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Review Article

The role of the 12(*S*)-HETE/GPR31/12-HETER axis in cancer and ischemia–reperfusion injury

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The G protein-coupled receptors (GPCRs) constitute a large superfamily of seven transmembrane-spanning receptors that are activated by several classes of ligands, including bioactive lipids. GPCRs are attractive therapeutic targets for the treatment of human diseases, as they finely regulate a wide array of cellular functions. In this minireview, we summarized what is currently known about the G protein-coupled receptor GPR31/12-HETER. We highlighted, in particular, its structural similarity with human homologs, the biological functions of its recognized ligand 12(*S*)-hydroxyeicosatetraenoic acid (HETE), an arachidonic acid metabolite, and the role that GPR31/12-HETER-mediated signals play in cancer cell growth, invasion and metastasis, and in liver ischemia–reperfusion (IR) injury. Recent studies shed light and interest on the 12(*S*)-HETE/GPR31/12-HETER-activated signaling pathways and functions. The full spectrum of GPR31/12-HETER-mediated biological functions has yet to be characterized. Further studies are needed to identify other potential ligands, i.e. other than 12(*S*)-HETE. Another important remaining question is whether the multiple 12(*S*)-HETE-induced biological activities, including its role in diabetes, neurodegeneration, neuroprotection, and platelet function, occur via GPR31/12-HETER and/or involve the activation of other receptor molecules and pathways.

Introduction

The G protein-coupled receptor (GPCR) family include a large number of structurally related seven transmembrane-spanning receptors that bind unique or shared ligands that can act as full-, partial-, inverse-agonists, or antagonists. GPCR ligands include hormones, neurotransmitters, bioactive lipids, smell-, taste-, light-, stimuli, biologic amines, and chemokines [1–3]. Many GPCRs are still orphan receptors, in search of ligands, some of them showing constitutive, ligand-independent activity [4]. GPCR activation regulates a multitude of basic cellular functions such as proliferation, apoptosis, migration/chemotaxis, adhesion, contraction, and differentiation. Furthermore, they play a pivotal role in fine-tuning inflammation, cancer, vision, taste, olfaction, cardiovascular and endocrine organ functions. GPCRs are classified in several families, the largest of which is represented by the rhodopsin-like receptor family A, which include adrenergic, angiotensin II, chemokine and bioactive lipid receptors. GPCR activities can be affected by the receptor status, such as oligomerization or heterodimerization, by conformational dynamics, or by subcellular localization [1–3]. GPCR signaling is typically activated by specific ligands that may alter receptor conformation and trigger intracellular pathways that involve heterotrimeric G proteins, GPCR kinases (GRK), and arrestins, and several other effector molecules that relay signals and promote biological activities and receptor turn off. GPCR signaling commonly induces the stimulation of adenylyl cyclase (AC), the production of cyclic AMP (cAMP), inositol triphosphate (IP_3), intracellular Ca^{++} mobilization, the activation of several kinases and enzymes such as phospholipase A and C (PLA/PLC) [1–3,5].

Lipid-binding GPCRs have an important role in cancer, allergy, tissue fibrosis, atherosclerosis, thrombosis, ischemia, and several other diseases and biological processes. Such GPCRs include leukotriene (LT), HETE/oxo-ETE, prostanoid, free fatty acid (FFA), and lysophospholipid, such as

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lysophosphatidic acid (LPA), receptors [6–9]. Lipid-binding GPCRs trigger multiple intracellular signaling pathways that activate molecules such as MAPK, Akt, PLA/C, PI3K, Ras, Rho, PKC, as is the case for BLT2-, cysteinyl-LT receptor 1-, OXER1-, and/or LPA-receptor [6–9].

GPR31 was originally cloned and identified by our group as an orphan Family A GPCR [10] and, several years ago, it was shown to bind and signal in response to 12(*S*)-hydroxyeicosatetraenoic acid (HETE); as such it was renamed GPR31/12-HETER [11]. HETEs are lipid mediators, that regulate several biological processes, and whose production is catalyzed, among other enzymes, by lipoxygenases (LO) that act on arachidonic acid (AA), at oxidation sites mostly located at position 5, 12, or 15 of the molecule [12–16]. Platelet-type ALOX12 gene, expressed by several cell types including platelets, keratinocytes, and tumor cells generates 12S-LO, that is the main enzyme responsible for 12(*S*)-HETE production; 12(*S*)-HETE can also be generated by the 12/15-LO enzyme (Figure 1).

Since, in the most recent years, studies on the 12(*S*)-HETE/GPR31/12-HETER axis have opened interesting perspectives in different research fields, we will review what is known on the 12(*S*)-HETE/GPR31/12-HETER axis and highlight its role in cancer and IR injury.

GPR31/12-HETER: phylogenetic tree and gene expression

GPR31/12-HETER is a human seven transmembrane-spanning GPCR, containing 319 amino acids [10,11]. A current NCBI Blastp human protein homology search of the human GPR31/12-HETER protein yielded the highest percentage of protein identity (up to 36%) with hydroxycarboxylic acid receptors (HCAR) 1–3 [17]. HCAR1/GPR81 is a lactate receptor with anti-lipolytic effect in adipose cells and promotes breast cancer cell growth and angiogenesis [18]. HCAR2/GPR109A and HCAR3/HM74/GPR109B are niacin/nicotinic acid receptors; they display antilipolytic activity [17,19] and play a role in skin cancer and epidermal differentiation [20]. A 25–30% amino acid homology was found with several others GPCR including (a) OXER1, which is a 5-oxo-ETE receptor with a role in prostate cancer cell growth and steroidogenesis [21,22]; (b) the GPR20 orphan receptor, which constitutively activates Gi proteins [23]; (c) proteinase-activated receptor (PAR) 4, a regulator of platelet aggregation [24]; (d) members of the chemokine receptor family [25], purinergic receptors [26], and leukotriene B4 (LTB4) receptors/BLTs [27]. A dendrogram, graphically representing the phylogenetic tree, generated following the Blastp homology search, shows that OXER1 and HCAR1–3 are closely related to GPR31 (Figure 2). Protein alignment between GPR31/12-HETER, OXER1, HCAR1, 2, and 3 is shown in Figure 3. GPR31 is very conserved among species and is 61% identical, at the protein level, with Gpr31b, its murine ortholog [31]. Human GPR31 was previously localized at chromosome 6p27 [10], while murine Gpr31b was shown to map to chromosome 17 (NCBI Reference Sequence: NM_001013832.2).

GPR31/12-HETER mRNA expression in multiple normal human tissues is shown in Table 1. This receptor is expressed at low levels in several tissues and is overexpressed by the spleen, small intestine, and testis. GPR31/12-HETER was also shown to be expressed in human endothelial cells (EC), in PC-3 and DU145 prostate cancer cell lines [11]. It is up-regulated in prostate cancer tissues, where it positively correlates with tumor

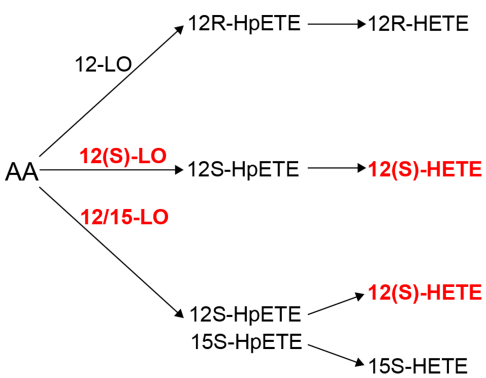

Figure 1. LOs involved in 12(*S*)-HETE generation.

12(*S*)-, 12(R)-, and 15(R)-HETE are formed by the enzymatic activity of LO, with the production of hydroperoxy product intermediates (HpETEs). AA, arachidonic acid.

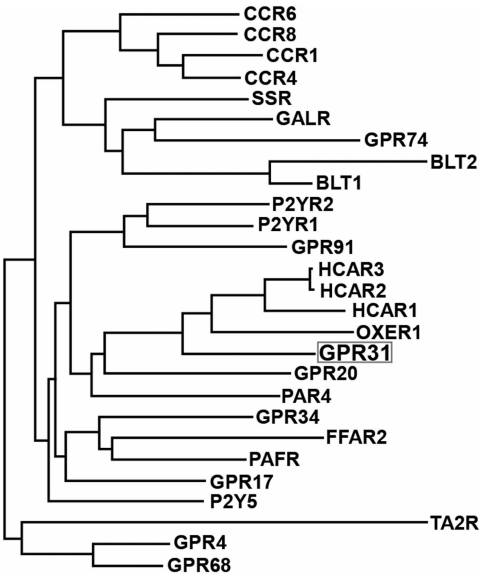

Figure 2. Phylogenetic tree diagram.

An amino acid similarity search to human GPR31, using the Blastp program of the NCBI database, yielded homologous human GPCR sequences utilized to generate a Phylogenetic tree diagram. The latter was obtained using the Phylogeny.fr web service [28] (that integrates various programs such as Muscle, PhyML, and TreeDyn). The Accession numbers of the GPCRs were: GPR31/12-HETER (NP_005290.2) (please note that such accession number replaced, in GenBank, our original submitted sequence (V to L amino acid at position 277); HCAR3/HM74/GPR109B (NP_006009.2); HCAR2/GPR109A (NP_808219.1); HCAR1/GPR81 (NP_115943.1); OXER1 (NP_683765.1); GPR20 (NP_005284.2); GPR17 (AAH31653.1); CCR1 (NP_001286.1); P2YR1 (NP_002554.1); P2YR2/P2Y2 (NP_002555); GPR91/SUCNR1 (NP_149039.2); CCR4 (NP_005499.1); GPR34 (AAD50531.1); CCR6 (NP_004358.2); P2Y5 (AAB62190.1); GALR1 (NP_001471.2); PAFR (AAA60108.1); GPR4 (NP_005273.1); SSR (AAA20828.1); BLT1/LTB4R1/P2Y7 (NP_858043.1); BLT2/LTB4R2 (NP_001158164.1); TBXA2R (NP_001051.1); GPR68/OGR1 (AAI28537.1); FFAR2 (NP_005297.1); PAR4/F2RL3(NP_003941.2); GPR74 (AAK58513.1); CCR8/TER1 (NP_005192.1 CCR8).

Figure 3. Multiple sequence alignment of GPR31, OXER1, HCAR1, 2, and 3 amino acid (aa) sequences using the MUSCLE [29] and MView programs [30], EMBL-EBI services.

Alignment starts at aa 3 (GPR31), aa 81 (OXER1), aa 16 (HCAR2 and HCAR3), and aa 4 (HCAR1). Colors show amino acid identity.

grades [32], in EBV (Epstein–Barr virus)-transformed lymphocytes (GTEx Analysis Release V7, not shown), and, at low levels, in cell lines such as K562, Jurkat, MCF-7, EJ, and Hut78 [10]. Furthermore, it is expressed by lung, colorectal, pancreatic, and bladder carcinoma cells [33,34].

The widespread GPR31/12-HETER expression in almost all normal tissues points at the role of this receptor in yet-to-be-described physiological processes.

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Table 1 GPR31/12-HETER gene expression in normal human tissues

GPR31/12-HETER mRNA EXPRESSION IN HUMAN TISSUES																									
	BM	WB	LN	TH	SP	KI	LI	LU	TY	HE	MU	AR	SM	AD	SI	CO	PA	SK	SG	AG	BR	OV	UT	TE	PR	PL
a)	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
b)					+++										+++									+++		

Data sources: (a) Genecard (Weissman, IL)/Microarray BioGPS; (b) GTEx Analysis release V7. Abbreviations: BM, bone marrow; WB, whole blood; LN, lymph node; TH, thymus; SP, spleen; KI, kidney; LI, liver; LU, lung; TY, thyroid; HE, heart; MU, skeletal muscle; AR, adrenal gland; SM, smooth muscle; SI, small intestine; CO, colon; AD, adipocyte; PA, pancreas; SK, skin; SG, salivary gland; AG, adrenal gland; BR, brain; OV, ovary; UT, uterus; TE, testis; PR, prostate; PL, placenta; –, not expressed; +, expressed; +++, overexpressed (x5–12).

12(S)-HETE-induced functions and its receptors

12(*S*)-HETE was hypothesized to bind to a GPCR and was shown to play an important role in cancer biology as it promoted cell invasion, adhesion, growth, survival, and metastasis [11,13,32,33] of many cancer cells including prostate, colorectal, epidermoid, breast, and melanoma [34–37], and to bind to Lewis lung carcinoma (LLC) cells [38]. 12-LO/LOX was reported to be expressed in human prostate cancer tissues, and its expression correlated with tumor aggressiveness [39,40]. Furthermore, in prostate cancer cells, 12(*S*)-HETE promoted NF-κB activation [41]. 12(*S*)-HETE/12-LOX was shown to act on EC by increasing vascular endothelial growth factor (VEGF), and metalloproteinase (MMP)-9 expression in prostate cancer cells, the latter via PI3K/Akt/NF-κB signaling [13,16,42]. Of note, a 12-LOX inhibitor impaired basic (b)FGF- and VEGF-induced EC proliferation, VEGF-induced EC migration, tube formation, and bFGF-induced angiogenesis *in vivo*, while 12-LOX overexpression promoted EC migration and tube formation *in vitro* [43].

12(*S*)-HETE was shown to bind to human skin epidermal Langerhans cells [44], and human keratinocytes [45]; and, interestingly, a marked increase in 12-HETEs has been observed in 'involved' vs. 'uninvolved', epidermis of psoriasis patients [46]. Moreover, 12-HETE was shown to have a role in skin cancers, in fact, 12(*S*)-HETE high-affinity binding to murine melanoma cells (B16) induced pertussis toxin-sensitive IP_3 hydrolysis, PKC-α activation, increased cellular adhesion to fibronectin (FN) [37], and melanoma cell motility [47]. 13(*S*)-hydroxyoctadecadienoic acid (HODE), derived from linoleic acid metabolism, was previously described as a putative 12(*S*)-HETE antagonist as it inhibited 12(*S*)-HETE binding to B16 melanoma cells, and the 12(*S*)-HETE-induced functional effects in these cells [37]. Furthermore, 12-HETE levels were shown to be strongly increased in human actinic keratosis and squamous cell carcinoma compared with nonlesional skin [48]. Previous studies in an epidermoid carcinoma cell line showed that 12(*S*)-HETE bound to such cells and activated multiple key signaling molecules such as ERK1/2, phospholipase Cγ1, PKCα, Shc, Grb2, and Ras, possibly via a GPCR [35].

Interestingly, 12-HETE was shown to have a role in diabetes as it inhibited glucose-stimulated insulin secretion and induced pancreatic islet cell death [13,49,50]; 12/15-LO induced insulin resistance on a high-fat diet [51] and ALOX12-KO mice were shown to be resistant to develop streptozotocin-induced diabetes [52].

The effect that 12-LO or 12(*S*)-HETE have on platelets is dual, with both positive and negative effects on platelet function/aggregation [53–55].

12(*S*)-HETE was proposed to have a role in neurodegeneration and Alzheimer's disease, as it induced c-Jun-dependent neuronal apoptosis and as 12-LOX inhibition resulted in impaired β-amyloid-induced apoptosis of cortical neurons [56]. On the contrary, 12(*S*)-HETE was shown to have a role in neuroprotection as it attenuated rat cortical neuron glutamate-induced cytotoxicity, via a pertussis toxin-sensitive mechanism [57], and as 12(*S*)-HETE infusion promoted neuroprotection in a rat model of brain ischemia [58].

12(*S*)-HETE-binding sites on LLC cells had a cytosolic-nuclear localization and were constituted by a 50 kDa protein, that interacted with steroid receptor co-activator-1, in the presence of 12(*S*)-HETE, and that were part of a high molecular mass complex of ∼650 kDa with heat shock protein hsp70 and hsp90 [59].

The identification of putative or bona fide receptors for this ligand [BLT2, thromboxane A2, and peroxisome proliferator-activated receptors (PPARγ), and the high-affinity receptor GPR31/12-HETER] has been reported by means of binding studies, ligand-induced intracellular calcium mobilization, chemotaxis experiments, specific receptor antagonist binding, or the use of cellular transfectants. Noteworthy, in contrast with the studies

that clearly identified GPR31/12-HETER as a 12(*S*)-HETE receptor, the experimental evidence that thromboxane A2 and PPARγ receptors may be 12(*S*)-HETE receptors is weak and still needs further investigation.

BLT2

12(*S*)-HETE was previously shown to act as an agonist of the low-affinity LTB4 receptor, i.e. BLT2, by inducing, at high ligand concentrations, intracellular calcium mobilization and chemotaxis of BLT2 transfectants. Furthermore, 12(*S*)-HETE competed with the binding of LTB4 to BLT2 [60]. Of note, 12(*S*)-HETE induced an itch-associated scratch response in mice that was in part mediated via BLT2, as demonstrated by treatment with a specific receptor antagonist [61]. In addition, 12(*S*)-HETE protected PC3 prostate cancer cells from anoikis and further decreased anoikis in detached normal epithelial prostate BLT2-overexpressing cell transfectants [62].

Thromboxane A2 receptor

The endothelial 12(*S*)-HETE-induced vasorelaxation in mouse mesenteric arteries treated with thromboxane agonists was shown to occur via thromboxane A2 (TP) receptor inhibition by 12(*S*)-HETE as it displaced the binding of a TP antagonist to mouse platelets, and inhibited intracellular Ca^{++} mobilization induced by a TP agonist in TP-overexpressing host cells [63]. 12(*S*)-HETE inhibited both the binding of a thromboxane-mimetic to the TXA2/PGH2 receptor in human platelets and thromboxane-mimetic-induced platelet aggregation [53]; moreover, it inhibited prostaglandin H2 (PGH2)-induced platelet aggregation [54]. In contrast, the inhibition of platelet 12(*S*)-LOX impaired FcγRIIa-mediated platelet aggregation, similarly to what was observed in 12-LOX-deficient platelets, but no data are yet available on the specific molecular mechanisms involved in this process [55].

PPARγ

12-HETE and other 12/15-LO-derived metabolites have been suggested to be ligands and/or activators of PPARγ, regulating zygote implantation in the uterus [64]. Furthermore, 12(*S*)-HETE was shown to induce PPARγ expression and activity in rat cortical neurons, inhibited the PPARγ-mediated COX-2, iNOS and NF-κB up-regulation exerted by brain ischemia and promoted *in vivo* brain neuroprotection from ischemia [58].

GPR31/12-HETER

GPR31 was found to be a 12(*S*)-HETE high-affinity receptor (K_d = 4.8 nM) and renamed it 12-HETER [11] and, later, 12-HETER1/GPR31 [32]. Briefly, 12(*S*)-HETE specifically bound to GPR31-transfected CHO cells, while no high-affinity binding was observed for the 12(R)-HETE stereoisomer. Furthermore, 12(*S*)-HETE binding was not displaced by many eicosanoids such as LTB4, 15(S)-HETE, LTB4, and few prostaglandins, and 12(*S*)-HETE binding to GPR31 transfectants stimulated GTPγS coupling. In host cellular transfectants, 12(*S*)-HETE could activate ERK 1/2, MEK, and NF-κB via GPR31/12-HETER, while no intracellular Ca^{++} mobilization was observed. The authors also showed that 12(*S*)-HETE bound to BLT2, although with a lower affinity. It cannot be definitively excluded, but it would need further characterization, that 12(*S*)-HETE may also bind to 5-oxo-ETE receptor OXER1 and to the structurally related HM74/HCAR3, as a small increase in specific binding in host cells was observed in CHO cell transfectants, although no NF-κB activation was observed [11]; further studies are needed to clarify such issue.

Very recently, it has been shown that either lactate or pyruvate induced (a) GPR31-dependent functional effects on chemokine receptor $CX3CR1^+$ intestinal cells (see section below); (b) increased intracellular cAMP levels of GPR31-overexpressing cellular transfectants and it was shown that the chemokine CX3CL1, that binds to the chemokine receptor CX3CR1, increased GPR31b expression of intestinal $CX3CR1^+$ cells [65].

As the finding that 12(*S*)-HETE is a high-affinity ligand for GPR31/12-HETER is relatively recent, only few studies have analyzed the role of such receptor in 12(*S*)-HETE-mediated functions in both *in vitro* studies and in disease, in particular in cancer and in IR injury, as described below in the following sections. Therefore, for all the above-mentioned 12(*S*)-HETE-induced functions, even if putative receptors have been shown to be involved, the role of GPR31/12-HETER-induced signaling pathways and potential cross-talk with the other receptors (and ligands) has still to be investigated.

The recent findings that lactate and pyruvate activate GPR31 signaling and the interplay shown with CX3CR1 and its ligand are very interesting and require further characterization also to address a putative role of 12(*S*)-HETE in this regulation.

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GPR31/12-HETER-mediated functions

12(*S*)-HETE/GPR31/12-HETER in cancer

Interestingly, 12(*S*)-HETE stimulated prostate cancer cell invasion *in vitro*, in a Matrigel assay, via GPR31/12-HETER, and MEK activation, as demonstrated by the use of GPR31-silenced vs. control cells [11]. GPR31/12-HETER is up-regulated in prostate cancer and such up-regulation correlated with progression and stage of prostate tumors, also by means of data mining analysis. Furthermore, GPR31 silencing impaired metastatic prostate cancer cell tumorigenesis in soft agar colony-forming cells and inhibited the tumor growth, *in vivo*, of subcutaneously implanted GPR31-silenced prostate cancer cells [32].

Very recently, it has been found that GPR31 expression is an independent predictive factor of colorectal cancer patient survival, as GPR31 overexpression is associated with poorer survival in such patients [34].

GPR31/12-HETER: a putative role as a molecular chaperone for KRAS

One of the key players regulating cell growth is represented by the small GTPase Kirsten RAS homolog (KRAS), which is often mutated in cancer cells of different origins such as pancreas, colon–rectum, and lung [66]. The KRAS splice variant KRAS4B is a farnesylated protein, that requires to be localized to the plasma membrane to exert its growth regulatory functions [33,67]. Very interestingly, it has recently been reported that GPR31 associates with KRAS4B, as it colocalizes with the latter, and is coimmunoprecipitated with farnesylated, or alternatively prenylated, KRAS4B [33]. The authors have shown that (a) either silencing GPR31 or forcing GPR31 to be retained in the endoplasmic reticulum, or (b) overexpressing GPR31, reduced or promoted, respectively, membrane KRAS4B expression on different types of host cells, thus suggesting a role for GPR31 as a 'trafficking chaperone' [33,68]. Noteworthy, 12(*S*)-HETE treatment did not affect either KRASB membrane association or ERK activation. Furthermore, GPR31 silencing of lung and bladder carcinoma cells slowed tumor cell growth and KRAS-promoted macropinocytosis (MP) [33]. Of note, binding of Harvey RAS homolog (HRAS) or neuroblastoma RAS homolog (NRAS) to GPR31 can also occur, although to a lesser extent [33].

The 12(*S*)-HETE/GPR31/12-HETER axis induces lymph endothelial barrier defects

Vascular ECs constitute the inner part of both arteries and veins of different caliber and structure and exhibit distinct patterns of gene expression. ECs play a crucial role in the traffic of blood components as well as in transendothelial migration of blood cells. Furthermore, they also express several genes affecting a variety of functions including lipid metabolism and transport [69,70]. Lymphatic endothelial cells (LEC) constitute a very permeable monolayer of cells with roles in tissue pressure homeostasis and cell transport toward lymph nodes for fine regulation of immune response and, in cancer, for lymphatic metastasis [69].

12(*S*)-HETE secretion by tumor cells, and/or the addition of this ligand to EC monolayers, plays an important role in cell invasion, not only by inducing cell adhesion to ECs but also by inducing reversible PKC-dependent EC retraction by cytoskeletal rearrangement [39,71,72]. Moreover, 12(*S*)-HETE, secreted by MCF-7 breast cancer cells, was shown to cause retraction of human LEC monolayers and was involved in the formation of circular chemorepellent-induced defects (CCID), thus favoring both tumor cell invasion of human mammary carcinoma cells and lymph node metastasis. The 12(*S*)-HETE-induced VE-cadherin down-regulation was hypothesized to be one of the mechanisms leading to CCID formation, possibly via NF-κB and ZEB1 signaling [73,74].

More recently, it has been shown that 12(*S*)-HETE, which is produced by breast cancer cells, activates/phosphorylates myosin light chain 2 (MLC2), a regulator of F-actin stress fibers and migration. MLC2 activation was shown to be a key player involved in LEC retraction [75]. Noteworthy, 12(*S*)-HETE-induced MLC2 phosphorylation/activity and CCID formation were GPR31/12-HETER-mediated, as demonstrated by GPR31/12-HETER silencing studies in LEC. The signaling molecules RHO and ROCK were shown to be crucial for MLC2 phosphorylation and CCID formation. It has been hypothesized that are involved in 12(*S*)-HETE/GPR31/12-HETER-mediated LEC retraction and CCID formation [75].

Overall, the above results point at a role of 12(*S*)-HETE/GPR31/12-HETER in cancer growth, invasion, and tumorigenesis both *in vitro* and *in vivo*. Furthermore, GPR31/12-HETER overexpression correlated with tumor progression and poorer patient survival, at least in prostate and colorectal cancers. Moreover, GRP31/12-HETER was shown to have a role in promoting KRAS localization to the plasma membrane, tumor growth and MP, see Table 2 and Figure 4.

Table 2 The 12(*S*)-HETE/GPR31/12-HETER axis in cancer

Activities	Signaling	References
12(*S*)-HETE stimulated prostate cancer cell Matrigel invasion *in vitro* via 12-HETER/GPR31	GPR31-dependent MEK activation in prostate cancer cells 12(*S*)-HETE induces a GPR31-dependent ERK-1/2 and NF-κB activation	[11]
GPR31/12-HETER silencing impaired metastatic prostate cancer cell tumorigenesis in soft agar, and inhibited prostate cancer cell growth, *in vivo*		[32]
GPR31/12-HETER overexpression is associated with poorer survival in colorectal cancer patients		[34]
GPR31 association with KRAS4B promoted membrane KRAS4B expression, acting as a putative 'trafficking chaperone'. 12(*S*)-HETE treatment had no effect on KRASB membrane association. GPR31 silencing of lung and bladder carcinoma cells slowed tumor cell growth and KRAS-promoted MP	12(*S*)-HETE treatment had no effect on ERK activation. RAS/RAC1 signaling is involved in MP	[33]
The 12(*S*)-HETE/GPR31/12-HETER axis induced lymph endothelial barrier defects, i.e. LEC retraction and CCID formation, favoring tumor cell invasion and lymph node metastasis of human breast cancer cells	12(*S*)-HETE/GPR31/12-HETER-mediated LEC retraction and CCID formation involved RHO- and ROCK-dependent MLC2 phosphorylation	[73]

12(*S*)-HETE/GPR31/12-HETER in IR injury

Reperfusion of ischemic tissues may induce tissue injury, i.e. IR injury, with massive cell damage, in organs such as heart, brain, lung, liver, and kidney subjected to blood flow deprivation, as is the case of myocardial infarction, stroke, shock, organ transplantation, or cardiopulmonary bypass. The mechanisms underlying IR injury include oxidative stress and a systemic and local inflammatory response. Ischemic preconditioning

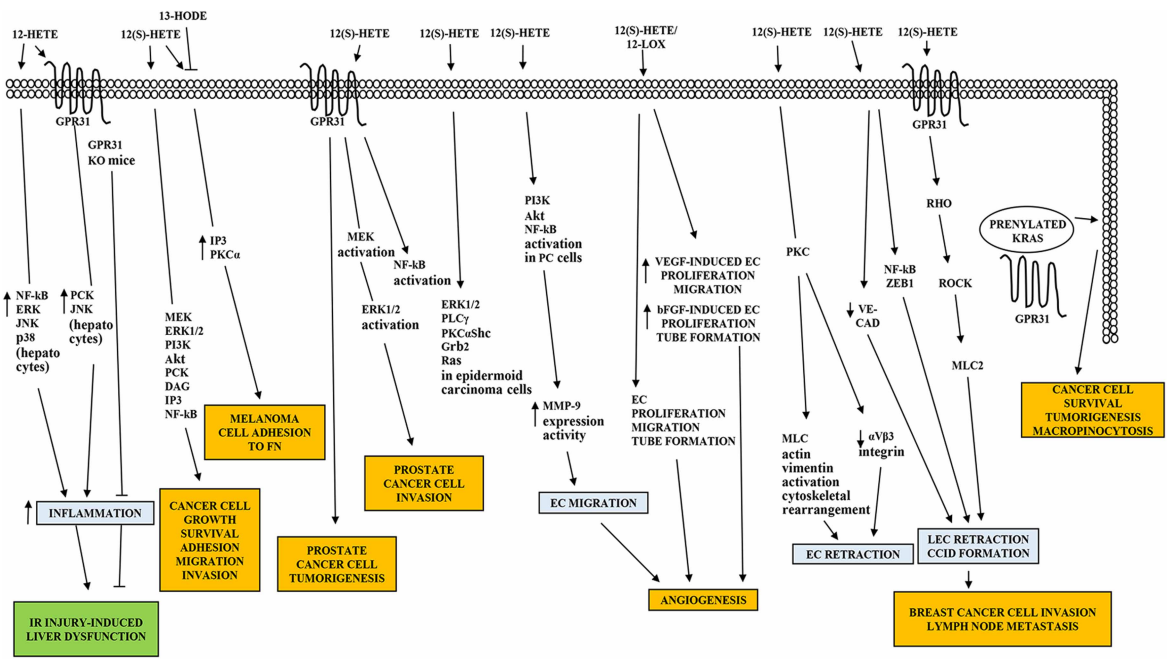

Figure 4. Biological functions and molecular targets of 12(*S*)-HETE/GPR31/12-HETER in cancer and IR injury.

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(including hypoxic- and acidic-), and preconditioning with molecules such as adenosine and chemokines, has been shown to protect tissues from subsequent ischemic conditions by adaptative responses at both the cellular and systemic level [76–78].

12-LO and/or its metabolites have a known role in the IR injury of different tissues. 12-HETE increases K^+ and decreases Ca^{2+} channel activities and treatment with LO inhibitors impairs the response to preconditioning of ischemic heart [79]. 12-LO-deficient mice showed impaired ischemic preconditioning-induced cardioprotection and an impaired increase in 12(*S*)-HETE in WT heart following preconditioning [80]. Furthermore, it was shown that the ALOX12/TRPV1 receptor/PKC pathway, activated by 12(*S*)-HETE, protected the heart from IR injury following hypoxic preconditioning and that 12-HETE/12(*S*)-HETE production occurred during cardiac ischemic preconditioning [81]. Noteworthy, 12/15-LOX and 12-HETE levels increased significantly in cerebral IR injury, with activation of PPARγ [82].

ALOX12-GPR31/12-HETER signaling in hepatic IR injury

Liver ischemia–reperfusion (IR) injury may occur following liver surgery and can lead to liver dysfunction. Very interestingly, Zhang et al. [83] demonstrated that ALOX12/12-HETE/GPR31 signaling plays a key role in IR injury the liver. They reported that ALOX12 expression is induced in the liver during IR, and leads to increased expression of 12-HETE, *in vitro*, in hepatocytes. Furthermore, ALOX12-KO or hepatocyte-specific ALOX12-TG mice following IR (60 min ischemia and 6 h reperfusion), showed a decreased or increased expression of 12-HETE *in vivo*, respectively, correlating with lower or higher liver dysfunction vs. control mice. Interestingly, following IR injury, ALOX12-KO mice showed a decreased expression of inflammatory molecules, including cytokines and chemokines, and a decreased hepatic activation of the p65 NF-κB subunit. Accordingly, in ALOX12-overexpressing primary hepatocytes, increased p65 NF-κB, ERK, JNK, and p38 phosphorylation was observed, similarly to the effect exerted by 12-HETE on primary hepatocytes [83,84]. Interestingly, GPR31/12-HETER silencing inhibited the 12-HETE-induced activation of JNK and PKC and of inflammatory responses in hepatic cell lines. Furthermore, in Gpr31b-KO mice, liver dysfunction and inflammation were strongly inhibited following IR injury when compared with control mice. The role of ALOX12/12-HETE in liver IR injury has also been clearly demonstrated by the use of selective inhibitors and different animal models [83].

GPR31/12-HETER in dendrite protrusion of intestinal CX3CR1$^+$ cells

Very recently, GPR31 has been shown to be a key molecule regulating dendrite protrusion of small intestinal CX3CR1$^+$ mononuclear cells for the uptake of luminal antigens [65]. In particular, it has been reported that orally administered lactic and pyruvic acid induced a GPR31-dependent dendrite protrusion of CX3CR1$^+$ intestinal cells and a positive regulation of immune responses in WT mice, as such responses were inhibited in Gpr31b$^{-/-}$ mice. Furthermore, lactate and pyruvate induced a GPR31-dependent increase in cytosolic cAMP levels. Such findings are very interesting not only for the newly discovered GPR31-mediated functions but as it point at yet-to-be-identified molecular mechanisms underlying such activities, that include a putative cross-talk between lactate receptors, GPR31 and CX3CR1. Of note, the role of 12(*S*)-HETE/GPR31 axis has not yet been investigated in those studies [65].

Conclusions and perspectives

The 12-LO/12(*S*)-HETE axis has been in the past years the focus of several studies in physiologic and diseased conditions. The identification of GPR31 as a high-affinity 12(*S*)-HETE receptor (12-HETER) has opened new promising and developing fields of research studies with exciting perspectives in cancer biology and tumorigenesis, as well as in IR injury (Table 2 and Figure 4). Studies that characterized GPR31/12-HETER as a key player and a putative molecular chaperone involved into KRAS plasma membrane localization and function suggest a potential role of such receptor in proliferation and cell migration, as well as tumorigenesis, of several other mutated KRAS cancer cell types, in addition to the functions shown in prostate, lung, bladder, and colorectal cancer. Besides the role shown by 12(*S*)-HETE/GPR31/12-HETER in prostate cancer progression and in the poor prognosis of colorectal cancer, it is possible that the activities shown by 12(*S*)-HETE in cancers such as melanoma, epidermoid carcinoma, and breast cancer, might be, at least in part, GPR31/12-HETER-mediated. The role of the 12(*S*)-HETE/GPR31/12-HETER axis in lymph endothelial barrier breaching further points at this axis as an important player in tumor cancer cell invasion and metastasis.

The 12-LO and/or its metabolites affect the response to ischemia and to IR in different tissues. 12-LO modulates ischemic/hypoxic preconditioning-induced cardioprotection and protection from heart IR injury.

Moreover, 12-LO metabolites were shown to have a role in neuroprotection of ischemic brain and 12/15-LOX and 12-HETE increased significantly in cerebral IR injury. As the 12-LO cascade is an important modulator of IR injury, and as the ALOX12-GPR31/12-HETER signaling axis has a role in IR-induced liver damage, the 12(*S*)-HETE/GPR31/12-HETER axis may be involved in the response of tissues, other than the liver, to IR injury.

Furthermore, it is possible that the GPR31/12-HETER axis may play a role in diabetes, neurodegeneration, neuroprotection, and Alzheimer's disease, and in the regulation of platelet function, if, in future studies, it will be demonstrated that 12(*S*)-HETE exerts such regulatory functions via GPR31/12-HETER.

Due to the complexity of the LO system, including the formation of several metabolites, and the evidence that 12(*S*)-HETE can bind to different bona fide or putative receptors, such GPR31/12-HETER, BLT2, PPARγ, or TPα receptors, more studies are needed to better characterize the 12(*S*)-HETE receptors and the 12(*S*)-HETE-induced signaling pathways.

The role that GPR31 plays in lactic and pyruvic acid-induced regulation of dendrite protrusion of intestinal $CX3CR1^{+}$ cells and of immune responses, and the up-regulation of Gpr31b expression exerted by CX3CL1, suggest putative cross-talks between lactate receptors, GPR31, CX3CR1 or, possibly, other chemokine receptors. It still remains to be assessed the potential contribution of 12(*S*)-HETE in the intestinal GPR31-mediated functions.

GPR31/HETER targeting, by selective drugs, will, therefore, constitute an interesting area for future drug development for clinical application in different diseases.

Abbreviations

AA, arachidonic acid; AC, adenylyl cyclase; cAMP, cyclic AMP; bFGF, basic fibroblast growth factor; CCID, circular chemorepellent-induced defects; COX, cyclooxygenase; EC, endothelial cell; ERK, extracellular signal-regulated kinase; FFA, free fatty acid; GRB2, growth factor receptor-bound protein 2; GPCR, G protein-coupled receptor; GRK, GPCR kinases; HCAR, hydroxycarboxylic acid receptor; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; HpETE, hydroperoxy product intermediate; HRAS, Harvey RAS homolog; JNK, c-Jun-N-terminal kinase; KO, knock-out; KRAS, Kirsten RAS homolog; IP, inositol triphosphate; IR, ischemia–reperfusion; LEC, lymphatic endothelial cell; LLC, Lewis lung carcinoma; LO/LOX, lipoxygenase; LPA, lysophosphatidic acid; LTB4, leukotriene B4 receptor 2; MEK, mitogen-activated protein kinase; MLC, myosin light chain; MMP, metalloproteinase; MP, macropinocytosis; NF-κB, nuclear factor kappa B; NOS, nitric oxide synthase; NRAS, neuroblastoma RAS homolog; PAR, proteinase-activated receptor; PKA, protein kinase A; PKC, protein kinase C; PLA/PLC, phospholipase A/C; PPAR, peroxisome proliferator-activated receptor; ROCK, RHO-associated protein kinase; TG, transgenic; TP/TXA2, thromboxane A2 receptor; TRPV, transient receptor potential vanilloid type; VEGF, vascular endothelial growth factor; ZEB, zinc finger E-box-binding homeobox.

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Competing Interests

The Author declares that there are no competing interests associated with this manuscript.

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