REIVEW ARTICLE

Neutrophil gelatinase-associated lipocalin (NGAL) role in diagnosis of complications of liver cirrhosis

[1]Dalia K. Nassar*, [1]Mohamed F. Elkenawy, [1]Mohammed M. El-Naggar, [2]El-Sayed A. Khalil, [1]Ghada I. Barakat

[1]Medical Microbiology & Immunology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[2]Department of Internal Medicine, Faculty of Medicine, Mansoura University, Mansoura, Egypt

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Corresponding Author:
Dalia Kamal Nassar
Medical Microbiology & Immunology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt
Tel.: 01006210518
dalianasser@mans.edu.eg

ABSTRACT

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa glycoprotein that is normally expressed at very low levels in several human tissues. NGAL comprises a critical component of innate immunity to bacterial infection acting as an acute phase protein. Also, it is one of the most promising markers for diagnosis of kidney injury.

Abbreviations:
NGAL: Neutrophil gelatinase-associated lipocalin**, AKI:** acute kidney injury, **kDa**: Kilo Dalton, **ACLF:** acute on chronic liver failure, **LPS:** lipopolysaccharides, **TNF α:** tumor necrosis factor alpha, **SBP:** spontaneous bacterial peritonitis.

INTRODUCTION

Liver cirrhosis is defined as diffuse hepatic fibrosis with nodules replacing the normal liver architecture, causing disturbance of normal physiology of the liver. Cirrhosis is associated with high mortality rates and particularly affects persons in the most productive years of their lives [1].

The major complications of liver cirrhosis include ascites, hepatic encephalopathy, hepatocellular carcinoma, bacterial infections and acute kidney injury (AKI), of which the latter two are commonly associated with mortality in patients with cirrhosis [2,3].

NGAL nomenclature and production

NGAL belongs to the lipocalin family that has gained wide attention from scientists in the past few years[4]. It is stored in the secondary granules of neutrophils [4,5].

NGAL is normally expressed in bone marrow, liver, lung and kidney. There is a low baseline production of NGAL that maintains its serum concentration to around 20 ng/ mL [5]. These normal levels of circulating NGAL are due to glomerular filtration as it possesses low molecular weight and positive charge [6].

NGAL is overexpressed in several tissues in response to tissue injury[7,8]. Urine NGAL is secreted from damaged kidney tubules [9]. Induced expression of NGAL is achieved through the binding of activated nuclear factor NF-kB to the promoter region of the NGAL gene [10]. Moreover, marked NGAL secretion occurs in human cancerous tissues due to induction of NGAL gene by tumor-promoting agents as polyoma virus, hepatocyte growth factor and transforming factor Neu[11].

NGAL structure

NGAL is a 25-kDa protein that is encoded by lipocalin-2 gene[12]. The gene contains 7 exons encoding for 5 functional transcripts [5].

Lipocalin family functions as transporter proteins that act by binding ligands and delivering small molecules to target cells. They are involved in many processes such as regulation of cell growth and immune response, transport of retinol and synthesis of prostaglandin [13].

Lipocalin family are present in prokaryotes and eukaryotes[14]. Lipocalins are quite diverse sharing only about 20% of sequence homology. However, they all have structurally conserved regions encoding a common tertiary structure called the lipocalin folds which compromise an 8 anti-parallel β-sheets enclosing a hydrophobic cavity for ligand binding [15].

Seven short loops (L1–L7) connect the β-sheets together. Also, the lipocalin fold compromises 3–10 helices at the N- terminus and an α- helix at the C-terminus. Various ligands can bind lipocalin fold in different members of lipocalin family due to different amino acids within the fold[12] (figure 1).

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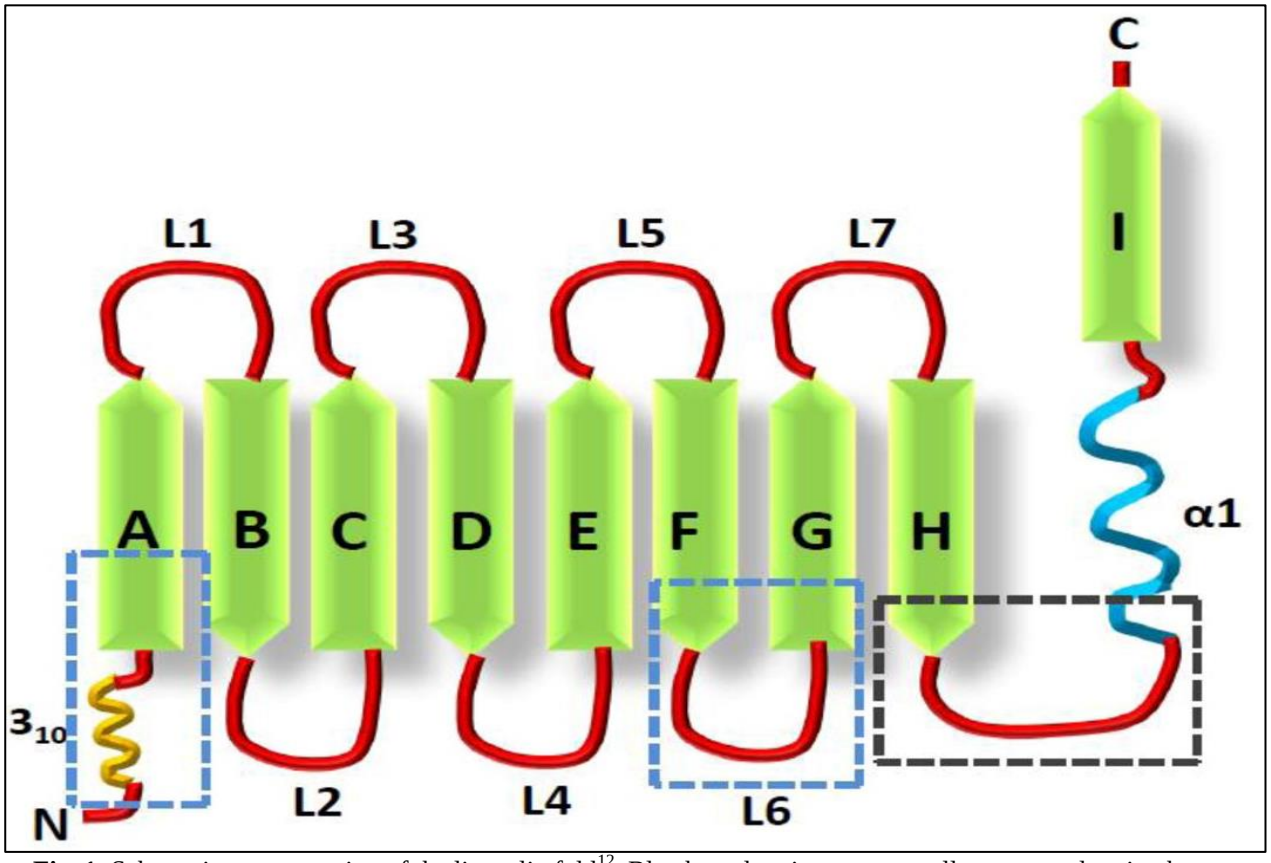

Fig. 1: Schematic representation of the lipocalin fold[12]. Blue boxed regions: structurally conserved region between different lipocalins, Black boxed region shows significant conservation in amino acid sequence.

Ligands for NGAL: Siderophores

Goetz et al.[13] reported that NGAL ligand is siderophores after studying X-ray crystallography of recombinant NGAL expression in *E. coli*. Siderophores are a group of iron binding chemicals that is produced in bacteria and fungi[16].

NGAL Expression and Induction

NGAL is an acute phase reactant that is rapidly induced by tissue damage[17]. Its increased expression occurs within few hours from toxic insults. This property of rapid and intense expression is of major value in identifying patients at risk of developing tissue damage [6]. Inducers of NGAL include IL-1β, LPS, TNF α, prostaglandin F2α and hypoxia [18].

NGAL rapidly increases in blood and urine in acute kidney disease [19]. Moreover, NGAL expression was reported to increase in case of bowel and respiratory inflammation [12,20].

Functions of NGAL

NGAL exerts many activities by binding to siderophores [4]**.** Enterochelin is a siderophore produced by bacteria that has high affinity to iron to provide bacteria with intracellular iron stores. So, it binds iron in the extracellular space and the siderophore-iron complex is delivered back to bacteria by specific transporters[21,22].

NGAL binds siderophores by ionic interactions between positively charged amino acids of NGAL pocket and negatively charged siderophores' side chains[23]. Thus, NGAL exerts a bacteriostatic function by capturing and depleting these siderophores consequently depleting the bacterial iron stores and preventing their growth [21,22].

This bacteriostatic role has been confirmed by studies on knockout mice without both copies of the NGAL gene. Comparing those animals with their wild-type counterparts, they were more susceptible to bacterial infections with higher mortality rate due to sepsis [24].

Therefore, NGAL is considered a critical element of innate immune response to exogenous bacterial infections [4].

Moreover, NGAL is responsible for apoptosis of pro-B cells and inhibition and erythropoiesis by chelating iron [25,26]. The mammalian siderophores are used by NGAL to provide cells with iron as iron plays an important role in regulation of cell cycle activities [27].

As for its role in promoting tumorigenesis, NGAL expression within carcinoma cells represents a poor

prognostic element, meanwhile its decreased expression delays tumorigenesis[4]. Zhang et al[28] concluded that NGAL is responsible for survival of tumor cell, enhanced cell proliferation and metastatic spread.

Mechanism of action of NGAL

NGAL binds with specific surface receptors as 24p3R which are present on surface of the kidney tubules [29].

Upon binding these receptors, NGAL enters the cell in one of two forms; Apo-NGAL (protein alone) or Holo-NGAL (NGAL-iron-siderophores complex **(figure 2)** [17].

Holo-NGAL is transported through the cytoplasm within endosomal vesicles and it regulates the activity of iron-dependent genes by releasing the siderophore-iron complex**.** Upon Apo-NGAL entry, it depletes intracellular stores of iron [17].

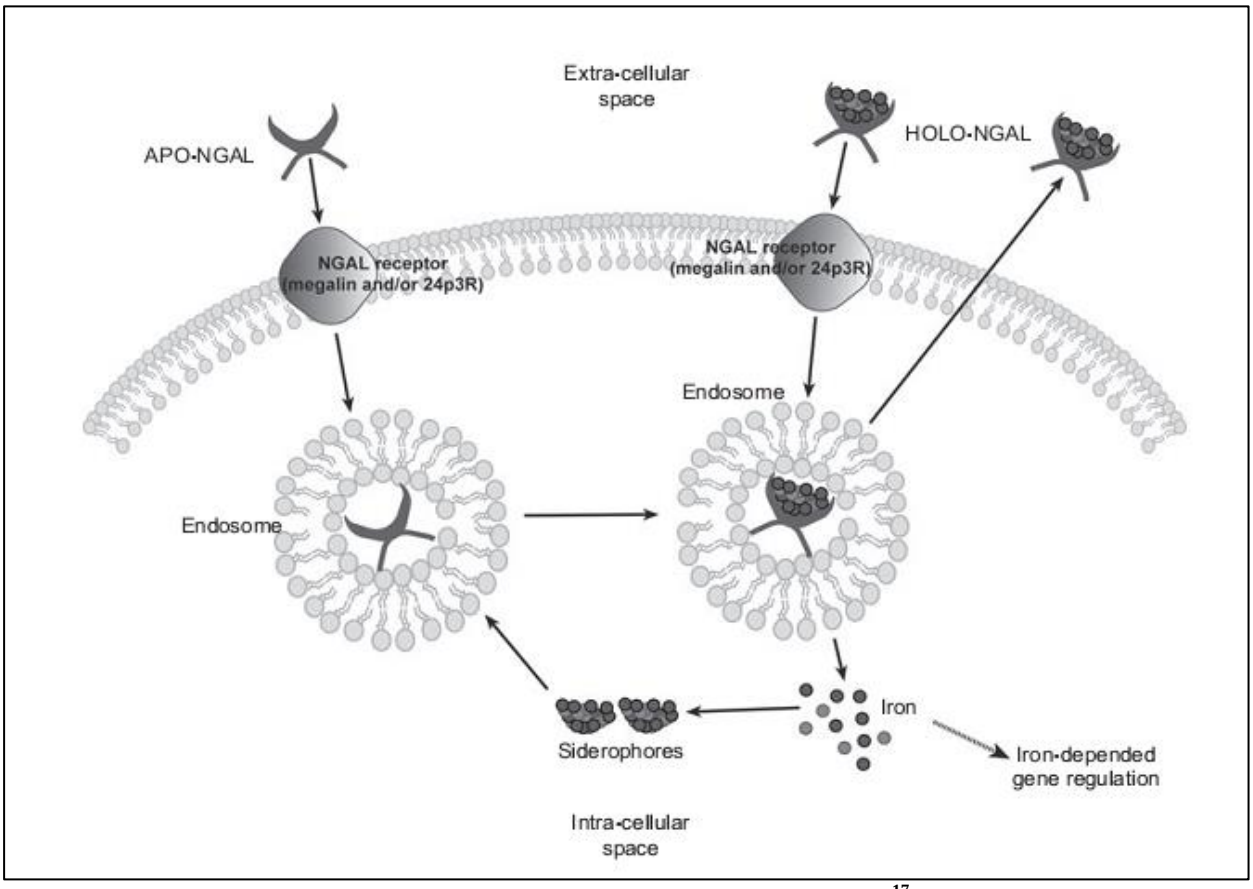

Fig. 2: Schematic model of the functions of NGAL[17]

NGAL role in liver diseases

It is not clear which cell types are primarily responsible for elevated serum NGAL, given that many cell types have been shown to produce it [8]. Serum NGAL expression increased in hepatocytes in patients with acute on chronic liver failure (ACLF) [30].

NGAL as a biomarker for infection

NGAL has been evaluated in diagnosis of infection in several studies. Guiddir et al [31] reported higher cerebrospinal NGAL levels in acute bacterial meningitis in comparison to viral meningitis.

Also, NGAL can distinguish between bacterial and non-bacterial peritonitis in peritoneal dialysis patients [32]. Cullaro et al [33] found that ascites NGAL in cirrhotic patients was higher in spontaneous bacterial peritonitis (SBP) than control patients suggesting that NGAL levels is a sensitive and specific biomarker for diagnosis of SBP. Moreover, serum NGAL was higher in septic than non-septic patients [34].

NGAL as a biomarker for AKI

Transcriptome profiling and proteomic studies have identified NGAL as 'ready to go gene' that is highly

induced and rapidly expressed upon sensing stress and/or kidney damage [35].

NGAL is regarded as an acute phase reactant such as IL-6 and C-reactive protein. NGAL protein was reported to increase up to 1000 fold in severe cases of AKI [16].

The discovery that urine NGAL was detected in experimental animals soon after AKI has encouraged conducting translational studies to assess its role in human AKI [36].

As NGAL is rapidly secreted from the nephron, its level increases 48 hours before serum creatinine changes [37]. Moreover, NGAL can detect subclinical or modest renal damage without significant variations in serum creatinine [38].

CONCLUSION

NGAL is an acute phase reactant that is induced in infection, injury and inflammation. It can be a valuable marker for diagnosis of multiple complication of liver cirrhosis as AKI and bacterial infection.

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