

Published in final edited form as:

Mol Cell Pharmacol. 2010 January 1; 2(3): 111–116.

G Protein-Coupled Receptor 87: a Promising Opportunity for Cancer Drug Discovery

Yanhong Zhang, Ariane Scoumanne, and Xinbin Chen

Center for Comparative Oncology, Schools of Medicine and Veterinary Medicine, University of California, Davis, California

Abstract

G protein-coupled receptors (GPRs) constitute one of the largest families of membrane proteins encoded by the human genome. Upon binding to various ligands, these seven-transmembrane receptors play an essential role in many physiological processes, including neurotransmission, immunity, inflammation, regulation of mood and behavior. In view of their important functions, aberrant expression and activity of GPRs have been implicated in a wide spectrum of diseases, including tumorigenesis. GPR87, a cell surface GPR related to the LPA receptor family, is overexpressed in diverse carcinomas and plays an essential role in tumor cell survival. In our recent work, we uncovered that GPR87 expression is regulated by the tumor suppressor p53 and by DNA damage in a p53-dependent manner. Moreover, we found that a lack of GPR87 triggers an increase in p53, concomitant with a decrease in Akt, which results in the sensitization of tumor cells to DNA damage-induced apoptosis and growth suppression. Altogether, we uncovered an essential function for GPR87 in p53-dependent cell survival in response to stress signals. Due to their unique structure, localization and ligand binding ability, GPRs have been extensively used for drug development and are the most common targets of commercial drugs. Although studies are required to determine GPR87 natural ligand(s) and signaling pathways, GPR87 is undoubtedly a very promising novel target for cancer prevention and treatment.

Keywords

p53; G protein-coupled receptor 87 (GPR87); Apoptosis; DNA damage; Cancer therapy

G protein-coupled receptors (GPRs) account for more than 2% of all genes encoded by the human genome. They constitute a large and burgeoning family of integral membrane proteins playing an essential role in a wide range of physiological processes including olfaction, neurotransmission, hormone and enzyme releases from endocrine and exocrine glands, immune responses, cardiac- and smooth-muscle contractions and blood pressure regulation (1). In order to accomplish these diverse functions, GPRs are activated by various stimuli, such as hormones, chemokines, nucleotides, lipid-derived messengers, divalent cations and light (2,3). Upon ligand binding, GPRs interact with specific heterotrimeric G proteins, which lead to the exchange of GDP for GTP on G protein α -subunit ($G\alpha$) and the dissociation of GTP-bound $G\alpha$ from G protein $\beta\gamma$ -subunits. This initiates a plethora of intracellular signaling cascades resulting in the activation of effectors such as adenylyl cyclases, small GTPases, phospholipases and kinases to ultimately regulate the expression of

Correspondence: Dr. Xinbin Chen, VM: Surgical and Radiological Sciences, University of California, 2128 Tupper Hall, Davis, CA 95616, USA. Tel. 530-754-8404, Fax. 530-752-6042. xbchen@ucdavis.edu.

Conflicts of Interest

No potential conflicts of interest to disclose.

genes involved in cell survival, proliferation, differentiation and other cellular processes (4–6). Given that a particular GPR can interact with several types of G proteins, it is not surprising that GPRs are implicated in many physiological functions and that alterations in GPR signaling contribute to many human pathologies, including diabetes, central nervous system disorders, cardiac dysfunction and cancer (6–8).

Since the cloning of the first GPR, bovine opsin, over two decades ago, nearly a thousand GPRs have been identified in organisms as diverse as bacteria, yeasts, plants, nematodes and animals (9). All GPR family members share a common structure composed of an extracellular amino terminus, a seven-transmembrane region containing α helices connected by three intracellular and three extracellular loops, and an intracellular carboxyl terminus (10). GPRs are classified into over a hundred sub-families according to sequence homology, ligand nature and receptor function (2). More than 400 GPRs are involved in olfactory signal transduction. Another 300 GPRs have known ligands and physiological functions. There are an additional 150 ‘orphan’ GPRs for which natural ligands and physiological functions remain to be defined (11). Due to their characteristic structure and specific ligand binding ability, many GPRs have been successfully used as targets for therapeutic drug development. Indeed, more than half therapeutic agents currently on the market, such as Clarinex® and Zantax® are targeting GPRs, which represents an annual worldwide sale exceeding \$30 billion (12,13). It is most reasonable to speculate that many orphan receptors are potential therapeutic targets. In this perspective, we will give a brief overview on the involvement of GPRs in human cancers. Then, we will focus on the orphan receptor GPR87 and its recently uncovered role in cell survival and regulation of tumor suppressor p53. Finally, we will discuss the promising use of GPR87 as a target for cancer drug development.

A large body of evidence links aberrant GPR expression and signaling to human cancers. Indeed, several GPRs are overexpressed in multiple types of cancers (14). For example, high levels of G protein-coupled estrogen receptor 1 (GPER, GPR30) are found in breast, prostate, ovarian, lung and brain cancers (15). Overexpression of lysophosphatidic acid (LPA) receptors, LPA2 and LPA3, are involved in many ovarian cancers (16). Aberrant activation of GPRs due to high levels of circulating or locally produced ligands also plays a role in tumorigenesis. In fact, many ligands are mitogenic (LPA, lysophospholipid shingosine 1P, chemokines) and therefore responsible for the abnormal activation of specific GPRs to mediate cell proliferation, transformation, angiogenesis, metastasis and drug resistance (14). Although less common, mutations of GPRs are associated with cancer development, such as activating mutations in the thyroid-stimulating hormone receptor, which are found in 30% of human thyroid adenomas and some carcinomas (17). Currently, several therapeutic agents targeting GPRs are used in cancer treatment, including Lupron® and Zoladex®, which target the gonadotropin-releasing factor receptor in the treatment of breast and prostate cancers, respectively (18). Another example is Sandostatin®, which targets the somatostatin receptor to therapy a variety of cancers including pancreatic carcinomas, hepatocellular carcinomas and recurrent meningiomas (19). Ongoing efforts to fully characterize the many orphan GPRs will certainly lead to the identification of novel cancer therapeutic targets in the near future.

Human GPR87, also known as GPR95, was first identified in 2001 through a search for novel GPR encoding genes using an Expressed Sequence Tag database (20). Human GPR87 is located on chromosome 3q24. The ORF of human GPR87 is comprised of 1077 bp framed by a stop codon upstream of the start ATG and the putative poly (A) signal AATAAA 40 bp downstream the stop codon. Human GPR87 codes for 358 amino acids, characterized by a typical seven-transmembrane structure and the presence of a consensus site (S/T-X-V) for binding of PDZ domain-containing proteins (Figure 1). The GPR87

protein is classified as a member of the P2Y receptor family based on sequence homology and the presence of conserved amino acids important for ligand binding and specificity in other P2Y family members (21). GPR87 is a cell surface GPR expressed at low levels in most tissues with the exception of prostate, placenta, head and neck. In contrast, GPR87 is highly expressed in squamous cell carcinomas (SCC) located in lung, cervix, skin, urinary bladder, testis, head and neck (22). Potential ligands for GPR87 have been suggested, such as UDP-glucose, cysteinyl-leukotrienes and most recently LPA (23). However, natural ligands and physiological roles for GPR87 still remain to be fully determined.

In our recent study, we found that p53 and its family members, p63 and p73 induce GPR87 expression in response to genotoxic stress (24). P53 is a master regulator in the cellular response to various forms of stresses, such as DNA damage, hypoxia, growth factor depletion and heat shock (25). As a sequence-specific transcription factor, the tumor suppressor p53 regulates a plethora of target genes, including p21, FAS, GADD45, Dec1 and its own negative regulator Mdm2 to initiate cell cycle arrest, DNA repair, senescence, apoptosis and other cellular processes (26). To date, only a handful of studies have reported the regulation of GPRs by p53 family members. Indeed, p53 represses the retinoic acid-induced protein 3, an orphan GPR involved in the proliferation of breast cancer cells (27–29). p53 also down-regulates Bradykinin B1 receptor, an important mediator of inflammatory responses upon tissue injury (30,31). In contrast, p53 up-regulates Bradykinin B2 receptor (BKB2R), a developmentally-regulated GPR involved in inflammatory responses as well as sodium excretion, vascular reactivity and cell growth (32). Interestingly, p73 is found to regulate BKB2R during nephrogenesis. Indeed, Δ Np73 isoform represses BKB2R in proliferating nephron precursors, whereas TAp73 isoform activates BKB2R during terminal differentiation of the developing nephron (33). Although regulation of GPRs by p63 has not yet been reported, p63 is involved in the modulation of several CC chemokine ligands, including thymus and activation-regulated chemokine (TARC/CCL17) (34). TARC/CCL17 is a ligand for CC chemokine receptor 4 (CCR4), a major player in the recruitment of specific T cells during the immune response. To our knowledge, GPR87 is the only GPR activated by all three p53 family members in response to DNA damage.

To further explore the physiological function of GPR87, we knocked down GPR87 in both breast and colon cancer cell lines. We found that the loss of GPR87 inhibits cell proliferation in response to DNA damage. This anti-proliferative effect is associated with an increase in p53 stability and transcriptional activity, which sensitizes cells to undergo apoptosis (24). Consistent with our findings, a recent study revealed that GPR87 is required for the proliferation of a SCC cell line (22). Taken together, this suggests that GPR87 plays a survival and anti-apoptotic role and that overexpression of GPR87 is essential for the development and maintenance of tumors. But, how does GPR87 regulate the expression of tumor suppressor p53? To mediate cell survival and proliferation, GPRs activate a plethora of downstream signaling pathways, including Rho GTPases, MAPK, and phosphatidylinositol 3-kinase (PI3K)/Akt pathways (6). Among those, activation of the PI3K/Akt survival pathway has been reported to prevent p53 activation and p53-dependent apoptosis (35,36). Indeed, following activation by PI3K, the serine/threonine kinase Akt phosphorylates and stabilizes the E3 ubiquitin ligase Mdm2, which then mediates the ubiquitination and degradation of p53 (37,38). Consistent with this, we found that the loss of GPR87 triggers a decrease in Akt levels, concomitant with an increase in p53 levels. Therefore, we postulate that the inhibition of Akt upon GPR87 knockdown is responsible for the stabilization and activation of p53 in response to genotoxic stress (Figure 2). Yet, additional signaling pathways may also be involved in GPR87-mediated survival and will need to be further explored in the future.

Cancer therapeutic agents usually act by inducing genotoxic stress in tumor cells. However, resistance to chemotherapy is becoming a major issue in long-term treatment of cancer patients. In this context, targeting GPR87 signaling pathway may provide new exciting approaches for cancer management. A major approach for future therapies would be to target GPR87 ligand binding capacity. To do this, studies are required to determine the *in vivo* structure of GPR87 and its natural ligand(s). These data will then be used for the identification and development of GPR87 antagonists using high throughput screenings. We anticipate that in subsequent studies, some GPR87 antagonists will be effective in activating the p53 pathway to induce antiproliferation and apoptosis. Because GPR87 is highly expressed in SCC, those tumors will be a first choice to further determine the therapeutic value of GPR87 antagonists. It will also be interesting to further confirm that LPA is a true *in vivo* ligand for GPR87. LPA is a binding ligand for several GPRs, such as LPA1-3, to promote proliferation, invasiveness, and angiogenesis in ovarian and other cancers types. Studies have reported that LPA signaling increases the proteasomal degradation of p53, resulting in a decreased p53 transcriptional activity in a lung carcinoma cell line (39). Similarly, LPA agonists induce cell proliferation and prevent apoptosis by decreasing p53 levels and transcriptional activity in chondrocytes (40). More importantly, treatment with the LPA1/3 antagonist VPC32183(S) prevents the effects of LPA and its agonists. Thus, selective LPA-GPR87 antagonists may also have a high therapeutic potential for the treatment of human cancers. Additional approaches will be to target mediators of GPR87 intracellular signaling pathways, such as yet-unknown G protein(s) coupled to GPR87, PI3K, Akt, and other downstream effectors. Further efforts should also be directed to completely elucidate GPR87 intracellular signaling as this will warrant the identification of other specific targets for cancer therapies.

Studies by us and others strongly support a role for GPR87 as a survival factor, and p53 regulator as well as its use as a target for anticancer drug discovery. Extensive studies are required to completely decipher GPR87 biology and provide the basis for designing therapeutic strategies to treat and cure human cancers. We believe that inhibition of GPR87 signaling pathway alone or in combination with known therapeutic agents, will increase the efficiency of current therapies and prevent the development of chemoresistance. Finally, as more data linking GPRs and p53 signaling networks emerge, there is no doubt that GPR87 and its associated factors will become increasingly used as cancer therapeutic agents.

Acknowledgments

We apologize to all authors whose work could not be cited due to space limitation. We also regret that we cited some reviews instead of the original reports due to the nature of this article. Work from the authors is supported by the National Institute of Health (CA076069 and CA102188).

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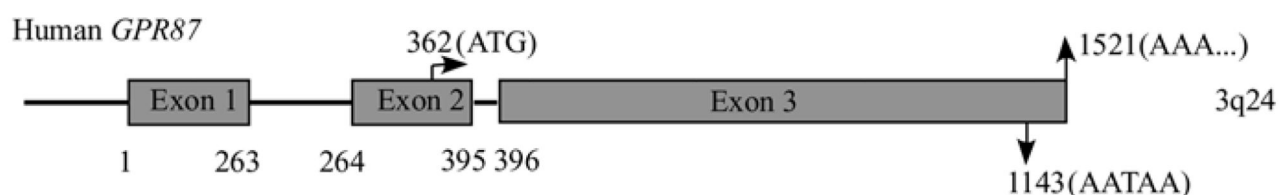
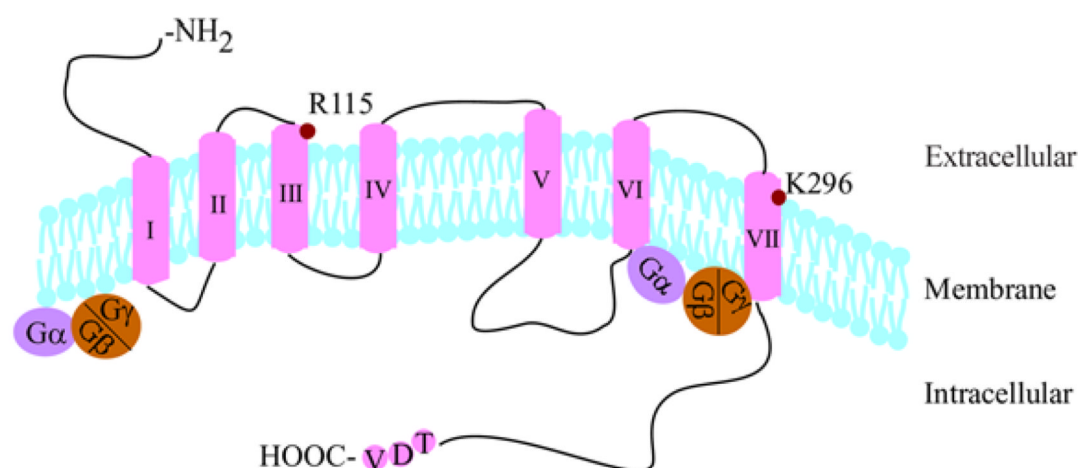
A**B**

Figure 1. Schematic representation of GPR87 genomic and protein structures

(A) Human GPR87 gene is located on chromosome 3q24. GPR87 is composed of a start ATG within a Kozak consensus sequence, an open reading frame (ORF) of 1077 bp, and a putative poly (A) signal AATAAA 40 bp located downstream the stop codon (20). **(B) Human GPR87 protein contains 358 AA arranged in a seven transmembrane (7TM) topology.** It is comprised of an extracellular N terminus, seven helices, three intracellular loops, three extracellular loops and an intracellular C terminus. The third intracellular loop and the C terminus might be important in G protein coupling. As a special feature, the C terminus of GPR87 bears consensus site S/T-X-V (T356, D357, V358) for binding of PDZ domain-containing proteins (20). LPA binding is suggested to involve residue 115 in TM3 and K296 in TM7 (23).

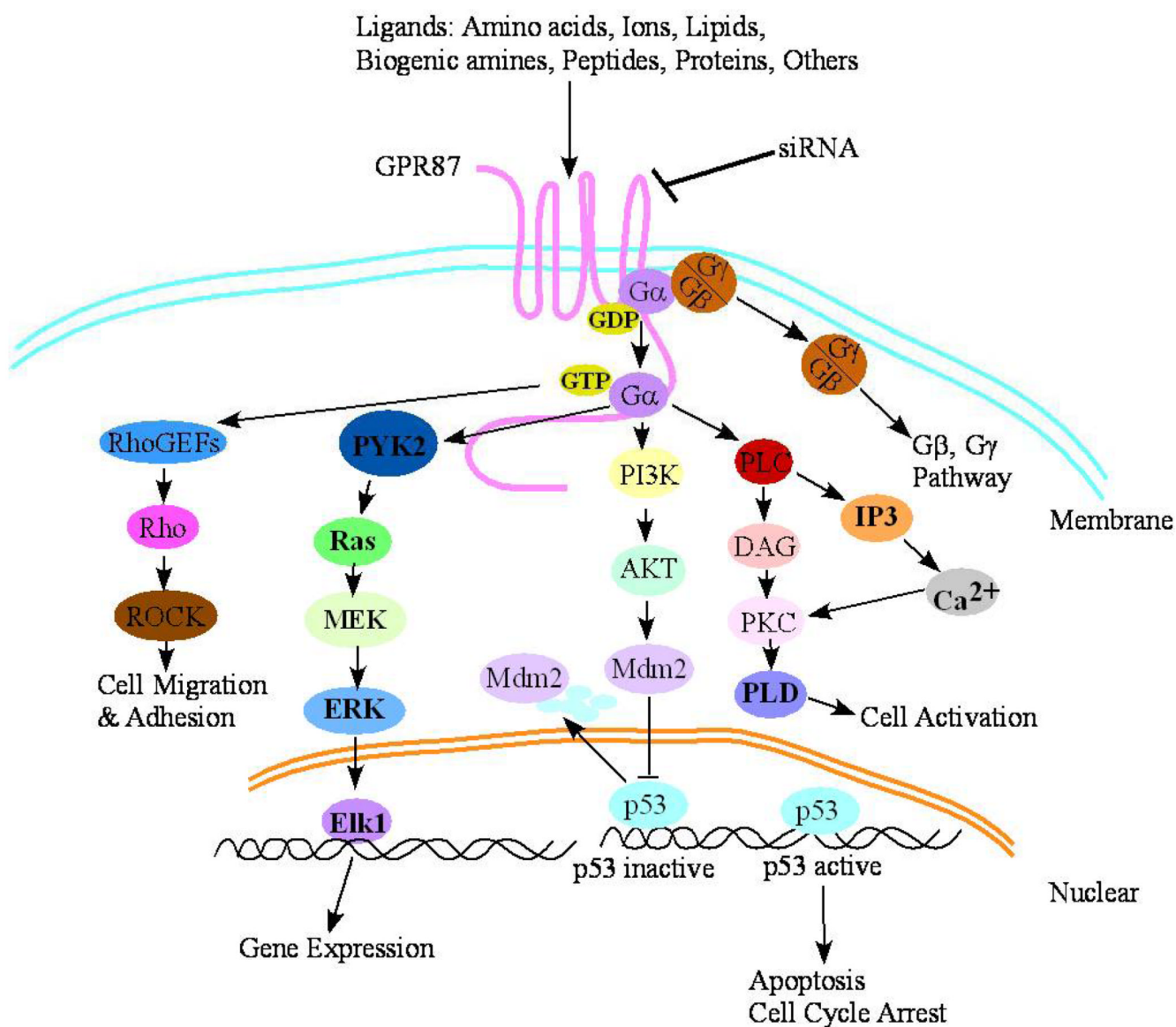


Figure 2. Putative GPR87 signalling pathway. Various ligands bind to GPR87 on the cell membrane

This binding results in conformation changes of the receptor, which induces the binding of GTP to G protein α subunit and the dissociation of α subunit from $G\beta$ and $G\gamma$ subunits. The activation of G protein induces downstream signalling pathways, such as PLC, DAG, PKA and PKC. $G\alpha$ subunit triggers the induction of effector molecule PI3K/Akt survival pathway, which in turn down-regulates apoptotic factors (BAD, caspase-9 and XIAP) and phosphorylates Mdm2, the major inhibitor of p53. Importantly, this phosphorylation enhances the nuclear accumulation of Mdm2, which then inhibits p53 activity and destabilizes p53 protein to promote cell survival. Upon GPR87 knockdown, PI3K/AKT survival pathway become inactive and leads to p53 stabilization and transcriptional activity to mediate anti-proliferation and apoptotic responses. Other potential prosurvival pathways activated by GPR87 are MAPK pathway and PLC/PKC pathways. **Abbreviations:** $G\alpha$, the α subunit of G proteins; PLC, phospholipase C; DAG, diacylglycerol; PKC, protein kinase C; PLD, phospholipase D; IP3, inositol 1,4,5-trisphosphate; PI3K, phosphoinositide 3-kinase;

PYK2, prolinerich tyrosine kinase 2; MEK, MAP kinase kinase; ERK, Extracellular Signal-Regulated Kinase; Elk2, a Elk1, member of ETS oncogene family; Rho, Ras homolog gene family; RhoGEFs, Rho GTP exchange factors; ROCK, Rho-associated kinase.