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Lipocalin 2: An Emerging Player in Iron Homeostasis and Inflammation

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Abstract

Lipocalin 2 (Lcn2), an innate immune protein, has emerged as a critical iron regulatory protein during physiological and inflammatory conditions. As a bacteriostatic factor, Lcn2 obstructs the siderophore iron-acquiring strategy of bacteria and thus inhibits bacterial growth. As part of host nutritional immunity, Lcn2 facilitates systemic, cellular, and mucosal hypoferremia during inflammation, in addition to stabilizing the siderophore-bound labile iron pool. In this review, we summarize recent advances in understanding the interaction between Lcn2 and iron, and its effects in various inflammatory diseases. Lcn2 exerts mostly a protective role in infectious and inflammatory bowel diseases, whereas both beneficial and detrimental functions have been documented in neurodegenerative diseases, metabolic syndrome, renal disorders, skin disorders, and cancer. Further animal and clinical studies are necessary to unveil the multifaceted roles of Lcn2 in iron dysregulation during inflammation and to explore its therapeutic potential for treating inflammatory diseases.

Contents

INTRODUCTION

Inflammation and its classical clinical symptoms were first described in Latin as *calor* (heat), *dolor* (pain), *rubor* (redness), and *tumor* (swelling) by the Roman scholar Cornelius Celsus. As studies on medicine and science evolved over the ages, our understanding of inflammation has unequivocally advanced beyond those four corporeal terms and begun to characterize their intricacy at the molecular level. Acute inflammation, for instance, is associated with the elevated expression of many acute phase proteins (APPs)—such as C-reactive protein, serum amyloid A, ferritin, and hepcidin—that drive or dampen the inflammation. Many of these APPs have found clinical use as diagnostic biomarkers, in addition to their potential to be exploited as therapeutic targets. Within the past two decades, another APP, lipocalin 2 (Lcn2; alias 24p3, SIP24, siderocalin) and its human ortholog, neutrophil gelatinase–associated lipocalin (NGAL), have emerged as new players in the regulation of host responses to inflammation, particularly in modulating iron homeostasis.

Iron is an essential nutrient for almost all aerobic organisms and its homeostasis is intimately tied to inflammation. A healthy 70-kg human adult has approximately 3–5 g of iron; most of the iron is enclosed within hemoglobin, and a small fraction is associated with iron storage proteins, such as ferritin and transferrin, to maintain extracellular free iron at concentrations below 10^{-24} M (42, 134). This scarcity of available iron serves as a natural nutritional immunity against pathogens and protects the host from the reactivity of free iron. If not regulated appropriately, iron can become a potent redox engine that generates labile iron (also known as catalytic iron), participates in Fenton and Haber–Weiss reactions, and results in the formation of free radicals that can damage cellular

APP: acute phase protein

Lcn2: lipocalin 2

biomolecules. Thus, the hypoferremic response during inflammation can be viewed as a primitive protective mechanism against iron reactivity and infection (18). Importantly, in the context of this review, Lcn2 has been demonstrated to mediate key roles in facilitating hypoferremia via modulating the labile iron pool (LIP) and sequestering mucosal and luminal iron. This review aims to summarize the physiological and pathological functions of Lcn2 during inflammation, and emphasizes iron homeostasis. In this review, both animal Lcn2 and human NGAL will be referred to as Lcn2 for convenience.

LIP: labile iron pool

LIPOCALIN 2 AND IRON REGULATION

Lipocalin 2: Discovery and Nomenclature

The discovery of Lcn2 was preceded in 1989 by the identification of its messenger RNA as 24p3 in an SV40-infected mouse kidney cell culture by Hraba-Renevey et al. (82). Based on its predicted protein sequence, Flower et al. (63) deduced that 24p3 encoded a lipocalin protein. In 1993, groups led by Borregaard (99) and Tschesche (177) independently codiscovered human Lcn2 as a 25 kDa protein covalently bound to the 92 kDa matrix metalloproteinase 9 (MMP9) from human neutrophils. Kjeldsen et al. (99) dubbed the protein "neutrophil gelatinase–associated lipocalin," whereas Xu et al. (195) described it as human neutrophil lipocalin (or HNL). Despite being named as a neutrophil protein, Lcn2 can also be secreted by other cell types (e.g., macrophages, hepatocytes, epithelia, and adipocytes).

Murine Lcn2 was also discovered as the superinducible protein (or SIP24) in 1995 by Liu & Nilsen-Hamilton (105). Their study was fundamental in establishing Lcn2 as an APP secreted by the liver, brain, and uterus. Liu et al. (106) subsequently renamed the protein uterocalin, due to its high expression in uterine luminal fluid and epithelium, especially around parturition. In 2002, Goetz et al. (71) made the landmark discovery that Lcn2 specifically binds to bacterial siderophores; thus, they proposed that the protein be renamed siderocalin. Aderem's group (62) eventually unraveled Lcn2 as an innate immune antibacterial protein against iron-dependent bacteria, and they proposed the name lipocalin 2, which is now widely accepted as the canonical term for murine Lcn2. In comparison, human Lcn2 is widely described as NGAL, although other nomenclature, including 24p3, HNL, and siderocalin, is still employed in recent literature. Other known Lcn2 homologs from diverse species are *neu*-related lipocalin (or NRL; rat), extracellular fatty acid binding protein (Ex-FABP; chicken), and Q83 (quail).

Structure of Lipocalin 2

Human and murine Lcn2 share 85% homology in amino acid composition and exhibit 70% similarity in nucleotide composition. Lcn2 belongs to the lipocalin superfamily, which comprises small secreted proteins (160–180 amino acids) with limited sequence homology, but they all display a highly conserved core tertiary structure of an eight-stranded, antiparallel β-barrel that defines the calyx, or ligand-binding site (**Figure 1**) (64). Notable members of the lipocalin family are lipocalin 1 in tears, retinol-binding protein, α-1 microglobulin, apolipoprotein D, prostaglandin D synthase, and many others. Yet unlike most other lipocalins, the calyx in Lcn2 is shallower, broader, and is large enough to accommodate macromolecular ligands, and it exhibits sites lined with polar (at the barrel opening), positively charged (centered in the calyx), and hydrophobic residues (at the base of the barrel) (71).

The majority of human Lcn2 is not constitutively associated with MMP9, but can be stored as monomers or homodimers in the neutrophil-specific granules. The unpaired cysteine residue

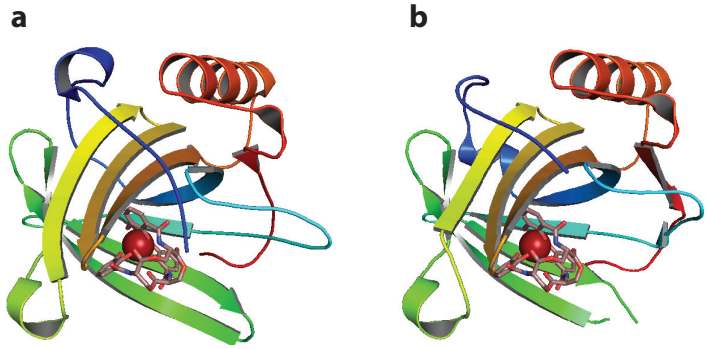

Figure 1

The crystal structure of the lipocalin 2 (Lcn2)–enterobactin–iron complex. Three-dimensional structures of Lcn2 complexed with enterobactin (colored by atom type: N, *blue*; C, *brown*; O, *red*) and ferric iron (*red sphere*) generated by the PyMol program (The PyMOL Molecular Graphics System, version 1.8, Schrödinger LLC). (*a*) Human Lcn2 (neutrophil gelatinase–associated lipocalin, or NGAL; Protein Data Bank identification number 3CMP). (*b*) Murine Lcn2 (*Mus musculus* and *Rattus norvegicus*; Protein Data Bank identification number 3U9P).

(Cys87) allows human Lcn2 to form protein linkages and to be secreted in multiple forms: monomer, disulfide-linked homodimer, and a disulfide-linked heterodimer with MMP9 (99, 177). Immune cells, such as neutrophils, predominantly secrete dimeric Lcn2, but epithelia secrete only monomeric Lcn2 (30). Molecular mechanisms dictating these Lcn2 forms are not clear; it is presumed that the long storage of Lcn2 in the neutrophil granule (30) allows the formation of dimers, whereas epithelia-derived Lcn2 is primarily composed of monomers, given their relatively rapid secretion. The disparity in form substantially influences Lcn2 physiology as, for instance, Lcn2 monomers undergo more rapid clearance from the circulation than the dimers (7). Moreover, research led by the Venge (30, 114) and Barasch (130) groups further illustrates that diagnosis based on urinary Lcn2 monomer or dimer forms has the potential to distinguish among acute kidney injury (AKI), chronic kidney disease (CKD), and urinary tract infection.

Whereas human neutrophils can generate both the Lcn2 monomer and dimer, mice produce Lcn2 only in its monomeric form (44). The inability of murine Lcn2 to form homodimers or heterodimers with MMP9 is explained by the lack of Cys87 required to form disulfide linkages (79). Using the apparent lack of a dimer to distinguish between immune-derived and nonimmune-derived Lcn2, however, does not preclude the possibility of functional differences between the two sources of murine Lcn2. For instance, our recent study using Lcn2-deficient bone marrow chimeric mice suggested that immune-derived Lcn2 has a larger role than nonimmune-derived Lcn2 in conferring mucoprotection against murine colitis (150, 161).

Lipocalin 2 Receptors

Two major membrane-bound receptors for Lcn2 have been identified: megalin (also known as LRP2) and 24p3R (also known as solute carrier SLC22A17 and brain-type organic cation transporter, or BOCT) (51). The first characterized receptor for Lcn2 was megalin, which is a multiligand endocytosis receptor that is primarily expressed by kidney epithelia to facilitate the renal reabsorption of Lcn2 (86). The second Lcn2 receptor characterized was 24p3R, which belongs to the organic cation transporter family and is expressed in many tissues (51).

Devireddy and colleagues (51), who discovered 24p3R, also found that the binding of 24p3R to either holo-Lcn2 or apo-Lcn2 could in cancer cells, respectively, promote iron uptake or induce apoptosis. It has been suggested that apo-Lcn2–24p3R-induced apoptosis is mediated through iron depletion by apo-Lcn2, resulting in iron deficiency that triggers cell death (51). Considering that 24p3R displays varying binding affinities to apo- and holo-Lcn2 (29), it is possible that 24p3R may also activate different signaling pathways, depending on the iron status of its ligand. For instance, the interaction between Lcn2 and 24p3R has been shown to induce extracellular signal–regulated kinase 1 (or ERK1) and ERK2 signaling in neutrophils, which, in turn, modulates neutrophil migration, adhesion, and function (155).

Ent: enterobactin

Siderophores: The Essential Cofactor for Interaction Between Lipocalin 2 and Iron

Several putative lipophilic ligands for Lcn2 have been investigated, including retinol, oleate, bacterial *N*-formyl-methionyl-leucyl-phenylalanine (known as f-MLP), leukotriene B4 (known as LTB4), and platelet-activating factor, yet their weak affinity to Lcn2 implies that these candidates are not the cognate ligands (28). The siderophore-binding property of Lcn2 was serendipitously discovered when Strong and coworkers (71) observed a red-colored complex formed between Lcn2 and ferric iron–bound enterobactin (Fe^{+3}-Ent). By employing a tryptophan fluorescence quenching analysis, they showed that Lcn2 has a K_d value of 0.41 nM in the presence of Fe^{+3}-Ent. This high affinity for Ent allows Lcn2 to effectively compete with FepA (the bacterial receptor for Ent) and thus to inhibit the uptake of Ent in *Escherichia coli*. Structural analysis of the Lcn2–Ent–Fe adduct (**Figure 1**) reveals that the 1:1:1 stoichiometry is mediated by a combination of simple ionic and cation–π interactions between the negatively charged Fe-Ent and the positively charged arginine 81 (Arg81), lysine 125 (Lys125), and Lys134 in the Lcn2 calyx (71). Such interaction mirrors the binding between Fe-Ent and FepA, and is apparently well conserved in mediating the binding between Ent and Lcn2 homologs from different species (71). Because a large portion of the Lcn2 calyx remains unfilled by Fe-Ent, it was presumed that Lcn2 may be capable of binding larger, and perhaps a diverse group of, siderophores (71).

Despite its role in iron homeostasis, Lcn2 per se does not bind to iron directly, but interacts with iron only by forming a ternary complex with a siderophore as its cofactor. By definition, siderophores (Greek: iron carriers) are secreted, low-molecular-weight (<1.0 kDa) iron chelators with high affinity for insoluble ferric iron but not for soluble ferrous iron. Siderophores are generally classified according to their functional chemical groups into catecholates, carboxylates, and hydroxamates. Lcn2 is primarily capable of sequestering siderophores that are catecholates and some carboxylates, but not hydroxamates. Depending on their origin, the siderophores can be further classified into three groups: microbial-, mammalian-, or plant-derived. The physiology of siderophores and their iron-chelating properties have been reviewed in Reference 78.

Warfare Between Lipocalin 2 and Microbial Siderophores

Iron is an essential micronutrient required by almost all aerobic microorganisms except *Borrelia* and *Lactobacillus* spp. (139, 188). It is estimated that a bacterial cell with a volume of 10^{-9} cm^3 requires 10^5–10^6 Fe^{+3} per generation to maintain the required internal concentration of 10^6 M. In the thrust and parry for iron between bacteria and their host, bacteria have evolved aggressive iron-acquiring mechanisms through the expression of siderophores to steal iron from host proteins (i.e., transferrin and ferritin). Ent is one of the most extensively studied prototypical catecholate siderophores, composed of three subunits of 2,3-dihydroxybenzoate (2,3-DHBA). These subunits

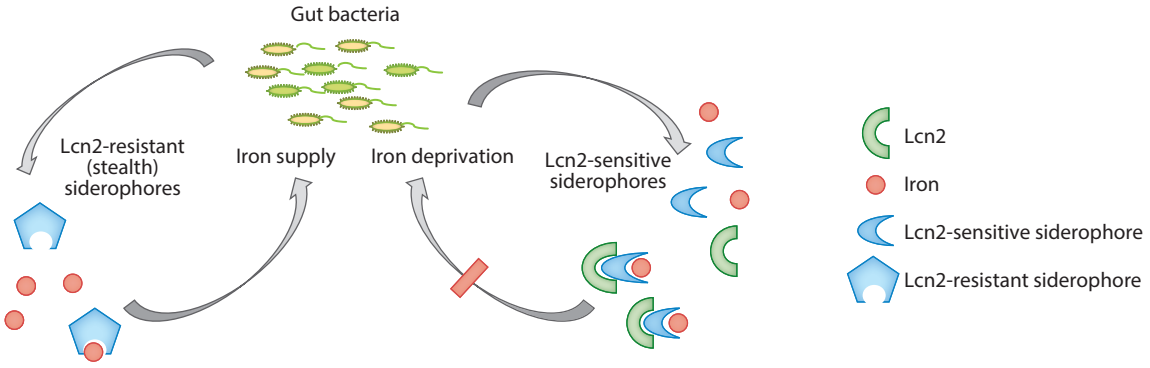

Figure 2

Biowarfare between lipocalin 2 (Lcn2) and microbial siderophores in the gut. Pathogenic bacteria synthesize and secrete siderophores to acquire iron. However, these processes can be counter-regulated by Lcn2. Some bacteria produce stealth siderophores, which are resistant to Lcn2, to circumvent inhibition.

are connected by a triserine lactone backbone to form a hexadentate ligand that has the highest known affinity for ferric iron ($K_d = 10^{-49}$ M) (110).

During inflammation, the condition of iron scarcity prompts *E. coli* to increase Ent production to meet its iron requirement. The unmatched affinity of Ent for iron allows it to effectively outcompete most host iron-binding proteins, including lactoferrin (60). In response, neutrophils and other host cell types secrete Lcn2 to sequester and neutralize Ent. Incidentally, the large calyx in Lcn2 allows it to bind to a spectrum of catecholate- and carboxylate-type siderophores and, thereby, to institute nutritional immunity against various siderophores and iron-dependent pathogens (**Figure 2**). However, several clinical isolates of *E. coli* have evolved to express stealth siderophores that can evade recognition by Lcn2 (**Table 1** and **Figure 2**). *Salmonella enterica* modifies its Ent via *C*-glycosylation to generate salmochelin, which cannot be sequestered into the Lcn2 calyx due to steric hindrance from the glucose groups (61). Petrobactin is another stealth siderophore, from *Bacillus anthracis*, which incorporates 3,4-DHBA as the iron-binding functional group instead of 2,3-DHBA and thus cannot be bound by Lcn2 (202). Certain Lcn2 homologs, such as the galline Ex-FABP, achieve detection of monoglycosylated salmochelin by having a modified calyx pocket to accommodate its binding (40). Despite this, avian pathogens such as *Salmonella* spp. can still evade Ex-FABP by producing the diglycosylated form of salmochelin.

Iron Scaffolding by Lipocalin 2 and Mammalian Siderophores

In the past, studies focused on microbial siderophores, and it was not until recently that research began to unravel the existence of mammalian siderophores. A study led by Barasch and Strong (199) discovered that Lcn2 can mediate the trafficking of radiolabeled iron into the ureteric bud cell line and embryonic kidney in vivo. The quandary that Lcn2 could not directly bind to iron implicated the involvement of a putative mammalian siderophore. In their subsequent study, these researchers (14) identified a group of simple catechols (i.e., catechol, 3-methylcatechol, 4-methylcatechol, pyrogallol) as the endogenous siderophores copurified with iron and Lcn2 from mouse urine. Iron-free catechol has a lower affinity for Lcn2; however, the iron-laden catechol bound to Lcn2 with a much higher affinity (2.1–0.4 nM) (14). Although the sources of catechol interacting with Lcn2 are yet to be elucidated, it is well established that catechols can be derived

Table 1 Lipocalin 2–sensitive siderophores and lipocalin 2–resistant (stealth) siderophores

Species	Siderophores	
	Lipocalin 2–sensitive	Lipocalin 2–resistant
Bacterial siderophores		
Escherichia coli	Enterobactin	Salmochelin
		Aerobactin
Klebsiella pneumoniae	Enterobactin	Yersiniabactin
		Salmochelin
		Aerobactin
Pseudomonas aeruginosa	Enterobactin	Pyochelin
		Pyoverdine
Mycobacterium tuberculosis	Mycobactin	–
	Carboxymycobactin	–
Bacillus subtilis	Bacillibactin	Petrobactin
Pseudomonas cepacia	Cepabactin	Pyochelin
Paracoccus denitrificans	Parabactin	–
Staphylococcus aureus	–	Staphyloferrin A
Brucella abortus	Brucebactin	–
Vibrio cholerae	Vibriobactin	–
Mammalian siderophores		
–	Catechol	Citrate
–	2,5-dihydroxybenzoate	–
–	Pyrogallol	–
Plant-derived siderophores		
–	Piperine	–
–	Epigallocatechin-3-gallate	–

from bacterial and mammalian metabolism of dietary compounds. Incidentally, the Barasch group (15) also discovered that epigallocatechin-3-gallate (EGCG; the major polyphenol in green tea) can also serve as a plant-derived siderophore that binds to both iron and Lcn2. Studies from our group have further demonstrated that EGCG and Ent are functionally similar in potently inhibiting myeloperoxidase (MPO), but lose their efficacy when bound to Lcn2 (200).

Devireddy's laboratory (138) discovered that one of the mammalian siderophores might be 2,5-DHBA, which is similar to the 2,3-DHBA component in Ent. Molecular analyses have supported the capability of 2,5-DHBA to bind ferric iron in a salicylic-binding mode, yet modeling of the interaction between 2,5-DHBA and Lcn2 indicated structural incompatibility due to steric clashes within the Lcn2 calyx (41). Despite the conundrum of whether 2,5-DHBA might be an Lcn2 ligand, the functional roles of 2,5-DHBA have been characterized to include mediation of cytosolic iron homeostasis, modulation of cellular levels of reactive oxygen species (ROS), facilitation of iron transport into mitochondria, and modulation of cellular apoptosis (46). At around the same time, Skerra's laboratory (118) found that host catecholamines might also be endogenous siderophores and ligands for Lcn2. Their in vitro study demonstrated that ferric iron–bound norepinephrine could augment the growth of iron-dependent bacteria (i.e., *E. coli*), but this was largely abrogated when the norepinephrine–iron complex is sequestered by Lcn2 (118).

ROS: reactive oxygen species

WT: wild type

The extent to which mammalian siderophores influence iron homeostasis in concert with other well-characterized iron-binding proteins is not completely understood. The endogenous siderophores may participate in iron transport, thus preserving iron solubility, keeping iron in a nonreactive state, and facilitating iron excretion. By themselves, mammalian siderophores are susceptible to being hijacked by bacteria to fuel their growth, and in this regard, it is fascinating that Lcn2 plays an essential part in safeguarding host-derived siderophores (108, 118). In turn, mammalian siderophores reciprocate by augmenting the antibacterial activity of Lcn2 (159).

Systemic and Cytosolic Euferremia

Iron is efficiently recycled and reabsorbed in the body to maintain euferremia. At the onset of inflammation, the acute phase response to proinflammatory cytokines [such as interleukin (IL) 6] initiates the upregulation of hepatic hepcidin. Hepcidin binds to ferroportin (SLC40A1) lining the basolateral side of the duodenum and to ferroportin on macrophages to induce ferroportin internalization and degradation (67). Because ferroportin is the sole protein known to mediate iron export from cells into the bloodstream, its downregulation impedes iron uptake from the gastrointestinal tract and promotes the so-called anemia of inflammation. The hepcidin–ferroportin axis has been regarded as the master regulator of inflammatory hypoferremia, and it was not until recently that studies began to unravel the contribution of hepcidin-independent mechanisms (50, 74). Compelling evidence has now revealed Lcn2 as a new player in facilitating hypoferremia via modulating the tissue LIP and sequestering mucosal and luminal iron during inflammation.

Systemic hypoferremia occurs within hours in mice treated with lipopolysaccharide (LPS). Genetic deletion of hepcidin in mice substantially blunted their hypoferremic response to LPS, yet they still exhibited an approximately 15% decrease in circulatory iron (50). The observation that hepatic Lcn2 levels are strikingly upregulated in hepcidin-deficient mice (50) raises the prospect that hepcidin-independent hypoferremia could be mediated by Lcn2. Indeed, our group (165) has demonstrated that the genetic loss of Lcn2 significantly delays systemic iron clearance and results in increased mortality in mice treated with LPS. Lcn2 deficiency also increases apoptosis of immune cells upon LPS challenge, thus causing immunosuppression. Moreover, we consistently observed higher levels of systemic LIP in Lcn2-deficient mice than wild-type (WT) mice, with and without LPS treatment (191). In this respect, Lcn2 helps to preserve the stable iron pool by quenching the reactivity of siderophore-bound iron (14, 15). Additionally, Lcn2 facilitates cytosolic hypoferremia by promoting iron export from *Salmonella*-infected macrophages as a mechanism to combat intracellular pathogens (126).

INTERPLAY BETWEEN LIPOCALIN 2 AND IRON IN INFLAMMATORY DISEASES

Infectious Diseases

The axiom that pathogen virulence is positively associated with iron availability can be traced to as early as the 1850s when Armand Trousseau warned physicians not to prescribe iron preparations for patients with tuberculosis. Indeed, excessive iron consumption has been shown to increase the virulence of iron-dependent pathogens and exacerbate overall mortality in patients infected with them (133). The role of Lcn2 in prohibiting the growth of Ent and iron-dependent bacteria was first demonstrated in vitro by Strong's group (71), whereas its in vivo relevance was established by Aderem and coworkers (62). The latter study demonstrated that Lcn2-deficient mice died at a higher rate after systemic administration of *E. coli* (62). In comparison, the upregulated Lcn2 in

WT mice mediated the bacteriostatic effects on *E. coli* and protected the mice from sepsis-induced mortality. Several follow-up studies have further confirmed that Lcn2 offers protection against *E. coli*–induced septicemia (22), pneumonia (76, 189), and urinary tract infection (167), but not under conditions of excess iron or in the presence of Lcn2-resistant siderophores.

Mycobacteria spp., including *Mycobacterium tuberculosis*, which is responsible for causing pulmonary tuberculosis, rely on their siderophores (i.e., mycobactin and carboxymycobactin) to acquire iron from the host (81). Mycobacteria-derived siderophores can be sequestered by Lcn2 (79), thus subjecting mycobacteria spp. to iron starvation. The Lcn2 expressed by alveolar macrophages and epithelia are crucial in controlling the intracellular replication of mycobacteria infecting the aforementioned host cells (89, 151), as well as upregulating their secretion of chemokines to recruit neutrophils (73). Moreover, the overexpression of Lcn2 and exogenously administered recombinant (rec) Lcn2 were both found to suppress the growth of *M. tuberculosis* within macrophages (89), whereas Lcn2 deficiency increases mycobacterial growth within infected alveolar epithelia (151).

Inflammatory cascades downstream of Toll-like receptor (TLR) activation have been shown to upregulate Lcn2 (62). Analysis of the Lcn2 promoter has unraveled the presence of a consensus site for NF-κB (nuclear factor κB), the transcription factor that is activated by various inflammatory cytokines (43). Lcn2 can also exert proinflammatory activity, which is, intriguingly, associated with its siderophore- and iron-binding activities. Nelson et al. (128) found that Lcn2 can deliver Ent into the cytosol of respiratory epithelia in vitro and elicit upregulation of IL-8, a neutrophil chemoattractant. The chelation of cellular iron by bacterial siderophores is sufficient to induce respiratory epithelia to secrete IL-6, IL-8, and CCL20 (chemokine ligand 20) in vitro, but such responses are further potentiated in the presence of Lcn2.

To countervail host Lcn2, certain pathogens can express Lcn2-resistant, or stealth, siderophores (**Table 1**). Despite this, studies have shown that Lcn2 can still mediate antimicrobial activity to some extent, independently of its siderophore-binding activity. For instance, Lcn2 provides partial protection against the systemic dissemination of yersiniabactin-expressing *Klebsiella* in WT mice, whereas such protection was completely lost in Lcn2-deficient mice (9–11). In another instance, Lcn2 facilitated iron export from *Salmonella*-infected macrophages to eliminate *Salmonella* infection (125), although Lcn2 did not neutralize their salmochelin. In *Salmonella* infection in the gastrointestinal tract, elevated Lcn2 levels may instead suppress the growth of the host microbiota, thus favoring the pathogen in colonizing the intestines (140). Exogenous rec-Lcn2 was shown to be effective in depleting free iron in the lungs, and it rescued mice from infection by *Staphylococcus aureus*, whose siderophores are not recognized by Lcn2 (62, 146). *Chlamydia pneumoniae* and *Streptococcus pneumoniae* are respiratory pathogens that do not express any siderophores; although rec-Lcn2 is effective in curtailing infection by *C. pneumoniae* (19), the Lcn2 response was instead shown to worsen *S. pneumoniae* infection (187).

Lcn2 expression in the gastric mucosa is also upregulated in response to *Helicobacter pylori* infection (4, 87), which suggests a possible antimicrobial role for Lcn2 on *H. pylori*. However, this has yet to be investigated mechanistically; likewise, it is also unclear whether *H. pylori* expresses any Lcn2-sensitive siderophores.

Lcn2 is also abundantly produced in response to parasitic infection. One study showed that during *Plasmodium* infection, upregulated Lcn2 controlled parasite growth by enhancing macrophage function, suppressing reticulocytosis, and modulating adaptive immune responses (204). This study also found that Lcn2 promoted the recycling of systemic iron to facilitate the host antiparasitic responses, whereas Lcn2-deficient mice displayed higher serum iron and less splenic iron (204).

Inflammatory Bowel Diseases

IBD: inflammatory bowel disease

Inflammatory bowel diseases (IBDs), such as ulcerative colitis and Crohn's disease, are characterized by chronic inflammation of the intestines with infiltration of immune cells into the lamina propria. Our published results (36, 181–183) have demonstrated that systemic and fecal Lcn2 levels increase drastically in murine models of colitis, including dextran sulfate sodium–induced colitis, *Salmonella*-induced gastroenteritis, and in spontaneously colitic *Tlr5* knockout mice. Lcn2 is likewise upregulated in human IBD (131). As such, the dynamic upregulation of fecal Lcn2 has been exploited as a sensitive biomarker for gut inflammation, both in animal models (36, 83) and for human IBD (172). If hepcidin is regarded as the master regulator of systemic hypoferremia, then one could consider Lcn2 to be the chief mediator of mucosal hypoferremia. In addition to restricting iron availability in the inflamed gut, Lcn2 also exerts a cytoprotective effect against mucosal damage (137), enhances bacterial phagocytosis by macrophages (176), and regulates the gut microbiota (123).

Acute hypoferremia is widely perceived to be a protective response against gastrointestinal infection, yet its perpetuation in IBD, in addition to chronic intestinal bleeding, would result in iron deficiency. In fact, iron deficiency anemia is the most common complication of IBD, affecting more than two billion people worldwide (12). Oral iron fortification may be effective in treating iron-deficiency anemia; however, concerns have been raised regarding its side effects. A large dose of orally administered iron, for instance, could escape absorption in the small intestine and reach the distal gut where its catalytic property might exacerbate colonic inflammation through the generation of ROS. Furthermore, iron absorption is largely blocked by inflammation-triggered hepcidin, and the iron withheld in the gut epithelia may further aggravate inflammation. In this regard, the depletion of colonic luminal iron may be an effective strategy for improving gastrointestinal health during IBD (80). Kortman et al. (100) found that restricting dietary iron intake substantially mitigated bacteria-induced inflammatory responses in the gut and lowered the levels of fecal and serum Lcn2. Surprisingly, treating mice with a high-iron diet also decreased the levels of fecal Lcn2 and lessened colitic symptoms (100). Although it is unclear how both the deficiency and excess of iron could alleviate colitis, it has been suggested that these results may be related to alterations in the composition of the gut microbiota in response to dietary iron. Considering the adverse side effects of oral iron, it is perhaps more feasible to administer intravenous iron supplementation instead. Comparisons between oral versus intravenous iron supplementation, and different iron formulations for treating IBD-associated anemia have been reviewed in Reference 132.

During the past decade, the gut microbiota have emerged as key players in dictating intestinal health by modulating a variety of host physiological responses. Innate immune dysfunction, however, can lead to dysbiosis of the microbiota (180) that increases host susceptibility to IBD (181). Our recent publication (161) provides evidence that Lcn2 has a vital role in preventing gut microbiota dysbiosis–induced colitis. Accordingly, we demonstrated that Lcn2 deficiency resulted in a more severe dextran sulfate sodium and IL-10R neutralization–induced colitis. The ablation of gut microbiota by antibiotics substantially mitigates colitis in Lcn2-deficient mice to levels that are comparable to WT mice, suggesting that their aggravated colitis is microbiota dependent (161). The mucoprotective feature of Lcn2 was further exemplified in another study, in which Lcn2 deficiency was shown to accentuate spontaneous colitis and colonic tumorigenesis in IL-10-deficient mice (124). Moschen et al. (124) revealed that exacerbated disease is largely attributable to the iron-dependent pathogen *Alistipes* spp., which expanded dramatically in the gut of mice lacking Lcn2. These findings were corroborated in a third study that indicated the impediment to bacterial clearance by Lcn2-deficient macrophages could also contribute to the exacerbation of colitis in *Lcn2–IL-10* double knockout mice (176).

Lcn2 is indispensable for the migration, adhesion, and function of neutrophils during inflammation (155). Our group (162) further uncovered that Lcn2 is essential for preserving the antimicrobial activity of MPO. MPO is a heme protein that utilizes H_2O_2 and a halide ion to generate antimicrobial hypochlorous acid. In our study (162), we demonstrated that *E. coli*–derived Ent substantially inhibited the activity of MPO, thus providing a potential survival advantage to *E. coli* during IBD. Rather counterintuitively, the mechanism of Ent-mediated inhibition is not via direct chelation of iron from the heme moiety of MPO; instead, the binding of iron diminishes the capacity of Ent to inhibit MPO. Nonetheless, the sequestration of Ent by Lcn2 rescues MPO from being inhibited, thus keeping MPO active and potent (162). We also determined that EGCG, which is a siderophore-like compound found in green tea, can inhibit MPO in a similar manner and that this inhibition is counter-regulated by Lcn2 and iron (200). Likewise, we also found that urolithin A (uroA; derived from microbial metabolism of dietary ellagic acid) can also inhibit MPO activity, but not in the presence of Lcn2. The similarity among Ent, EGCG, and uroA in their functional interactions with Lcn2 suggests that uroA may be an Lcn2 ligand, although further molecular studies are required to confirm this notion. Nonetheless, these findings suggest that Lcn2 has a role in safeguarding host proinflammatory responses from being inhibited by bacteria or diet-derived molecules. However, this also suggests that future clinical studies are needed to understand the possible interference by host Lcn2 when developing Ent or EGCG as a therapeutic treatment for IBD.

E. coli often blooms and dominates the gut ecology in various types of IBD. In addition to serving as a putative indicator of gut dysbiosis, the blooming of *E. coli* may also set the stage for pathogen evolution (166). Such a notion coincides with the findings that numerous clinical isolates of virulent *E. coli* tend to express varying combinations of stealth siderophores (157, 170), which are likely to be acquired via horizontal gene transfer. Furthermore, the high levels of Lcn2 in the inflamed gut may exert selective immune pressure on bacteria to express more stealth siderophores. *E. coli* Nissle 1917 is a prime example of an *E. coli* strain that has acquired the ability to express four types of siderophores: Ent, aerobactin, yersiniabactin, and salmochelin. Although the presence of stealth siderophores often indicates pathogen virulence, *E. coli* Nissle 1917 has, surprisingly, been shown to be a nonpathogenic probiotic bacterium capable of reducing the colonization of pathogens during IBD by competing for the iron sources restricted by Lcn2 (48, 152, 156). In a similar fashion, we envision that probiotics genetically engineered to express Lcn2 may have a potential benefit in treating IBD or could be employed to complement other siderophore-based strategies.

Neurodegenerative Diseases

Maintaining iron homeostasis in the central nervous system (CNS) is important because iron is an indispensable factor for oxygen transport, neuronal transmission, and neuronal metabolism. The brain itself is highly susceptible to oxidative damage due to its high content of unsaturated lipids and constant exposure to high concentrations of oxygen. If not regulated adequately, the labile iron could react with H_2O_2 to generate ROS via the Fenton reaction, which is a major cause of lipid peroxidation in the CNS. In fact, abnormalities in CNS iron metabolism have been observed in neurodegenerative diseases (8, 53, 70, 149), such as Alzheimer's disease and Parkinson's disease, in which cerebral iron overload is universal. Excess iron can also promote mitochondrial dysfunction and activate microglia, thus potentiating CNS disorders (116). Accordingly, iron chelation therapy has been shown to be effective in preventing and treating a murine model of Parkinson's disease by reducing the levels of labile iron (96). Yet aside from iron dysregulation, progression in CNS disease is also associated with neuroinflammation driven primarily by activated

CNS: central nervous system

DFO: deferoxamine

microglia (5). Neuroinflammation and iron overload have been concurrently reported in brain injury and ageing, both of which finally lead to neurodegenerative diseases (92, 185). Although the factors triggering the pathogenesis of neurodegenerative diseases are not completely understood, it is certain that both iron dysregulation and neuroinflammation are tightly involved in these complicated processes.

Lcn2 has emerged as a new player in neurodegenerative diseases, given its features as an inflammatory protein and iron regulator in the CNS. Under physiological conditions, the normal brain displays a low-to-undetectable level of Lcn2 (37, 88, 112). However, Lcn2 expression is substantially upregulated by injury or inflammation (37, 88, 113) and is likely to exert modulatory effects on iron homeostasis. Under pathological conditions, Lcn2 is expressed in major components of the CNS, including neurons, the choroid plexus, microglia, and astrocytes (88, 113, 193). The Lcn2 secreted in the CNS serves as a help-me signal to activate astrocytes and microglia (193); promotes neurovascular recovery, such as after stroke and brain injury (190); and mediates anti-inflammatory effects against sepsis-induced brain damage and behavioral changes (95). However, Lcn2 has also been shown to induce proinflammatory cytokines as well as inducible nitric oxide synthase, which might cause secondary damage and impede recovery (142). Indeed, upregulated Lcn2 in the substantia nigra has been shown to promote neurotoxicity and neuroinflammation (24), and to contribute to the damage of nigrostriatal dopaminergic projections and abnormal locomotor behavior in WT mice (98). In contrast, these symptoms were ameliorated in Lcn2-deficient mice (98).

Suk and colleagues (98, 103, 104) demonstrated that upregulated Lcn2 sensitizes astrocytes and microglia to self-regulatory apoptosis in an autocrine manner. Apo-Lcn2, but not holo-Lcn2, was shown to promote apoptosis in astrocytes and microglia by depleting intracellular iron; these findings are consistent with the proapoptotic aspect of apo-Lcn2 reported previously (51). Conversely, holo-Lcn2 transported iron into astrocytes and microglia (103, 104), and potentially contributed to the accumulation of intracellular iron in the substantia nigra of patients with Parkinson's disease (186). Furthermore, Lcn2-induced neurotoxicity in vivo is increased when treated with exogenous iron, but decreased when treated with an iron chelator, deferoxamine (DFO) (98). This is reversed in vitro in the context of Lcn2-sensitization to nitric oxide–induced apoptosis, whereby neuronal cell death is partially protected by exogenous iron, but augmented when treated with DFO (103). Despite this, most of these studies have examined only the acute effects of Lcn2; thus, future studies are needed to establish the role of Lcn2 in chronic models that are more relevant to human neurodegenerative diseases.

Iron overload and inflammation are also tightly associated with intracerebral hemorrhage (ICH) caused by brain trauma or stroke. ICH-induced signaling causes erythrocyte lysis, resulting in the release of large amounts of iron into the CNS (25). Exposure to an abnormal level of free iron and inflammation are detrimental to the brain and could potentially result in perihematomal edema, neuronal death, and brain atrophy. The expression of Lcn2 in the brain appears to be positively regulated in an iron-dependent manner (98), and in a rat model was found to be elevated by 70–80-fold in a model of ICH and 136-fold during intracerebral iron administration (54). The upregulation of brain Lcn2 has also been reported in clinical settings, thus indicating its potential for utilization as a biomarker for assessing the severity of brain injury (205). Yet the findings that Lcn2-deficient mice display alleviated symptoms of brain injury and reduced ICH-induced iron storage (129) implicate Lcn2 as having a pathological role. Intracerebral thrombin-induced brain injury was markedly reduced in Lcn2-deficient mice when compared with WT mice, whereas exogenous administration of rec-Lcn2 intensified injury, such as neural death, brain swelling, and blood–brain barrier disruption (111). Similarly, associations among heightened Lcn2 expression, accumulation of cellular nonheme iron, and a worse clinical outcome were reported in studies on

ischemic stroke patients. Thus, further in-depth studies are needed to clarify the pathophysiology of Lcn2 and iron in the CNS.

Both the protective and harmful roles of Lcn2 are documented in multiple sclerosis (MS), which is an inflammatory, demyelinating disease of the CNS. Studies in a murine model of autoimmune encephalomyelitis (EAE) have demonstrated that Lcn2 is upregulated throughout the spinal cord and within the choroid plexus (20, 112). Such induction of Lcn2 can be reversed upon treatment with natalizumab (Biogen, Research Triangle Park, North Carolina), which blocks leukocyte entry into the CNS (20, 112). The role of Lcn2 in MS, however, is rather controversial because one study observed increased inflammation and EAE severity in Lcn2-deficient mice (20), but this was contradicted by another study wherein Lcn2 deficiency was shown instead to ameliorate EAE (127). Despite this conundrum in mouse studies, one study on a clinical cohort posited that Lcn2 could be pathological, as patients with higher Lcn2 levels in cerebrospinal fluid (CSF) were more likely to transition from clinically isolated syndrome or early MS to clinically definite MS (97). Elevated Lcn2 in CSF was also shown to be positively correlated with high levels of transferrin in CSF and iron accumulation in the basal ganglia (97), thus raising the prospect that perhaps iron dysregulation explains the contradictory role of Lcn2 in the pathophysiology of EAE.

Neurodegenerative disorders with brain iron accumulation (NBIA) are rare, inherited neurological disorders characterized by abnormal iron accumulation in the basal ganglia, substantia nigra, thalamus, and cerebellum (72). Treatments focus on reducing the accumulation of iron, for instance, by using the iron chelator DFO, which is the first bacterial siderophore (produced by *Streptomyces pilosus*) to be used in a clinical setting. Currently, synthetic forms of iron chelators, deferiprone and deferasirox, are also used in treating iron overload. However, it has been demonstrated that none of these iron chelators can prevent NBIA. Lcn2 may have the therapeutic potential to treat NBIA. However, it remains to be determined whether Lcn2 can cross the blood–brain barrier and whether it will target the abnormal accumulation of iron in brain.

Obesity and Metabolic Syndrome

Metabolic syndrome (MeS) describes a constellation of metabolic abnormalities, including obesity, dyslipidemia, hypertension, hyperglycemia, hyperinsulinemia, and insulin resistance (IR). Studies on the molecular mechanisms of MeS have uncovered its tight association with low-grade chronic inflammation. Such a link is exemplified by the fact that numerous adipocyte-derived adipokines involved in modulating metabolic homeostasis (IL-6, tumor necrosis factor-α, adiponectin) also display immunomodulatory properties. From a metabolic standpoint, Lcn2 is also considered to be an adipokine, given its high expression in adipose tissue and its putative role in promoting IR (184).

Levels of Lcn2 in serum, liver, and adipose tissue are elevated in multiple murine models of obesity (197, 203). Our group also demonstrated the upregulation of Lcn2 in the serum of mice lacking TLR5 and displaying microbiota-dependent MeS (180). One human study reported that obese individuals with a BMI >30 kg/m^2 had 60% higher levels of serum Lcn2 when compared with lean individuals with a BMI <23 kg/m^2 (184). Another study did not observe differences in serum levels of Lcn2 between lean and obese individuals, but instead detected increased serum Lcn2–MMP9 complex and significant upregulation of Lcn2 in the visceral adipose tissue of obese individuals (33). Studies led by Ntambi (66) have demonstrated that mice with a skin-specific deficiency of stearoyl-coenzyme A desaturase 1 (Scd1) are protected from high-fat diet–induced adiposity; one of their studies found that Lcn2 expression is highly upregulated in the skin of these mice. However, global deletion of Lcn2 in skin-Scd1-deficient mice did not reverse their resistance to obesity, thus indicating that Lcn2 may not directly mediate resistance to high-fat

diet–induced obesity in the *Scd1* knockout model (66). Controversial results have been reported regarding the function of Lcn2 as an adipokine. In one study, Guo et al. (75) observed that Lcn2 deficiency aggravated high-fat diet–induced IR. However, this is disputed by the results of another study wherein Jun et al. (90) observed no difference in insulin sensitivity between WT and Lcn2-deficient mice fed a high-fat diet. Yet these two studies were further contradicted by a third study in which Law et al. (101) contended that *Lcn2* knockout mice were protected from ageing and high-fat diet–induced IR, and that the genetic deletion of Lcn2 also protected obese leptin-receptor-deficient *db*/*db* mice from IR. The paradoxical effects of Lcn2 on IR are far from being resolved, although more recent publications appear to support a detrimental role for Lcn2. For instance, Lcn2-mediated IR was shown to be exacerbated by the glucocorticoid dexamethasone in premenopausal females (94). Moreover, Lcn2 has been suggested to augment cardiac IR by inhibiting autophagic flux, contributing to the incidence of myocardial infarction and heart failure (35). In agreement with the adverse role of Lcn2, blocking the expression of Lcn2 in 3T3-L1 adipocytes in vitro was shown to reduce IR, whereas adding exogenous Lcn2 to hepatocytes promoted IR (197).

A large body of evidence has shown that iron dysregulation is also involved in the development of IR, dyslipidemia, and arterial hypertension (27). However, few reports have linked iron homeostasis to Lcn2 in obesity and MeS. Intriguingly, Catalan et al. (32) observed that heightened Lcn2 expression in adipose tissue was positively correlated with circulating levels of ferritin but negatively correlated with serum iron levels. Given that Lcn2 and ferritin are APPs involved in inflammatory hypoferremia, it is possible that reduced iron levels are the consequence of hypoferremia associated with the low-grade chronic inflammation underlying MeS.

Approximately one-third of patients with obesity and MeS suffer from iron dysregulation. Such comorbidity has been described as dysmetabolic iron overload syndrome (DIOS) (2), and it is characterized by hyperferritinemia and mildly increased iron stores in the body. The etiology of this disorder is unclear and understanding of the pathology is only in its infancy, although DIOS is likely to be associated with a state of chronic inflammatory hypoferremia (121). A recent study has suggested that DIOS may be related to hepcidin resistance (141), whereas, hitherto, Lcn2 has not been discussed in relation to this condition. Due to its multifaceted roles in inflammation and iron regulation, Lcn2 could be explored as a potential therapeutic target for DIOS.

Kidney Diseases

Urinary Lcn2 has been extensively studied as a sensitive, real-time biomarker for renal diseases (1, 34), whereby the composition of Lcn2 monomers and dimers in the urine could potentially distinguish between AKI, CKDs, and urinary tract infection (30, 130). In addition to its role as a biomarker, Lcn2 also actively protects against AKI, such as ischemia–reperfusion injury, but it is detrimental in that it aggravates the progression of CKD.

Ischemia–reperfusion kidney injury is typically caused by an initial restriction of blood supply followed by the abrupt restoration of blood flow and oxygenation. During ischemia, the substantial amount of iron released from iron-binding proteins by superoxide-induced reactions (39), and heme degradation by heme oxygenase 1 (59), enhances the LIP, which, in turn, promotes oxidative stress and tissue damage (164, 201). Furthermore, the ensuing reperfusion delivers more iron to the kidney, thus exacerbating oxidative stress–induced damage. In animal studies the use of iron chelators or scavengers, such as DFO, apo-transferrin, and Lcn2, has been shown to mitigate ischemia–reperfusion AKI (23, 47, 119). Administering the Lcn2–siderophore–iron complex was also found to alleviate ischemia–reperfusion AKI (122) and attenuate acute rejection of renal transplants (6). Further, the holo-Lcn2 complex may deliver iron to the kidney epithelia, function

as a growth factor, and potentially expedite recovery from damage (154, 199). A study found that the use of IL-10-overexpressing macrophages to treat renal ischemia in rats, through the induction of Lcn2, improved cell regeneration and repair (91).

In contrast to its beneficial effect in AKI, Lcn2 is mainly considered to be a proinflammatory factor that aggravates CKD. One study in a murine model of nephritis observed elevated levels of urinary Lcn2 in WT mice with nephrotoxic nephritis (135). However, attenuated pathological symptoms were observed in Lcn2-deficient mice, which displayed improved histopathology and lessened proteinuria when compared with WT mice (135). In another animal study, Lcn2 was shown to be upregulated in response to the activation of epidermal growth factor receptor (EGFR) that stimulated CKD progression, whereas the severity of renal damage in Lcn2-deficient mice was significantly reduced (179). This study suggested that Lcn2 could mediate the mitogenic effects of EGFR and contribute to renal deterioration (179). Yet another study proposed that increased Lcn2 may also be mediated by endoplasmic reticulum stress that is induced by proteinuria in CKD (56). The albumin in urine has been shown to upregulate Lcn2 expression via ATF4 and then to promote apoptosis and tubulointerstitial damage (56). Although iron is an important pathogenic factor during CKD, it is not completely understood how Lcn2 is involved in iron metabolism.

Both AKI and CKD are associated with kidney iron overload; hence, iron clearance is deemed to be a critical step in treating kidney disorders. As such, an intervention that could induce iron excretion through the urine is quite attractive because it is more direct and noninvasive to treat iron overload–related diseases than to use phlebotomy or iron-chelation therapy. Despite this preference, the prevailing paradigm dictates that iron is a highly recycled micronutrient and thus is largely reabsorbed by the kidney (115). A recent innovative study by Barasch et al. (16) proposed that renal reabsorption of iron can be circumvented by utilizing a patented mutant form of Lcn2 (K3Cys; patent application number: 20150329607) that cannot bind to megalin in the kidney. In addition to stabilizing the urinary LIP, the K3Cys Lcn2 mutant was effective in clearing iron from systemic circulation into the urine as a new means of iron excretion. Such novel application of Lcn2 coincides with the abundance of diverse groups of siderophores present in the urine (e.g., catechol) that could assist K3Cys Lcn2 in potentially treating a spectrum of renal, inflammatory, and other iron overload–related disorders.

Wound Healing and Skin Disorders

The skin is the largest organ of the human body, serving as a physical barrier and thus protecting our internal body from foreign organisms. Comparable to the intestines, the skin is likewise colonized by a diverse milieu of commensal and pathogenic microorganisms. As such, the skin epithelia and neutrophils are well armed with the capacity to upregulate or release their Lcn2 to curtail potential microbial incursion (26). Aside from depriving the invading pathogens of iron, Lcn2 also facilitates the process of cutaneous wound healing in response to growth factors (163). Miao et al. (117) demonstrated that the overexpression of Tcf3, a transcription factor regulating adult skin stem cell functions, also upregulated Lcn2 expression in wounded epithelia. The efficacy of wound repair, however, was notably diminished upon topical treatment with an Lcn2-blocking antibody (117).

Despite its positive effects on wound healing, Lcn2 upregulated via Tcf3 has been associated with dysregulated keratinocyte differentiation in several skin disorders (194). Moreover, serum and tissue Lcn2 were reported to be elevated in patients with psoriasis (55, 93), which is a hyperproliferative inflammatory skin disease. The highly expressed Lcn2 in psoriatic lesions may exacerbate the pathogenesis of psoriasis by activating neutrophils to produce proinflammatory cytokines, inducing neutrophil chemoattractants (158), and enhancing T helper- 17–mediated inflammation

(77). However, anti-Lcn2 antibody treatment was shown to alleviate the disease in a mouse model of psoriasis (158). A study by Shiratori-Hayashi et al. (160) demonstrated that STAT3-dependent upregulation of Lcn2 expression in the spinal cord might be critical in amplifying the chronic itching associated with skin diseases; they showed that intrathecal injection of rec-Lcn2 promoted gastrin-releasing peptide–induced scratching. Lcn2 has also been suggested to underlie the link between psoriasis and MeS; however, the association is not clear due to contradictory reports (55, 148). Although the iron-regulatory role of Lcn2 has not yet been explored in regards to skin biology, it is likely that Lcn2 may serve not only as an antimicrobial or inflammatory factor but may also facilitate the transport of iron, which is indispensable for rapidly proliferating and differentiating epithelial cells.

Cancer

Lcn2 is highly upregulated in various cancers, including but not limited to colorectal, liver, breast, ovarian, pancreatic, prostate, renal, and gastric cancers (reviewed in 34). Initial studies on Lcn2 in cancers mostly centered on its feature as a biomarker, in which its high expression is often indicative of malignancies. However, recent studies have begun to document both anti- and procancer roles for Lcn2, depending on the tumor type, stage (21), and location (143).

In most cancer studies, Lcn2 has been shown to facilitate tumorigenesis by enhancing cancer cell survival, growth, and metastasis (147, 174), and to augment cellular resistance to iron-induced toxicity (85) and chemotherapeutics (120). In this respect, the knockdown of the *Lcn2* gene was shown to substantially diminish the growth of prostate cancer cells, but Lcn2 overexpression enhanced tumor cell proliferation and migration (178). In a study in the $APC^{min/+}$ model of intestinal tumorigenesis, Lcn2 deficiency was reported to reduce the average size of tumors in the distal intestines but increase the load and multiplicity of duodenal tumors, which implies, respectively, the oncogenic and tumor-suppressing roles of Lcn2, depending on the location. Most importantly, Lcn2-deficient tumors display a substantially reduced ferrous iron (Fe^{2+}) content, thus indicating the role of Lcn2 in facilitating iron uptake into tumors.

As a vital nutrient, iron is critical in tumor initiation, proliferation, and metastasis (175). Consistent with this notion, a higher cancer risk was reported to be positively associated with higher dietary iron intake (65), an increase in body iron stores (168, 169), and genetic conditions of iron overload, as occur in individuals with hereditary hemochromatosis (84). Molecular studies have shown that an increased abundance of iron can assist tumor growth, whereas reducing iron by using iron chelators or blocking iron import suppresses tumor growth (45, 144). In fact, distinct iron regulatory programs have been documented in malignant cells; for example, breast cancer cells can express hepcidin to downregulate ferroportin expression, thus decreasing cellular iron efflux (136). The overexpression of Lcn2 in tumors could be a mechanism to facilitate the delivery of iron into the tumors via 24p3R (51, 58) to meet their iron demand (17).

The oncogenic role of Lcn2 has also been associated with its ability to complex with MMP9 (196), the activity of which is linked to angiogenesis and tumor progression (21, 49). Lcn2 binding stabilizes MMP9 and prevents its autodegradation, assists in its gelatinolytic activity on the extracellular matrix (196), and potentially enhances the protumorigenic activity of MMP9. Upregulation of the Lcn2–MMP9 complex in tissue or in circulation has been reported to exacerbate the development of urothelial bladder, breast, lung, and colon cancers (34). One study proposed that Lcn2 could promote the epithelial–mesenchymal transition (198), which is the critical process contributing to tumor metastasis (171). Yang et al. (198) overexpressed Lcn2 in MCF-7 breast cancer cells and demonstrated that these cells developed an epithelial–mesenchymal transition phenotype, characterized by decreased expression of E-cadherin (an anchor protein for

cell-to-cell adhesion). Moreover, another study suggested that Lcn2 decreased E-cadherin and its upstream regulator Rac1 in an iron-dependent manner, and this phenotype could be abrogated if treated with the iron chelator DFO (85).

Admittedly, the role of Lcn2 on tumors appears to be modulated in an iron-dependent manner, but although ferric-Lcn2 (holo-Lcn2) fuels tumor growth, iron-free Lcn2 (apo-Lcn2) promotes apoptosis instead. The proapoptotic feature of apo-Lcn2 is not completely understood, although apo-Lcn2 may trigger apoptosis via downregulation of Bcl2 and upregulation of Bax (38), or it may induce iron efflux from cancer cells, leading to a state of iron deficiency that triggers cell death (51, 52, 173). The proapoptotic aspect of Lcn2, however, has been a subject of contention (41), as recent studies have given more support to the prosurvival role of Lcn2 in cancers (51, 58, 85, 174). Regardless of whether Lcn2 is pro- or antiapoptotic, its links to iron and cancer indicate that Lcn2 could be potentially targeted to disrupt the iron supply to cancerous cells.

However, the upregulation of Lcn2 has also been suggested to mediate tumor suppression, although the literature in this area is limited. For instance, Lcn2 was reported to impede proliferation and invasion of hepatocellular carcinoma cells via inhibiting the JNK and PI3K/Akt signaling pathways (102). A recent study by Moschen et al. (124) demonstrated that Lcn2 has an important role in restraining the growth of the procarcinogenic bacteria *Alistipes* spp., which bloom during conditions of Lcn2 deficiency and promote tumorigenesis in *Lcn2–IL-10* double knockout mice. *Alistipes* spp. are characterized as iron dependent; thus, their regulation by Lcn2 would be beneficial in mitigating bacterial-induced colon tumorigenesis.

POTENTIAL CLINICAL APPLICATIONS OF LIPOCALIN 2 AS IRON CHELATOR AND AN IMMUNOCALIN

The bacterial siderophore DFO has been used clinically to treat iron overload–related disorders due to its slow, but durable and robust, iron-chelating functions (192). However, there are limitations to using DFO because it cannot cross the cell membrane and, in addition, there is the risk of potentially delivering DFO-bound iron to bacteria and fungi. The synthetic iron chelators deferiprone and deferasirox are also being used in the clinic, yet they are not without side effects. Accordingly, considerable efforts have been made to exploit not only Lcn2 as the next-generation chelator of iron but also other candidate ligands. For instance, Barasch et al. (16) developed a novel mutant Lcn2 that could escape reabsorption in the kidney and, therefore, could facilitate iron excretion through the urine. This innovative exploit could pave the way for novel uses of mammalian siderophores, such as catechol, to complement Lcn2 in eliminating excess iron. The synergistic function of the Lcn2–siderophore complex could also be investigated for possible use in mitigating iron-mediated diseases, such as hereditary hemochromatosis, DIOS, NBIA, ischemia–reperfusion injury, and ICH. Aside from iron chelation, the Lcn2–siderophore complex can also bind radioactive actinide (3), tetravalent zirconium, and thorium (31), implying that Lcn2 could be exploited to treat radioelement contamination or could be engineered as a vehicle to deliver radioisotopes into tumors for imaging or radiotherapy.

Lcn2 is an attractive candidate for synthetic biology research, given its small size (25 kDa), simple molecular architecture (one polypeptide chain), and that it does not require posttranslational modification for its bioactivity. Moreover, the scaffold of Lcn2 may be feasibly modified to alter its target specificity, and its structure can be tuned to increase its plasma half-life, reduce immunogenicity, and enhance tissue penetrance (69). These properties allow lipocalins to be engineered into anticalins, which are regarded as an alternative to monoclonal antibodies for binding to specific ligands. The idea behind anticalins, originally pioneered by the Skerra group (57, 153), has led to the development of digoxigenin-binding anticalins, which can reverse the

toxicity associated with digitalis, a drug widely used for treating cardiac disorders. Two other Lcn2-based anticalins that are being studied for potential use are anticalins that bind to oncofetal fibronectin (68) and VEGFR (vascular endothelial growth factor receptors) (145).

Lcn2 is also regarded as part of the protein subfamily known as immunocalins (109), which comprises lipocalins with immunoregulatory functions. As an immunocalin, Lcn2 may present a potential therapeutic target for treating patients with specific granule deficiency (SGD) (107). SGD is a rare, congenital disease characterized by the complete deficiency of secondary granule proteins, including Lcn2. As such, SGD patients are prone to infectious diseases due to impaired neutrophil functions. In this regard, Lcn2 supplementation may be a viable treatment option. As an antibacterial factor, rec-Lcn2 has been shown to reduce bacterial contamination in platelet supplementation delivered to platelet-deficient patients (13). If future technologies allow the generation of mutant Lcn2 that can bind and neutralize LPS, it may have tremendous therapeutic potential to benefit human health.

CONCLUSIONS AND DIRECTIONS FOR FUTURE STUDIES

To summarize, the major iron-regulating functions of Lcn2 during inflammation are to (*a*) scavenge iron, thus exerting a bacteriostatic effect against infection and microbiota dysbiosis; (*b*) facilitate systemic and mucosal hypoferremia of inflammation; (*c*) modulate cytosolic iron levels and reduce oxidative stress; (*d*) transport iron among cells, tissues, and organs; and (*e*) stabilize the LIP (**Figure 3**). Although Lcn2 has been shown to be mostly protective against infectious diseases and IBD, disparities in its pathophysiological effects were observed in other disorders, including neurodegenerative diseases, MeS, kidney diseases, and cancers. Yet given its multifaceted functions, Lcn2 presents a promising new target for treating iron- and inflammation-related disorders, and future research on Lcn2 may lead to a novel therapeutic avenue for iron regulation in various diseases.

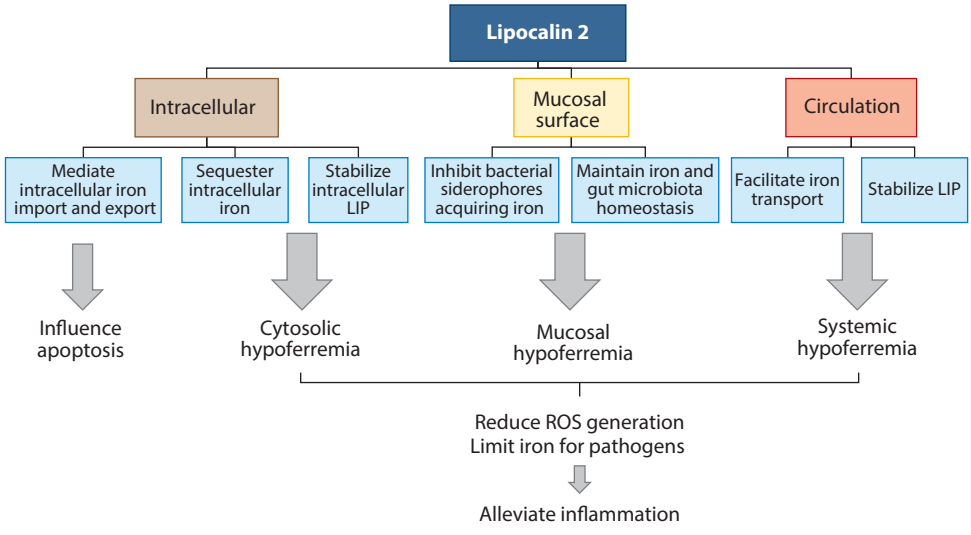

Figure 3

Overview of lipocalin 2–mediated iron homeostasis. Abbreviations: LIP, labile iron pool; ROS, reactive oxygen species.

Although knowledge regarding Lcn2 has accumulated dramatically during the past several years, there are still many questions that need to be answered. Studies showing that mammalian siderophores can transport iron into the mitochondria, for instance, raise new questions about whether Lcn2 may also be involved in the process. Lcn2 may be transported into mitochondria and, perhaps, also influence the iron redox engine catalyzed by members of the electron transport chain. Likewise, it is unknown whether red blood cells, which contain high levels of iron, may also express or retain Lcn2 as a mechanism to withstand iron-induced oxidative stress. Perhaps, Lcn2 may also play a part in stabilizing the membrane-bound iron present on red blood cells and other cell types. In addition to the bacteriostatic role of Lcn2, which is relatively well established, improved insights into other iron-regulation functions are needed, and these require both basic research and clinical evidence. This knowledge may pave the way for using Lcn2 as a novel treatment for iron homeostasis and inflammation.

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