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Nutritional and metabolic signalling through GPCRs

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Deregulated metabolism is a well-known feature of several challenging diseases, including diabetes, obesity and cancer. Besides their important role as intracellular bioenergetic molecules, dietary nutrients and metabolic intermediates are released in the extracellular environment. As such, they may achieve unconventional roles as hormone-like molecules by activating cell surface G-protein-coupled receptors (GPCRs) that regulate several pathophysiological processes. In this review, we provide an insight into the role of lactate, succinate, fatty acids, amino acids as well as ketogenesis-derived and β-oxidation-derived intermediates as extracellular signalling molecules. Moreover, the mechanisms by which their cognate metabolite-sensing GPCRs integrate nutritional and metabolic signals with specific intracellular pathways will be described. A better comprehension of these aspects is of fundamental importance to identify GPCRs as novel druggable targets.

Keywords: extracellular signalling molecules; G-protein-coupled receptors; lactate; metabolites; nutrients; succinate

With regard to cellular, tissue and systemic functions, nutrients and metabolic intermediates are generally considered as either energy sources or building blocks involved in metabolic pathways. Beyond these conventional roles, mounting evidence highlights that many nutrients/metabolites may act as intracellular signalling mediators by regulating the activity of both metabolic and non-metabolic enzymes (*e.g.* prolyl-

Abbreviations
5-HIAA, 5-hydroxyindoleacetic acid; AC, adenylate cyclase; ATM, adipose tissue macrophage; BM, bone marrow; cAMP, cyclic-adenosine monophosphate; CasR, calcium-sensing receptor; COX-2, cyclooxygenase 2; CREB, cAMP-response element-binding protein; DC, dendritic cells; EMT, epithelial–mesenchymal transition; ERK1/2, extracellular signal-regulated kinase; FFAs, free fatty acids; GPCRs, G-protein-coupled receptors; HCARs, hydroxy-carboxylic acid receptors; HIF-1α, hypoxia-inducible factor-1α; HMGB1, high-mobility group box 1 protein; HSCs, hepatic stellate cells; IL-1β, interleukin-1β; IP3, inositol 1,4,5-trisphosphate; JNK, c-Jun N-terminal kinases; KLF4, Kruppel-like factor 4; KYN, kynurenic acid; LC-FFAs, long-chain free fatty acids; LNs, lymph nodes; LPA, lysophosphatidic acid; LPS, lipopolysaccharide; MAPKs, mitogen-activated protein kinases; MCTs, monocarboxylate transporters; MEK1/2, mitogen-activated protein kinase; MHC, major histocompatibility complex; MPs, mononuclear phagocytes; NAFLD, non-alcoholic fatty acid liver disease; NASH, non-alcoholic steatohepatitis; NF-kB, nuclear factor kappa-light- enhancer of activated B cells; NLRP3, NLR family pyrin domain containing 3; NSCs, neural stem cells; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1; PGE2, prostaglandin E2; PHDs, prolyl hydroxylases; pHSL, phosphorylated hormone-sensitive lipase; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PKB/AKT, protein kinase B; PLC, phospholipase C; ROCK, Rho-associated protein kinase; ROS, reactive oxygen species; SC-FFAs, short-chain free fatty acids; SDH, succinate dehydrogenase; SLC13A3/5, solute carrier family 13-member 3/5; STAT3, signal transducer and activator of transcription 3; SUCNR1, succinate receptor 1; T1AM, 3-iodothyronamine; TAAR1, trace amine-associated receptor 1; TAB, (TAK)1-binding proteins; TAK, transforming growth factor β-activated kinase; TCA, tricarboxylic acid cycle; Th, T helper; TME, tumour microenvironment; VEGF, vascular endothelial growth factor; α-SMA, α-smooth muscle actin.

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hydroxylases (PHDs), de-methylases, methyltransferases, de-acetylases, etc.) and/or by establishing feedback loops [1,2]. Moreover, their ability to regulate epigenetic traits and gene expression by acting within the nucleus as substrates for chromatin-modifying enzymes is being recognized, particularly concerning cancer cell metabolic flexibility [3]. Noteworthy, it is becoming increasingly clear that key metabolites may also act as extracellular signalling molecules, similarly to neurotransmitters and hormones, but, at variance with them, only with localized (*i.e.* autocrine/paracrine) effects on specific receptors. These receptors are mainly G-protein-coupled receptors (GPCRs) that trigger signal transduction events upon recognition of and binding to nutrient-derived, microbiota-synthesized or intermediary metabolism-derived molecules. Metabolite-sensing GPCRs activate different intracellular signal transduction pathways depending on their coupling to different G proteins. Indeed, metabolite-activated GPCR signalling can be mediated by G_i, G_q, G_s or $G_{12/13}$ proteins, resulting in the activation/recruitment of a plethora of downstream effector molecules, such as the cyclic adenosine monophosphate (cAMP)-generating enzyme adenylate cyclase (AC), phospholipase C (PLC) or Rho small GTPases respectively. Moreover, most GPCRs also interact with β-arrestins, which ultimately leads to the activation of the mitogen-activated protein kinase (MAPK) pathways. Interestingly, the downstream signalling activated by these GPCRs may differ in a tissue- or cell-specific manner.

In this review, we will focus on nutrients/metabolites, including succinate, lactate, ketogenesis-derived and β-oxidation-derived intermediates, free-fatty acids (FFAs) and amino acids as extracellular signalling molecules. Moreover, we will discuss how activation of their cognate GPCRs, GPR91 (or SUCNR1), hydroxy-carboxylic acid receptor 1 (HCAR1 or GPR81), HCAR2 (or GPR109A), HCAR3 (or GPR109B), short-chain and long-chain FFA receptors (GPR40, GPR43, GPR41, GPR120) and amino acid receptors (calcium-sensing receptor (CasR), trace amine-associated receptor 1 (TAAR1), GPR35 and GPR142), respectively, is associated with certain physiological and pathological scenarios.

GPR91 mediates extracellular succinate-dependent signalling

Succinate is a key metabolic intermediate of the tricarboxylic acid (TCA) cycle, generated from succinyl-CoA by succinyl-CoA synthetase. It acts as a substrate for succinate dehydrogenase (SDH), a crucial link between TCA cycle and the mitochondrial respiratory chain [4]. Mitochondrial metabolic deregulation, anaerobic glycolysis and glutamine-dependent anaplerosis are the major causes of cytosolic succinate accumulation, primarily occurring under stress conditions, such as hypoxia, ischaemia, tumourigenesis and immune cell activation [5–7]. Intracellular succinate metabolism is intertwined with different metabolic pathways, including heme synthesis, ketone bodies utilization, GABA shunt, branched-chain amino acid metabolism and reactive oxygen species (ROS) homeostasis regulation [4]. Moreover, succinate is responsible for protein succinylation at lysine residues, a post-translational modification occurring on both mitochondrial and extramitochondrial proteins, such as histones [8–10]. Elevated succinate acts as an intracellular messenger by inhibiting 2-oxoglutarate-dependent dioxygenases [11]. Among these enzymes are PHDs, which hydroxylate the hypoxia-inducible factor-1α (HIF-1α) leading to its degradation. Succinate-mediated inhibition of PHDs generates a status of pseudo-hypoxia and promotes HIF-1α-dependent gene expression [12]. Moreover, high succinate levels establish a hyper-methylator phenotype by inhibiting 2-oxoglutarate-dependent oxidative de-methylation of histones and DNA, thus affecting epigenetic marks and gene expression [13,14]. Besides its intracellular functions, succinate is released in the extracellular milieu, where it acts as a signalling molecule, resembling hormones or cytokines [13,15]. Given its role as both energy metabolite and signalling molecule, the regulation of intra- and extracellular succinate concentrations is strictly modulated by specific membrane carriers. Succinate is transported from mitochondria to cytosol by dicarboxylate carrier, located in the mitochondrial inner membrane, and the voltage-dependent anion channel, in the mitochondrial outer membrane. Succinate efflux in the extracellular environment is dependent on organic/anion dicarboxylate transporters or monocarboxylate transporters (MCTs) [16,17]. As a signalling molecule, succinate is sensed by its cognate receptor succinate receptor 1 (SUCNR1), belonging to GPCR family, and also known as GPR91 [15,17]. SUCNR1 was deorphanized in 2004 [18], and although it binds several carboxylic acids other than succinate, these agonists display a lower affinity and no physiological relevance. GPR91 is characterized by an extracellular N-terminal region that binds the ligand and can be phosphorylated, and a C-terminal intracellular region, with two N-glycosylation sites. This latter couples with heterotrimeric GTPases, specifically $G_{\alpha q}$, $G_{\alpha i}$ or $G_{\alpha s}$ proteins, thereby sustaining the synthesis of a plethora of intracellular second messengers, with cell-specific mechanisms (Fig. 1A) [17,19,20]. Under steady-state conditions, circulating succinate levels range from 2 to 20 μM, but strongly increase under certain pathophysiological

states, up to 100 μM [5,21,22]. Remarkably, succinate concentrations in the range 20–50 μM induce half-maximal effective response for GPR91, suggesting that the receptor functions as a sensor of homeostasis perturbations [5,18].

Elevated succinate levels have recently been reported during physiological conditions, such as endurance exercise. Indeed, the metabolic signature of exercise-trained muscle is characterized by mitochondrial reprogramming, which includes increased succinate levels, and is correlated with whole-body insulin sensitivity [23]. Selective succinate secretion through pH-sensitive MCT1 in local interstitium and circulation is promoted by succinate transient protonation, occurring during muscle cell acidification. Once secreted, succinate binds to SUCNR1 in non-myofibrillar cells in muscle tissue to control muscle-remodelling transcriptional programmes and regulate muscle innervation, matrix remodelling and strength in response to exercise training (Fig. 2A) [24]. In addition, dietary succinate supplementation, by activating SUCNR1 and its downstream calcium/nuclear factor of activated T-cells signalling pathway, increases endurance exercise ability, myosin heavy-chain I expression, aerobic enzyme activity and mitochondrial biogenesis in mouse skeletal muscle [25].

Remarkably, given its widespread distribution throughout the body, GPR91 is activated in different pathological contexts. It plays key roles in inflammation [26–28], rheumatoid arthritis [29], cardiomyocyte hypertrophy [30] and tumourigenesis [31], but also strongly contributes to metabolic diseases, such as obesity [22,32,33], type 2 diabetes [21], inflammatory bowel disease [34], hepatic fibrosis [35,36] and diabetes-related complications, including renin–angiotensin system activation [37,38], bone dysregulations [39] and proliferative ischaemic retinopathy [40].

During chronic inflammation, succinate is rapidly released in the extracellular compartment where it binds to GPR91-expressing immune cells. GPR91 upregulation in immune populations highlights its important role in inflammatory cellular responses, following tissue damage or pathogen exposure [41]. While succinate is usually released by pro-inflammatory immune cells, GPR91 activation can either elicit pro-inflammatory responses or participate in the natural resolution of inflammation (Fig. 2B). In this scenario, $G_{\alpha q}$ and $G_{\alpha i}$ subunit activation has been associated with pro-inflammatory polarization of myeloid cells [20], while $G_{\alpha s}$ subunit activation results in an anti-inflammatory phenotype [32]. Specifically, in human primary M2 macrophages, physiological extracellular succinate acts through GPR91-activated G_q signalling to regulate the transcription of immune function genes in order to hyperpolarize M2 towards M1 phenotype [20]. In the innate immune system, GPR91 is expressed in dendritic cells (DCs) and mononuclear phagocytes (MPs). An early study demonstrated that lipopolysaccharide (LPS)-activated bone-marrow (BM)-derived macrophages promote succinate accumulation from glutamine-dependent anaplerosis. Intracellular succinate acts as a pro-inflammatory immunometabolite by inhibiting PDH, thus promoting HIF-1α stabilization and consequently activating pro-inflammatory gene expression, including interleukin (IL)-1β production [6]. Interestingly, LPS-mediated macrophage activation supports a strong succinate release in the extracellular milieu, where it stimulates GPR91-expressing macrophages to further increase IL-1β production, maintaining a chronic inflammatory state. In this context, in an *in vivo* model, $SUCNR1^{-/-}$ mice are protected from antigen-induced experimental arthritis [28]. Besides, *in vivo* models of allergic contact dermatitis experience increased reactions in the absence of SUCNR1, related to hyperreactive mast cells in $SUCNR1^{-/-}$ mice, suggesting that succinate receptor may affect mast cell differentiation and maturation capacities [42]. Moreover, SUCNR1 augments LPS-induced production of proinflammatory cytokines in DCs. In this context, GPR91 acts as a chemotactic gradient sensor, which drives SUCNR1 expressing DCs into lymph nodes, strongly contributing to the expansion of pathogenic T helper (Th) 17 cells and leading to the development of experimental antigen-induced arthritis [29]. Furthermore, SUCNR1 is activated following hypoxic ischaemic injury and inflammatory damage leading to central nervous system-intrinsic dysfunction development. Following hypoxia ischaemia, SUCNR1 upregulation in cortical neurons and astrocytes induces pro-angiogenic and inflammatory factor secretion [43]. Similarly, in rodent retinal ganglion cells, SUCNR1 activation leads to increased release of prostaglandin E2 (PGE2) and vascular endothelial growth factor (VEGF), favouring vascularization through extracellular signal-regulated kinase (ERK1/2) (c-Jun N-terminal kinase (JNK)/cyclooxygenase-2 (COX-2) signalling) [44]. Recent evidence highlighted that transplanted neural stem cells (NSCs) ameliorate chronic neuroinflammation in mice with experimental autoimmune encephalomyelitis. *In vitro*, inflammatory MPs release succinate in the cerebrospinal fluid, which activates SUCNR1 on NSCs, with consequent release of PGE2. SUCNR1 signalling in NSCs boosts extracellular succinate uptake by upregulating the expression of two succinate co-transporters solute carrier family 13 member 3 (SLC13A3) and solute carrier family 13 member 5 (SLC13A5). In turn, PGE2 release and succinate

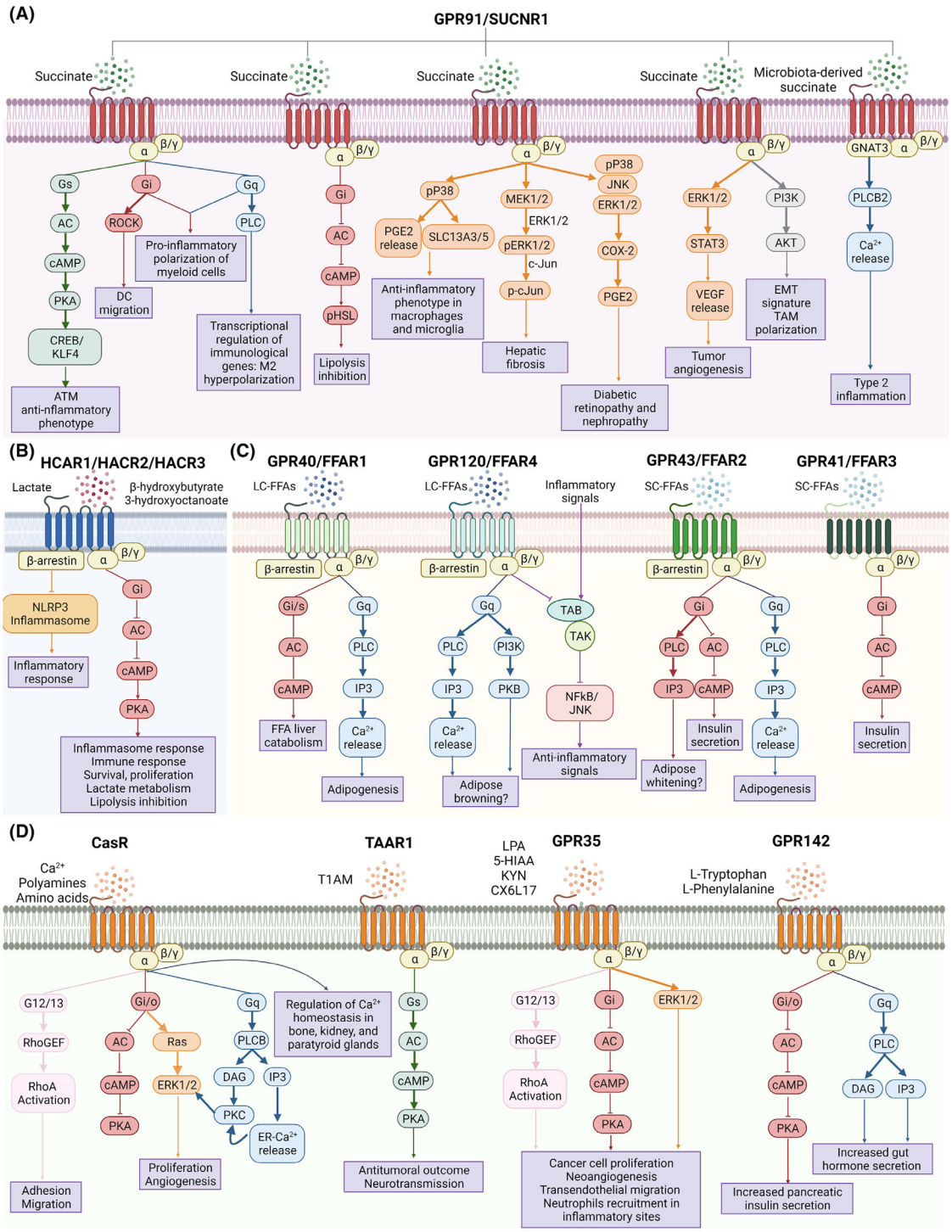

Fig. 1. Intracellular signalling pathways activated by metabolite GPCRs. Metabolite GPCRs display a conserved heptahelical structure, with a N-terminal extracellular region, that binds the ligand, and a C-terminal intracellular region that couples with two protein families (heterotrimeric G proteins and arrestin), thereby sustaining the synthesis of a plethora of intracellular second messengers, with cell-specific mechanisms. Metabolite GPCRs recognize and bind extracellular nutrients/metabolites, including succinate- (A), lactate-, ketogenesis- and β-oxidation-derived metabolites, (B), free fatty acids (C) and amino acids (D), to elicit the activation (bold arrows) or inhibition (lines carrying a perpendicular bar at the end) of various intracellular transduction mechanisms, correlated with several pathophysiological effects (purple boxes). Created with Biorender.com.

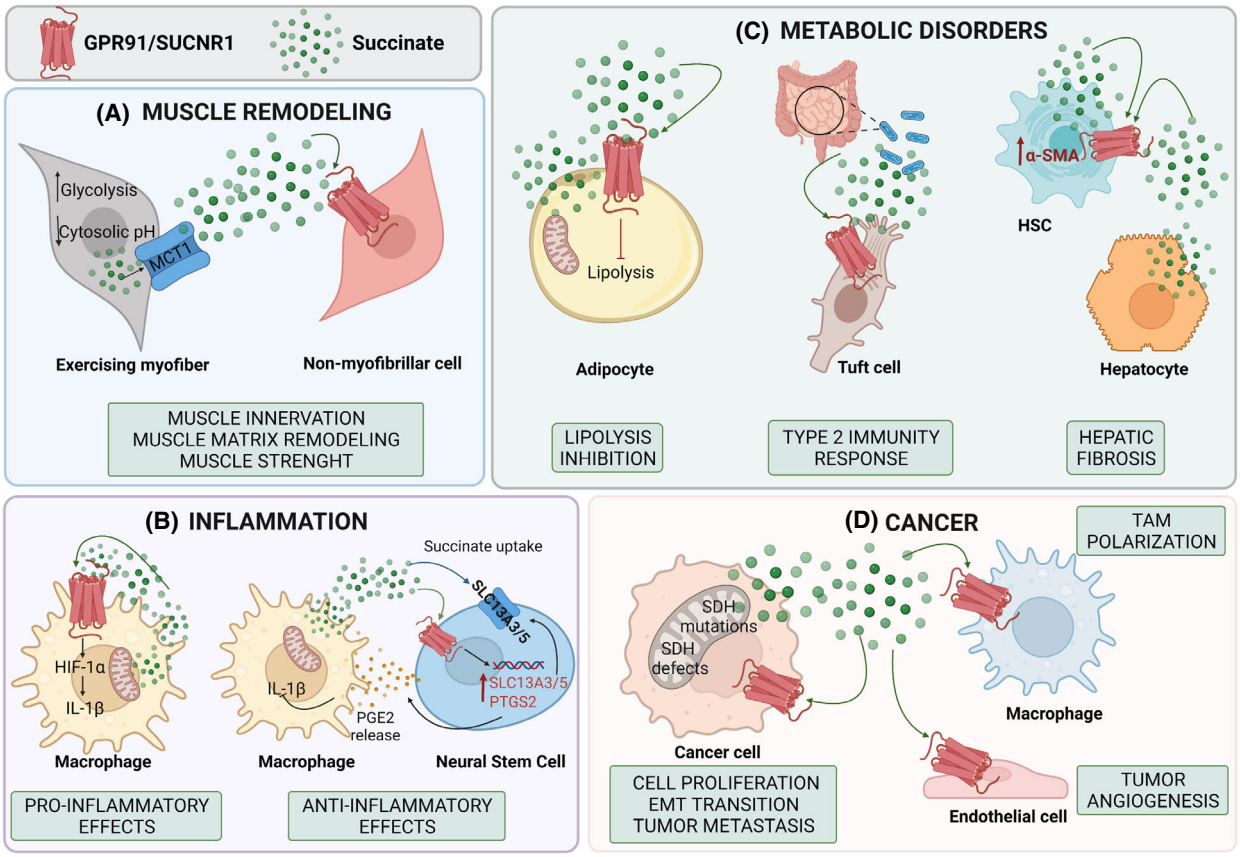

Fig. 2. Succinate regulates pathophysiological processes in muscle, macrophages, adipocytes and cancer cells through GPR91 activation. (A) GPR91 signalling pathway is required for succinate-induced skeletal muscle remodelling. Particularly during exercise, active muscle cells release succinate by pH-regulated MCT1 transport and this paracrine secretion acts on non-myofibrillar cells to control muscle-remodelling transcriptional programmes driving muscle innervation, muscle matrix remodelling and muscle strength. (B) Succinate acts as an inflammatory molecule, particularly on macrophages. In an autocrine manner, GPR91 activation triggers an HIF1α-dependent pro-inflammatory signature. However, in the chronically inflamed brain, neural stem cells respond to succinate released by inflammatory macrophages by activating succinate receptor, thus initiating PGE2 secretion and extracellular succinate scavenging and consequently repressing neuroinflammation. (C) GPR91 is expressed at the surface of adipocytes of the white adipose tissue, and stimulation by succinate in these cells inhibits lipolysis and prevents the release of free fatty acids. GPR91-expressing tuft cells in the small intestine sense succinate derived from resident microbes thus initiating an innate type 2 immune response. In the hepatic fibrosis, upon stimulation of circulating succinate derived from hepatic cells, hepatic stellate cells undergo activation following succinate binding to GPR91, resulting in higher α-SMA expression, a marker of fibrogenic response (D) Accumulation and release of succinate in cancer cells – due to SDH defects and/or TCA cycle rearrangement promoted by glutamine anaplerosis – drives autocrine GPR91 activation and tumour growth, invasive programme and metastasis, as well as paracrine GPR91 stimulation on endothelial cells and macrophages, leading to angiogenesis and pro-tumour polarization respectively. Created with Biorender.com.

scavenging in the cerebrospinal fluid drive anti-inflammatory effects and contribute to the resolution of inflammation by repressing IL-1β transcription and inducing MP metabolic reprogramming towards oxidative phosphorylation, further limiting succinate accumulation [27]. The anti-inflammatory SUCNR1 effects were further confirmed in mice BM-derived macrophages lacking SUCNR1, which display an increased pro-inflammatory phenotype, with enhanced release of IL-6, tumour necrosis factor-α and nitric oxide [45].

Emerging evidence underlines a pivotal role of succinate–SUCNR1 axis in controlling metabolic homeostasis (Fig. 2C) [15] and succinate levels have been found increased in type 2 diabetes (~47–125 μM) and obesity (~80 μM) [5,21,22]. SUCNR1 is highly expressed in white adipose tissue-mediating antilipolytic effects [33]. Indeed, the binding of exogenous succinate to SUCNR1 in adipocytes inhibits lipolysis by dampening the cAMP-phosphorylated hormone-sensitive lipase (pHSL) pathway, thereby preventing the release of

FFAs from adipocytes to the liver and suppressing obesity-associated liver lipotoxicity [46]. Several recent data underlined that SUCNR1 serves as a link between metabolic stress and inflammation. In particular, while succinate–SUCNR1 axis sustains a pro-inflammatory response during acute inflammation, it also supports a negative feedback mechanism for the resolution of inflammation. In this context, conditional inactivation of Sucnr1 in myeloid cells is associated with a higher percentage of pro-inflammatory macrophages in subcutaneous white adipose tissue, and leads to increased insulin levels and impaired glucose tolerance. IL-4 treatment induces expression of Sucnr1 in peritoneal macrophages, which stimulates an anti-inflammatory phenotype *via* stimulation of $G_{\alpha s}$ subunit. In addition, SUCNR1 alters intracellular signalling in adipose tissue macrophages (ATMs) depending on white adipose tissue location, with Sucnr1-deficient ATMs from subcutaneous white adipose tissue showing a pro-inflammatory status. Overall, these results attribute an anti-inflammatory effect to SUCNR1-mediated signalling. [32]. Opposite results were obtained using Sucnr1$^{-/-}$ mice, in which a decreased macrophage infiltration into adipose tissue and improvement of glucose tolerance were reported, thereby indicating that SUCNR1 induces a pro-inflammatory phenotype in succinate-producing adipose tissue during obesity [21]. These contradictory results point out the necessity to underscore the specific role of SUCNR1 in individual cell subsets.

Recently, succinate has been also recognized as a microbiota-derived metabolite, representing a primary cross-feeding metabolite that is produced by primary fermenters, as a catabolic metabolite of microbial oligo-/polysaccharide or amino acid fermentation, and consumed by secondary fermenters in the gut. During gut dysbiosis, associated with inflammatory pathologies, an increase in succinate accumulation in the intestinal lumen and in the serum has been reported [22,47]. Dietary succinate acts as a primary substrate for intestinal gluconeogenesis, thus improving glucose and energy metabolism [47,48]. In this scenario, recent works revealed that small intestine tuft cells express SUCNR1, which senses pathogen-secreted succinate, resulting in tuft cell hyperplasia and promoting microbiota-triggered type 2 immune response [49,50]. SUCNR1 is also overexpressed in intestinal tissues of Chron's disease patients and is involved in both intestinal inflammation and fibrosis [34].

Moreover, succinate–SUCNR1 signalling regulates liver fibrosis by activating hepatic stellate cells (HSCs) [51], possibly *via* SIRT3-dependent mechanisms [35]. HSCs isolated from an animal model of non-alcoholic steatohepatitis (NASH)/non-alcoholic fatty acid liver disease (NAFLD) display an increase in succinate concentration and SUCNR1 expression, correlating with upregulation of fibrogenic response markers [52]. The metabolic cross-talk in NASH between succinate-secreting hepatocytes and GPR91-expressing HSCs also leads to fibrotic deposition [35,36]. In NAFLD, uncoupling protein 1-knockout mice experience increased liver succinate, which binds to SUCNR1 in macrophages and resident stellate cells, thus driving liver immune cell infiltration and glucose intolerance in an obesogenic environment [53].

In cancer cells, succinate accumulates in the cytosol as a consequence of TCA cycle dysfunction. As an intracellular oncometabolite, succinate alters DNA and histone methylation patterns [14] and stabilizes HIF-1α, thus leading to overexpression of genes involved in cancer progression [12,54]. Alongside, circulating succinate also acts in a paracrine and endocrine manner by activating GPR91, overexpressed in different types of cancers (Fig. 2D) [55,56]. Importantly, loss-of-function SDH mutations are mainly responsible for succinate intracellular accumulation in paragangliomas and phaeochromocytoma, gastrointestinal stromal tumours, thyroid cancer and renal cell carcinoma [4,57], and often correlate with elevated SUCNR1 expression [58]. Specifically, autocrine stimulation of SUCNR1 by secreted succinate increases cancer cell viability, thus playing a pivotal role in the pathogenesis of SDHx mutation-derived paragangliomas and phaeochromocytoma [58]. Moreover, succinate binding to GRP91 on endothelial cells sustains tumour angiogenesis both *in vitro* and *in vivo* through the activation of a signalling cascade, involving signal transducer and activator of transcription 3 (STAT3)/ERK1/2 activation and consequent VEGF upregulation [59]. Remarkably, SUCNR1 is crucial for glutamine-addicted cancer cells, where SUCNR1 signalling, through activation of $G_{\alpha i}$, ERK and protein kinase B (AKT/PKB), is involved in a feedback mechanism limiting TCA cycle, mitochondrial respiration and ROS production [60]. Moreover, bioinformatic analyses revealed that SUCNR1 expression correlates with immune cell infiltration and T-cell exhaustion in ovarian cancer [55]. Recent data demonstrate that succinate represents a new class of circulating oncometabolite with potential value for predicting non-small-cell lung cancer outcomes. Indeed, serum levels of circulating succinate are significantly higher in non-small-cell lung cancer patients, when compared to healthy subjects. *In vitro*, lung cancer cells accumulate intracellular succinate due to SDH reduced activity

and then release it in the extracellular milieu. Tumour-derived succinate promotes cancer metastasis through SUCNR1 activation *in vivo*, as determined in a syngeneic murine model of lung cancer. Secreted succinate acts as a paracrine signalling molecule by activating SUCNR1 on macrophages, thus promoting their polarization into tumour-associated macrophages, and accounting for macrophage recruitment and migration. Mechanistically, succinate–SUCNR1 axis activates phosphatidyl-inositol 3-kinase (PI3K)-AKT signalling cascade that leads to HIF-1α stabilization, with consequent upregulation of tumour-associated macrophage-specific markers and metastasis-related genes, specifically activating an epithelial–mesenchymal transition (EMT) signature [31].

Overall, targeting succinate–SUCNR1 axis might exert relevant therapeutical outcomes, especially for the treatment of cancer, metabolic diseases or chronic inflammation states. To this aim, SUCNR1 antagonists have been recently developed [61–63], and found to ameliorate *in vivo* hypertension and impair collagen production in succinate-activated HSCs [62]. However, given the widespread distribution of SUCNR1 along diverse tissues and the different (and sometimes opposing) effects resulting from its activation, targeting SUNCR1 still represents an unmet challenge and systemic effects should be considered when designing SUCNR1 agonists/antagonists. Thus, in order to achieve a better comprehension of SUCNR1 activity and validate it as a drug target, recent efforts have been addressed to the synthesis of potent and selective agonists, devoid of any effects on intracellular SDH [64,65].

GPR81 mediates extracellular lactate-dependent signalling

Lactate has long been considered as a waste product of cellular metabolism, namely fermentative glycolysis. This concept was challenged during the last decades when it has been proposed as a metabolic fuel; firstly, in neuron–astrocyte shuttle and then in a plethora of cell types, particularly relevant in the tumour microenvironment (TME) [66]. Physiological levels of lactate reach millimolar concentrations, oscillating between 1 mM under physiological conditions in both plasma and healthy tissues, and 10–20 mM after an intense physical exercise. Alongside, lactate accumulates in disease-related environments, such as solid tumours and chronically inflamed tissues, with a concentration of up to 40 mM [67]. In tumours, the hypoxic areas, originating from the poorly oxygenated cancer cells that are more distant from functional blood vessels, are highly enriched in lactate released by the glucose-consuming malignant cells. However, lactate has recently emerged as a major source of carbons for the TCA cycle, even in excess of glucose, both in normal and cancerous tissues. In this scenario, the lactate gradient, together with the activity of specific solute carrier transporters that perform proton-lactate symport (*i.e.* MCT1-4) or sodium-dependent transport (*i.e.* SLC5A8 and SLC5A12), drives cellular lactate import. This led to the concept of a metabolic symbiosis between lactate-generating and lactate-consuming cells in solid cancers, where lactate is re-used for anabolic and transcriptional purposes [68–70]. Also, lactate shapes immune cell functions leading to specific metabolic alterations associated with the recruitment and the activation of specific immune cell populations (*e.g.* macrophages, T regulatory and Th17), thereby drastically affecting the immune response in both inflamed and malignant tissues.

Besides lactate transporters, GPCRs associated with lactate as their endogenous ligand have been identified, namely the hydroxy-carboxylic acid receptors (HCARs), consisting of three members: HCAR1 (GPR81), HCAR2 (GPR109A) and HCAR3 (GPR109B). As the most evolutionarily conserved among the three receptors, HCAR1/GPR81 was initially described as being strongly expressed in adipocytes, but recent evidence highlights that it is also regulated in immune cells of both the innate and the adaptive immune system as well as in cancer cells. GPR81 could functionally signal through both $G_{\alpha i}$ and $G_{\beta\gamma i}$ subunits. $G_{\alpha i}$-protein pathway was the first signal identified in adipocytes, where G_i-dependent inhibition of AC and thus of cAMP accumulation is able to suppress lipolysis (Fig. 3B). Moreover, HCAR1 can act through arrestin-β2 to protect against inflammasome-mediated cell damage. Besides, it can even activate ERK1/2 through pertussis toxin-sensitive pathways, independently on arrestin (Fig. 1B).

As the brain is a well-known lactate-rich environment, lactate sensing *via* membrane receptor has emerged in both physiological and pathological conditions. Neurons sensing lactate can activate GPR81 resulting in a decrease in their excitability [71]. Also, ischaemia or intense physical exercise leads to the hypoperfusion of the brain, and lactate acts as a neuroprotective agent. Thus, GPR81-activating leptomeningeal fibroblast-like cells are bathed by extravascular lactate and stimulate angiogenesis through VEGF production thereby resulting in an important neurotrophic effect [72].

GPR81 is highly expressed in cancer cells and the increasing roles of lactate within the TME have highlighted the potential involvement of this receptor in

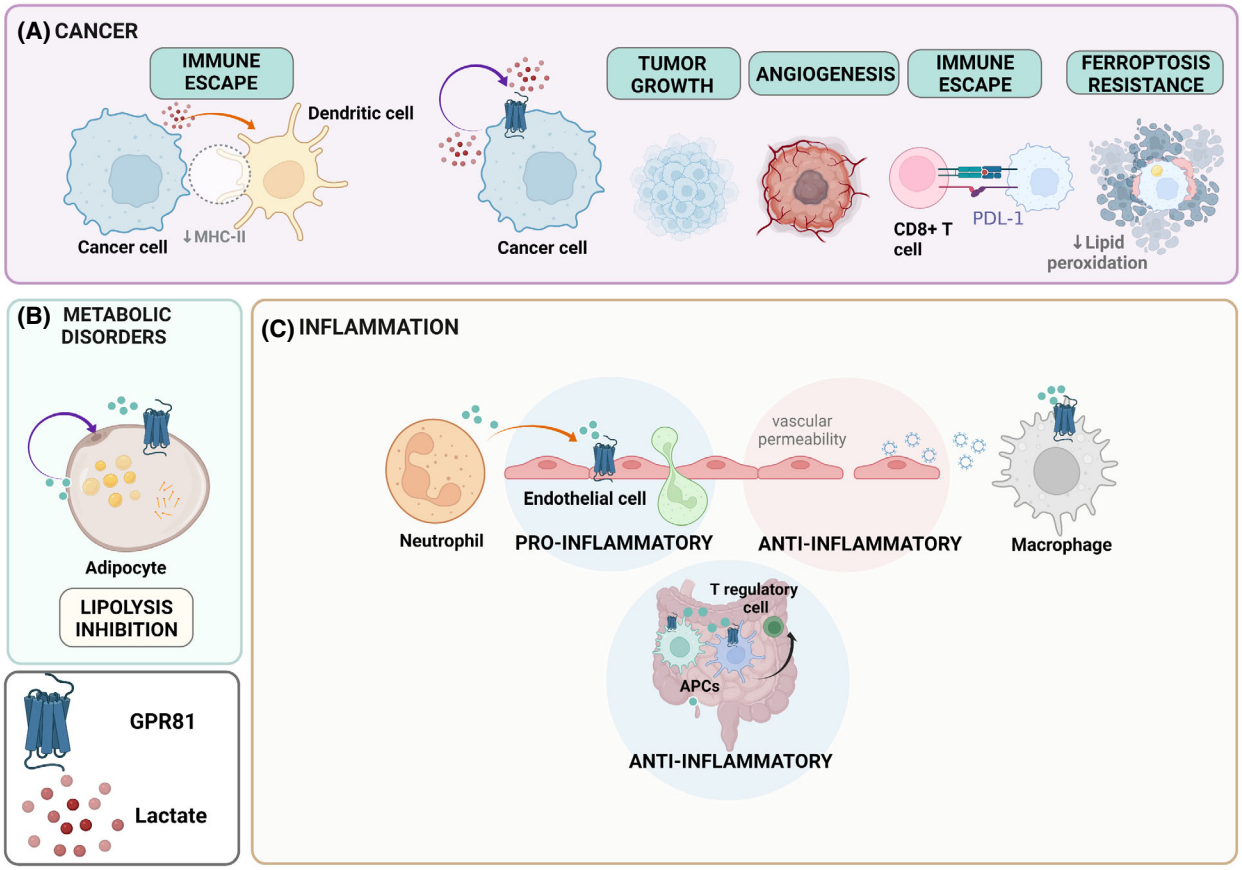

Fig. 3. Lactate drives several processes including cancer progression, metabolic disorders and inflammation through GPR81 activation. (A) Autocrine stimulation of GPR81 by lactate upregulates a plethora of cancer cell-associated mechanisms including MCTs associated with tumour growth, amphiregulin associated with angiogenesis, inhibitory checkpoint ligands like PD-L1 involved in immune escape and ferroptosis resistance linked to lipid metabolic rewiring. Paracrine activation of GPR81 in DCs leads to the downregulation of MHC, and consequently decreased tumour antigen presentation and activation of T cells. (B) Activation of GPR81 in adipocytes leads to the inhibition of lipolysis resulting in the metabolic regulation of insulin action within the adipose tissue and also the muscle. (C) Inflammatory conditions following bacterial infection result in lactate accumulation in the BM, revealing metabolic crosstalk between lactate-producing neutrophils and BM endothelium: endothelial GPR81 signalling promotes BM vascular permeability and this supports neutrophil mobilization and function at the inflammation site. In parallel, the endothelial barrier dysfunction is triggered by lactate-sensing macrophages that release exosomes, able to disrupt vascular permeability, upon GPR81 activation and consequently underlying the macrophage pro-inflammatory action. In the gut, macrophages and antigen-presenting cells sense environmental lactate and activate a GPR81-dependent anti-inflammatory immune response. Created with Biorender.com.

the disease progression (Fig. 3A). GPR81 expression is required for cancer cell survival, and this induces the transcriptional regulation of the major players involved in lactate metabolism, such as MCT1, MCT4 and peroxisome proliferator-activated receptor-gamma coactivator-1α [73]. Notably, GPR81-silenced pancreatic cancer cells delay the xenograft tumour growth and metastasis *in vivo*, making GPR81 a new potential therapeutical target. Similarly, GPR81-expressing breast cancer cells rely on GPR81 activation to initiate tumourigenesis and stimulate angiogenesis through the production of the angiogenic mediator amphiregulin [74].

Tumours facing harsh microenvironmental conditions take advantage of extracellular lactate to resist oxidative stress and promote lipid biosynthesis. This is orchestrated by GPR81 sensing which promotes MCT1-dependent incorporation of extracellular lactate in hepatocellular carcinoma cells. Lactate in turn deactivates the adenosine monophosphate-activated protein kinase and sustains the lipid desaturation process, responsible to increase the levels of anti-ferroptotic monounsaturated fatty acids, thus avoiding lipid peroxidation and cell death, through the so-called ferroptosis [75]. Moreover, lipid species induction is regulated by lactate-induced STAT3 signalling [76].

Such transcription factor has been found to sustain the expression of GPR81 receptor itself in lung cancer cells, as the lactate-promoted GPR81 induces another transcription factor Snail, which, in turn, upon complexing with STAT3 itself, binds to the GPR81 promoter thereby enhancing its expression [77].

The TME creates a niche that favours tumour progression over anti-tumour immune surveillance. One of the main mechanisms of tumour immune escape involves the immune regulatory pathway-programmed cell death protein 1/PD-1 ligand (PD-L1). Lactate has been found to induce PD-L1 expression in lung cancer cells *via* GPR81. In particular, activation of GPR81 by tumour lactate decreases intracellular cAMP levels and protein kinase A (PKA) activity, leading to the activation of the transcriptional coactivator TAZ and the consequent induction of PD-L1 expression in cancer cells, which in turn become resistant to cytotoxic T-cell targeting [78]. In a such lactate-rich environment, other cell types can be sensitive to lactate and consequently to its relative signalling. Autocrine GPR81 induction in lactate-releasing DCs leads to decreased secretion of pro-inflammatory IL-6 and IL-12, downregulation of major histocompatibility complex (MHC) and consequently decreased tumour antigen presentation [79].

Lactate metabolism is an important regulator of inflammatory processes. In this scenario, macrophages together with other resident or recruited stromal cells undergo metabolic changes due to the presence of environmental lactate, thereby affecting their function. It has been proven that lactate can shape macrophage polarization towards an anti-inflammatory immunosuppressive phenotype in tumours and other pathologies [80]. In this regard, lactate suppresses macrophage response to inflammatory stimuli by inactivating yes-associated protein/nuclear factor kappa-light-chain enhancer of activated B cells (NF-kB) molecular axis *via* GPR81 signalling. In addition, targeting GPR81 alleviates inflammation promoted by innate immune cells, such as monocytes and macrophages (Fig. 3C). These cellular populations orchestrate inflammatory responses by releasing soluble factors [81]. Among them, high-mobility group box 1 protein (HMGB1) is secreted during sepsis. Interestingly, high levels of circulating lactate promote HMGB1 epigenetic modifications, such as lactylation and acetylation, both recently associated with lactate metabolism. HMGB1 acetylation is driven by GPR81 signalling, through β-arrestin2-mediated recruitment of p300/CBP acetylases, thereby resulting in the increased release of macrophage HMGB1-containing exosomes and leading, consequently, to the endothelial barrier dysfunction [82]. In the gut environment, lactate levels can be arisen by microbiota. This plays a pivotal role in promoting the epithelial development of the intestinal stem cells *via* GPR81-Wnt signalling pathway [83]. Interestingly, during the early phases of bacterial infection, increasing levels of lactate are released by the bone marrow-resident glycolytic neutrophils. Endothelial cell GPR81 stimulation is crucial for enhancing bone marrow vascular permeability through cAMP-mediated VE-cadherin downregulation, resulting in the mobilization and anti-inflammatory action of neutrophils (Fig. 3C) [84].

In an experimental model of gut inflammation, the activation of GPR81 and its signals culminate in the modulation of T-cell subsets. Genetic deletion of GPR81 leads to the increase in inflammatory cues derived from Th1/Th17 cells disrupting the intestinal homeostasis. However, pharmacological activation of GPR81 is crucial for the induction of Treg cells, resulting in the suppression of intestinal inflammation (Fig. 3C) [85].

Although targeting lactate metabolism has increasingly led to the development of many compounds, *e.g.* blocking the MCTs transport, no GPR81 antagonists are available. Even if some reports show a putative antagonist, namely 3-hydroxy-butyrate acid, there is no evidence for its antagonistic activity against GPR81. To date, only RNAi or knockout/knockdown approaches are consistent in investigating GPR81-mediated signalling effects [86].

GPR109A is regulated by extracellular ketogenesis-derived metabolites

Similar to lactate, butyrate and β-hydroxy-butyrate, produced by the oxidation of fatty acids, are exported *via* MCT4, imported into cells through MCT1/2 and used as alternative energy substrates. They have been reported to activate HCAR2 (also known as GPR109A), a G_i protein-coupled receptor previously identified as responsible for mediating the pharmacological actions of nicotinic acid (Fig. 1B). HCAR2/GPR109A expression has been demonstrated in adipocytes, immune cells and the epithelia of gastrointestinal tissues as well as in the brain. In this latter site, HCAR2 is expressed selectively by microglia and is robustly induced by amyloid pathology in Alzheimer's disease. Indeed, HCAR2 activation leads to protective anti-inflammatory role of microglia resulting in a reduced plaque burden and neuronal dystrophy, attenuation of neuronal loss and rescue of working memory deficits [87]. In keeping, HCAR2 signalling promotes anti-inflammatory properties in the colon lumen,

where macrophages and dendritic cells are induced to stimulate the differentiation of anti-inflammatory Treg cells and IL-10-producing T cells [88]. In the context of tumorigenesis, HCAR2 has been hypothesized to have dual role: (a) its activation induces cellular differentiation in normal cells, while (b) it inhibits cell survival or induces apoptosis in tumour cells. Indeed, HCAR2 activation is correlated with the reduction in the growth of solid cancers such as breast and colon tumours [89].

GPR109B mediates β-oxidation metabolic intermediates

The 3-hydroxy medium-chain fatty acid, 3-hydroxy-octanoic acid, is a β-oxidation intermediate that participates in energy metabolism and also serve as a signalling molecule. In fact, 3-hydroxy-octanoic acid affects metabolic and immune responses by acting as a specific agonist of HCAR3 (also known as GPR109B), with an EC50 of 4–8 μM [90,91]. Noteworthy, shortening or extension of 3-hydroxyoctanoic acid by more than two carbon atoms leads to molecules incapable of activating HCA3 receptor [91]. In addition, despite HCAR3 displays a high degree of similarity to HCAR2, being derived from a recent event of gene duplication, it does not bind HCAR2 agonists, such as nicotinic acid and β-hydroxy-butyrate [92]. HCA3 receptor can be found only in humans and higher primates and it is mainly expressed in adipocytes [91,93], immune cells [94–96] and epithelial cells of the colon [89]. During fasting or prolonged starvation, as well as under ketogenic diet, mitochondrial fatty acid β-oxidation disorders and diabetic ketoacidosis [91,97], lipolysis-derived FFAs are increasingly synthesized from triglycerides, thereby promoting β-oxidation flux. Under these conditions, the plasma levels of the β-oxidation intermediate 3-hydroxyoctanoic acid strongly increase, reaching concentrations sufficient for HCAR3 activation (5–20 μM) [91,98]. Interestingly, HCAR3 couples to $G_{i/o}$-type G proteins and its activation by 3-hydroxyoctanoic acid leads to AC inhibition and decreased cAMP levels. In adipocytes, cAMP activates PKA, that in turn phosphorylates and activates several lipolytic enzymes. Thus, HCAR3 activation in adipocytes ultimately results in anti-lipolytic effects and in the inhibition of FFA synthesis (Fig. 1B). It is noteworthy that this negative feedback mechanism activated by 3-hydroxyoctanoic acid further limits its own synthesis and may serve to preserve energy during fasting [91,98]. In immune cells, HCAR3 activation leads to the increase in intracellular Ca^{2+} levels in a G_i/G_o-dependent manner [95]. It has been highlighted that HCAR3 activation in human macrophages and adipocytes suppressed LPS-induced pro-inflammatory cytokine production (*e.g.* IL-6, TNF-α and IL-8), partly through the NF-kB pathway, without affecting adipogenesis, thereby underlining the anti-inflammatory effects of HCAR3 and its potential role in numerous inflammatory-related pathologies [96].

Moreover, it has been reported that several aromatic D-amino acids and their derivatives, including D-phenylalanine, D-tryptophan and D-kynurenine, but not the corresponding L-enantiomers, act as HCAR3 agonists, eliciting a transient increase in intracellular Ca^{2+} levels, through inhibition of AC activity. HCAR3 activation by aromatic D-amino acids resulted in chemotactic response in human neutrophils [95]. However, the pathophysiological relevance of aromatic D-amino acid binding to HCAR3 has to be investigated. HCAR3 expression has also been found to increase in human breast cancer patient tissues and primary breast cancer cells. Interestingly, siRNA-mediated knock-down of HCAR3 reduced the viability of breast cancer cells, while the inhibition of fatty acid oxidation (FAO) rescued their viability. In this scenario, HCAR3 activates a negative feedback mechanism to control FAO rate in breast cancer cells [99]. Several synthetic compounds have been found to act as HCAR3 ligands and inhibit lipolysis in human adipocytes [100–102]. Nevertheless, since HCA3 receptor can be found only in higher primates, the availability of animal models to study its pathophysiological role and identify synthetic agonists is limited and further investigations to elucidate its effects are necessary.

Free fatty acids function as extracellular ligands for plasma-membrane GPCRs

Besides other saccharide-derived molecules, FFAs have also been indicated as ligands for GPCRs on the surface of cells, impacting key physiological processes. FFA-activated receptors include FFAR1 (GPR40), FFAR2 (GPR43), FFAR3 (GPR41) and FFAR4 (GPR120). GPR41 and GPR43 are activated by short-chain fatty acids, including acetate, propionate and butyrate, whereas GRP40 and GPR120 are sensitive to medium- and long-chain fatty acids, like palmitate, oleate, linoleate, etc., irrespectively of their saturation. FFA-GPCRs fascinated the scientific community over the last few years and several lines of evidence underline their role in the regulation of metabolic processes, like insulin and incretin secretion, regulation of food intake and adipose tissue biology [103]. These FFA receptors are mainly expressed in adipose tissues,

pancreatic and enter endocrine cells, neurons and immune cells, with a selectivity of GPR120 for liver, bone and lung.

After their de-orphanization, the contribution of these receptors to metabolic and energy homeostasis has been comprehensively recognized, and they are now considered attractive therapeutic targets for metabolic disorders, like obesity, type 2 diabetes and cardiovascular diseases.

Although data are still incomplete, GPR120 has been reported to play a key role in adipose tissue homeostasis and colon cancer carcinogenesis. In humans, the expression of GPCR120 is significantly higher in obese patients compared to lean healthy individuals, and a variant of GPR120 (R270H) is associated with an increased risk of obesity and enhanced fasting glucose levels [104]. Targeting of GPR120 inhibits insulin sensitivity and glucose tolerance, acting mainly through $G_{\alpha q}$ and the regulation of PI3K, impairment of GLUT4 translocation and insulin-receptor substrate-1 phosphorylation (Fig. 1C) [105]. GPR120 is also highly expressed in brown adipose tissue, being sensitive to cold exposure and increasing thermogenesis *via* regulation of transcriptional pathway for browning, including uncoupling protein 1 [106].

Activation of GPR120 can also explain the beneficial systemic anti-inflammatory effects of ω3-FFAs on dysmetabolic diseases. Indeed, although the whole mechanism is not fully described yet, it has been shown that stimulation of ω3-FFA is followed by GPR120 receptor phosphorylation, interaction with β-arrestin-2 and inhibition of NF-KB-mediated pro-inflammatory signals through regulation of transforming growth factor-β-activated kinase (TAK) pathway (Fig. 1C).

Moreover, the loss of epithelial GPR120 represents an early event of colorectal carcinogenesis and results in increased intestinal permeability, microbiota translocation and dysbiosis, culminating in the regulation of β-catenin signalling and the increase in epithelial cell proliferation. Hence, the role of GPR120 is mandatory to maintain the mucosal barrier integrity, as well as to weaken tumorigenesis during inflammation-driven colorectal cancer development [107].

The receptor GPR40/FFAR1, upon activation by long-chain fatty acids, couples to G-protein subunit $G_{\alpha q11}$ and $G_{\alpha i/s}$ signalling, leading to phospholipase C metabolism and Ca^{2+}/protein kinase C activation. The activity of cAMP metabolism is strictly dependent on tissue context and association with $G_{\alpha i}$ or $G_{\alpha s}$, leading to the upregulation of insulin secretion and the improvement of fatty acid metabolism in the liver (Fig. 1C). Similarly to its companion GPR120, also sensing long saturated and unsaturated fatty acids, GPR40 is involved in metabolic and neoplastic diseases. Indeed, enhanced GPR40/FFAR1 expression/signalling reduces diabetes symptoms, while it enhances tumour malignancy traits of various cancer types, including melanoma and prostate cancer. Hence, GPR40/FFAR1 and GPR120/FFAR4 have opposed functions in cancer progression, likely due to their opposing ability to regulate matrix metalloprotease-2 (MMP-2) activity. Indeed, GPR120/FFAR4 knockdown causes decreased levels of MMP-2, whereas GPR40/FFAR1 knockdown leads to increased MMP-2 levels [108].

Short-chain fatty acids selectively activate GPR43 and GPR41, mainly through the pre-coupled $G_{\alpha q}$ and $G_{\alpha i}$ proteins, leading to cytoplasmic Ca^{2+} increase, ERK activation and the inhibition of cAMP production (Fig. 1C) [109]. The role of GPR43/FFAR2 in adipogenesis is still a matter of debate, as some studies reported the ability of propionate and other agonists to activate adipogenesis, but many others indicate the opposite behaviour on both adipocyte whitening and mesenchymal stem cell differentiation [110]. GPR43 is also involved, through stimulation of AKT and ERK phosphorylation, in sensing environmental signals and gut immune cell regulation, modulating gut homeostasis and pathogen defence [111].

GPCRs sense amino acids and amino acid-derived metabolites

Several amino acids or amino acid-derived metabolites have been described to signal through GPCRs, including CasR, TAAR1, GPR35 and GPR142, thereby modulating physiological functions or affecting pathological processes.

CasRs are highly expressed in the parathyroid glands and kidneys, where they carry out their calciotropic effects by regulating systemic Ca^{2+} homeostasis. The CasRs are also expressed in lung, intestine, pancreatic islets, brain, skin and vasculature, where they are involved in non-calciotropic actions, including regulation of cell proliferation, differentiation and apoptosis, as well as in the physiological regulation of enteroendocrine hormone secretion, cardiac function, vascular tone and lung and neuronal development.

CasRs can be activated by different ligands, *i.e.* L-amino acids, cations and polyamines, and stimulate several signalling pathways mediated by $G_{q/11}$, $G_{i/o}$ and $G_{12/13}$ proteins, depending on the cell type and the ligand (Fig. 1D). The gastrointestinal-expressed CasRs are activated by dietary amino acids and peptides, which in turn influence digestion, satiety and glucose metabolism [112,113]. Moreover, polyamines, such as

spermine, can activate CasRs signalling in colon and brain, thereby modulating physiological processes, including colonic fluid secretion and synaptic transmission [114,115]. Abnormal CasR function or expression in non-calciotropic organs is associated with cardiovascular diseases, gastrointestinal disorders, Alzheimer's disease, impaired glucose tolerance and cancer [116]. High CasR expression has been observed in aggressive prostate cancers, such as metastatic castration-resistant and neuroendocrine tumours and correlates with decreased survival [117]. Interestingly, the proliferation and migration of human prostate and gastric cancer cells were impaired by CasR antagonists, suggesting a possible therapeutic effect of CasR targeting in cancer [118,119]. In breast cancer, CasR stimulates cell proliferation, inhibits apoptosis [120] and promotes the secretion of pro-angiogenic and chemotactic factors that are implicated in breast cancer skeletal metastases [121]. The involvement of CasR in bone metastasis was also observed in renal cell carcinoma [122]. In contrast, in colorectal cancer, CasR elicits tumour suppressor roles since it inhibits proliferation, induces apoptosis and prevents epithelial–mesenchymal transition [123]. An anti-tumoural effect of CasR was also observed in neuroblastoma, where CasR expression correlates with low clinical stage [124].

TAAR1 is a $G_{\alpha s}$-protein-coupled receptor, which acts by the binding of 3-iodothyronamine (T1AM) and some endogenous monoamines synthetized after the decarboxylation of aromatic amino acids [125]. Along with the expression of TAAR1 in pancreas, intestine and central nervous system, where it regulates the classical monoamine neurotransmission [126], a deregulation of the receptor has been observed in different cancers [127]. In leukaemia and lymphoma, TAAR1 has been proposed as a possible pharmacological target, since its activation with several agonists induced apoptosis of tumour cells [128]. TAAR1 expression is also a positive prognostic factor for overall survival in ovarian [129] and breast cancer patients [130]. In keeping, in patients with both oestrogen receptor-negative and -positive breast cancer, the TAAR1 agonist cadaverine resulted in a beneficial effect against tumour development [131], suggesting that TAAR1 might represent a promising pharmacological target (Fig. 1D).

GPR35 is expressed in various tissues including gastrointestinal tissues and several types of immune cells. Kynurenic acid (KYN), a tryptophan metabolite, is one of the first reported ligand for GPR35, although also lysophosphatidic acid (LPA) and the chemokine CXCL17 were shown to activate the receptor [132]. The concentration of KYN and the expression of GPR35 have been found deregulated in different cancers [133]. GRP35 is highly expressed in human colorectal cancer, and deletion of GPR35 in the intestinal epithelium was sufficient to reduce epithelial cell proliferation and tumour incidence [134]. Recently, an additional tumour-promoting mechanism of GPR35 has been reported, identifying macrophage GPR35, through a Na/K-ATPase-dependent activation of Src kinase, as a key driver of neo-angiogenesis and tumour growth [135]. Importantly, the T108M variant of GPR35 has been associated with inflammatory bowel diseases (IBD), leading to the definition of GPR35 as an IBD risk gene [136]. An elegant recent study from De Giovanni et al. [137] strengthened the role of GPR35 in inflammatory diseases, reporting 5-hydroxyindoleacetic acid (5-HIAA) as a novel GPR35 ligand that is secreted by activated platelets and mast cells and that promotes neutrophil recruitment and trans-endothelial migration to sites of inflammation (Fig. 1D). Treatment with serotonin uptake inhibitor fluoxetine or with a monoamine oxidase inhibitor overcomes GPR35-mediated neutrophil recruitment and sheds new light on the use of these drugs as novel therapeutic strategies to counteract the onset of chronic inflammatory disease and cancer.

GPR142 is a G_q- and G_i-coupled GPCR, which is highly and almost exclusively expressed in pancreatic and enteroendocrine cells [138]. GPR142 is activated by L-tryptophan and at a lower extent by L-phenylalanine. The binding of L-tryptophan stimulates insulin secretion and improves glucose tolerance, thereby suggesting a role for GPR142 in the regulation of glucose homeostasis and improving the design of synthetic GPR142 agonists for the treatment of metabolic diseases such as obesity or diabetes. One of these has reached phase 1 in clinical trials for type 2 diabetes treatment (Fig. 1D) [139]. Moreover, GPR142, along with CasR, GPR35 and TAAR1, act as sensors of dietary amino acids and gut microbiota amino acid metabolites and control hormone secretion in enteroendocrine cells. Indeed, CasR stimulates cholecystokinin and glucagon-like peptide-1 secretion [140], while GPR142 stimulates secretion of gastric inhibitory polypeptide and glucagon-like peptide-1, thus improving glucose metabolism [141,142].

Conclusions

Growing evidence draws attention to the unconventional roles of nutrients and metabolic intermediates as autocrine or paracrine extracellular signalling molecules that act by binding to metabolite-sensing GPCRs on the plasma membrane of target cells. It is noteworthy that these metabolite–GPCR axes lead to the

activation of various downstream intracellular signalling pathways, depending on the specific metabolite and cognate receptor, cell type and microenvironmental context, thereby resulting in different pathophysiological effects. Therefore, given the involvement of metabolite-sensing GPCRs in a spectrum of diseases that includes metabolic disorders, inflammation and cancer, their targeting is emerging as a novel promising therapeutical approach. However, the systemic distribution of metabolite-sensing GPCRs and their disparate effects make GPCR druggability very challenging. The vulnerabilities related to nutrients/metabolites-associated extracellular signalling should thus be further dissected.

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Author contributions

All the authors performed a literature search and wrote and supervised the manuscript. EP and LI prepared the figures.

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