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Recent Advances on the Role of G Protein-Coupled Receptors in Hypoxia-Mediated Signaling

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Abstract. G protein-coupled receptors (GPCRs) are cell surface proteins mainly involved in signal transmission; however, they play a role also in several pathophysiological conditions. Chemically heterogeneous molecules like peptides, hormones, lipids, and neurotransmitters activate second messengers and induce several biological responses by binding to these seven transmembrane receptors, which are coupled to heterotrimeric G proteins. Recently, additional molecular mechanisms have been involved in GPCR-mediated signaling, leading to an intricate network of transduction pathways. In this regard, it should be mentioned that diverse GPCR family members contribute to the adaptive cell responses to low oxygen tension, which is a distinguishing feature of several illnesses like neoplastic and cardiovascular diseases. For instance, the G protein estrogen receptor, namely G protein estrogen receptor (GPER)/GPR30, has been shown to contribute to relevant biological effects induced by hypoxia via the hypoxia-inducible factor (HIF)-1α in diverse cell contexts, including cancer. Likewise, GPER has been found to modulate the biological outcome of hypoxic/ischemic stress in both cardiovascular and central nervous systems. Here, we describe the role exerted by GPCR-mediated signaling in low oxygen conditions, discussing, in particular, the involvement of GPER by a hypoxic microenvironment.

KEYWORDS: angiogenesis; GPCRs; GPER; hypoxia; signal transduction.

INTRODUCTION

G protein-coupled receptors (GPCRs) are seven transmembrane-spanning receptors that regulate many cellular functions upon ligand activation (1). The biological responses mediated by GPCRs involve the recruitment of proteins prompting the receptor internalization and desensitization, like arrestins and GPCR kinases (GRKs) as well as membrane-bound partners, namely heterotrimeric G proteins (1,2). In the inactive state, G proteins consist of a Gβγ monomer which maintains a high affinity for a guanine diphosphate (GDP)-bound Gα subunit (1,2). On the basis of the sequence identity, four subtypes of Gα subunit (Gαs, Gαi, Gαq, and Gα12) have been extensively characterized (1,2). Ligand binding promotes conformational modifications that result in the exchange of GDP for GTP on the Gα subunit, leading to a decreased affinity of Gα for the Gβγ subunit. The dissociation of the heterotrimer allows that both GTP-bound Gα and free Gβγ activate numerous transduction pathways like mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3-K), small GTP-binding proteins (Ras and Rho GTPases), and other mediators that contribute to various physiopathological responses (1,2). For instance, an aberrant expression of GPCRs and/or their activation have been associated to several types of tumors (3,4). Consequently, the pharmacological manipulation of certain GPCR-mediated signaling may represent a promising anti-cancer strategy (3,4). As demonstrated for many GPCRs (3,4), the G protein estrogen receptor (GPER, also known as GPR30) may trigger oncogenic signaling (5,6). GPER binds to estrogens, phyto- and xenoestrogens, and also estrogen receptor (ER) antagonists that may act as GPER agonists (7–12). GPER mediates the activation of a network of transduction pathways; however, the actual role elicited by GPER in tumorigenesis is still controversial. Previous studies have shown that GPER may induce cell cycle arrest and inhibition of cancer cell growth (13–16). Nevertheless, other *in vitro* and *in vivo* studies have revealed that GPER triggers cancer cell migration and proliferation (5,17). In addition, GPER expression was associated to inflammatory breast tumor (18), was found reduced during breast cancer tumorigenesis (19), and was related to a poor relapse-free survival in breast cancer patients treated with tamoxifen (20). The lack of GPER in the plasma membrane was linked to a

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favorable prognosis in breast cancer (21), whereas its expression was associated with aggressive features of breast, endometrial, and ovarian tumors (22–24). In this context, we have demonstrated that GPER is upregulated by EGF, insulin-like growth factor (IGF)-I, insulin, and a main factor contributing to tumor aggressiveness like hypoxia (25–31). A low oxygen tension characterizes the growth of solid tumors, where it promotes adaptive responses like anaerobic glycolysis, reduction of macromolecule synthesis, and angiogenesis (32). In addition, hypoxia is critical for the pathogenesis of heart disease and stroke, the major causes of human mortality (33). The effects of hypoxia are mainly mediated by hypoxia-inducible factor (HIF) family members, which orchestrate the complex responses to low oxygen tension (34). In particular, HIF-1α regulates the expression of several pro-angiogenic factors involved in tumor angiogenesis progression (34,35). Many signaling cascades are engaged by hypoxia toward HIF-1α activation such as receptor tyrosine kinases (RTKs) and GPCRs (30,31,36). Here, we discuss the involvement of certain GPCRs, including GPER, in hypoxia-mediated signaling toward cancer development and cardiovascular diseases.

GPCR INVOLVEMENT IN HYPOXIA-MEDIATED SIGNALING

A low oxygen tension characterizes relevant pathophysiological conditions like cancer and cardiovascular diseases (32–34). Multiple mechanisms for oxygen sensing have been developed and conserved in both prokaryotic and eukaryotic organisms (32–34). In particular, HIF-1 acts as a master regulator of the adaptive cell response to limited oxygen availability mainly by activating the transcription of genes that regulate physiological processes as glycolysis, survival, and angiogenesis (34–37). HIF-1 is a heterodimer of two helix-loop-helix-PAS proteins, namely HIF-1α and HIF-1β or ARNT (38). Upon hypoxia, HIF-1α and HIF-1β dimerize and bind to the hypoxia-responsive elements (HREs) located within the promoter region of target genes (38). Several factors contribute to HIF-1α-mediated action in hypoxic conditions, including diverse GPCRs (30,39). For instance, GPR41 was shown to be a hypoxia-induced receptor that drives p53-dependent apoptosis in rat cardiomyocytes subjected to ischemia and reoxygenation injury (40) whereas GPR22 was involved in cardioprotection as its ablation increased the susceptibility to functional decompensation following hemodynamic stress (41). The adrenergic signaling axis, consisting of catecholamines and their adrenergic receptors, has been included among GPCRs that play a primary role in oxygen-related diseases like hypertension, cardiac hypertrophy, and heart failure (42). Moreover, the adrenoreceptors have been shown to functionally interact with opioid receptors (43), which elicit protective actions in response to pre- and post-conditioning stimuli upon cardiac and cerebral damages (44,45). It is worth noting that certain ligand-activated GPCRs induce HIF-1 expression and function (46–48), thus mimicking hypoxic conditions. For instance, the recruitment of transcription factors to the promoter sequence of HIF-1 as well as the stabilization of HIF-1 protein levels may occur upon activation of GPCRs by endothelin-1 (ET-1), β-adrenoceptor agonists, and lysophosphatidic acid (46–48).

GPCR INVOLVEMENT IN TUMOR ANGIOGENESIS UPON HYPOXIA

Tumor microenvironment is often characterized by hypoxia, which is a distinguishing feature of an aggressive cancer phenotype and disease recurrence (32). The metabolic changes occurring in rapidly growing cells, the increasing diffusion distances between the blood vessels and certain tumor areas, and the compressive action elicited by the expanding mass on local blood vessels may cumulatively account for low intra-tumor oxygenation (32). The effects of hypoxia on the malignant progression are mediated by complex mechanisms that allow tumor cells to survive and/or escape their oxygen-deficient environment (32,34). Moreover, the adaptive responses to hypoxic stress in the tumor microenvironment trigger the formation of new blood vessels stimulated by pro-angiogenic factors (35,37). Along with the activation of endothelial cells (ECs) and the subsequent degradation of the basement membrane, the angiogenic response leads to the migration and proliferation of ECs, which then form tubes generating new blood vessels (49). Moreover, tumor angiogenesis prompts cancer cells to grow, evade the host surveillance, form the pre-metastatic niche, and invade distant sites (49); hence, the molecular players driving this complex process are intensively investigated toward effective anti-tumor strategies (49). To date, the major growth factors involved in the formation of blood vessels are members of the vascular endothelial growth factor (VEGF) family (50). It includes placental growth factor (PlGF), VEGF-A VEGF-B, VEGF-C, VEGF-D, and VEGF-E, which bind to the tyrosine kinase receptors, namely VEGF receptor (VEGFR)-1, VEGFR-2, and VEGFR-3 (50). VEGF-A mainly mediates new blood vessel formation within the tumor mass as its binding to VEGFR-2 promotes EC proliferation, migration, and vascular permeability (50). Hormones, cytokines, and growth factors have been shown to boost VEGF-dependent tumor angiogenesis; however, hypoxia represents the primary stimulus for VEGF production and release in the tumor microenvironment (50). Diverse members of the GPCR family are involved in the angiogenic action induced by thrombin, prostaglandins, lysophosphatidic acid, chemokines, and sphingosine 1-phosphate in different pathophysiological conditions, suggesting that certain GPCRs contribute to the development of blood vessels (51–54). In addition, the heterotrimeric G proteins Gαq and Gα11 may contribute to angiogenic responses by interacting with VEGFR-2 (55) and the G protein-coupled receptor kinase 2 (GRK2) has recently emerged as an integrative node toward the development of cancer-associated vascularization (56). In the tumor microenvironment, chemokines and their receptors elicit relevant paracrine actions, as suggested by the ability of CCL2, CCL5, and CXCL8/IL-8 to recruit within the tumor mass leukocytes and macrophages, which release VEGF and other angiogenic factors (57). Furthermore, cytokines may stimulate the production of prostaglandin E2 (PGE2), which increases the secretion of VEGF, CXCL8, and CXCL5 by tumor and stromal cells (57). Overall, these data suggest that GPCR-mediated signaling may modulate the angiogenic

process together with the VEGF/VEGFR axis. Further corroborating these observations, the anti-tumor activity exhibited by several GPCR antagonists has been correlated with their anti-angiogenic properties and anti-proliferative effects (58). Among the GPCRs contributing to the formation of new blood vessels in hypoxic conditions, the chemokine receptor CXCR4 that binds to the stromal cell-derived factor-1 (SDF-1)/CXCL12 has been shown to stimulate tumor outgrowth and metastasis as well as angiogenesis upon hypoxia (59). The angiogenic factor named adrenomedullin (ADM) signals through the calcitonin receptor-like receptor (CRLR), which is a GPCR expressed in several tumors like the high-vascular clear renal cell carcinoma (RCC) (60,61). A functional consensus HRE was identified within the promoter region of the human CRLR gene, thus corroborating the role of CRLR in the formation of new blood vessels upon hypoxic conditions (60,61). Among the vasoactive pro-angiogenic molecules, ET-1 and the cognate receptors (ETRs) are aberrantly activated in diverse malignancies and regulated by low oxygen tension through HIF-1α (62). In this regard, HIF-1α/VEGF signaling has been considered as a downstream transduction pathway activated by the ET-1 axis (62). For instance, in human chondrosarcoma cells, ET-1 promoted the expression of VEGF, angiogenesis, and cell migration by activating integrin-linked kinase (ILK), Akt, and HIF-1α-mediated signaling cascades (63). In ovarian carcinoma, in both normoxic and hypoxic conditions, ET-1 induced the transcription and accumulation of HIF-1α and the upregulation of VEGF, suggesting that ET-1 action may be linked to hypoxia and HIF-1α-dependent angiogenesis (64). In our recent study (65), we also found that ET-1 may trigger GPER expression and function leading to angiogenic responses. Recently, the adrenergic system has been shown to boost tumor angiogenesis and aggressive features through the upregulation of diverse angiogenic factors like VEGF, IL-6, IL-8, matrix metalloproteinase (MMP)-2, and MMP-9 (66,67). The involvement of HIF-1α in the aforementioned biological responses to catecholamine-mediated stress was also evidenced in other studies showing that the β2-adrenergic receptor (AR)/HIF-1α axis regulates angiogenesis and stress-induced pancreatic tumor growth in mouse models (68). In hypoxic melanoma cells, β3-ARs have been found to be upregulated and involved in the increase of VEGF, as evidenced by using two β3-AR blockers (69). Additionally, in ovarian cancer cells, the α1-AR blocker doxazosin prevented VEGF-mediated cell migration, proliferation, and capillary-like structure tube formation (70). These effects were dependent on the activation of VEGFR-2 and downstream signaling including HIF-1α (70). Altogether, these observations may suggest that the adrenergic system plays a role in tumor angiogenesis and progression, in particular through HIF-1α-mediated responses and VEGF expression in hypoxic conditions. Virally encoded GPCRs may also contribute to cancer angiogenesis and progression as evidenced by the human herpesvirus-8 (HHV-8 or Kaposi's sarcoma-associated herpesvirus (KSHV))-encoded G protein-coupled receptor (vGPCR) (71). In this regard, it has been demonstrated that KSHV stimulates the expression of the angiogenic factor angiopoietin-like 4 (71) as well as the production of

VEGF through HIF-1α (72). Accordingly, the expression of vGPCR in human umbilical vein endothelial cells (HUVECs) triggered cell immortalization together with a constitutive expression and activation of VEGFR-2, thus proposing a role for vGPCRs in the acquisition of the KS-angiogenic phenotype in the model system used (73).

GPER IS INVOLVED IN HYPOXIA-MEDIATED SIGNALING

GPER has been recently characterized toward its ability to mediate estrogen action in reproductive, immune, skeletal, cardiovascular, and central nervous systems (5). In addition, our and other studies have largely demonstrated the involvement of GPER in the stimulatory effects elicited by estrogens in cancer cells and tumor microenvironment (6,9–11). Significantly, several studies performed in different cell and animal models have ascertained the role exerted by GPER in certain pathological conditions characterized by oxygen deficiency (30,31,74–78). In this regard, it has been demonstrated that GPER activation may decrease myocardial damage and increase functional recovery after ischemia-reperfusion (I/R) injury, which often induces dangerous complications like arrhythmia in patients with myocardial infarction (74–78). Likewise, in rat hearts of both sexes exposed to I/R injury, the activation of GPER reduced myocardial inflammation and infarct size as well as improved immunosuppression and myocardial mechanical performance (79–81). Interestingly, the expression levels of both GPER and HIF-1α were found to be increased in spontaneously hypertensive rat hearts compared to normotensive controls, suggesting that HIF-1α/GPER signaling may represent a transduction mediator in certain conditions characterized by elevated blood pressure (74), which is tightly linked to hypoxia (82). Of note, the selective GPER agonist G-1 markedly lowered blood pressure in normotensive and hypertensive rats (83,84), thus supporting the hypothesis that GPER may be a valuable pharmacological target for the prevention/treatment of certain cardiovascular diseases. Further supporting the role elicited by GPER in hypoxic conditions, previous studies have reported that its activation may attenuate the detrimental effects induced by oxygen deficiency in some areas of the central nervous system like the hypothalamic-pituitary axis, hippocampal formation, brainstem autonomic nuclei, and spinal cord (85,86). For instance, GPER activation promoted neuronal survival after global ischemia through the activation of pro-survival and anti-apoptotic signaling cascades (86). An improvement in cerebral microvascular function upon hypoxia/reoxygenation injury was also observed upon GPER activation in male and female rats (87), although sex-dependent protective effects mediated by GPER have been also shown to influence the outcome of ischemic stroke (88). In this regard, it has been demonstrated that GPER expression increases after stroke in the brain of male but not female mice, thus suggesting that a gender-specific regulation of GPER may occur and influence the recovery from cerebral I/R (88). The regulation of GPER expression following hypoxia has been evaluated in breast cancer cells as well as in cancer-associated fibroblasts (CAFs) obtained from breast malignancies (30,31). In these cells, hypoxia-stimulated HIF-1α was found recruited to the HRE sequences located

within the promoter region of the human GPER gene (30,31). Accordingly, HIF-1α was required for both the transactivation of a GPER promoter reporter gene as well as for the upregulation of GPER expression upon hypoxia (30,31). These observations were further corroborated by the involvement of HIF-1α/GPER signaling in VEGF expression toward tumor angiogenesis and progression (31,89). In addition, HIF-1α/GPER/VEGF transduction pathway was triggered in cancer cells upon exposure to copper, which showed the ability to mimic the hypoxia-mediated signaling (90). Interestingly, the copper-chelating agent TEPA exerted an inhibitory action on the activation of the aforementioned pathway (90), in accordance with previous studies demonstrating that copper-chelating agents can exert anti-tumor effects (91). Altogether, these results indicate that diverse stimuli including hypoxia may trigger relevant biological responses through GPER, which was recently shown to be also involved in the stimulatory effects exerted by aldosterone in breast cancer cells and breast tumor-derived endothelial cells (92) as well as in pregnancy-induced vasodilation of rat uterine arteries (93).

GPCRS AND HYPOXIA: IMPLICATIONS FOR DRUG DISCOVERY

The multifaceted mechanisms of oxygen sensing mainly orchestrated by HIF-1 represent an essential response to cope with hypoxic stress, which often occurs in cancer, heart disease, and stroke (32,33). As many members of the GPCR family elicit a role in the intricate cell adaptation to oxygen deficiency, a cross talk between HIF-1 and GPCR-mediated pathways may be involved in the biological responses to hypoxia in the aforementioned pathological conditions. In recent years, the discovery and development of several different strategies to block HIF-1 action directly or indirectly has been suggested as a promising tool to overcome the resistance to conventional chemotherapeutic agents in hypoxic microenvironment (34,94). In this vein, HIF-1 inhibitors may be regarded as golden candidates in combination treatment targeting the molecular mediators activated by hypoxia. For instance, a further approach toward new therapeutic strategies may combine the pharmacological manipulation of both HIF-1- and GPCR-mediated signaling. In addition to the therapeutic purposes, GPCRs along with HIF-1 may be regarded as further hallmarks of hypoxia signature in different pathophysiological conditions. As it concerns GPER, on the basis of its involvement in biological responses to low oxygen tension, new GPER-targeted therapies might pioneer for innovative drug discovery strategies aimed to improve the efficacy of HIF blockers and conventional angiogenic inhibitors.

CONCLUSIONS

A significant progress has been made in the past few years toward the characterization of the molecular mechanisms involved in GPCR action. In particular, many members of the GPCR family have been shown to contribute to the adaptive cell responses to low oxygen tension, which is a distinguishing feature of tumor development and certain cardiovascular diseases. In this regard, GPER may be included among the HIF-1α target genes that drive cancer cell survival and malignant progression. In addition, HIF-1α/ GPER signaling may play a relevant role toward VEGF stimulation, angiogenesis, and cancer development. Furthermore, the role elicited by GPER in heart failure, stroke, and hypertension has been largely elucidated, paving the way for novel therapeutic approaches in these relevant illnesses that are characterized by hypoxia and ischemia.

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