

Genomics and Biological Activity of Neutrophil Gelatinase-Associated Lipocalin in Several Clinical Settings

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Key Words

Acute kidney injury • Lipocalin • Neutrophil gelatinase-associated protein • Siderophore

Abstract

Recent literature has shown that neutrophil gelatinase-associated lipocalin (NGAL) is one of the most interesting and promising biomarkers in case of acute kidney injury. However, several studies indicated that this protein may be applied beyond the boundaries of renal pathophysiology and may be used in other pathophysiological settings since it is also expressed in neutrophils, and respiratory, bowel and prostate epithelia. In this review, we report NGAL genomics and biology and its possible use in several clinical settings. In particular, we review the genomic organization of the NGAL gene, the lipocalin family structure, the interaction between NGAL and ligands, and the induction and expression of NGAL in different conditions.

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Introduction

Recent literature has shown that neutrophil gelatinase-associated lipocalin (NGAL) is one of the most interesting and promising biomarkers in case of acute kidney injury (AKI). However, several studies have demonstrated that this protein may also be used in other pathophysiological settings, since it is also expressed in neutrophils, and respiratory, bowel and prostate epithelia.

In this review, we report NGAL genomics and biology and its possible use in several clinical settings.

NGAL: Genomic Organization and Homologous Gene

The NGAL gene (NC_000009.11) consists of 7 exons that produce at least 5 functional transcripts (i.e. mRNAs translated into proteins), the most common of which encodes a 198-amino acid secreted protein. The NGAL mouse homologue is called lipocalin 2 (lcn2); Lcn2 (NC_000068.7) is encoded by a gene located on chromosome locus 2 [1]. The lcn2 gene has 6 exons and encodes two functional transcripts.

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The NGAL gene is a rapid response gene whose expression is driven in a dose-dependent manner by different stimuli which generally induce tissue damage. Several studies have shown that its expression usually increases a few hours after toxic insults. The rapidity and the intensity of its expression are useful tools for the identification of patients at particular risk of developing tissue damage [2]. These basic clinical observations have been reproduced both in human populations and animal models.

NGAL: The Lipocalin Family and Structure

NGAL is a ubiquitous glycoprotein which belongs to the lipocalin superfamily [3]. Lipocalins generally act as transporters binding a number of ligands and carrying small molecules to specific cells. They have been shown to be involved in retinol transport, invertebrate cryptic coloration and olfaction, pheromone transport, prostaglandin synthesis, cell growth and metabolism modulation, immune response regulation, tissue development and animal behavior regulation [4]. The lipocalin protein family is extremely widespread, with members in bacteria, insects and mammals, including human beings [5]. The members of this family share little matched sequence homology (approximately 20%), but all confine to a common tertiary structure determined by highly conserved segments of the individual lipocalin proteins, termed the lipocalin folds. These folds organize the lipocalins in 8 anti-parallel β -sheets surrounding a hydrophobic pocket, which is fundamental for their function as transport or carrier proteins of a variety of ligands [6]. The β -sheets are connected to another sheet through 7 short loops (L1–L7). Loop L1 forms a lid-like structure to close the ligand binding cavity. The difference in specific amino acids within the lipocalin fold gives rise to the wide diversity in ligands that can be bound by lipocalins [7]. In addition, outside the β -barrel there are 3–10 helices at the NH2 terminus and an α -helix at the COOH terminus [3].

Immunoprecipitation assays revealed an interaction between NGAL and MMP-9 gelatinase, covalently conjugated via a disulfide linkage. However, such a linkage is not observed in the murine Lcn2 which lacks the corresponding cysteine residues [8, 9]. By forming this complex, NGAL seems to protect MMP-9 from autodegradation, thus preserving its activity in extracellular matrix remodeling [10]. MMP-9 is, indeed, secreted by neutrophils and degrades basement membrane and extracel-

lular matrix components (including cartilage proteoglycan, type I gelatin and collagens type I, IV, V and XI) [11]. NGAL exists in different forms: a 25-kDa monomer, a 45-kDa disulfide-linked homodimer with MMP-9 and a 135-kDa heterodimeric form. The monomeric form, and to some extent the heterodimeric forms, are the predominant forms produced by tubular epithelial cells, whereas the dimeric form seems unique to the neutrophils. The monomeric form is also produced by neutrophils [12]. Neutrophils are not the only cells producing NGAL; monocytes/macrophages and adipocytes also present abundant NGAL expression (fig. 1) [13]. Importantly, immature neutrophils show a high expression of NGAL mRNA, whereas mature neutrophils/granulocytes are deprived of mRNA but contain a large amount of NGAL protein, which is localized in specific granules [14, 15].

Ligands for NGAL: Siderophores

Goetz et al. [4] carried out an epoch work on the X-ray crystallography of recombinant human NGAL protein expression in *Escherichia coli*. They demonstrated that the NGAL ligand is siderophore [4], a diverse group of small (1-kDa or less) nonpeptide iron (Fe^{3+})-binding chemicals produced in bacteria, fungi and plants [16–19]. NGAL has been found to work as a bacteriostatic agent by sequestering siderophore-bound iron. The binding of siderophores to the pocket of NGAL is largely mediated by ionic strength where positively charged amino acids (Arg^{81} , Lys^{125} and Lys^{134}) interact with negatively charged side chains of siderophores (fig. 2) [8, 20]. NGAL seems to prevent the growth of the bacterial strains that rely on siderophore production to satisfy their iron demands [4]. NGAL is also known to be protease resistant [9], but it can be degraded [21]. Its long-term bacteriostatic effect depends on the ability of epithelial cells not only to synthesize NGAL in response to bacterial infection, but also to take it up from the extracellular milieu, thus protecting it from microbial proteases [20]. The iron-binding capacity of NGAL has also been shown to facilitate iron delivery into cells, thus potentially playing an important role in the regulation of iron-sensitive genes that participate in the mesenchymal-epithelial transition during the development of proximal parts of the mammalian nephron [22]. Iron regulates cell cycle activities, and signaling and development genes at transcriptional level, but the mechanism of remains to be elucidated [23–25].

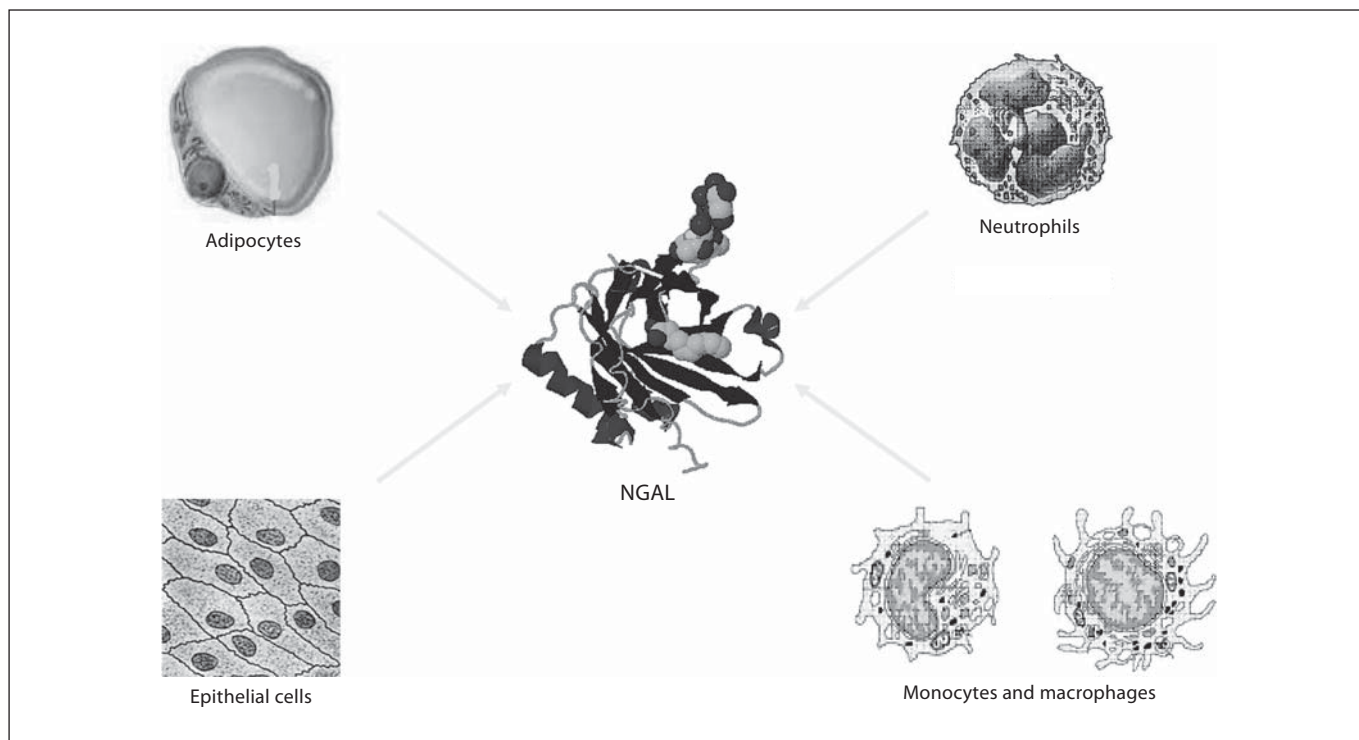


Fig. 1. Cell types that produce NGAL.

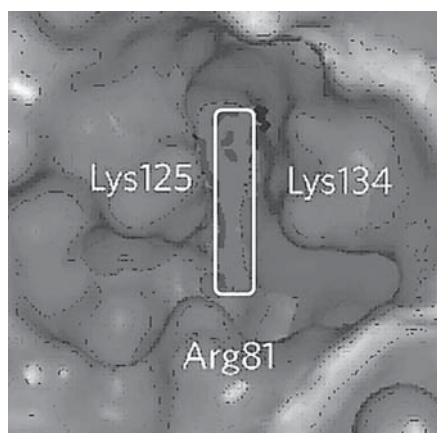


Fig. 2. The binding pocket of NGAL: positively charged amino acids (Arg81, Lys125, and Lys134) interact with the negatively charged side chains of siderophores.

Baseline Levels

Biological fluids contain very low levels of NGAL protein at steady state. NGAL serum concentration is about 20 ng/ml and it is probably related to physiological neu-

trophil production and limited liver, spleen and kidney expression [26]. Renal clearance is a main regulator of this steady state level, because circulating NGAL undergoes glomerular filtration given its low molecular weight and positive charge [2].

Similar to serum, urine contains approximately 20 ng/ml NGAL at steady state. The origin of this protein is not clear, but it may derive in part from serum NGAL which bypasses capture in the proximal tubule (approximately 1/200 molecules). Alternatively, NGAL may be derived from neutrophils or even from bladder epithelia [2].

NGAL Expression and Induction

NGAL expression is increased in case of bowel and respiratory inflammation, in particular in the setting of bacterial infections [15]. Viral infections are also associated with significant NGAL upregulation [20].

The NGAL-siderophore complex without iron [23, 27] can chelate iron from cells, thus inducing cellular iron deprivation and apoptosis [27], bacterial growth inhibition and erythropoiesis suppression [4, 15, 28]. The biologic significance of this finding has been demonstrated

in genetically modified mice deficient in both copies of the NGAL gene. These animals were more sensitive to certain Gram-negative bacterial infections and died of sepsis more frequently than their wild-type counterpart [29, 30]. In fact, mounting evidence points towards growth factor effects of NGAL and its involvement in various cellular responses, such as proliferation, apoptosis and differentiation [31]. In contrast, other authors believe that NGAL may enhance cellular survival, because decreased NGAL levels seem to be associated with increased apoptosis [27].

Furthermore, immunohistochemistry has recently shown massive staining for NGAL protein in epithelial cells in case of diverticulitis, appendicitis, ulcerative colitis and morbus Crohn, as well as in neoplastic conditions. Indeed, NGAL has been reported to be highly expressed in malignant tumors arising from several organs, including the skin, thyroid, breast, ovary, endometrium, colon, lung, liver, bile ducts, esophagus, stomach and pancreas [7]. Zhang et al. [32] evidenced the important role of NGAL in promoting tumorigenesis via enhanced tumor cell survival, activation of cell proliferation and metastatic spread. In particular, in several cancer types, decreased NGAL expression delays or even abrogates tumorigenesis.

NGAL expression increases in case of AKI. Mishra et al. [33] demonstrated high NGAL upregulation in mouse models of renal ischemia reperfusion injury. Its levels correlated with the duration of ischemia. It was also identified as a marker of cisplatin nephrotoxicity in an animal model [34]. In a study of 71 children undergoing cardiopulmonary bypass surgery, urinary NGAL and plasma

NGAL 2 h after surgery were found to be powerful independent predictors of AKI [35]. In a prospective study of 91 children with congenital heart disease undergoing elective cardiac catheterization with contrast enhancement, both urinary NGAL and plasma NGAL predicted contrast-induced nephropathy within 2 h of contrast agent administration [36]. Wheeler et al. [37] reported a high sensitivity of NGAL in detecting AKI in 143 critically ill children with the systemic inflammatory response syndrome or septic shock within 24 h of admission to the intensive care unit. Zappitelli et al. [38] studied urinary NGAL in 140 critically ill patients requiring mechanical ventilation. A significant rise in urinary NGAL levels occurred 2 days earlier than a 50% increase in serum creatinine levels.

Conclusion

The small size of NGAL makes it an attractive target as a molecular imaging tool and as a diagnostic and follow-up marker in several diseases. Identification of the relevant NGAL receptors in different cells and tissues and their characterization, combined with genomic analysis and bioinformatics, will greatly improve our understanding of its action in the setting of various renal and nonrenal diseases.

Disclosure Statement

None of the authors has any conflict of interest.

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