linkage analysis, the region of the chromosome containing GPR30 is thought to be related to familial hypertensive disease in humans [15].

It has been reported that the AP-1 site that exists in the second intron is a transcriptional regulatory region of GPR30 activated in response to EGF [16]. There is a possibility that some of the transcript products for GPR30 are expressed in response to stimulation, because the AP-1 site in intron 2 cannot transcribe Exons1 and 2 of GPR30. GPR30 is a G protein-coupled seven-transmembrane receptor, and human GPR30 comprises 375 amino acids with a theoretical molecular mass of approximately 41 kDa. It is thought that the N-terminus is located outside of the cell, and that aspartic acid residues in the terminal region might be modified by glycosylation if GPR30 is localized in the plasma membrane. It is speculated that the ligand associates with the N-terminal domain to activate the receptor. Trimeric G protein is presumed to bind to the 3rd loop of the intracellular domain based on the molecular structure. A PDZ domain appears to be in the C-terminal region of GPR30, but its physiological role is unknown.

B. Intracellular localization of GPR30

There are reports that GPR30 localizes in both the endoplasmic reticulum and Golgi apparatus [12, 17], and that GPR30 is present in the plasma membrane [13, 14], although a consensus about this has yet to be reached. In general, the data on the localization of GPCR in the endoplasmic reticulum and Golgi apparatus have to be evaluated carefully because the over-expression of exogenous genes often leads to an accumulation in the endoplasmic reticulum due to the use of a strong promoter and the effects of fusion proteins such as GFP. It is also necessary that the epitope sequences of the antibody present in the N-terminal region of GPCR should be noted. In the case of an antibody that recognizes a tag protein introduced into the N-terminal region of GPCR, it is possible that the antibody will be unable to recognize the epitope sequence following glycosylation of the N-terminal region in the Golgi apparatus. Unless GPC on the plasma membrane is detected in some way, unmodified GPCR in the Golgi apparatus and endoplasmic reticulum will be stained, while that in the plasma membrane will remain unobserved. The anti-GPCR antibody has to be confirmed to recognize glycosylated GPCR through the use of immunoblotting. The localization of GPR30 in the plasma membrane has been reported by several groups, including ours, but it is not mysterious that GPR30 is observed in the endoplasmic reticulum and Golgi apparatus during the process of protein synthesis of the receptor. We could not find a commercially available antibody that accurately recognizes GPR30, and so we produced our own antibody for use in this study. Another group has suggested that many commercial antibodies for GPR30 react with proteins other than GPR30 by using KO mice, indicating that it is difficult to detect the GPR30 protein in tissues. There is a possibility that the use of insufficiently specific antibodies has led to the confusion concerning the localization of GPR30. At the least, the specificity of the antibody has to be confirmed by observing the disappearance of staining in the presence of an antigen peptide.

C. Expression of GPR30 mRNA

Up to now, data on the expression of the GPR30 mRNA in tissues has not necessarily produced a consensus result, although various methods were used including northern blotting, RT-PCR methods, and RNase protection assays [9, 10, 18-21] (Table 1). The mRNA for GPR30 appears to be expressed extensively in most tissues as judged from the overall reports.
Recently, Isensee et al. have succeeded in producing GPR30 KO mice with a LacZ gene introduced downstream of the GPR30 promoter, causing research in this field to be greatly advanced [19]. In these mice, GPR30 expression is limited to the blood vessels in the brain and the arteries in skeletal muscles and adipocytes. The expression of GPR30 shows a unique distribution. The expression pattern in blood vessels may produce the impression that there is ubiquitous expression in most tissues. In peripheral blood vessels, the GPR30 mRNA is expressed in the arterial endothelium, consistent with the physiological effect in which E2 induces the relaxation of blood vessels through NO produced in the endothelium. It is necessary to note the occurrence of high pressure and oxidative stress in the tissues during sampling, because the GPR30 mRNA is rapidly induced in response to stress. Other than blood vessels, the data obtained from LacZ-expressing mice indicates the GPR30 mRNA to be highly inducible in digestive tissues including the stomach, pancreas, and duodenum. An interesting observation that the expression of GPR30 is mainly observed in the exocrine cells of the digestive tissues has been obtained in mice [19]. The expression of GPR30 in sexual organs has not been analyzed in detail up to now.

### D. Signal transduction of GPR30

Since breast cancer cells proliferate in response to E2, E2 antagonists have been used for breast cancer therapy. However, relapse and metastasis have frequently been observed during therapy involving E2 antagonists, suggesting the possibility that a signal pathway other than in the ER in response to E2 may be present in breast cancer cells. It has been noted that GPR30 acts as a receptor in an alternative pathway of E2 activation. In studies of the signal pathways of GPR30, much of the data have been obtained.
using the breast cancer cell line MCF-7 and SkBr (Fig. 1). E2 associates with GPR30, a membrane receptor, leading to the activation of the trimeric G protein in breast cancer cell lines. The α subunit in the activated trimeric G protein induces the activation of adenylyl cyclase, which results in the production of c-AMP. On the other hand, the β and γ subunits of the G protein activate Src tyrosine kinase, which binds to integrin α5β1 through an adaptor protein, Shc. This complex activates matrix metalloproteinase (MMP), and the activated MMP degrades pro-HB-EGF, which releases HB-EGF into the extracellular space. HB-EGF activates the EGF receptor via an autocrine/paracrine mechanism, leading to the activation of PI-3K. The activated EGF receptor also induces ERK activation [22, 23]. In a recent paper, it was reported that the activation of ERK through GPR30 after E2 stimulation results in the secretion of a growth factor, CTGF, into the extracellular space, and that this secretion is involved in the proliferation of breast cancer cells [24]. These signal pathways in response to E2 appear to lead to the proliferation of breast cancer cells.

In other cell types, it has been reported that GPR30 is involved in apoptotic cell death and cell cycle arrest. Therefore, a pathway unrelated to the signal that leads to cell proliferation in cancer cells might be activated by GPR30 in other cell types. Further investigation is needed to clarify GPR30 signal transductions [25, 26].

II. Circulatory system

It has been reported that G-1 reduces systolic blood pressure in ovariectomized female mRen2 Lewis rats, an estrogen-sensitive model of hypertension, suggesting that the renin-angiotensin system is involved in the reduction of blood pressure through GPR30 [29]. In normal rats, the infusion of G-1 results in an acute reduction in mean arterial blood pressure within 2 min [30]. The effect of G-1 has been confirmed using GPR30-/- mice. The deletion of GPR30 from mice attenuates the G-1-induced relax of the carotid arteries, and there is no observed sex difference in the reductive effect of GPR30 [30]. The expression of the GPR30 mRNA is observed in the endothelium of the peripheral vessels of LacZ-introduced mice [19]. The production of NO by E2 in endothelial cells may be mediated

E. Biological functions of GPR30

The discovery of G-1, an artificial agonist of GPR30 [27], and the production of GPR30 KO mice have led to progress in clarifying the biological functions of GPR30 (Table 2).

I. Immunological system

The phenotype of GPR30 KO mice was first shown in the immunological system. Wang et al. demonstrated that GPR30 was required for thymocyte apoptosis that occurs preferentially in T cell receptor β chain -/- double-positive thymocytes using GPR30-deficient mice, and that G-1 induced thymotic atrophy and thymocyte apoptosis [25]. They also showed that the GPR30-induced suppression of CD4^+Foxp3^+ T regulatory cells has a protective effect on experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis [28]. These findings have been confirmed by other groups, and it is expected that the mechanism of the induction of T cell apoptosis by GPR30 will be clarified in the near future.
by GPR30, thus leading to the reduction in blood pressure. Clarification of the NO production pathway by E2 is an issue that needs to be addressed further.

III. Glucose homeostasis

GPR30 is expressed in pancreatic islet cells in mice, and adult female GPR30−/− mice do not exhibit the E2-induced release of insulin [20]. This result is consistent with experiments using isolated pancreatic islet cells in vitro [20]. There are no differences in the expressions of glucose-related genes such as GLUT2 and glucokinase in GPR30−/− mice as compared with wild type mice. Thus GPR30 may act in regulating the process of insulin release after E2 stimulation. GPR30 mRNA is also expressed in secretory gland cells and mammary gland epithelial cells, and GPR30 may be involved in the secretion pathway of breast fluid and hormones including insulin. It has been reported that the regulation of blood glucose levels is unaffected in GPR30−/− mice. In reports in which differences in glucose levels were observed, mice older than 3 months age were used, and it would be interest-

### Table 2. GPR30-related diseases

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ing if only aged mice show the regulation of glucose levels through GPR30 activation.

Concluding remarks

The data obtained using GPR30-/- mice and the G-1 agonist indicate that the roles of GPR30 in the cardiovascular and immunological systems are mostly consistent with previous findings observed by E2 treatment, probably through its activity as a membrane receptor. It is expected that GPR30 plays important roles in early E2-induced events that take place on the membrane. On the other hand, the concentration of E2 needed for GPR30 activation is higher than that required ER activation. In our laboratory, we have found that high concentrations of E2 and G-1 are needed to activate GPR30 as shown in Figure 2. Further investigations are needed to clarify whether these E2 concentrations are physiological in living animals, or whether another physiological ligand is present in cells.

There are also reports that GPR30 is a classical ER cofactor; however, experimental evidence for this is inadequate [31]. If GPR30 acts as a cofactor in the ER, the E2 concentration for GPR30 activation might be equal to that for ER activation. Direct evidence such as crystal structure analysis is necessary to clarify this point.

References


