Trends in Immunology

CellPress

Review

Succinate Receptor 1: An Emerging Regulator of Myeloid Cell Function in Inflammation

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The rapidly evolving area of immunometabolism has shed new light on the fundamental properties of products and intermediates of cellular metabolism (metabolites), highlighting their key signaling roles in cell-to-cell communication. Recent evidence identifies the succinate–succinate receptor 1 (SUCNR1) axis as an essential regulator of tissue homeostasis. Succinate signaling via SUCNR1 guides divergent responses in immune cells, which are tissue and context dependent. Herein, we explore the main cellular pathways regulated by the succinate–SUCNR1 axis and focus on the biology of SUCNR1 and its roles influencing the function of myeloid cells. Hence, we identify new therapeutic targets and putative therapeutic approaches aimed at resolving detrimental myeloid cell responses in tissues, including those occurring in the persistently inflamed central nervous system (CNS).

Highlights

The signaling properties of the G-protein-coupled SUCNR1 depend on cell and tissue specificity, as well as pathophysiological context.

SUCNR1 activation can not only drive proinflammatory responses, but also directly participate in the resolution of inflammation.

Succinate signaling via SUCNR1 has a role in the progression of tissue damage and resolution of inflammation in the CNS.

Careful consideration of these aspects must be adopted when targeting SUCNR1 signaling to develop new candidate therapies aimed at mitigating the effects of persistent CNS inflammation.

The Succinate–SUCNR1 Axis in Inflammation

The past decade has seen an impressive increase in our understanding of the wider role of metabolites in the regulation of cellular functions [1]. In innate immune cells, extensive intracellular metabolic reprogramming controls cell behavior, phenotype, and differential states of activation in response to external stimuli [2]. This is a fine-tuned process that requires the coordination of several metabolic networks that ultimately modulate intracellular and extracellular signaling pathways.

The metabolite succinate, an intermediate of the tricarboxylic acid (TCA) cycle, has emerged as a key modulator of innate immune responses in mammals (Box 1). Proinflammatory macrophages accumulate succinate intracellularly as a consequence of TCA cycle breaks [3].

Intracellular succinate acts as an **immunometabolite** (see Glossary) to guide macrophage effector functions via the transcription of proinflammatory cytokines [3] and the production of reactive oxygen species (ROS) [4]. In chronic inflammation, such as in rheumatoid arthritis (RA), succinate is released into the extracellular compartment, where it regulates cell-to-cell communication. In mouse disease models, extracellular succinate acts as both a **metabokine** and an **alarmin** to modulate immune cell function by binding to its cognate receptor SUCNR1 *in vitro* and *in vivo*, thus eliciting complex responses that are tissue and context dependent [5,6].

Herein, we explore the main cellular pathways regulated by the succinate–SUCNR1 axis and focus on the biology of SUCNR1 and its roles in modulating adaptive and innate immune responses. We then focus on myeloid cells, describing evidence of the dual role of SUCNR1 in these cells.

We anticipate that unraveling the complex mechanisms regulating this novel extracellular signaling pathway may be crucial to understanding the mechanisms sustaining innate immune responses during persistent inflammation. This understanding should be instrumental to developing new

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Box 1. Intracellular Succinate Signaling in Mammals

In response to *in vitro* stimuli, such as lipopolysaccharide (LPS) and hypoxia, myeloid cells switch their metabolism from oxidative phosphorylation (OXPHOS) to aerobic glycolysis for energy production (i.e., Warburg effect) in a similar manner to tumor cells**.** Inflammatory mononuclear phagocytes (MPs) display a dysfunctional mitochondrial respiratory chain, and a breakdown of the Krebs cycle (also known as TCA cycle) at succinate dehydrogenase (SDH), resulting in intracellular succinate accumulation [3]. Accumulated succinate is then oxidized by SDH to initiate reactive oxygen species (ROS) generation via complex I by reverse electron transport (RET) [4]. In mouse bone marrow-derived macrophages (BMDMs), succinate acts as a stabilizing signal for the transcription factor hypoxia-inducible factor 1 alpha (HIF-1α) via prolyl hydroxylases enzyme (PHD) inhibition, to induce transcription of interleukin (IL)-1β and drive a proinflammatory phenotype [3].

Intracellular succinate can also affect chromatin structure and function. Succinate promotes protein succinylation of lysine residues, altering protein conformation and changing chromatin–histone interactions [63]. Histone succinylation can then be regulated by mitochondrial sirtuin 5 (SIRT5)-dependent desuccinylation, which also suppresses SDH activity [64]. Finally, succinate accumulation also results in epigenetic reprograming by inhibiting tet methylcytosine dioxygenase 2 (TET2) and Jumonji c domain-containing (JMJD) protein families, leading to histone and DNA methylation [65].

candidate therapeutic approaches aimed at resolving detrimental myeloid cell responses in tissues, such as those occurring in the persistently inflamed CNS.

Molecular Biology of SUCNR1

SUCNR1, initially named P2U2 due to its homology with the purinergic receptor P2Y2/P2U, was discovered in 1995 in a human megakaryocytic cell line [7]. In 2001, human *SUCNR1* and its mouse ortholog were identified using an expressed sequence tag data-mining strategy for novel **G-protein-coupled receptors** (GPCRs) [8]. SUCNR1 was initially classified as GPR91, an orphan receptor, and in 2004, succinate was identified as its cognate ligand [9]. Since then, SUCNR1 has been recognized as a key regulator of numerous and diverse physiological processes in cells [10].

The assessment of SUCNR1 molecular structure is key to understanding the variety of its intracellular signaling pathways [11,12]. SUCNR1 has seven transmembrane domains connected by three hydrophilic extracellular loops, two N-glycosylation sites (Asn4 and Asn164), and one phosphorylation site (Ser326) [8,13]. These are sites of post-translational modifications, the role of which in regulating receptor function and stability in different cell types remains largely unknown [8,10].

As a GPCR, the activity of SUCNR1 is dependent on its coupling to different G-proteins. Specifically, SUCNR1 intracellular signaling relies on the associated α subunit type (i.e., $G\alpha_i$, $G\alpha_s$, and $G\alpha_q$). While the activation of the $G\alpha_q$ and $G\alpha_i$ subunits has been associated with proinflammatory polarization of myeloid cells [e.g., platelets and dendritic cells (DCs)] [14,15], $G\alpha_s$ subunit activation mostly results in an anti-inflammatory phenotype [16] (Figure 1). Therefore, both the post-translational modifications and α subunit expression dynamically regulate the activity of SUCNR1 in cells. This leads to a highly complex regulation that results in different, and somewhat opposing, signaling cascades in cells, as discussed later.

SUCNR1 Expression in the CNS

Most **metabotropic receptors** are highly expressed in metabolically demanding organs, such as the kidneys, liver, and heart. Tissue-level expression data identified SUCNR1 expression on human kidney *macula densa* cells [13], quiescent hepatic stellate cells of the rat liver [17], and cardiomyocytes of the rat heart [18]. In these cells, SUCNR1 regulates blood pressure and contributes to the pathophysiology of liver injury and cardiomyocytes viability [13,17,18]. SUCNR1 is also expressed in the musculoskeletal system [19] and in certain cancer and stem cells where it promotes proliferation, migration, and tissue remodeling [20,21].

Glossary

Adaptive immune system: antigen-dependent immune response involving clonal expansion and activation of lymphoid cells, including T and B lymphocytes (days to weeks to be established).
Alarmin: rapidly released endogenous molecule following disease, trauma, or infection; recruits and activates various types of immune cell.
Bone-morphogenetic protein (BMP)-6: secreted signaling protein able to induce the growth of cartilage and bone.
Chemotaxis: movement of cells (i.e., phagocytotic) towards an increasing gradient (e.g., ligand) to elicit inflammatory responses, antigen presentation, and phagocytosis.
Experimental antigen-induced arthritis: animal model reproducing certain aspects of clinical rheumatoid arthritis by immunization of a joint with methylated bovine serum albumin.
Experimental autoimmune encephalomyelitis (EAE): murine model of MS, induced either by injected myelin protein or by myelin-activated T cells.
G-protein coupled receptors (GPCRs): largest family of membrane receptors with diverse intracellular signaling properties resulting from G-protein subunit activity.
Half maximal effective concentration (EC_{50}): drug concentration needed to induce half of a maximum biological response (e.g., upon a ligand binding to a receptor).
Half maximal inhibitor concentration (IC_{50}): drug concentration needed to reduce a biological response by 50%.
Hemochromatosis: disease of iron homeostasis causing accumulation of iron in the body.
Hypoxia-ischemia injury: here, brain injury (e.g., stroke resulting from cardiac arrest) leading to oxygen deprivation in the brain (brain hypoxia).
Immunometabolite: intermediate product generated during cellular metabolism that can act on intracellular and extracellular signaling pathways to shape and modulate immune responses.
Innate immune response: immediate, nonspecific immune response mediated by innate immune cells (e.g., myeloid cells); based on myeloid cell recognition of evolutionary-conserved molecular features of pathogens (e.g., LPS) to elicit an immune response.

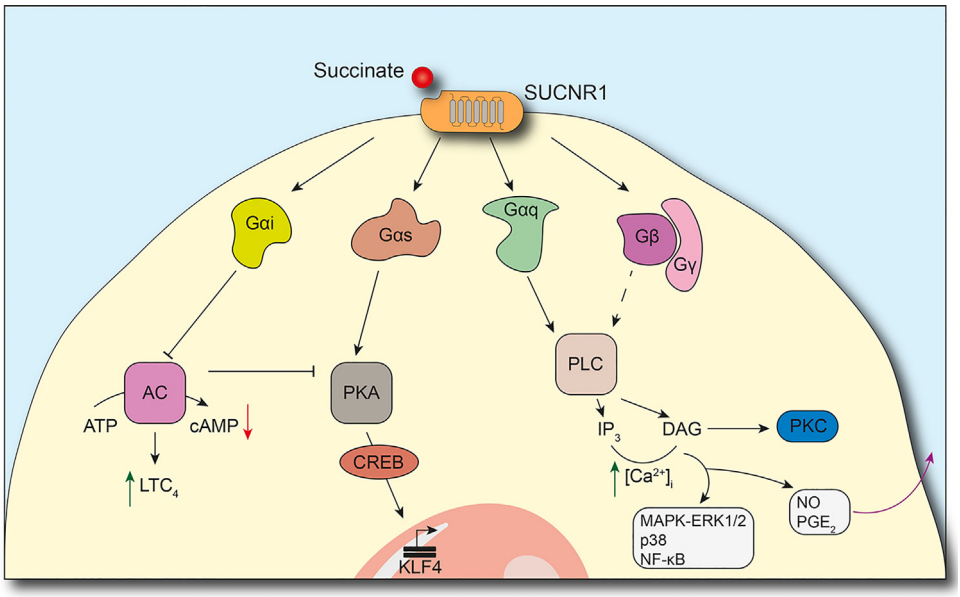

Figure 1. Diverse Signaling Functions of the G-Alpha Subunit of Succinate Receptor 1 (SUCNR1) in Mammals. SUCNR1 activation of the $G\alpha_i$ subunit leads to inhibition of adenylyl cyclase (AC), which results in decreased cAMP concentrations, decreased activity of protein kinase A (PKA), and, ultimately, the release of proinflammatory cytokines [14,15]. By contrast, SUCNR1 activation of the $G\alpha_s$ subunit has opposite effects, leading to increased PKA activity and regulation of transcription of anti-inflammatory mediators via cAMP response element binding protein (CREB) activity [16]. Finally, SUCNR1 activity via the $G\alpha_q$ subunit instead enhances the activity of phospholipase C (PLC), which produces the secondary messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG). While IP_3 increases Ca^{2+} in the cytosol leading to the activation of Ca^{2+}-dependent mitogen-activated protein kinase–extracellular signal-regulated kinases1/2 (MAPK-ERK1/2) pathways, DAG activates protein kinase C (PKC). Both of these pathways lead to proinflammatory cascades [such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), MAPK-ERK1/2, and p38], nitric oxide (NO) production, and prostaglandin E_2 (PGE_2) secretion [40,69]. Of note, recent evidence suggests that PLC activation in human cells may be mediated by the βγ subunits of SUCNR1 [70]. Red and green arrows indicate downregulation and upregulation, respectively. Abbreviation: KLF4, Krüppel-like factor 4.

Despite the brain being the most metabolically active organ of the body, basal SUCNR1 expression is nearly undetectable in the healthy mouse CNS [22]. Publicly available transcriptomics databases of healthy human and mouse samples confirmed that basal *SUCNR1/Sucnr1* expression is low throughout the CNS, with the highest expression observed in the olfactory bulbs, hippocampus, lateral ventricles, and cerebellum (Table 1) [23,24].

Regardless of its low basal expression in the healthy CNS, it appears that SUCNR1 activation is key in the response of CNS cells to **hypoxic-ischemic injury** and inflammatory damage (Figure 2). For example, in rodent retinal ganglion cells (RGCs), the activation of the succinate–SUCNR1 axis leads to increased release of vascular endothelial growth factor (VEGF) and prostaglandin E_2 (PGE_2) *in vitro*, thus favoring vascularization via the activation of the mitogen-activated protein kinase–extracellular signal-regulated kinases1/2 (MAPK-ERK1/2) signaling pathway [25,26]. In fact, the small interfering (si)RNA-mediated retinal downregulation of *Sucnr1* in wild-type (WT) rats abolished neovascularization in the presence of succinate, indicating that SUCNR1 expression in RGCs is a key regulator of the rodent retinal vascular network [27]. This could be of particular relevance in pathological settings, such as in hypoxic-ischemic injury [28]. While on the one hand, succinate–SUCNR1 signaling is beneficial, because it promotes neovascularization through PGE_2-dependent release of angiogenic factors [29], on the other hand, succinate signaling through SUCNR1 can

Metabokine: signaling product derived from cellular metabolism that binds to and regulates dedicated cell surface G-protein-coupled and ionotropic receptors.
Metabotropic receptors: G-protein-coupled receptors; membrane-bound receptors that act via signaling on secondary messengers, such as G-proteins.
Microglia: resident MPs responsible for CNS surveillance in an 'immune-privileged' tissue behind two anatomical 'barriers' (i.e., the blood–brain barrier and the blood–cerebrospinal fluid barrier); they restrict the free movement of circulating peripherally derived immune cells and soluble factors, such as metabolites, into the CNS parenchyma.
Mononuclear phagocytes (MPs): innate immune myeloid cells residing within the tissue (e.g., microglia) or are blood borne (e.g., monocytes).
Naphthyridine-based: molecular entities based on a fused double-ringed aromatic diazanaphthalene core.
Nonalcoholic steatohepatitis (NASH): inflammatory liver disease marked by the accumulation of fat in the organ, often accompanied by fibrosis.
T helper (Th)17 cells: subset of proinflammatory $CD4^+$ T cells that produce proinflammatory interleukin (IL)-17.
Th2-like: response, generally anti-inflammatory, resembling that of T helper (Th)2 cells, a subset of $CD4^+$ T cells characterized by the production of IL-4, IL-5, IL-10, and IL-13.
Toll-like receptor: single-pass membrane-spanning receptor proteins that commonly recognize structurally conserved molecular patterns from infectious pathogens; they have a significant role in innate immunity.

Table 1. SUCNR1 Expression and Signaling Pathways in Rodent and Human Neural and Immune Cells

Cell type (SUCNR1 expression)	Function of SUCNR1	Activated G-protein	Activated pathway	Studied model	Refs
Retinal ganglion cells	Increased expression of proangiogenic factors (*Vegf*, *Ang1*, *Ang2*) and proinflammatory cytokines (*Il1b*, *Il6*); increased secretion of PGE_2 and VEGF	Unknown	MAPK-ERK1/2; PGE_2-EP_4-dependent mechanism	*Ex vivo Sucnr1*$^{-/-}$ mice; *in vitro* rat RGC-5 cells	[29]
	Vascularization in diabetic retinopathy mediated by VEGF induction and secretion	Unknown	MAPK-ERK1/2–C/EBP β (c-Fos, HIF-1α); MAPK-ERK1/2–COX2–PGE_2	*In vitro* rat RGC-5 cells; *in vitro* rat primary retinal ganglion cells	[25,26,59]
	Retinal angiogenesis in development, ischemia, and proliferative ischemia; production of proangiogenic factors AGP1, AGP2, and VEGF	Unknown	Unknown	*In vitro* rat RGC-5 cells	[27]
	Axonal growth and visual system development	Unknown	MAPK-ERK1/2	*Ex vivo Sucnr1*$^{-/-}$ mice	[77]
Cortical neurons	Unknown	Unknown	Unknown	*Ex vivo Sucnr1*$^{-/-}$ mice	[29]
Retinal pigment epithelium (RPE)	Iron homeostasis; VEGF secretion; increased expression in juvenile hemochromatosis	Possibly $G\alpha_i$	BMP6-Smad1/5/8-pSmad5	*In vitro* primary mouse RPE cells; *in vitro* human RPE cell lines ARPE-19 and HRPE	[30,31]
Astrocytes	Increased expression of proangiogenic factors (*Vegf*, *Ang1*, *Ang2*) and proinflammatory cytokines (*Il1b*, *Il6*); increased secretion of PGE_2	Unknown	PGE_2-EP_4-dependent mechanism	*In vitro* rodent brain cortex astrocytes	[29]
Neural stem cells (NSCs)	Increased succinate uptake; increased *Slc13A3/5* and *Ptgs2*; release of PGE_2	Unknown	P-p38 MAPK	*In vivo* mouse EAE model; *in vitro* mouse NSCs/iNSCs	[32]
Erythroblasts (EBs) (mRNA, protein)	Unknown	Unknown	Unknown	*In vitro* human EBs from hematopoietic $CD34^+$ cells	[37]
Platelets (mRNA, protein)	Stimulates aggregation, enhances platelets-derived eicosanoid release	$G\alpha_i$ and $G\beta_\gamma$	cAMP–PKA pathway; Src kinase activation and PI3K*β*/Akt1	*In vitro* human platelets	[14,70]
T cells ($CD4^+$) (protein)	Possibly regulate T cell-dependent B cell activation	Unknown	Unknown	*Ex vivo* human umbilical cord blood	[37]
B cells ($CD8^+$) (protein); B cells ($CD19^+$, IgD^+CD27^-, IgD^+CD27^+, and IgD^-CD27^+) (protein)	IgG and IgM secretion in synergy with IL-10 from naïve B cells	Unknown	Unknown	*Ex* vivo human blood from patients with systemic lupus erythematosus	[38]
Immature monocyte-derived dendritic cells (iMoDCs) (mRNA)	Promotes chemotaxis, proinflammatory augmentation, T cell activation enhancement	$G\alpha_q$	MAPK-ERK1/2	*Ex vivo* human iMoDCs; *ex vivo Sucnr1*$^{-/-}$ mouse DCs	[40]
Bone marrow-derived DCs (BMDCs) (mRNA)	Promotes proinflammatory phenotype and chemotaxis	Unknown	Unknown	*In vitro* mouse BMDCs; *in vivo Sucnr1*$^-$ mouse antigen-induced arthritis (AIA) model	[41]
Bone marrow-derived macrophages (BMDMs)	Modulates inflammatory response	Unknown	Unknown	*In vivo Sucnr1*$^{-/-}$ mouse model; *in vitro* mouse BMDMs	[39,46]
Monocyte-derived macrophages [M2 (IL-4), M1 (IFNy + LPS)] (mRNA)	Enhances chemotaxis in white adipose tissue	Unknown	Unknown	*In vitro* human blood monocytes	[43]
Peripheral blood mononuclear cells [anti-inflammatory (IL-4, IL-13)] (mRNA)	Decreases expression of IL-10, TLR4 and TLR5 while upregulating TNF-1α	Unknown	Unknown	*Ex vivo* human blood mononuclear cells	[45]
Adipose tissue macrophages (ATMs) (mRNA, protein)	Promotes anti-inflammatory phenotype via IL-4	$G\alpha_s$	PKA–CREB–KLF4 pathway	*In vivo* LysM-Cre *Sucnr1*$^{fl/fl}$ mice; *in vitro* human monocyte THP1 cell line	[16]

Table 1. (continued)

Cell type (SUCNR1 expression)	Function of SUCNR1	Activated G-protein	Activated pathway	Studied model	Refs
Tumor-associated macrophages (TAMs) (mRNA, protein)	Promotes tumor metastasis via IL-6	Unknown	PI3K–HIF-1a pathway	*In vivo* mouse cancer models; *in vitro* mouse peritoneal macrophages; *in vitro* mouse *Sucnr1*$^{-/-}$ peritoneal macrophages	[21]
Mast cells (MCs) (mRNA)	Hyper-reactive phenotype of *Sucnr1*$^{-/-}$ MCs	Unknown	Unknown	*In vitro* human MCs; *in vitro* mouse bone marrow derived MCs; *in vitro* mouse *Sucnr1*$^{-/-}$ dermatitis model	[42]

also be detrimental by leading to vessel hyperproliferation, such as in the context of proliferative ischemic retinopathy [27].

The apical membrane of the retinal pigment epithelium (RPE) also expresses *Sucnr1* under homeostatic conditions [30,31]. Here, SUCNR1 has a putative role in iron homeostasis, because both the *in vivo* genetic disruption of the iron regulatory genes *Hfe* (*Hfe*$^{-/-}$) or *Hfe2* (*Hjv*$^{-/-}$) in mice, and the *in vitro* exposure of primary RPE cells to soluble iron, led to increased SUCNR1 expression [30,31]. This evidence is clinically relevant to **hemochromatosis,** an iron storage disorder associated with excessive iron accumulation. In fact, when human RPE cells are exposed *in vitro* to succinate and soluble iron, SUCNR1-dependent increased *VEGF* expression is observed [30]. Moreover, in the mouse model of juvenile hemochromatosis (*Hjv*$^{-/-}$), enhanced **bone-morphogenetic (BMP)-6** signaling in combination with succinate, facilitated the interaction of pSmad4 with the *Sucnr1* promoter sequence, promoting angioma formation through a *Sucnr1*- and *Vegf*-dependent mechanism [31].

Active SUCNR1 expression and signaling is also present in both mouse and human induced neural stem cells (iNSCs) *in vitro* [32]. However, its precise roles in regulating endogenous NSC function, differentiation, and how it could interact with their metabolism in conditions of inflammation [33] are still poorly understood. Similarly, rat astrocytes express *Sucnr1* [29] and, when exposed to succinate *in vitro*, there is increased expression of proangiogenic factors (*Vegf, Ang1,* and *Ang2*) and proinflammatory cytokines [interleukin (*IL*)*1b* and *IL6*] [29]. Succinate also increases the secretion of PGE_2, but not of VEGF, from RCA cells [29].

These data, together with the recent finding of genome-wide variation in *SUCNR1* function in humans [34], suggest that SUCNR1 activity is a predisposing factor for the development of CNS-intrinsic dysfunctions, although this remains to be thoroughly investigated.

SUCNR1 Signaling in Adaptive and Innate Immune Cells

Human hematopoietic progenitor cells (HPCs) are known to express SUCNR1 [35]. SUCNR1 has been linked with the maintenance of the self-renewing pool of HPCs, mostly via the activation of the MAPK signaling pathway [35,36]. Megakaryocytes and erythroblasts also express SUCNR1 [37], as do many cells of the **adaptive** and **innate immune systems** across species (Table 1). The conserved expression of SUCNR1 in immune cells suggests a key role in immune system function, acting as a regulator of both homeostatic and inflammatory cellular responses following exposure to pathogens or after tissue damage.

Within the adaptive immune system, SUCNR1 is detected on human T lymphocytes (both $CD4^+$ and $CD8^+$ subsets) and all major human B lymphocyte subsets [37,38]. Although the exact

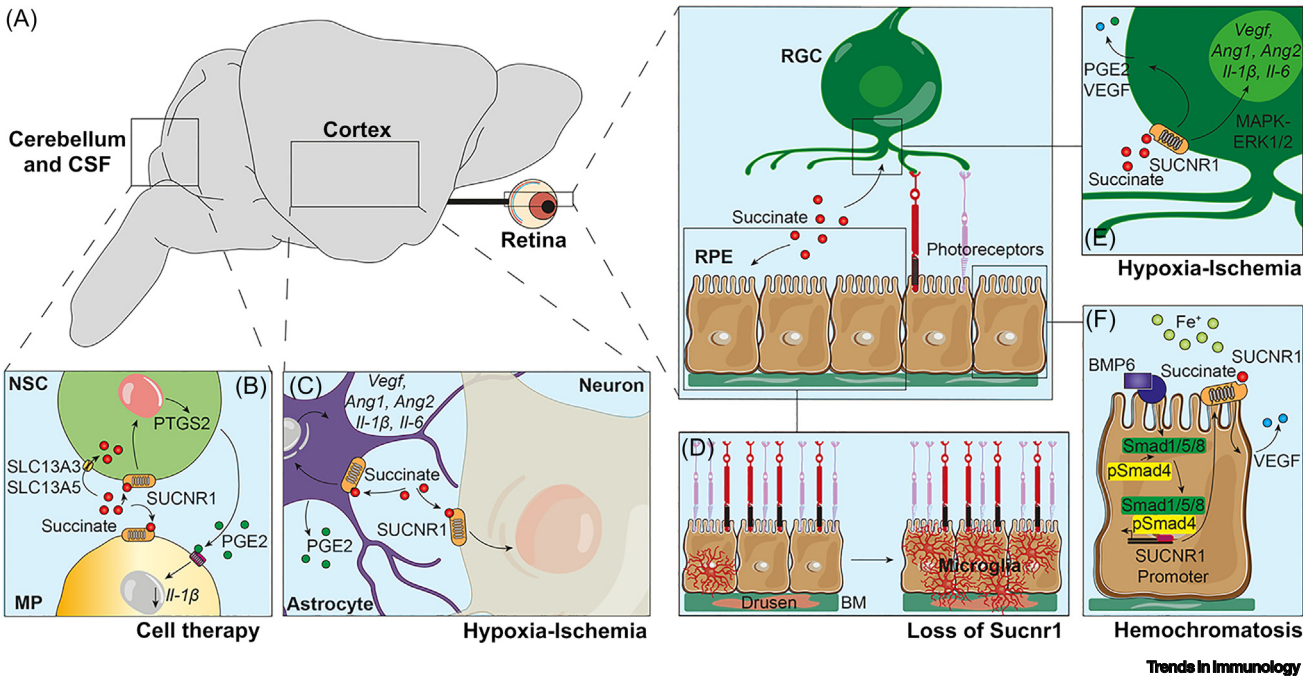

Figure 2. Succinate Receptor 1 (SUCNR1) Expression in Non-Immune Cells in the Mammalian Central Nervous System (CNS) (A). During cell therapy to suppress chronic inflammation, excessive succinate (SUC) accumulation in the cerebrospinal fluid (CSF) activates SUCNR1 on grafted neural stem cells (NSC) leading to the release of prostaglandin E_2 (PGE_2), SUC uptake, and downregulation of proinflammatory mononuclear phagocytes (MP) (B) [32]. In hypoxia-ischemia, SUCNR1 is expressed in cortical neurons and astrocytes, inducing the secretion of proangiogenic and inflammatory factors (C) [29]. Within the retina, microglia, retinal ganglion cells (RGC), and the retinal pigmented epithelium (RPE) express SUCNR1 [25,26,30,44]. Global loss of *Sucnr1*$^{-/-}$ leads to microgliosis in healthy mice (D), SUCNR1 on RGC leads to increased expression of proangiogenic and proinflammatory factors during hypoxia-ischemia (E), and SUCNR1 on RPE induces vascular endothelial growth factor (VEGF) release through bone-morphogenetic protein 6 (BMP6)-Smad signaling in hemochromatosis (F) [31]. Abbreviations: Ang, angiopoietin; BM, Bruch's membrane; ERK, extracellular signal-regulated kinase; IL, interleukin; MAPK, mitogen-activated protein kinase; PTGS2, prostaglandin-endoperoxidase synthase 2; SLC13A3/5, solute carrier 13A3/5.

function of SUCNR1 in T lymphocytes is yet to be fully characterized, a study on *ex vivo* expanded T cells from patients with systemic lupus erythematosus (SLE) suggested that T cells release large amounts of IL-10 and succinate following activation with oxidized mitochondrial DNA (mtDNA) *in vitro* [38]. In these oxidized mtDNA-stimulated SLE T cells with naïve B cell co-cultures, co-incubation with neutralizing anti-SUCNR1 antibodies partially inhibited the T cell-dependent activation of B cells and subsequent production of immunoglobulin G (IgG), relative to controls; this suggested that SUCNR1 expression on B cells was required for their activation [38]. Whether succinate, in synergy with other cytokines and chemokines, is relevant to the enhancement and amplification of adaptive immune responses in other contexts, is open for investigation.

The functional effects of SUCNR1 activation in innate immune cells are not always straightforward, because they are both cell and context dependent [16,39]. Within the innate immune system, SUCNR1 is expressed on DCs, mast cells, and **mononuclear phagocytes** (MPs) [such as bone marrow-derived macrophages (BMDMs), tumor-associated macrophages (TAMs), adipose tissue macrophages, and **microglia**] (details on species and cells are given in Table 1).

Among the innate immune cells, human immature DCs show maximal expression, and SUCNR1 activity controls their **chemotaxis** in a succinate concentration-dependent manner [40]. Here, SUCNR1 acts synergistically with **toll-like receptor** (TLR)-3 and 7, but not TLR-2 or TLR-4, to increase the expression of the proinflammatory cytokines tumor necrosis factor (TNF)-α and IL-1β.

Overall, this leads to enhanced antigen-specific presentation and subsequent $CD4^+$ T cell activation [40]. Of relevance, this response appears to be time limited**,** because SUCNR1 is swiftly downregulated once DCs become fully activated [40]. Also in mouse DCs, SUCNR1 enhances lipopolysaccharide (LPS)-induced (i.e., TLR-4-dependent) production of proinflammatory cytokines [41]. Indeed, in mice with **experimental antigen-induced arthritis**, SUCNR1-mediated chemotaxis guided mouse DCs into lymph nodes *in vivo*, leading to the expansion of pathogenic **T helper (Th)17 cells**, which in turn contributed to autoimmunity [41].

The activity of mast cells also relies on the presence of functional SUCNR1. In *Sucnr1*$^{-/-}$ mice, mast cells displayed a hyperreactive phenotype, as evidenced from increased TNF-α production in response to oxazolone-induced allergic contact dermatitis, relative to controls [42]. However, the hyper-reactive response of mast cells within this context does not correlate with an equally hyperreactive adaptive immune response, as measured by either T cell infiltrates or the expression of the **Th2-like** (anti-inflammatory) cytokines IL-4 and IL-13 [42]. These findings suggest a key physiological role for succinate–SUCNR1 signaling in mast cells that impacts their differentiation and maturation capacity, although this warrants further studies.

Overall, these data suggest that coordinated expression and activation of SUCNR1 among multiple cell types of the adaptive and innate immune systems is necessary for modulating homeostatic and inflammatory responses via this newly recognized metabolic signaling pathway.

Context-Dependent Role of SUCNR1 in MPs

While the succinate–SUCNR1 axis is rather defined in DCs and mast cells [40–42], its role in the chemotaxis and activation of MPs remains controversial.

In *Sucnr1*$^{-/-}$ mice, macrophage infiltration into succinate-producing adipose tissue is significantly reduced *in vivo* relative to controls [43]. However, the lack of a clear chemoattractant effect for succinate alone on macrophages *in vitro* questions the specificity of these findings. In fact, *Sucnr1*$^{-/-}$ macrophages show reduced migration only in response to tissue culture media derived from apoptotic/hypoxic adipocytes, which suggests that the presence of receptors other than SUCNR1 [e.g., reduced C-C chemokine receptor type I (CCR1) expression] has a major role in MP chemotaxis [43]. Nevertheless, recent data demonstrated that succinate alone can promote the chemotaxis of SUCNR1-expressing TAMs in both syngeneic and xenogeneic lung cancer grafts models in mice, because this effect was abrogated by pretreatment with anti-SUCNR1 antibodies *in vitro,* suggesting a direct role of SUCRN1 in TAM chemotaxis [21].

Engagement of SUCNR1 has also been linked to microglial chemotaxis, as suggested by the increased accumulation of microglia in the retina of *Sucnr1*$^{-/-}$ mice relative to controls [44]. However, this phenotype only occurs when the hosts are globally deficient in *Sucnr1*, suggesting that the SUCNR1-mediated control of retinal microglia chemotaxis is in fact dispensable [44].

Besides chemotaxis, the effects of the succinate–SUCNR1 axis on inflammatory responses of MPs have also led to conflicting results (Figure 3, Key Figure). For example, a proinflammatory effect of SUCNR1 was observed in alternatively activated (anti-inflammatory) macrophages derived from human peripheral blood mononuclear cells (PBMCs) [45]. Here, when macrophages were challenged *ex vivo* with IL-4 and IL-13, they significantly upregulated *SUCNR1*. However, subsequent stimulation with succinate, or the SUCNR1 agonist compound 131, decreased the expression of the anti-inflammatory cytokine IL-10, while upregulating the proinflammatory cytokine TNF-α, thus enhancing proinflammatory responses [45]. These findings suggest a prominent role for the succinate–SUCNR1 axis in maintaining inflammation by dampening anti-

Key Figure

Pro- and Anti-Inflammatory Effects of Succinate Receptor 1 (SUNCR1) Activation in Mammalian Macrophages

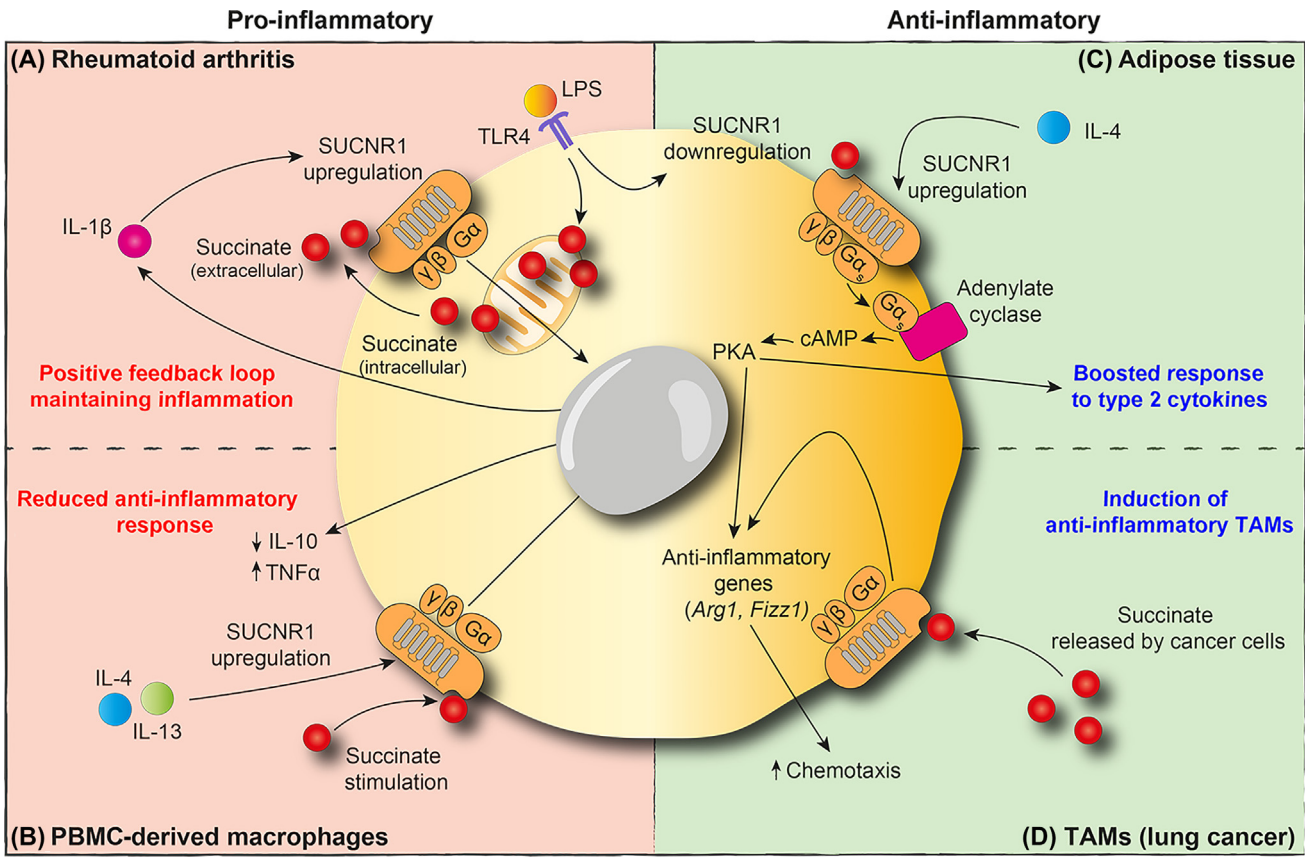

Figure 3. Current evidence suggests that the succinate–SUCNR1 axis on macrophages induces tissue- and disease-specific polarization. (A,B) Proinflammatory effects are seen in rheumatoid arthritis, where a 'positive feedback' loop between interleukin (IL)-1β and succinate maintains chronic inflammation [39], and also in human peripheral blood mononuclear cells (PBMCs) and alternatively activated macrophages, where stimulation of SUCNR1 induces tumor necrosis factor (TNF)-α while suppressing IL-10 [45]. (C,D) Anti-inflammatory effects have been described in the adipose tissue, where activation of SUCNR1 promotes the response of these cells to type 2 cytokines [16], and in tumor-associated macrophages (TAMs), where the succinate–SUCNR1 axis promotes an anti-inflammatory gene signature and chemotaxis within the lung cancer microenvironment [21]. Abbreviations: Arg 1, Arginase 1; Fizz1, found in inflammatory zone protein 1; LSP, lipopolysaccharides; PKA, protein kinase A; TLR-4, toll-like receptor 4.

inflammatory responses. In addition, while previous evidence has shown that the absence of SUCNR1 on mouse peritoneal macrophages has no effect on intracellular IL-1β concentrations, or on the secretion of TNF-α and IL-6 (in response to 24-h stimulation with 10 ng/ml LPS [43]), recent data suggested that, when BMDMs are stimulated for 24 h with higher doses (100 ng/ml) of LPS, or with a combination of LPS (1 ng/ml) and monosodium urate (180 ug/ml) *in vitro*, the absence of SUCNR1 led to significantly lower IL-1β production [39]. In addition, IL-1β treatment per se increased *Sucnr1* mRNA expression in mouse BMDMs *in vitro* [39]. This finding anticipates a possible bidirectional crosstalk between proinflammatory mediators and the activity of SUCNR1, and the existence of a positive feedback mechanism in inflammation. In this model, IL-1β would trigger the production and release of succinate from macrophages, while succinate in

turn would stimulate SUCNR1-expressing MPs to increase IL-1β, thus maintaining a chronic inflammatory state via an autocrine and paracrine loop [39]. Accordingly, *in vivo* data suggest that *Sucnr1*$^{-/-}$ bone marrow chimeric mice are protected from antigen-induced experimental arthritis as early as 2 days after challenge, relative to controls [39].

However, recent work has challenged the prevalent proinflammatory role of SUCNR1 by showing that mouse BMDMs lacking SUCNR1 (*Sucnr1*$^{-/-}$) exhibit an increased proinflammatory phenotype relative to controls, characterized by increased release of IL-6, TNF-α, and nitric oxide (NO), in response to LPS (100 ng/ml) or LPS + interferon (IFN)-γ (10 ng/ml + 10 U/ml, respectively) for 24 h *in vitro* [46]. Since the *Sucnr1*$^{-/-}$ mice used to derive BMDMs were identical in both of the aforementioned studies [39,46], discrepancies in published results might be related to the use of different BMDM maturation protocols (i.e., recombinant M-CSF + IFN-γ pulse [39] versus L-929 conditioned media [46]), as well as the age of the animals (age-matched [39] versus an average of 28 weeks difference between WT and *Sucnr1*$^{-/-}$ mice [46]). However, the exact mechanisms behind these apparent discrepancies are yet to be fully addressed.

Further data supporting the anti-inflammatory effect of SUCNR1 come from a study of an inflamed CNS, where SUCNR1 stimulation can directly participate in the resolution of inflammation *in vivo* [32]. Specifically, delayed accumulation of succinate occurred in the cerebrospinal fluid (CSF), but not in peripheral blood of mice with **experimental autoimmune encephalomyelitis** (EAE), a model of chronic multiple sclerosis (MS) [32]. In this study, CSF succinate, most likely released by proinflammatory macrophages and microglia, signaled to grafted NSCs via SUCNR1; NSCs, in response, initiated the secretion of PGE_2 and the scavenging of extracellular succinate, both contributing to the resolution of neuroinflammation [32].

Subsequent work confirmed the anti-inflammatory role of SUCNR1 *in vivo*, showing that SUCNR1 in mouse macrophages induced a prevalent anti-inflammatory phenotype [16]. Specifically, in a LysM^{Cre}*Sucnr1*$^{fl/fl}$ myeloid cell-specific *Sucnr1* conditional knockout mouse line, a significant increase in CD11b^{+}/CD11c^{+}/CD206^{-} proinflammatory macrophage numbers and proinflammatory genes (*Il12b*, *Tnf*, and *Nos2*) was reported in white adipose tissue (WAT) *in vivo* relative to controls [16]. *In vitro*, treatment with IL-4 (30 ng/ml) induced *Sucnr1* in mouse peritoneal macrophages [while treatment with LPS (250 ng/ml) had the opposite effect (decreased *SUCNR1* expression) in macrophages derived from human PBMCs] [16]. Of note, the SUCNR1-dependent induction of an anti-inflammatory phenotype in macrophages occurred in response to IL-4 via an alternative cAMP-dependent protein kinase A (PKA) phosphorylation signaling cascade, subsequent to $G\alpha_s$ stimulation [16].

Finally, recent evidence in syngeneic and xenogeneic lung cancer grafts models in mice provides further confirmation of the role of succinate–SUCNR1 signaling in skewing macrophages into anti-inflammatory TAMs [21]. In this setting, peritoneal macrophages treated with succinate (1 mM) for 24 h showed a significant increase in the expression of anti-inflammatory mRNAs *Arg1*, *Fizz1*, *Mgl1*, and *Mgl2 in vitro* relative to controls, which was completely abolished via siRNA-mediated *SUCNR1* downregulation. Furthermore, succinate released by the tumor induced a significant increase in the number of VCAM1^{+}/CD11c^{+}/CD11b^{low} TAMs *in vivo*, suggesting that the succinate–SUCNR1 axis was indispensable for inducing the expansion of anti-inflammatory TAMs [21].

Altogether, these findings suggest that succinate, produced (and released) by proinflammatory MPs, can act on SUCNR1 via competing feedback loops regulating both proinflammatory and anti-inflammatory MPs. However, the exact circumstances and mechanisms guiding the prevalence of either response remain to be fully elucidated.

SUCNR1 as a Potential Pharmacological Target for Chronic Neuroinflammation

While the role of acute inflammation is well known, the transition towards a chronic state of inflammation has now become a therapeutic focus, particularly in the context of inflammatory and degenerative neurological diseases [47,48]. Under persistent neuroinflammation, the activation of innate immune cells leads to a state of 'sterile' inflammation, wherein the proinflammatory phenotype of MPs continues in the absence of triggering stimuli, thus leading to constant damage of neurons and oligodendrocytes [5].

As described earlier, there is evidence supporting a key role for the succinate–SUCNR1 axis in regulating the phenotype and function of innate immune cells, and the response of CNS cells to damage; thus, pharmacological targeting of this pathway may be therapeutically relevant for certain conditions associated with persistent (detrimental) innate immune responses in the CNS, such as progressive MS. Unfortunately, the expression of SUCNR1 on a range of CNS and immune cells, as well as the seemingly disparate functional effects that can result from activating this pathway, complicate the study and putative development of therapeutics targeting SUCNR1. Major considerations regarding the cell- and context-specific effects of SUCNR1 signaling must be taken into account when designing and interpreting experimental results, as should the CNS permeability of any prospective succinate analogs [46,49]. Ideally, attention to these key points might not only lead to a better understanding of the succinate–SUCNR1 axis, but also inform the development of potential therapies that might target chronic/persistent neuroinflammation.

Most work in this area has been related to the identification of SUCNR1 agonists. By using a structural model based on the crystal structure of the closely related P2Y1 receptor (27% homology to SUCNR1), the binding site of.SUCNR1 was characterized and virtual screening was used to identify its putative ligands [45]. Several non-metabolite agonists were identified, with the most potent candidates having structures based on the succinate backbone with an amide-linked hydrophobic moiety capable of occupying a side pocket at the SUCNR1 binding site [45]. Certain agonists were determined to have 10–100 times higher potency than succinate, exhibiting specificity for either human or mouse SUCNR1, and displaying similar signaling effects to succinate in human *ex vivo* macrophages [45]. Further structure–function studies of this agonist series identified nanomolar potency ligand candidates with excellent *in vitro* stability, but high hydrophilicity [50]. The most potent succinate analog is *cis*-epoxysuccinic acid, with a **half maximal effective concentration** (EC_{50}) of 2.7 μM; this compound is not succinate dehydrogenase active but has the same effect as the canonical SUCNR1 ligand, because it increased blood pressure in rats upon intravenous administration [51]. For reference, EC_{50} for succinate for the activation of SUCNR1 has been reported in the range of 17–56 μM [9,45], while typical plasma concentrations of succinate in humans are on the order of 2–30 μM (see Clinician's Corner) [45]. Of note, the xanthone natural products vinaxanthone and xanthofulvin have also been found to be positive allosteric modulators of SUCNR1, enhancing the affinity of the receptor for succinate [52].

Based on the predominant role of SUCNR1 as a positive regulator of innate immune cell chemotaxis and chronic inflammation, it is not a stretch to consider that SUCNR1 antagonists might be protective in environments where inflammation is exacerbated, such as in certain autoimmune or neuroinflammatory conditions, including progressive MS. Several small-molecule inhibitors of human SUCNR1 were developed from a single hit identified in a high-throughput *in vitro* screen of a commercial compound library [53]. Through structure–activity relationship-guided systematic modification of the **naphthyridine-based** hit, potent and selective antagonists were identified, most notably those labeled '2c' and '4c'. These compounds exhibited promising **half maximal inhibitor concentration** (IC_{50}) values of 30 nM and 7 nM, respectively, and were >1000 times

more selective for human SUCNR1 over hGPR99 (which shares 33% protein sequence homology) [53][i]. While both 2c and 4c exhibit poor oral bioavailability, intraperitoneal administration into succinate-treated Wistar rats of either compound readily ameliorated hypertension relative to controls [53]. Other compounds synthesized in the study traded improved oral bioavailability for decreased affinity/selectivity [53]. Assays using another compound in the series, 2d, demonstrated the ability of the SUCNR1 inhibitor to suppress type I collagen induction in rat hepatic stellate cells subjected to activation by high glucose (700 mg/dl) or high succinate (3 mM) conditions modeling **nonalcoholic steatohepatitis** (NASH) *in vitro* [54]. An alternative series of SUCNR1 inhibitors was identified through high-throughput screening with one compound, NF-56-EJ40, demonstrating an impressive selectivity for human SUCNR1 (IC_{50} = 25 nM), over rat SUCNR1 (IC_{50} >100 μM) [12]. The structural basis of this species selectivity was elaborated through crystallography and molecular modeling, with comparisons to the structurally similar P2Y1 receptor providing leads for prospective allosteric inhibitors. While this series of antagonists demonstrated poor permeability due to their highly polar zwitterionic nature, systematic optimization of the structural scaffold, including incorporation of an internal salt bridge, yielded potent inhibitors with good bioavailability and oral exposure [55].

Follow-up studies of the therapeutic utility of SUCNR1 antagonists have yet to be reported in the scientific literature, but several of the co-authors of the naphthyridine-based antagonist study are listed as inventors on various patents describing a complementary series of SUCNR1 inhibitors with prospective applications in treating NASH and related conditions. Indeed, both fibroblast growth factor 21 and its recombinant peptide analog have lowered α-smooth muscle actin production through inhibition of the succinate–SUCNR1 signaling axis, thus reducing liver fibrosis in mouse models [56]. The mitochondrial fission-activating type 2 diabetic drug metformin has likewise been ascribed an inhibitory role in succinate–SUCNR1 signaling, ameliorating inflammation and fibrosis in a mouse model of NASH [57].

RNAi-mediated downregulation of SUCNR1 has also been used to explore the physiological ramifications of succinate–SUCNR1 signaling (and, thus, its potential therapeutic modulation). SUCNR1 is alleged to be a key target of miR-758, with oxidized low-density lipoprotein-induced overexpression of this miRNA associated with suppression of SUCNR1 and its downstream signaling pathways, resulting in damage to human vascular endothelial cells *in vitro* [58]. Alternatively, short hairpin RNA-mediated knockdown of *Sucnr1* expression in a rat model of retinopathy attenuated the avascular area, abnormal neovascularization, and loss of pericytes through regulation of VEGF relative to controls [59]. One novel approach envisions an enhanced-affinity recombinant SUCNR1 mutant able to sequester elevated concentrations of succinate in the gut, such as that associated with inflammatory bowel disease[ii].

There may be a significant opportunity to therapeutically intervene in conditions of chronic inflammation through amelioration of SUCNR1 overactivity in the innate immune system. The benefits of such an approach are perhaps most immediately achievable in metabolic conditions, such as NASH, obesity, and type 2 diabetes mellitus, which are marked by a strong inflammatory basis to their pathology and in which GPCR-targeting drugs have shown significant potential [60,61]. Nonetheless, the need remains to elucidate the pathological relevance of the succinate–SUCNR1 axis in human disease (given that preclinical animal models of GPCR signaling modulation have not always been successfully translated) [60,62].

Beyond systemic metabolic conditions, applications for SUCNR1 inhibitors in the treatment of neuroinflammatory diseases are feasible and worth pursuing. In conditions of elevated CNS succinate, potent and selective antagonists offer the potential to restore homeostasis to the

Clinician's Corner

The physiological concentration of plasma and serum succinate in healthy human individuals can vary from 2 to 20 μM [11] and 2 to 30 μM [9,45], respectively. Such concentrations under physiological conditions are insufficient to activate SUCNR1 signaling (EC_{50} for succinate-induced activation of SUCNR1 is 17–56 μM [45]).

A several-fold increase in succinate concentration in local tissues and biological fluids can be detected in patients with rheumatoid arthritis (RA) (74-fold increase in the synovial fluid in patients with RA) [71], myocardial infarction (succinate concentration in blood: 2.73 ±0.11 μM versus 1.38±0.14 μM control) [72], obesity (succinate in serum of obese 43.93 ± 6.16 μM versus 23.2 ± 1.57 μM in lean individuals) [73], lung cancer (serum concentration of patients with lung cancer: 0.53 ± 0.03 mM versus 0.30 ± 0.02 mM in healthy individuals) [21], and Crohn's disease (twofold increase of succinate in serum) [74]. Additionally, increased expression of SUCNR1 mRNA and protein occurs in the intestines of patients with Crohn's disease [74] and in lung cancer tissue [21].

Therapeutically, one study indicated that direct brain succinate infusion (12 mmol/l) by microdialysis catheters in patients with traumatic brain injury enhanced brain metabolism by recycling NADH to NAD^+, leading to improved postinjury recovery [75].

Diagnostically, ^{13}C-labeled succinate and ^{99m}Tc- and ^{18}F-labeled SUCNR1 compounds [76], which allow for human *in vivo* nuclear magnetic resonance (NMR) or positron emission tomography (PET) tracing of succinate and SUCNR1 expression (e.g., in the CNS), might open the possibility of using both succinate and SUCNR1 as putative biomarkers of chronic inflammatory disease, such as progressive MS [5].

Box 2. Constitutive SUCNR1-Knockout Mice

Constitutive knockout technology provides a powerful *in vivo* tool to understand the functional and behavioral phenotype of gene deletion. To this extent, *Sucnr1*$^{-/-}$ mice have been useful because they display normal development and maturation, despite a significantly lower body weight, compared with their WT littermates [66]. *Sucnr1*$^{-/-}$ mice are fertile, show no overt blood pressure anomalies, no abnormal brain vascularization [29,67], no alteration of food intake, and no deposition of fat or lean mass compared with WT littermates [66]. However, *Sucnr1*$^{-/-}$ mice do show deficits in conditions of stress and disease. Their endurance on a forced treadmill running test is significantly impaired, as indicated by overall lower average running speed and decreased muscle strength relative to WT mice [19]. When fed a high-fat diet (HFD), *Sucnr1*$^{-/-}$ mice showed increased fat deposition, hyperglycemia, reduced insulin secretion, and augmented hepatocyte damage compared with WT littermates [66]. Moreover, *Sucnr1*$^{-/-}$ mice intravenously supplemented with succinate showed abnormal cardiac parameters related to the function of the left ventricle, such as decreased systolic volume and ejection fraction [68]. In the future, to circumvent the well-known limitations of global gene knockout (such as compensatory mechanisms and off-target cellular effects), cell-specific gene knockout-targeting strategies should be used to provide more accurate insights into the function of SUCNR1 [16].

succinate–SUCNR1 axis in resident or infiltrating MPs, alleviating chronic inflammation driving neurodegeneration and disability. Towards this goal, there is an unmet need for orally bioavailable SUCNR1-inhibiting compounds capable of effectively permeating the blood–brain barrier (e.g., those with a low molecular weight and moderate lipophilicity while minimizing interactions with brain efflux transporters). The structural and pharmacokinetic optimization of SUCNR1 antagonists (described earlier) point to substantial opportunities for novel compounds to be explored in clinical studies, pending *in vivo* validation in appropriate animal disease models. Notably, while the apparent limited activity of SUCNR1 in a nonpathological context suggests that such a compound would have a high therapeutic index, a greater understanding of the systemic effects of pharmaceutical modulation of the succinate–SUCNR1 axis is essential for ruling out any unanticipated off-target effects.

Concluding Remarks

Overall, SUCNR1 has thus far been largely overlooked as a putative therapeutic target for certain inflammatory pathologies, perhaps due to its complex and tissue/context-dependent functions. While large pharmaceutical companies have previously filed patents on methods for screening SUCNR1 modulators or the use of SUCNR1 as a marker/target for immune cells, these have been seemingly neglected. Several research groups and small companies are furthering the development of more bioavailable agonists and antagonists; however, future therapeutic exploitation of the succinate–SUCNR1 axis will necessitate greater elucidation of its complexities.

The succinate–SUCNR1 axis appears to be relevant in conditions of stress and damage, while its role in homeostatic conditions is negligible (Box 2). This might be advantageous in developing candidate therapeutics to treat certain pathologies, given that the off-target effects of SUCNR1 modulation are likely to have minimal effects on nonactivated SUCNR1 cells in the host, although this remains to be rigorously tested (see Outstanding Questions). In our view, understanding the complex role of SUCNR1 in regulating neuro-immune interactions in health and disease can uncover the right window of opportunity to modulate this axis, where its targeting could contribute to anti-inflammatory and proregenerative responses that might resolve chronic neuroinflammation.

Outstanding Questions

Cellular cues, such as hypoxia, inflammation, and necrosis, stimulate succinate release. Mechanistically, accumulated succinate in the mitochondrial matrix is transported to the mitochondrial inner space by SLC25A10 mitochondrial dicarboxylate carrier (DIC). Succinate transport from mitochondria to the cytosol is controlled by voltage-dependent anion channel (VDAC). The extracellular release of succinate is mediated by solute carrier family 13 group (SLC13) transporters, expressed by neurons, astrocytes (SLC13A), and NSCs (SLC13A3/5), or the proton-coupled monocarboxylate transporter (MCT) 1, expressed by muscle cells. How is this mechanism of succinate release (and uptake) modulated in different cells and pathological conditions?

SUCNR1 activation leads to receptor internalization and desensitization via β-arrestin signaling [e.g., after either succinate (200 μM for 2 h) or LPS (250 ng/ml for 6 h) treatment]. In kidney cells, desensitization with 200 μM succinate lasted for 15 min, after which rapid resensitization occurred within 15 min. How stable is SUCNR1 activity and how does the cycling of SUCNR1 influence the pro- and anti-inflammatory phenotype of myeloid cells?

In the healthy CNS, basal SUCNR1 expression is nearly undetectable. However, during neuroinflammation, blood-borne and tissue-resident macrophage activation is linked to the upregulation of SUCNR1. Similarly, neural cells upregulate SUCNR1 in response to tissue damage. How are the expression dynamics of SUCNR1 in immune cells and neural cells regulated, and what is the overall contribution of these dynamics to the maintenance of chronic inflammation in the CNS?

Acknowledgments

The authors wish to acknowledge the contribution of past and present members of the Pluchino laboratory, who have contributed to (or inspired) this review. We are grateful to Giovanni Pluchino for critical insights into this manuscript. This work received support from the National MS Society (USA; grant RG-1802-30200 to S.P.), the Italian Multiple Sclerosis Association (AISM, grant 2018/R/14 to S.P.), the US Department of Defense (DoD) Congressionally Directed Medical Research Programs (CDMRP) (grant MS-140019 to S.P.), and the Bascule Charitable Trust (RG 75149 and RG 98181 to S.P.). L.P.J. was supported by a senior research fellowship Fondazione Italiana Sclerosi Multipla (FISM cod. 2017/B/5 and financed or co-financed by the '5 per mille' public funding), a clinical research career development fellowship from the Wellcome Trust (220711/Z/20/Z), and the Addenbrooke's Charitable Trust (RG 97519).

Disclaimer Statement

S.P. is cofounder, CSO, and shareholder of CITC Ltd and iSTEM Therapeutics, and cofounder and non-executive director at Asitia Therapeutics. L.P-J. is a shareholder and director of strategy and innovation at CITC Ltd. J.A.S. is a project manager and senior research associate at CITC Ltd, head of research at iSTEM Therapeutics, and CSO of Asitia Therapeutics.

Resources

[i]https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2018167800

[ii]https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2019053731

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