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Lipocalin 2 regulation and its complex role in inflammation and cancer

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ABSTRACT

Lipocalin 2 is a protein that has garnered a great deal of interest in multidisciplinary fields over the last two decades since its discovery. However, its exact function in metabolic processes remains to be completely characterized. More recently, it has come to light as a highly upregulated protein in the setting of injury and infection. This review focuses on lipocalin 2 regulation and its relationship to cytokine and endocrine signaling pathways.

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1. Introduction

Lipocalin 2 (aka SIP24, 24p3, NGAL, uterocalin, siderocalin) was first discovered and characterized in a search for oncogenes in SV 40 transformation of G0-arrested mouse kidney cell cultures [1,2]. The human form was copurified and found to be covalently associated with neutrophil gelatinase (matrix metalloproteinase (MMP)-9) [3,4]. As its name suggests, it belongs to a larger family of similarly structured molecules with shared motifs but varying sequence homology. They have diverse putative roles in lipophilic small molecule transport and are known to bind retinols [5], prostaglandins [6] and pheromones [7]. The molecular ligand for lipocalin 2 was serendipitously deduced using crystallographic methods – it binds catecholate-type siderophores, small molecules elaborated by bacteria to avidly scavenge scant soluble ferric iron for vital bacterial cellular processes [8]. This seminal work presented an elegant and distinct antimicrobial mechanism that distinguishes lipocalin 2 from conventional antimicrobial proteins. More recently, an endogenous mammalian siderophore binding target has been identified, implicating an even broader role for lipocalin 2 in host iron trafficking and metabolism [9].

2. Lcn2 in various disease states

Lipocalin 2 has basal expression in bone marrow and mucosal and epithelial barriers [10,11] and a diverse number of physiologic conditions lead to increased message or protein levels of lipocalin 2 in nearly all tissues. Its response can be classified with respect to three major categories of stress:

a. *Environmental:* these include hypoxia, ischemia–reperfusion, and pathogen-associated molecular pattern (PAMP) activation by infections or lipopolysaccharide (LPS).
b. *Metabolic:* encompassing conditions associated with hyperlipidemia, obesity and insulin resistance.
c. *Developmental:* the most diverse, including conceptus attachment and reproductive tissue involution, bone plate maturation, epithelial–mesenchymal transformation and malignancy.

As such, lipocalin 2 has become a surrogate marker of interest for clinical monitoring of tissue response to a variety of injuries or insults. It is being explored as a serum and urinary biomarker for various renal injuries [12–17], and as a prognostic biomarker in severe sepsis [18] and malignancies due to its upregulation in various tumors [19–21]. Increasingly, it is clear that lipocalin 2 plays a pathophysiologic role in seemingly diverse states including infectious disease [8,22–36], renal and cardiac disease or injury [37–39], metabolic syndrome and thermal dysregulation [40–47], dermatitides [48,49], and inflammatory lung diseases of the lung and bone [50–52] just to name a few. This list only touches the surface and bespeaks the fundamental and global tissue role that lipocalin 2 plays in the post-natal organ response to stress or injury.

3. Infection and acute lipocalin 2 regulation

Lipocalin 2 is rapidly upregulated in the setting of a growing list of infection models which now include Group B *Streptococcus* [53],

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Escherichia coli [22,23,26], *Klebsiella pneumoniae* [27–29], *Chlamydia pneumoniae* [30], *Salmonella typhimurium* [31–33], and *Mycobacteria* [34–36]. While a great deal of lipocalin 2 protein may be sourced from pre-formed, pre-packaged neutrophil granule proteins released at the site of infection, a major mechanism of de novo upregulation also involves signaling via Toll/IL-1 receptor (TIR) signaling by LPS stimulation and IL-1β release [22,27,53–55]. This mode of acute upregulation is crucially dependent on NF-κB binding to its consensus binding sequence upstream of the lipocalin 2 promoter. Work done by Cowland et al. and others demonstrated the necessary and sufficient role of IL-1β in stabilizing IκB-ζ as a binding partner for NF-κB and initiating transcription [55–57].

Compared to IL-1β, a relatively subacute mechanism of upregulation associated with infection is via IL-17 signaling. IL-17 pathways are important in the host defense against Gram negative, Gram-positive and fungal organisms and likely play a role in developing the later adaptive immune response. In the process of interrogating antimicrobial products downstream of IL-17, Shen et al. also discovered strong induction of lipocalin 2 and this effect was potentiated by TNFα in a mouse bone marrow cell line [58]. In this case, lipocalin 2 was also regulated at the level of transcription by IL-17 and this was highly dependent on NF-κB. Additionally, they and others identified the importance of the CCAAT/enhancer-binding protein (C/EBP) family, specifically C/EBPβ and C/EBPδ, in initiating transcription [57,59].

However, in contrasting results using human A549 cells synergistically stimulated with IL-17 and TNFα, Karlsen et al. found no dependence on C/EBPβ or C/EBPδ, when these protein effects were knocked down by siRNA or promoter mutation [60]. They reasoned that this difference in *cis*-activating promoter elements might be attributed to IL-17 alone stabilizing IκB-ζ in this tissue culture model and that this was sufficient. Vexingly, there are also other C/EBP binding proteins that can activate lipocalin 2 expression [61]. Likely, this contrast may be attributed to sequence differences in the promoters of mouse and man as variable spacing of transcriptional elements may dramatically affect activation. An already known example of human and mouse Lcn2 promoter differences is the presence of vitamin D response elements present only in the promoters of human antimicrobial protein promoters [62]. Fig. 1 illustrates the promoter mapping and various results on lipocalin 2 expression after binding motif mutation.

Further work on the IL-17 receptor complex (IL-17RA and IL-17RC) revealed that their cytoplasmic domains bore resemblance to TIR domains (termed "SEFIR"), and also analogously orchestrated complementary pathways. The IL-17RC SEFIR domain interacting with phosphorylated Act1 leads to upregulation of C/EBPβ, C/EBPδ and activation of NF-κB, all initiating lipocalin 2 transcription [63]. Meanwhile, the IL-17RA SEFIR domain triggers sequential phosphorylation of C/EBPβ that subsequently inhibits IL-17 target genes including IL-6 and lipocalin 2 [64]. Thus, C/EBP binding proteins exhibit both activating and inhibitory characteristics and are further regulated at the level of phosphorylation, translation and dimerization. A recent review in this journal elaborates on the intricacies of C/EBP elements [65]. Taken together this demonstrates a high level of complexity involving redundant upstream pathways to ensure activation and amplify response, delayed temporal auto-regulation of inflammation within the same receptor signaling complex, and possible idiosyncrasies in species-specific response.

Synthesizing findings from various bacterial infection models, we propose a model of inter- and intracellular cytokine crosstalk during host defense and resolution of inflammation (Fig. 2). There is acute innate immune activation via PAMP receptor activation in both non-immune and first-responder immune cells, eliciting TNFα and IL-1β release. This immediately leads to IL-1β and NF-κB pathway activation and subsequent induction of lipocalin 2 (among other antimicrobial products). Together, these early antimicrobial mediators directly kill microorganisms or inhibit growth by iron deprivation [32]. This provides a key front line barrier defense and anti-colonization effect which might be sufficient in preventing advancing infection [28,29,35,62]. Macrophages and non-immune

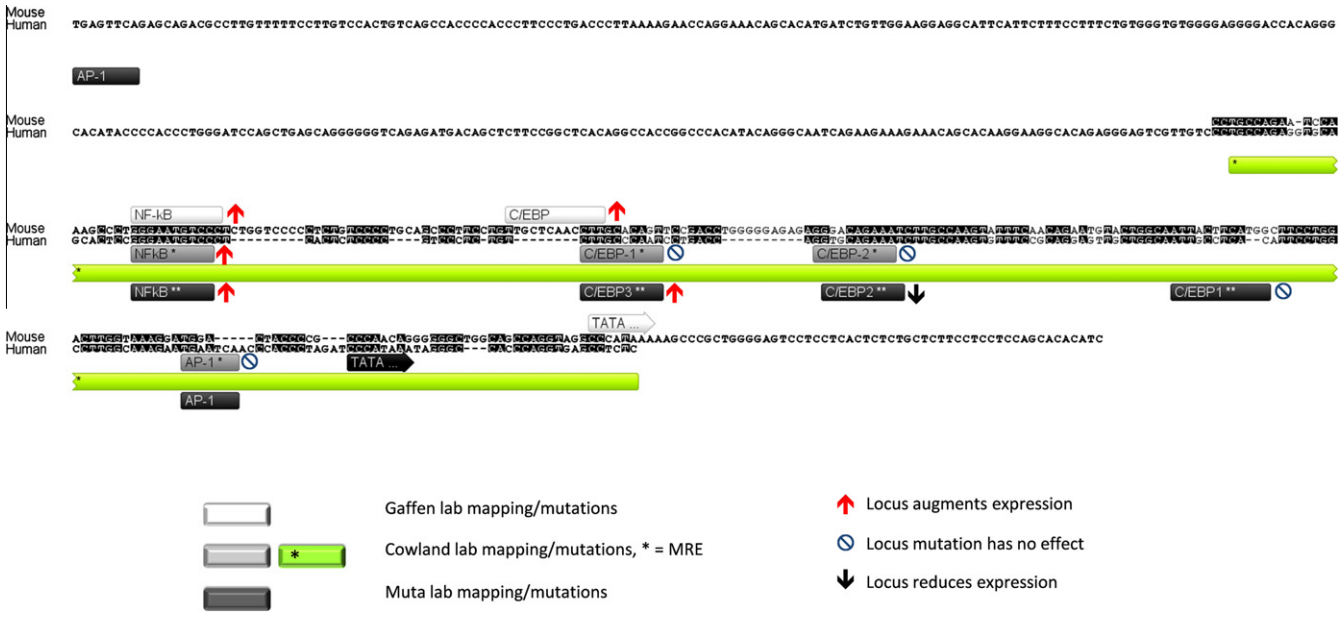

Fig. 1. Analysis of lipocalin 2 promoter elements. The sequence and mapping of promoter minimal response elements (MRE) used in reporter constructs by three groups studying the promoter regulation of murine and human lipocalin 2. Conserved consensus sequences for various binding motifs are highlighted in the sequence. Top sequence is the murine MRE used in reporter assays with NF-κB and C/EBP binding sites that were mutated labeled above it (white boxes) [59]. Bottom sequence is the MRE used by a second group with their NF-κB and C/EBP binding site mutations indicated (gray boxes) [57]. Green box indicates the MRE used by a third group along with their binding site mutations (dark gray boxes) [60]. Up, down or null symbols indicate whether the group found the sequence to be activating, repressive or to have no effect on lipocalin 2. Figure was created using Geneious software [117]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)