16

Neutrophil Gelatinase Associated Lipocalin: Structure, Function and Role in Human Pathogenesis

Subhankar Chakraborty[1], Sukhwinder Kaur[1], Zhimin Tong[2],
Surinder K. Batra[1] and Sushovan Guha[2]
[1]University of Nebraska Medical Center, Omaha, NE
[2]The University of Texas MD Anderson Cancer Center, Houston, Texas
USA

1. Introduction

Glycoproteins have a unique position in the pathogenesis of human diseases. Most of the commonly employed protein biomarkers are glycoproteins. Examples include CA19-9 (carbohydrate antigen 19-9) to follow-up patients with pancreatic cancer, CEA (carcinoembryonic antigen) for multiple solid tumors and CA125 (carbohydrate antigen 125) used in the diagnosis, follow-up and therapy of patients with ovarian cancer. Most of these glycoproteins are large molecules. However, there is a family of smaller, secreted glycoproteins (called lipocalins) that are important in the maintenance of health and in combating diseases effectively. A prototype of this family is a small, secreted glycoprotein called Neutrophil gelatinase associated lipocalin or NGAL. In recent years, NGAL has emerged as a novel biomarker in several benign and malignant diseases. Further, studies in cultured cells and in murine models have revealed a pivotal role for this molecule in several physiological processes and pathological conditions. In this Chapter, we review the biology of lipocalins, focusing specifically on NGAL and its role in human health and disease.

2. The lipocalin family

Lipocalins are a family of small (160-180 amino acids long) secreted proteins that mediate (among other functions) a common functional role- i.e., transportation of small hydrophobic ligands. Most protein families are classified on the basis of similarity in amino acid sequences, domain architecture and three-dimensional protein structure. The lipocalins however are a unique exception in that the percentage of sequence identity among different members of the lipocalin family is sometimes even lower than the minimum (20%) identity required to call it a reliable alignment (Flower *et al.*, 2000; Flower, 1996; Flower, 1995; Flower, 1994). Despite this lack of a sequence similarity, the lipocalins share three short stretches of amino acid sequences (or motifs) that are part of the structural conservation among different lipocalin sequences. These motifs, which are aptly termed as "structurally conserved regions or SCRs" are used to classify this large family into two main sub-families-the "kernel" and the "outlier" lipocalins (**Table 1**). The kernel lipocalins contain all three motifs while the outlier lipocalins contain only one or two of these (but not all three) SCRs.

Abbreviation	**Protein name**
Kernel Lipocalins	
Alpha 1M	Alpha 1-microglobulin
ApoD	Apolipoprotein D
A2U	α2micro-Globulin
BBP	Bilin binding protein
Blg	β1-Lactoglobulin
C8γ	C8γ
CPP	Choroid plexus protein
CRABP2	Cellular Retinoic acid binding protein
ACC	α-Crustacyanin
MUP	Major Urinary Protein
NGAL	Neutrophil gelatinase associated Lipocalin
PGDS	Prostaglandin D synthase
PP14	Pregnancy protein 14
PURP	Purpurin
	Lazarillo
Outlier Lipocalins	
AAAG	Alpha 1-Acid glycoprotein
	Aphrodisin
OBP	Odorant binding protein
	Probasin
VEGP	von Ebner's-gland protein

Table 1. List of known Kernel and Outlier Lipocalin proteins

The secondary and tertiary structure of the lipocalin family members is very similar and is characterized by the presence of a "lipocalin fold"- a symmetrical structure comprised almost entirely of β-pleated sheets closed at the two ends by two α helices (**Figure 1)**. There are eight antiparallel β sheets linked to each other by seven short loops. The N-terminal region of the protein forms the ligand binding cavity which is closed by a conserved 3_{10} helix. The cup-shaped cavity of the lipocalins enclosed within the β-pleated sheets is well adapted for binding to a wide array of hydrophobic ligands- for instance, while retinol binding protein (RBP) binds primarily to its endogenous ligand retinol, it can also bind to several other hydrophobic ligands including β-ionone, β-carotene, cholesterol, terpenoids and long chain esters of retinol and retinoic acid.

Many lipocalins have also been shown to bind to specific receptors including RBP, alpha1-microglobulin, major urinary protein (MUP) and Odorant-binding protein (OBP). A third property of lipocalins is their tendency to form complexes with soluble macromolecules. Some of the well-known protein-protein complexes involving the lipocalin family members include the complex of RBP with transthyretin, NGAL with human neutrophil gelatinase, C8γ with C8α as part of the membrane attack complex (MAC) and α1-microglobulin (A1M) with immunoglobulin A (IgA). These associations may either serve to transport proteins

(RBP), stabilize the interactor (NGAL), protect normal cells from damage (C8γ) or alter the biological activity of the interacting protein (A1M).

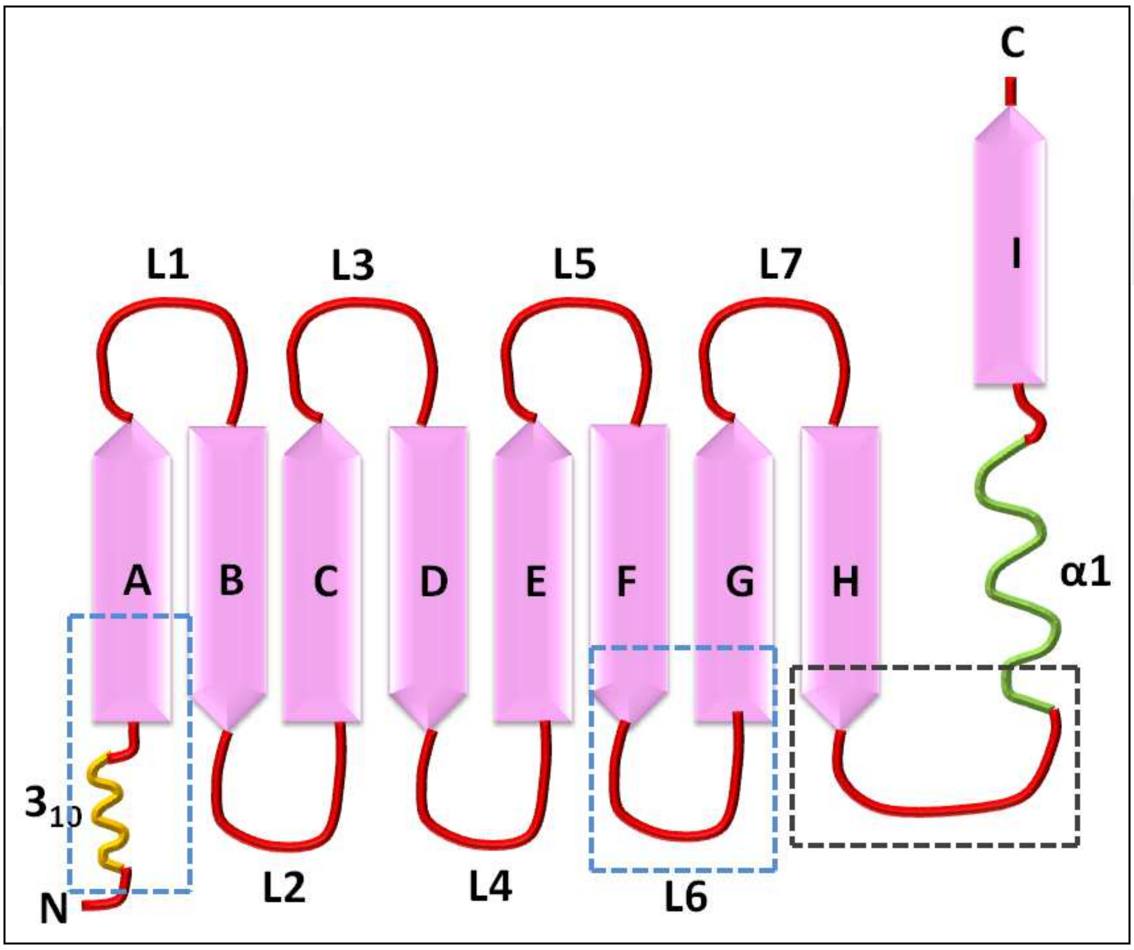


Fig. 1. Schematic representation of the lipocalin fold. The characteristic feature of lipocalins is the "lipocalin fold" which comprises of an N-terminal 3-10 helix followed by eight beta sheets (A-I) arranged in an antiparallel orientation. The eighth beta sheet is connected to an alpha helix (denoted as α1), which is in turn connected to a C-terminal beta sheet. The beta sheets are connected by loops (L1-L7). Loops L1,L3,L5 and L7 form the open end of the molecule (i.e. the opening to the ligand binding site of NGAL). The portion of the lipocalin fold that are structurally conserved between different lipocalins is indicated by the blue boxed regions while the region that shows significant conservation in amino acid sequence is indicated by the black boxed region.

Functionally, the lipocalins are generally well known as transport proteins that exist in the extracellular space. The prototypical lipocalin involved in transport of cargo in the body is RBP which binds to and transports retinol (Vitamin A) from the liver to various organs. Binding to RBP not only protects retinol from being excreted by the kidneys but also from being oxidized within the body. Further, it also permits the transport of a relatively insoluble retinol in the form of a soluble complex to the tissues to mediate its effects. Other functions attributed to lipocalins including regulation of cell ageing, survival and differentiation (probasin and purpurin), modulation of immune response (NGAL, AGP, PP14 and AIM), prostaglandin synthesis (glutathione-independent PGD2 synthase), coloration (insecticyanin, BBP and crusticyanin), smell (OBP), taste (VEGP) and as sex hormones or pheromones (MUP and Aphrodisin) (Flower *et al.*, 2000; Flower, 1996; Flower, 1995; Flower, 1994). We will examine the role of a member of the lipocalin family (NGAL) that has been extensively studied particularly as a potential marker for diagnosis and prognosis in several human diseases. We will investigate its biology, functions and clinical applications both in benign and malignant diseases.

3. Neutrophil gelatinase associated lipocalin (NGAL)-genomic organization and protein structure

Neutrophil gelatinase associated lipocalin (NGAL) also known as migration stimulating factor inhibitor (MSFI), human neutrophil lipocalin (HNL), alpha-1 microglobulin related protein, siderocalin or uterocalin is a 198 amino acid glycoprotein encoded by a gene located on the chromosome locus 3p11. It was first isolated from mouse kidney cells infected with a simian virus (SV-40) (Hraba-Renevey *et al.*, 1989). Triebel and colleagues identified a novel association between NGAL and the gelatinolytic enzyme MMP-9 (matrix metalloproteinase 9 or gelatinase B) known to degrade several components of the basement membrane including type I gelatin and collagen types I, IV, V and XI (Triebel *et al.*, 1992). Human NGAL protein (or LCN2) is represented in upper case, while the murine and rat homologues are usually represented in lower case (Ngal or Lcn2).

Structurally, human NGAL contains a 20-amino acid signal peptide at the N-terminal end of the protein followed by the "lipocalin" domain. This domain which is responsible for binding of lipocalins to their ligands, is structurally comprised of an eight stranded β barrel with its loops running in an antiparallel direction. A comparison of the amino acid sequence between NGAL homologues in different species reveals that human NGAL is highly similar to the homologue present in chimpanzees (98% identity) but shows much less identity to the mouse and rat homologues (62% and 63% identity respectively). Despite the limited identity of their amino acid sequences, the mouse and human homologue are remarkably similar in their domain architecture (**Figure 2**) and three-dimensional structure. This feature, i.e. marked structural identity in the absence of significant sequence identity is a common feature of the lipocalin-family of proteins and underlies the conserved function of the lipocalin domain. Studies carried out on mouse Ngal (also called mLcn2) have revealed that it can bind hydrophobic ligands like retinol, cholesterol oleate and oleic acid (Chu *et al.*, 1998). NMR (nuclear magnetic resonance) studies have revealed that the NGAL molecule contains eight antiparallel β strands that form a barrel shaped structure (Coles *et al.*, 1999). Three β bulges present in this barrel (one formed by the 1st and two by the 6th β strand) have been suggested to contribute to the ligand binding site of NGAL. Hydrophobic residues (Tryptophan, Valine and Phenylalanine) present at the base of this barrel-like structure have been shown to be involved in direct binding to the ligand. A patch of positively charged amino acid residues (Lysine and Arginine) present near the mouth of the barrel and projecting into the open end of the molecule has also been suggested to be also important in binding to ligands. Further, a negatively charged "pit" present at the base of the barrel formed by the amino acids Aspartate and Glutamate and a nearby unpaired Cysteine residue have been suggested to be crucial for binding of NGAL to the gelatinase MMP-9. Based on whether NGAL is free or bound to a ligand, it is termed as "apo" or "holo" NGAL respectively. The conformational change between these two forms of the protein is affected by a conformation change occurring at the open end of the NGAL protein.

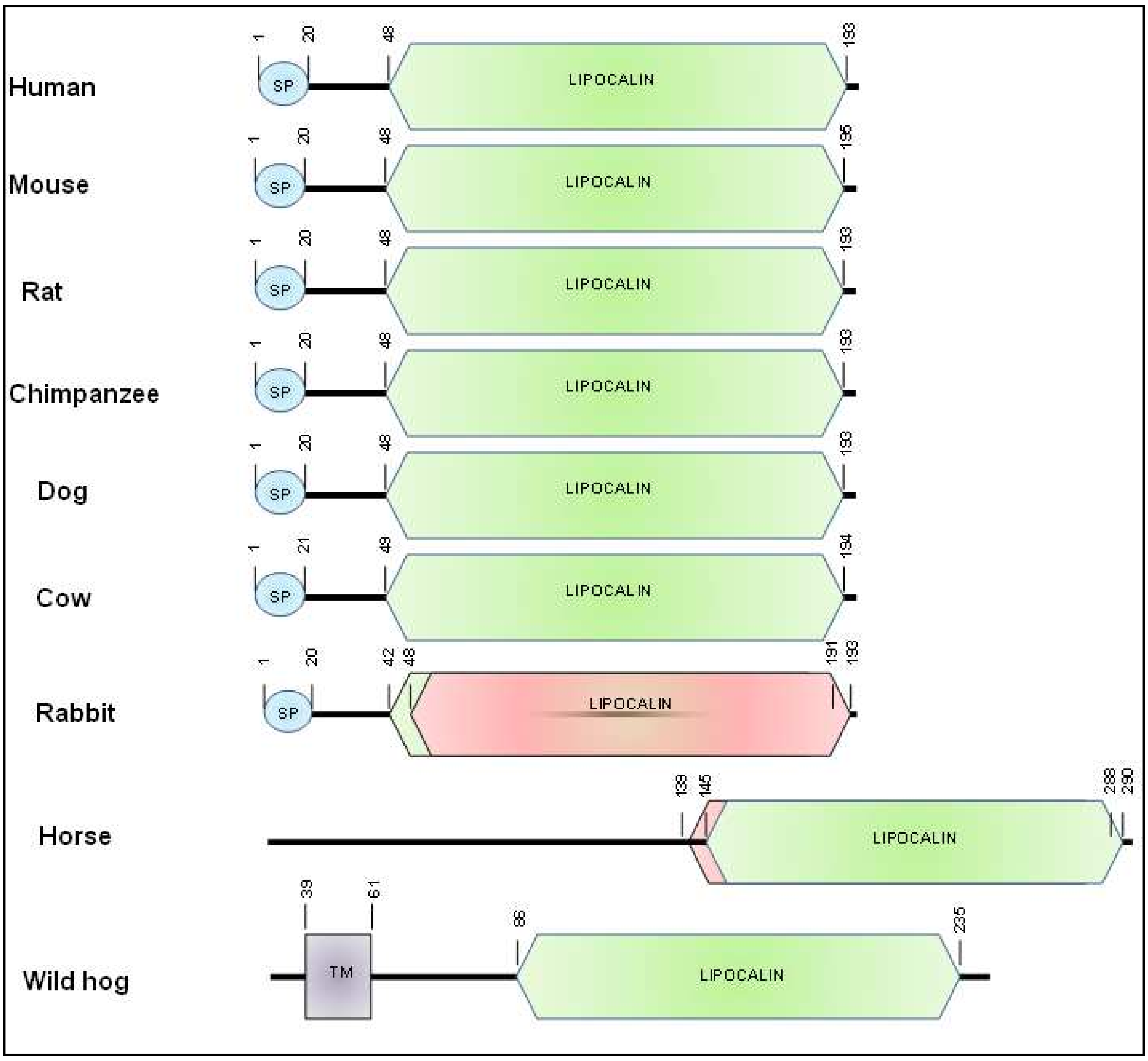


Fig. 2. Domain architecture of human NGAL and its homologues. Human NGAL and its homologues are characterized by a high degree of similarity in their domain architecture. All NGAL homologues (with the exception of the horse and wild hog) contain an N-terminal signal peptide which is cleaved prior to secretion of the protein. This is followed by the lipocalin domain (IPR000566 Lipocln_cytosolic_FA-bd) which makes up most of the length of the protein. This domain which has the consensus sequence [DENG] - {A} - [DENQGSTARK] - x(0,2) - [DENQARK] - [LIVFY] - {CP} - G - {C} - W - [FYWLRH] - {D} - [LIVMTA] is characteristic of proteins belonging to the lipocalin family. Structurally, the lipocalin domain is an eight stranded antiparallel beta barrel enclosing a ligand binding site which can bind to small hydrophobic molecules (e.g. steroids, retinoids, bilins and lipids). The rabbit and horse NGAL proteins also contain a second domain called the "lipocalin-2" domain (Accession number: IPR013208 Lipocalin_2). This domain is present in a small number of proteins (calycin, apolipoprotein D and Lipocalin 2) and is believed to be involved in the transport of hydrophobic ligands (Source: http://www.ebi.ac.uk/interpro/).

Recent data on the ligand binding pocket of NGAL has revealed that it is significantly larger and more polar than a similar pocket in other lipocalin proteins (Goetz *et al.*, 2000). This has led to the speculation that NGAL may bind to large, macromolecular and relatively less hydrophobic ligands including mammalian proteins. However, the identity of one or more of these mammalian ligands for NGAL remains to be elucidated. However, NGAL has been shown to bind to bacterial proteins. Specifically, it binds to iron-chelating proteins released by both gram negative bacteria (i.e. enterobacteria) and mycobacteria (e.g. *Mycobacterium tuberculosis*) (Nairz *et al.*, 2009; Holmes *et al.*, 2005). These proteins, termed as enterochelin and carboxymycobactin (CMB) respectively have been shown to bind to the ligand binding cavity of NGAL. The latter (i.e. CMBs) however, forms a better fit into the ligand binding pocket of NGAL. This has been in part attributed to the larger number of hydrophobic residues in CMBs which permits the formation of a larger number of hydrogen bonds and Van Der Walls interactions with the ligand binding cavity of NGAL.

3. Expression of NGAL in normal tissues

Although first discovered as a component of the late granules of neutrophils, NGAL is also strongly expressed in several normal adult human tissues including the non-neoplastic breast ducts, kidney, liver, lungs, trachea, small intestine, bone marrow, thymus, prostate, adipose tissue and macrophages. A weak to no expression of NGAL is observed in the normal pancreas, endometrial glands, and the thymus and peripheral blood leucocytes. NGAL is however completely absent from the normal brain, heart, skeletal muscle, spleen, testes, ovary and colon (Le, V *et al.*, 1997; Seth *et al.*, 2002; Moreno-Navarrete *et al.*, 2010; Cowland *et al.*, 2003; Furutani *et al.*, 1998; Cowland and Borregaard, 1997).

Limited studies done in human fetal tissues reveal that NGAL is expressed in trophoblast cells of the placenta, cartilage forming cells (chondrocytes) and in the epithelial cells present in the developing lung and small intestine. NGAL expression has also been reported in the epidermis of the fetal skin beginning around the 20th week of gestation. With increasing age of the fetus, the expression spreads to the lower layers of the skin and becomes progressively concentrated around the hair follicles (Mallbris *et al.*, 2002).

4. Altered expression of NGAL in benign and malignant diseases

4.1 Inflammatory diseases

The expression of NGAL is altered in several benign disease conditions including inflammatory and metabolic disorders. Inflammatory conditions associated with an increase in NGAL expression include pancreatitis, meningitis, myocarditis, psoriasis and periodontitis.

Acute pancreatitis (AP) is a reversible, acute inflammation of the pancreas that affects nearly 210,000 patients every year in the United States alone (Banks and Freeman, 2006). While nearly 85% of these patients have a mild form of the illness (termed as mild acute pancreatitis or MAP) and make an uneventful recovery, about 15%-20% of them develop features of severe acute pancreatitis (SAP) (Carroll *et al.*, 2007). SAP is associated with significant morbidity and mortality primarily owing to multi-organ failure (MOF, usually in the first week following onset of disease) and infected pancreatic necrosis (2 weeks or later after onset of disease). We have reported that compared to healthy individuals and those with MAP, plasma NGAL levels are significantly elevated (nearly 15-fold) within 48 hours (of onset of symptoms) in patients with SAP (Chakraborty *et al.*, 2010). The NGAL levels showed a tendency to decline when measured in blood samples collected at later time points (i.e. 72 hrs, 96 hrs and 120 hrs). To get a better idea of the time course of the rise in NGAL levels in the blood, we induced either mild or severe acute pancreatitis in mice and followed the change in plasma Ngal levels over time. We found that Ngal levels in mice rose early (within 6 hours following induction of AP) and remained elevated in those with SAP while trending down to levels in control mice (no pancreatitis) and in those with MAP.

Meningitis, particularly that caused by gram negative bacteria can cause a systemic inflammatory response syndrome (SIRS) associated with sepsis and significant mortality. A key mediator of this inflammatory response is lipopolysaccharide (LPS), a structural biomolecule present in the outer wall of gram negative bacteria. LPS is a potent stimulator of the immune response and a central player in septic shock in patients with gram negative bacterial infections. Ngal mRNA levels were found to be significantly induced in the choroid plexus (a structure in the ventricles of the brain that produces cerebrospinal fluid) within 12 hours following intraperitoneal injection of LPS while the levels of circulating Ngal (in the cerebrospinal fluid) was strongly induced within 6 hours after injection of LPS (Marques *et al.*, 2008).

A significant increase in the level of circulating Ngal was also noted in rats induced to develop features of autoimmune myocarditis by injection of purified porcine myosin (Ding *et al.*, 2010). However, compared to the acute inflammations described earlier, the elevation in Ngal in the rats was significantly delayed, being first evident 9 days following immunization with the foreign protein. A significant increase in NGAL mRNA levels has also been reported in the heart tissues from patients with myocarditis (Ding *et al.*, 2010).

Psoriasis, a chronic inflammatory disease of the skin is characterized by the formation of plaques covered by variable amount of scales. NGAL mRNA levels were shown to be significantly upregulated (nearly 10-fold) in the psoriatic lesions (Nomura *et al.*, 2003; Mallbris *et al.*, 2002). Further, NGAL levels were also upregulated in lesions that like psoriasis, exhibited a dysregulation in the process of keratinocyte differentiation (termed as parakeratosis). These diseases include pityriasis rubra pilaris, porokeratosis and chronic (not acute) eczema. Significantly, NGAL expression (from the affected areas of skin) disappeared once the psoriatic lesions had healed with treatment, suggesting that NGAL may play a role in the pathogenesis of diseases with dysregulated epidermal differentiation.

NGAL levels were significantly elevated in gum (i.e. alveolar mucosa) tissue from patients affected with localized juvenile periodontitis (LJP), a chronic inflammatory disease of the gums affecting young adults and associated with severe destruction of both soft tissues and bone (Van Dyke *et al.*, 1985). In addition to bacterial infections, NGAL, particularly circulating levels are significantly altered in certain viral infections. For instance, circulating NGAL levels were significantly lower in patients infected with the human immune deficiency virus (HIV) who had not received any therapy (i.e. treatment naïve) compared to non-HIV controls. Significantly, treatment of these patients with the highly active anti-retroviral therapy (HAART) was associated with a progressive increase in circulating NGAL levels (Landro *et al.*, 2008). In vitro studies using neutrophils isolated from the three groups of patient revealed that in healthy and HAART-treated HIV positive patients, the neutrophils released NGAL normally into the culture medium. However, in untreated HIV positive patients, this process was defective suggesting that the release of NGAL from neutrophils is inhibited by HIV infection and can be restored following therapy. What proteins of the virus mediate this inhibition, and if there is a prognostic significance of the restoration of NGAL levels following HAART therapy remain unanswered questions.

4.2 Metabolic diseases

Metabolic diseases associated with a dysregulation in NGAL expression include obesity, pre-eclampsia and kidney disease.

Obesity, defined as a body mass index (BMI) of $\geq 30 kg/m^2$ is now recognized to be associated with low grade chronic inflammation and insulin resistance (Pi-Sunyer, 2002). Studies have shown that NGAL levels are significantly elevated in the adipose tissues of both overweight mice and obese human subjects (Wang *et al.*, 2007). Studies in healthy human subjects have revealed that insulin can significantly increase the level of circulating NGAL in the blood (Tan *et al.*, 2009). Similar results have emerged from studies in pregnant women with gestational diabetes who had significantly higher levels of circulating NGAL than those without this complication of pregnancy (D'Anna *et al.*, 2009a).

Another pregnancy associated complication associated with a rise in circulating NGAL levels is pre-eclampsia. This condition, characterized by the development of hypertension in or after the 20th week of pregnancy is associated with significant maternal and fetal complications. The elevated levels of NGAL in pre-eclampsia were positively correlated with both systolic and diastolic blood pressure and presence of proteinuria but showed a significant negative correlation with maternal age, pre-pregnancy BMI and weight (D'Anna *et al.*, 2008; D'Anna *et al.*, 2009b). Other conditions where NGAL levels are significantly elevated include atherosclerosis (Anwaar *et al.*, 1998; Elneihoum *et al.*, 1997; te Boekhorst *et al.*, 2011) and hepatitis (Bu *et al.*, 2006). Both hyperlipidemia and chronic alcohol ingestion are associated with significant metabolic perturbations and the aforementioned correlative studies suggest that NGAL has a role to play in the pathogenesis of these diseases. With the development of animal models to study the function of NGAL (discussed later), it is expected that the mechanisms by which NGAL modulates metabolic pathways in health and disease will become better delineated in the near future.

The kidney is essential for our survival as it performs the crucial function of maintaining the fluid and mineral balance in our body. This in turn maintains the osmolarity of inter and intracellular fluids crucial for cellular metabolism. Both acute and chronic damage to the kidneys causes metabolic perturbations associated with a significant increase in the expression of NGAL.

Chronic kidney disease (CKD) is a disease characterized by a progressive decline in renal function and associated with significant morbidity. Studies in a mouse model that mimics the pathophysiology of CKD in humans revealed that NGAL is the most highly upregulated gene in the kidneys of mice with severe CKD (Viau *et al.*, 2010). It appears that the damaged kidneys synthesize a significantly higher amount of NGAL than usual, particularly in the proximal convoluted tubules (PCT) and the ascending limb of the loop of Henle. NGAL being a secreted protein is then released into the ultrafiltrate and subsequently excreted in urine. The level of Ngal expression in the diseased kidneys correlated positively with the severity of renal lesions (in mice) suggesting that Ngal may promote renal damage in CKD (Viau *et al.*, 2010). A similar observation was also noted in an animal model of autosomal polycystic kidney disease (ADPKD), another cause of CKD in humans (Lau *et al.*, 2000).

NGAL levels are elevated in acute kidney injury (AKI) resulting from a wide variety of insults to the kidney ranging from ischemia to toxins. This rise occurs early and depends on both the cause and the extent of renal damage. For instance, following ischemic injury, NGAL levels in the kidney tissue rises by nearly 10-fold within only 3 hours (Mishra *et al.*, 2003). Further, the rise appears to be sustained, being evident for several days following the initial insult. This pattern, an early rise and persistence, makes NGAL a highly sensitive marker of early AKI. Like in CKD, the kidney is the major source of NGAL release in AKI. However, other sources of NGAL that have been suggested include organs that synthesize and release mediators of an acute inflammatory response (e.g. the liver) and immune cells that contain NGAL in their granules (neutrophils and macrophages) (Hvidberg *et al.*, 2005).

4.3 Malignant diseases

The expression of NGAL is significantly increased in several solid and hematological tumors and been shown to correlate with both tumor characteristics and disease outcome. Solid tumors that express high levels of NGAL arise in a variety of organs including the digestive (e.g. esophagus, stomach, liver, bile ducts and pancreas), respiratory (lungs), endocrine (thyroid gland and ovaries), reproductive (breast and endometrium) organs and even in the skin.

Pancreatic cancer (PC) is the most lethal of all malignant solid tumors in humans (Chakraborty *et al.*, 2011). Several groups including ours have now shown that while NGAL is either completely absent or weakly expressed in the normal pancreatic ducts, a strong expression of NGAL is seen in the malignant cells (Moniaux *et al.*, 2008; Furutani *et al.*, 1998; Tong *et al.*, 2008; Tong *et al.*, 2011). We also observed that the expression of NGAL first appears in pre-malignant lesions (termed as Pancreatic intraepithelial neoplasia or PanINs), with a progressive increase from low to high grade dysplasia. In invasive pancreatic cancer, NGAL expression was significantly higher in well-differentiated PC while poorly differentiated cancer cells showed no expression of the protein (Moniaux *et al.*, 2008). Functional studies to investigate the role of NGAL in PC (discussed later) suggest that it plays a role in the progression of this deadly malignancy. Further, quantitative measurement of NGAL levels in plasma revealed that there was a significant increase in the levels of NGAL in the plasma compared to healthy individuals. These results suggest that NGAL might be potentially useful as a novel biomarker for the diagnosis of PC. Similar to PC, NGAL was weakly expressed by the normal liver cells but strongly expressed in hepatocellular carcinoma (Lee *et al.*, 2011). In gastric cancer however, NGAL expression remains controversial with two groups reporting opposing results- one reporting overexpression and the other a significant downregulation of NGAL in pre-malignant and malignant gastric tissues (Alpizar *et al.*, 2009; Wang *et al.*, 2010).

Ngal, the mouse homologue of human NGAL was strongly upregulated in mice that overexpress Insulin like growth factor (IGF-2), a major growth promoting hormone that acts during *in utero* development. These mice developed lung tumors spontaneously which showed a significant upregulation of Ngal mRNA.

Among the endocrine tumors, papillary, follicular and anaplastic thyroid cancers all express NGAL (Iannetti *et al.*, 2008). Notably, the expression of NGAL increases with the loss of tumor differentiation. While NGAL is not detected in the normal ovarian follicles and weakly expressed in benign ovarian tumors, a strong expression of NGAL is noted in borderline and grade 1 (well differentiated) malignant ovarian tumors (Lim *et al.*, 2007). Interestingly, its expression decreased in grade 2 (moderately differentiated) and 3 (poorly differentiated) ovarian cancer (Cho and Kim, 2009).

Of the malignancies arising from reproductive organs, ductal carcinoma of the breast and endometrial cancer express high levels of NGAL (Miyamoto *et al.*, 2011; Bauer *et al.*, 2008).

NGAL appears to promote the progression of breast cancer, being higher in actively proliferating cells, in lymph node metastasis and cells positive for the human epidermal growth factor receptor-2 (HER-2/ErbB2). Studies in cell lines suggest that NGAL expression in breast cancer may be regulated by HER-2 (Stoesz and Gould, 1995). In fact, another name for the Ngal protein in rats is Neu-related lipocalin (Neu being the other name for HER-2).

Microarray analysis using microdissected normal and malignant endometrial glands revealed that NGAL was strongly upregulated in the malignant endometrial glands . Immunohistochemical analysis of formalin fixed and paraffin embedded tissues also revealed that while the normal endometrium showed a weak expression of NGAL, a strong expression was noted in the areas of endometrial carcinoma (Miyamoto *et al.*, 2011).

NGAL is also secreted by the malignant cells in hematological malignancies. Studies done on the murine homolog of NGAL (24p3/Ngal) suggest that in leukemias, particularly those where the cells expresses BCR-ABL, *24p3/Ngal* is strongly expressed by the malignant cells (Devireddy *et al.*, 2005; Arlinghaus and Leng, 2008). BCR-ABL is a fusion protein that is found in association with certain leukemias, chiefly chronic myelogenous leukemia (CML) and to a lesser extent in acute lymphoblastic leukemia (ALL) and rarely in acute myelogenous leukemia (AML). This fusion results from a reciprocal translocation event occurring between the long arms of chromosomes 9 and 22. The resultant protein encodes for a protein kinase, specifically a tyrosine kinase that is constitutively active (i.e. does not require any external activation signal). As shown in **Figure 3**, normal hematopoietic cells (but not the BCR-ABL positive leukemia cells) in mice express the receptor for Ngal (called NgalR or 24p3R). The leukemia cells release large quantities of 24p3/Ngal into the bloodstream which acts via its receptor to induce cell death in the normal hematopoietic cells. Since the leukemic cells do not express the receptor, they remain unaffected. This

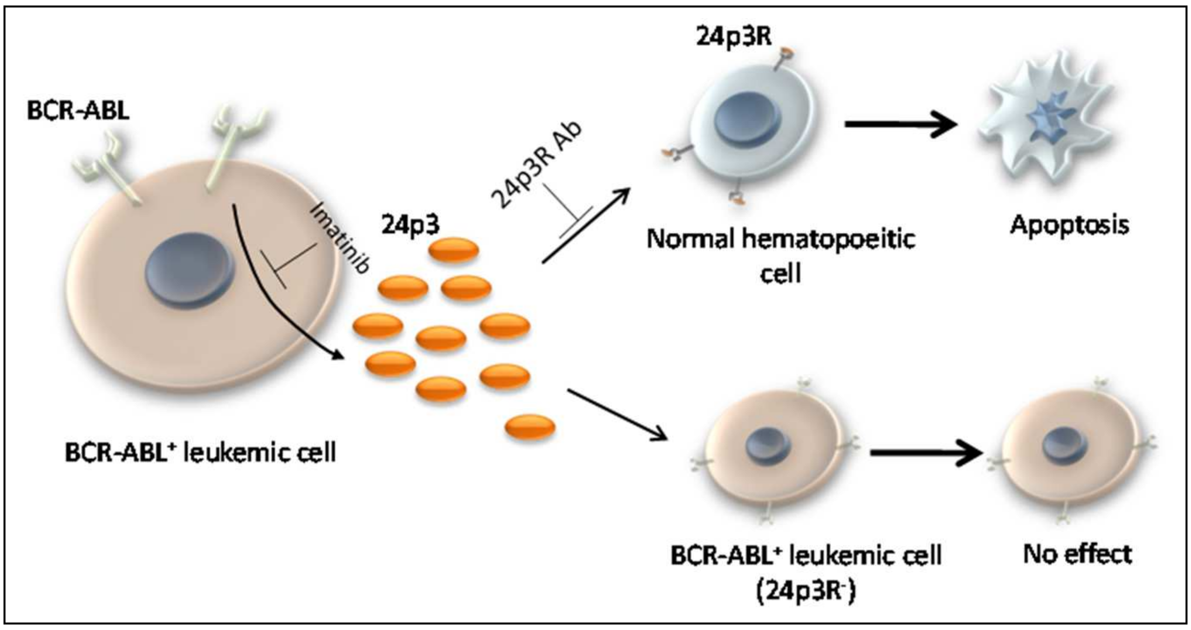


Fig. 3. Role of NGAL in hematopoietic malignancies: 24p3, the mouse homologue of human NGAL is secreted from BCR-ABL expressing mouse leukemic blast cells, a process that can be blocked using Imatinib, a specific inhibitor of the BCR-ABL tyrosine kinase activity. The 24p3 in the conditioned medium induces apoptosis in normal hematopoietic cells (express the 24p3 receptor or 24p3R) but not in the BCR-ABL+ leukemic cells that are devoid of 24p3R.

mechanism has been suggested to be responsible for the spread of leukemia cells through the healthy bone marrow (Hu *et al.*, 2009; Leng *et al.*, 2008; Lin *et al.*, 2005). Further, studies in mice also suggest that while 24p3/Ngal is required for the establishment of BCR-ABL$^+$ leukemia cells in the spleen and bone marrow, it is the *BCR-ABL* but not *Ngal* was the gene ultimately driving the progression of the disease. Studies in CML patients have confirmed the findings in mice. Both NGAL mRNA and plasma levels are significantly elevated in patients with CML (Leng *et al.*, 2008; Villalva *et al.*, 2008). Further, patients who responded to treatment with Imatinib (a specific inhibitor of BCR-ABL tyrosine kinase) showed a significant decrease in NGAL mRNA (but not in plasma NGAL levels). (Owen *et al.*, 2008)

5. Functions of NGAL

An important function of NGAL under physiological conditions is to act as bacteriostatic agent, thus protecting the body against both gram negative bacteria and mycobacterial infection. It mediates this function by binding with strong affinity to bacterial iron binding proteins called siderophores. Iron, particularly ferrous form is required by bacteria for their growth. However, the extremely low levels of free iron in the body make it an essential nutrient required by bacterial cells. To circumvent this requirement for iron, bacteria have evolved a mechanism involving the expression of proteins (termed siderophores) that bind to free iron (present in the intestinal fluid and inside macrophages) particularly in its ferric form. NGAL has a strong affinity to bind to siderophores, both when free and when laden with iron. Upon binding to these proteins, the NGAL siderophore complex is taken up by cells expressing the cognate receptor for NGAL and thus these iron laden proteins are sequestered away. In this way, by depriving bacteria of an essential nutrient, NGAL inhibits their proliferation (Nairz *et al.*, 2009; Holmes *et al.*, 2005).

The recent discovery that catechols, a family of plant derived metabolites present in our diet, can bind NGAL has opened new possibilities for the functional role of human NGAL in health and disease. Interestingly, while catechols itself bound weakly to NGAL, their binding was significantly enhanced in the presence of iron, specifically ferric iron. More detailed studies have revealed that three catechol molecules form a complex (a triscatecholate complex) with iron acting as a stabilizing agent through formation of pi interactions and electrostatic interactions with the catechol molecules. This ferric-triscatecholate complex then binds to the ligand binding cavity (calyx) of NGAL. In mice, it has been observed that this complex of Ngal and iron-catechol is filtered through the kidneys before being re-absorbed in the proximal convoluted tubules through specific Ngal receptors (Bao *et al.*, 2010; Backhed *et al.*, 2005). However, the function of these complexes alone and in combination with Ngal still remains to be elucidated.

Other functions attributed to Ngal include acting as a chemoattractant for neutrophils and as an inhibitor of cellular oxidative stress. The former function is based on observations that mice in which the Ngal gene is knocked out show a significant decrease in the number of neutrophils infiltrating transplanted heart tissue (compared to wild type mice with both copies of the gene intact). This suggests that neutrophils, the primary effectors of acute inflammatory response in the body, require NGAL to home in to the target organs (in this case the transplanted heart). The latter role is suggested from observations made in both Chinese hamster ovary (CHO) and human embryonic kidney (HEK) cells that an aberrant expression of 24p3/Ngal leads to a significant upregulation in the expression of the antioxidant enzymes superoxide dismutase (SOD) and heme oxygenase (HO) (Bahmani *et al.*, 2010; Roudkenar *et al.*, 2007; Roudkenar *et al.*, 2008c; Roudkenar *et al.*, 2008a; Roudkenar *et al.*, 2008b).

NGAL also appears to play a role in wound healing. In a study of patients with chronic venous ulcers (CVUs), an analysis of the exudate from the wounds of those with either healing (H) or non-healing (NH) CVUs revealed that the former (H-CVU) group had a significant decline the level of exudate-NGAL levels compared to the latter (NH-CVU) group of patients (Pukstad *et al.*, 2010).

NGAL also appears to modulate proliferation and synthesis of cartilage by murine chondrocytes and promotes proliferation of renal tubular epithelial cells (Owen *et al.*, 2008). Apart from healthy cells, NGAL also modulates proliferation and survival of certain types of malignant cells (thyroid cancer and hepatocellular carcinoma). In the former type of cancer cells, NGAL appears to promote proliferation and survival, while it had the opposite effect in hepatocellular carcinoma cells (Iannetti *et al.*, 2008; Lee et al., 2011). In other types of cancer cells (e.g. pancreatic, breast and colon cancer) however, NGAL does not seem to have any effect on proliferation. Instead, it significantly inhibits the ability of these cancer cells to invade and metastasize (Tong *et al.*, 2008; Lee *et al.*, 2006). Two possible mechanisms have been suggested to explain this anti-invasive effect of NGAL. One mechanism involves inhibition of the non-receptor tyrosine kinase FAK (focal adhesion kinase). FAK interacts with Src (another non-receptor tyrosine kinase) and provides a scaffold for activation of the Ras-MAPK (Mitogen activated protein kinase pathway), a key mediator of metastasis in cancer cells. NGAL, by inhibiting FAK would in turn inhibit the Ras-MAPK pathway and thus block both invasion and metastasis of cancer cells. A second mechanism, based on studies by our group, suggests that E-cadherin when ectopically expressed can increase expression of NGAL (Tong *et al.*, 2011). E-cadherin is a calcium dependent cell adhesion molecule that is expressed in and promotes epithelial cells to adhere to one another (thus preventing metastasis). Further, studies by us in human pancreatic cancer cells also suggest that NGAL inhibits angiogenesis (formation of blood vessels) (Tong *et al.*, 2008). A clue to the underlying mechanism comes from observations that conditioned media from pancreatic cells in which endogenous NGAL had been silenced (using short hairpin RNAs) significantly decrease formation of capillary like structures by human vascular endothelial (HUVEC) cells *in vitro*. This is associated with a significant decrease in the release of vascular endothelial growth factor (VEGF), a key promoter of angiogenesis from the NGAL silenced cells.

Knockout of both copies of the murine Ngal homologue (i.e. Ngal$^{-/-}$ mice) did not result in any phenotypic abnormalities in the knockout mice compared to their wild type littermates. However, the Ngal knockout mice were significantly more sensitive to infection with gram negative bacteria, exhibiting a nearly 1000-fold greater bacteremia (upon infection with *Escherichia coli,* a gram negative enteric pathogen) compared to their wild type littermates. Further investigation revealed that there was no difference in the absolute number of different leukocyte subpopulations between the wild type (Ngal$^{+/+}$) and knockout mice (Ngal$^{-/-}$) (Flo *et al.*, 2004). As discussed earlier, Ngal is crucial to prevent growth of bacteria, particularly within the macrophages and also for homing of neutrophils to sites of acute inflammation (including bacterial infection). Both these mechanisms would be severely affected in the *Ngal* knockout mice, thus contributing to the enhanced bacteremia and resulting mortality. These observations suggest that lack of Ngal produces a qualitative rather than a quantitative defect in the immune system that in turn leads to an increased susceptibility to gram negative sepsis.

6. Regulation of NGAL expression

Given the observation that NGAL is differentially expressed in a wide array of benign and malignant diseases, it is extremely important to investigate the mechanisms that regulate its expression. Most studies have focused on the regulation of transcription of its mRNA. Several cytokines, hormones, vitamins, minerals, synthetic drugs and growth factors have been shown to influence the expression of NGAL (**Table 2**). Each of these probably acts in a context dependent manner to influence the expression of this glycoprotein.

NF-κB , a transcription factor and regulator of several key pathways in the cell has emerged as a front runner in the regulation of NGAL gene expression. The NF-κB family comprises five transcription factors (p50, p52, p65 (or RelA) and c-Rel and RelB). These proteins are related to one another by the presence of the Rel homology domain (RHD), present in the N-terminal region of each member of this family. The C-terminal region contains either a transcription activation domain (TAD, in case of RelA, c-Rel and RelB) or a transcription repression domain (TRD in case of p50 and p52). These proteins form homo or heterodimers with each other through the RHD domain and then bind to short (9-10 bases long) sequence in the promoter or enhancer regions of target genes (termed as kB response elements). RelA, c-Rel and RelB activate gene expression (owing to presence of the TAD) while p50 and p52 inhibit gene expression (through the TRD) unless they associate with another member that has a TAD in its C-terminus. NGAL too has κB elements in its promoter region and has been shown to be positively regulated by agents that induce NF-κB (like insulin, IL-1β and TLRs). Another pathway that has been shown to promote NGAL expression is the JNK (c-jun N-terminal kinase) MAPK pathway. Cytokines like IL-1 and IL-17 can both activate the JNK pathway. A cross talk between the JNK and NF-κB has been suggested to act together to upregulate NGAL expression (Florin *et al.*, 2004; Yang *et al.*, 2008; Park *et al.*, 2005). Dexamethasone, a synthetic corticosteroid induces NGAL mRNA expression acting through the glucocorticoid receptor (GRs) and glucocorticoid response elements (GREs) present in the promoter region of NGAL (Garay-Rojas *et al.*, 1996).

The epidermal growth factor receptor (EGFR) when activated initiates signaling events that promote cell proliferation, survival and enhance migration and invasiveness of a variety of cancer cells. We have reported that EGF treatment decreases the expression of NGAL mRNA. This was associated with a significant downregulation of the epithelial marker E-cadherin. Mechanistically, EGF mediated downregulation of NGAL expression occurs by inhibition of the NF-κB pathway (Tong *et al.*, 2011). However, the role of EGF in regulating NGAL appears to be dependent on the cell type. For instance, treatment of mouse renal tubular epithelial cells with EGF led to a significant upregulation of *Ngal* expression. The mechanism of upregulation of *Ngal* by EGF (in mice renal cells) appears not to be through the NF-κB pathway but rather through stabilization of the transcription factor HIF-1α (hypoxia inducible factor-1 α).

Epigenetic mechanisms have been shown to be central to the process of *de novo* induction or repression of genes. Treatment of endometrial cancer cells that express a low level of NGAL with a demethylating agent 5-aza cytidine led to a significant upregulation of NGAL mRNA. However, the same agent had no effect on cells that had a high basal level of NGAL (Miyamoto *et al.*, 2011). This suggests that methylation may be a mechanism to differentially turn on or off the expression of NGAL in disease states including cancer.

Factors	Comments	Effect on NGAL expression
Cytokines		
GM-CSF	Granulocyte Monocyte colony stimulating factor	↑
IL-1α	Interleukin 1 alpha	↑
IL-1β	Interleukin 1 beta	↑
IL-6	Interleukin 6	↔
IL-17	Interleukin 17	↑
IL-22	Interleukin 22	↑
TGF-α	Transforming growth factor alpha	↑
TNF-α	Tumor necrosis factor alpha	↔/↓ [a]
bFGF	Basic fibroblast growth factor	↔
Growth factors		
IGF-1	Insulin like growth factor-1	↑
EGF	Epidermal growth factor	↓/↑ [a]
Synthetic drugs		↑
Dexamethasone	Synthetic corticosteroid	↑
Deferoxamine	Iron-chelator	↑
Diethylstilbestrol	Synthetic nonsteroidal estrogen	↑
5-aza cytidine	Demethylating agent	↑
Hormones		
Insulin		↑
Estrogen		↓/↑ [a]
Progesterone		↔
Bacterial components		
LPS	Lipopolysaccharide (TLR-4 ligand)	↑
Lipotechoic acid	Gram +ve bacterial cell wall component	↑
Peptidoglycan	Gram +ve bacterial cell wall component	↑
Others		
HIV-tat	HIV virus tat protein	↑
Pam3CSK4	TLR-1/2 ligand	↑
Flagellin	TLR-5 ligand	↑
pIC	Polyinosinic polycytidylic acid (TLR-3 ligand)	↑
CpG	Unmethylated CpG oligonucleotides (TLR-9 ligand)	↑
Calcium	Promotes cell differentiation	↑

Footnote 1 ↑ Indicates upregulation, ↓ indicates downregulation and ↔ indicates no effect on NGAL expression. [a] The same factor can have opposite effects on NGAL expression in a context dependent manner. HIV (Human Immune deficiency virus), TLR (Toll like receptor).

Table 2. Summary of factors regulating NGAL expression

These reports taken together suggest that multiple stimuli, acting through a relatively small number of pathways regulate the expression of NGAL. However, many of these studies have been done *in vitro* and need to be examined *in vivo* particularly using knockout mouse models. Nonetheless, they shed important light on the complex regulation of this protein and will be important in future attempts to modulate its expression for therapeutic purposes.

7. Role of NGAL as a diagnostic and prognostic marker

The differential expression of NGAL in disease states has been utilized as a rationale to investigate the potential of NGAL as a biomarker in the diagnosis of these diseases. Among benign diseases, acute kidney injury (AKI) represents the disease where this secreted protein has been most extensively studied for its diagnostic performance. In one study, urine NGAL levels rose significantly within as early as 6 hours following elective cardiac surgery in patients admitted to the intensive care units (ICU) who met the criteria for AKI, suggesting that it is an early marker of renal injury (Koyner *et al.*, 2010). Further, corrected urine NGAL levels (corrected for serum creatinine) when measured at the time of the patient's arrival to the ICU was a good predictor of severe AKI post-cardiac surgery (Area under the curve or AUC being 0.88, 95% CI: 0.73-0.99). Another study found that among post-cardiac surgery patients, plasma NGAL levels rose upto 24 hours prior to patients meeting the current clinical criteria for AKI (i.e. the RIFLE or *R*isk, *I*njury, *F*ailure, *L*oss and *E*nd stage renal disease criteria) (Cruz *et al.*, 2010). Further, plasma NGAL was a good predictor of the need for renal replacement therapy (RRT) during the stay of the patients in the ICU (AUC 0.82, 95% CI 0.70-0.95). NGAL is also an early predictor of DGD (delayed graft dysfunction), a type of renal dysfunction in kidney transplant recipients that often develops within a week after transplant and is often severe enough to require dialysis (Korbely *et al.*, 2011; Shapiro *et al.*, 2010).

Pre-renal azotemia refers to reversible renal injury which is completely reversible within 24-72 hours if the cause is removed. Distinguishing pre-renal azotemia from AKI is one of the major challenges for nephrologists and critical care physicians today. A comparison of urine NGAL levels between patients with pre-renal azotemia and AKI revealed that urine NGAL levels were significantly higher in patients with AKI (mean±SEM level being 416+387 µg/g of creatinine) compared to those with pre-renal azotemia (mean±SEM being 30+92 µg/g creatinine) (Nickolas *et al.*, 2008). The results were validated in another independent cohort of 107 patients, 32 of whom had pre-renal azotemia (Singer *et al.*, 2011).

Diagnosis of AKI in children presents a special challenge. Owing to the incomplete development of their nephrons, they have higher baseline levels of creatinine than adults. Hence, serum creatinine may not be an appropriate marker to detect AKI in these patients. A comparison of NGAL and creatinine in pediatric patients (both neonates and older patients) revealed that urine NGAL levels were elevated within 2 hours following cardiopulmonary bypass (CPB) surgery and remained elevated for even upto 48 hours. Significantly, urine NGAL >185 ng/ml or a plasma NGAL >95 ng/ml was 100% sensitive (88% for plasma) and 93% specific (both urine and plasma) in identifying AKI in neonatal patients. The corresponding cut-off for older children was >45ng/ml (urine) or >48ng/ml (plasma). The corresponding sensitivity and specificity were 85% (90% for plasma) and 86% (88% for plasma) respectively (Krawczeski *et al.*, 2011).

Elevation in NGAL, particularly in combination with an elevation in the levels of IL-1 receptor and protein C was the best predictor of the risk of developing severe sepsis among a panel of nine potential markers in a cohort of 506 patients admitted with systemic inflammatory response syndrome (SIRS) (AUC 0.75, 95% CI 0.72-0.78 vs. those who did not develop severe sepsis). This panel of three markers was also fairly accurate in predicting both septic shock (AUC 0.77) and death (AUC 0.79) within 24 hours of hospital admission in critically ill patients (Endre *et al.*, 2011).

In addition to its role as a diagnostic marker, NGAL has also emerged as an important prognostic indicator in several diseases. Studies in obese type-2 diabetics revealed that serum NGAL levels were negatively correlated with the levels of total cholesterol, an important determinant of coronary artery disease (CAD). Further, the level of serum NGAL was an independent predictor of insulin resistance and hyperglycemia in these patients. In patients with acute decompensated heart failure (ADHF), serum NGAL levels (at admission) were significantly higher in those patients who had worsening of renal function (WRF) compared to those whose renal function was intact. At a cut-off of ≥140 ng/ml, serum NGAL was 86% sensitive and 54% specific in predicting the development of WRF in these patients (Chertow *et al.*, 1998).

NGAL has been widely investigated as a prognostic indicator in AKI from various causes. In patients undergoing cardiovascular surgery, AKI is always a potential complication predominantly due to ischemia during the procedure. Urine NGAL levels have been shown to be fairly accurate (AUC 0.59-0.65) in early identification (within 3 hours) of AKI following CPB surgery (Shapiro *et al.*, 2009). We have demonstrated that an elevated plasma NGAL level among patients with SAP is associated with a significantly poor outcome (i.e. increased risk of death). This effect appears to be specific to SAP as no such correlation was observed in MAP patients. Further, in a small study, NGAL appeared to perform equal or better than other established clinical prognostic indicators in SAP (i.e. serum creatinine, Ranson's score and the APACHE-II score) (Chakraborty *et al.*, 2010).

As techniques continue to evolve, it is expected that assays for NGAL will become increasingly sensitive. One study comparing ELISA (enzyme linked immunosorbent assay) with RIA (radioimmunoassay) found that the latter was between 5-10 fold more sensitive than ELISA in detecting a rise in NGAL among patients undergoing cardiac surgery, with the sensitivity being the highest in the first 2 hours (Aghel *et al.*, 2010).

NGAL has also emerged as a potential biomarker in several epithelial malignancies. In gastric cancer patients, serum NGAL could distinguish patients with cancer from non-cancer patients with an AUC of 0.93 (Wang et al., 2010). NGAL was better than either CA19-9 or CEA (carcinoembryonic antigen) in identifying early gastric cancer (i.e. Stage 1 and 2) patients suggesting that it could be a potential biomarker for early stage gastric cancer. NGAL was also fairly accurate in discriminating between benign and malignant biliary obstruction (AUC 0.76 with a sensitivity and specificity of 96% and 56%) (Klapper *et al.*, 2008). In ovarian cancer however, NGAL was 72% sensitive but only 50% specific in distinguishing ovarian cancer from non-cancer patients. In comparison, CA125 was 80% sensitive and 79% specific in distinguishing between the two groups (Argani *et al.*, 2001).

NGAL also appears to correlate with prognosis of cancer patients. For instance, patients whose tumors expressed NGAL had a significantly shorter survival (both disease specific and disease free) compared to those with NGAL non-expressing tumors (12.2 years in NGAL expressing vs. 17.1 years in NGAL non-expressing tumors). Multivariate analysis revealed that NGAL was an independent predictor of disease free survival (hazard ratio 1.85). In gastric cancer too, patients whose tumors expressed NGAL had a significantly shorter survival compared to tumors whose tumors did not express any NGAL (35.6 .months vs. 54.4 months respectively) (Alpizar *et al.*, 2009).

8. Conclusions

The preceding discussion suggests that NGAL, despite its low molecular weight is an important protein particularly from the standpoint of protecting against bacterial infections. Further, it appears that NGAL has other novel functions in disease states, particularly in epithelial malignancies where it can modulate cell proliferation, cell death, invasion and metastasis of cancer cells. In other diseases, particularly in renal injury, the role of NGAL remains to be elucidated.

NGAL expression appears to be regulated by a variety of stimuli through mainly the NF-κB pathway although epigenetic modifications may be important, particularly in initiating its *de novo* expression during diseases like cancer. NGAL has also emerged as a biomarker with immense clinical potential, both in benign and malignant diseases. Further, while limited studies exist, it also appears to be capable of predicting the outcome of patients with multiple diseases.

All these features make NGAL an important diagnostic target. An interesting observation is that the Ngal knockout mice do not exhibit any obvious defects. However, they are extremely sensitive to infection by gram negative bacteria. These results clearly suggest that NGAL may be an important protective agent in gram negative bacterial infections. Further studies using these mice will be crucial to delineate its role in determining sensitivity to other inflammatory and malignant diseases. Taken together, NGAL has emerged as a clinically important member of the lipocalin family and holds immense potential for diagnostic and therapeutic applications in the future.

9. Acknowledgements

Surinder K Batra, Subhankar Chakraborty and Sukhwinder Kaur are supported in part by grants from the US Department of Defense (BC074631, BC083295 and PC074289) and the NIH (RO1 CA78590, UO1 CA111294, RO1 CA131944, RO1 CA133774, RO1 CA 138791 and P50 CA127297). Sushovan Guha is supported by grants from MDACC McNair Foundation Scholar Award and the Cyrus Scholar Award. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. No writing assistance was utilized in the production of this manuscript.

10. References

Aghel A, Shrestha K, Mullens W, Borowski A, Tang WH (2010) Serum neutrophil gelatinase-associated lipocalin (NGAL) in predicting worsening renal function in acute decompensated heart failure. *J Card Fail* 16, 49-54

Alpizar YA, Ramirez BS, Fernandez DR, Capote AR, Hidalgo GG, Rodriguez RP, Molina LE (2009) HER1-ECD vaccination dispenses with emulsification to elicit HER1-specific anti-proliferative effects. *Hum Vaccin* 5, 158-165

Anwaar I, Gottsater A, Hedblad B, Palmqvist B, Mattiasson I, Lindgarde F (1998) Endothelial derived vasoactive factors and leukocyte derived inflammatory mediators in subjects with asymptomatic atherosclerosis. *Angiology* 49, 957-966

Argani P, Rosty C, Reiter RE, Wilentz RE, Murugesan SR, Leach SD, Ryu B, Skinner HG, Goggins M, Jaffee EM, Yeo CJ, Cameron JL, Kern SE, Hruban RH (2001) Discovery of new markers of cancer through serial analysis of gene expression: prostate stem cell antigen is overexpressed in pancreatic adenocarcinoma. *Cancer Res* 61, 4320-4324

Arlinghaus R, Leng X (2008) Requirement of lipocalin 2 for chronic myeloid leukemia. *Leuk Lymphoma* 49, 600-603

Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI (2005) Host-bacterial mutualism in the human intestine. *Science* 307, 1915-1920

Bahmani P, Halabian R, Rouhbakhsh M, Roushandeh AM, Masroori N, Ebrahimi M, Samadikuchaksaraei A, Shokrgozar MA, Roudkenar MH (2010) Neutrophil gelatinase-associated lipocalin induces the expression of heme oxygenase-1 and superoxide dismutase 1, 2. *Cell Stress Chaperones* 15, 395-403

Banks PA, Freeman ML (2006) Practice guidelines in acute pancreatitis. *Am J Gastroenterol* 101, 2379-2400

Bao G, Clifton M, Hoette TM, Mori K, Deng SX, Qiu A, Viltard M, Williams D, Paragas N, Leete T, Kulkarni R, Li X, Lee B, Kalandadze A, Ratner AJ, Pizarro JC, Schmidt-Ott KM, Landry DW, Raymond KN, Strong RK, Barasch J (2010) Iron traffics in circulation bound to a siderocalin (Ngal)-catechol complex. *Nat Chem Biol* 6, 602-609

Bauer M, Eickhoff JC, Gould MN, Mundhenke C, Maass N, Friedl A (2008) Neutrophil gelatinase-associated lipocalin (NGAL) is a predictor of poor prognosis in human primary breast cancer. *Breast Cancer Res Treat* 108, 389-397

Bu DX, Hemdahl AL, Gabrielsen A, Fuxe J, Zhu C, Eriksson P, Yan ZQ (2006) Induction of neutrophil gelatinase-associated lipocalin in vascular injury via activation of nuclear factor-kappaB. *Am J Pathol* 169, 2245-2253

Carroll JK, Herrick B, Gipson T, Lee SP (2007) Acute pancreatitis: diagnosis, prognosis, and treatment. *Am Fam Physician* 75, 1513-1520

Chakraborty S, Baine MJ, Sasson AR, Batra SK (2011) Current status of molecular markers for early detection of sporadic pancreatic cancer. *Biochim Biophys Acta* 1815, 44-64

Chakraborty S, Kaur S, Muddana V, Sharma N, Wittel UA, Papachristou GI, Whitcomb D, Brand RE, Batra SK (2010b) Elevated serum neutrophil gelatinase-associated lipocalin is an early predictor of severity and outcome in acute pancreatitis. *Am J Gastroenterol* 105, 2050-2059

Chertow GM, Levy EM, Hammermeister KE, Grover F, Daley J (1998) Independent association between acute renal failure and mortality following cardiac surgery. *Am J Med* 104, 343-348

Cho H, Kim JH (2009) Lipocalin2 expressions correlate significantly with tumor differentiation in epithelial ovarian cancer. *J Histochem Cytochem* 57, 513-521

Chu ST, Lin HJ, Huang HL, Chen YH (1998) The hydrophobic pocket of 24p3 protein from mouse uterine luminal fluid: fatty acid and retinol binding activity and predicted structural similarity to lipocalins. *J Pept Res* 52, 390-397

Coles M, Diercks T, Muehlenweg B, Bartsch S, Zolzer V, Tschesche H, Kessler H (1999) The solution structure and dynamics of human neutrophil gelatinase-associated lipocalin. *J Mol Biol* 289, 139-157

Cowland JB, Borregaard N (1997) Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans. *Genomics* 45, 17-23

Cowland JB, Sorensen OE, Sehested M, Borregaard N (2003) Neutrophil gelatinase-associated lipocalin is up-regulated in human epithelial cells by IL-1 beta, but not by TNF-alpha. *J Immunol* 171, 6630-6639

Cruz DN, de CM, Garzotto F, Perazella MA, Lentini P, Corradi V, Piccinni P, Ronco C (2010) Plasma neutrophil gelatinase-associated lipocalin is an early biomarker for acute kidney injury in an adult ICU population. *Intensive Care Med* 36, 444-451

D'Anna R, Baviera G, Corrado F, Giordano D, Recupero S, Di BA (2009a) First trimester serum neutrophil gelatinase-associated lipocalin in gestational diabetes. *Diabet Med* 26, 1293-1295

D'Anna R, Baviera G, Giordano D, Russo S, Dugo N, Santamaria A, Corrado F (2009b) First trimester serum PAPP-A and NGAL in the prediction of late-onset pre-eclampsia. *Prenat Diagn* 29, 1066-1068

D'Anna R, Baviera G, Giordano D, Todarello G, Corrado F, Buemi M (2008) Second trimester neutrophil gelatinase-associated lipocalin as a potential prediagnostic marker of preeclampsia. *Acta Obstet Gynecol Scand* 87, 1370-1373

Devireddy LR, Gazin C, Zhu X, Green MR (2005) A cell-surface receptor for lipocalin 24p3 selectively mediates apoptosis and iron uptake. *Cell* 123, 1293-1305

Ding L, Hanawa H, Ota Y, Hasegawa G, Hao K, Asami F, Watanabe R, Yoshida T, Toba K, Yoshida K, Ogura M, Kodama M, Aizawa Y (2010) Lipocalin-2/neutrophil gelatinase-B associated lipocalin is strongly induced in hearts of rats with autoimmune myocarditis and in human myocarditis. *Circ J* 74, 523-530

Elneihoum AM, Falke P, Hedblad B, Lindgarde F, Ohlsson K (1997) Leukocyte activation in atherosclerosis: correlation with risk factors. *Atherosclerosis* 131, 79-84

Endre ZH, Pickering JW, Walker RJ, Devarajan P, Edelstein CL, Bonventre JV, Frampton CM, Bennett MR, Ma Q, Sabbisetti VS, Vaidya VS, Walcher AM, Shaw GM, Henderson SJ, Nejat M, Schollum JB, George PM (2011) Improved performance of urinary biomarkers of acute kidney injury in the critically ill by stratification for injury duration and baseline renal function. *Kidney Int*, *in press*

Flo TH, Smith KD, Sato S, Rodriguez DJ, Holmes MA, Strong RK, Akira S, Aderem A (2004) Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. *Nature* 432, 917-921

Florin L, Hummerich L, Dittrich BT, Kokocinski F, Wrobel G, Gack S, Schorpp-Kistner M, Werner S, Hahn M, Lichter P, Szabowski A, Angel P (2004) Identification of novel AP-1 target genes in fibroblasts regulated during cutaneous wound healing. *Oncogene* 23, 7005-7017

Flower DR (1994) The lipocalin protein family: a role in cell regulation. *FEBS Lett* 354, 7-11

Flower DR (1995) Multiple molecular recognition properties of the lipocalin protein family. *J Mol Recognit* 8, 185-195

Flower DR (1996) The lipocalin protein family: structure and function. *Biochem J* 318 (Pt 1), 1-14

Flower DR, North AC, Sansom CE (2000) The lipocalin protein family: structural and sequence overview. *Biochim Biophys Acta* 1482, 9-24

Furutani M, Arii S, Mizumoto M, Kato M, Imamura M (1998) Identification of a neutrophil gelatinase-associated lipocalin mRNA in human pancreatic cancers using a modified signal sequence trap method. *Cancer Lett* 122, 209-214

Garay-Rojas E, Harper M, Hraba-Renevey S, Kress M (1996) An apparent autocrine mechanism amplifies the dexamethasone- and retinoic acid-induced expression of mouse lipocalin-encoding gene 24p3. *Gene* 170, 173-180

Goetz DH, Willie ST, Armen RS, Bratt T, Borregaard N, Strong RK (2000) Ligand preference inferred from the structure of neutrophil gelatinase associated lipocalin. *Biochemistry* 39, 1935-1941

Holmes MA, Paulsene W, Jide X, Ratledge C, Strong RK (2005) Siderocalin (Lcn 2) also binds carboxymycobactins, potentially defending against mycobacterial infections through iron sequestration. *Structure* 13, 29-41

Hraba-Renevey S, Turler H, Kress M, Salomon C, Weil R (1989) SV40-induced expression of mouse gene 24p3 involves a post-transcriptional mechanism. *Oncogene* 4, 601-608

Hu L, Hittelman W, Lu T, Ji P, Arlinghaus R, Shmulevich I, Hamilton SR, Zhang W (2009) NGAL decreases E-cadherin-mediated cell-cell adhesion and increases cell motility and invasion through Rac1 in colon carcinoma cells. *Lab Invest* 89, 531-548

Hvidberg V, Jacobsen C, Strong RK, Cowland JB, Moestrup SK, Borregaard N (2005) The endocytic receptor megalin binds the iron transporting neutrophil-gelatinase-associated lipocalin with high affinity and mediates its cellular uptake. *FEBS Lett* 579, 773-777

Iannetti A, Pacifico F, Acquaviva R, Lavorgna A, Crescenzi E, Vascotto C, Tell G, Salzano AM, Scaloni A, Vuttariello E, Chiappetta G, Formisano S, Leonardi A (2008) The neutrophil gelatinase-associated lipocalin (NGAL), a NF-kappaB-regulated gene, is a survival factor for thyroid neoplastic cells. *Proc Natl Acad Sci U S A* 105, 14058-14063

Klapper JA, Downey SG, Smith FO, Yang JC, Hughes MS, Kammula US, Sherry RM, Royal RE, Steinberg SM, Rosenberg S (2008) High-dose interleukin-2 for the treatment of metastatic renal cell carcinoma : a retrospective analysis of response and survival in patients treated in the surgery branch at the National Cancer Institute between 1986 and 2006. *Cancer* 113, 293-301

Korbely R, Wilflingseder J, Perco P, Kainz A, Langer RM, Mayer B, Oberbauer R (2011) Molecular biomarker candidates of acute kidney injury in zero-hour renal transplant needle biopsies. *Transpl Int* 24, 143-149

Koyner JL, Vaidya VS, Bennett MR, Ma Q, Worcester E, Akhter SA, Raman J, Jeevanandam V, O'Connor MF, Devarajan P, Bonventre JV, Murray PT (2010) Urinary biomarkers in the clinical prognosis and early detection of acute kidney injury. *Clin J Am Soc Nephrol* 5, 2154-2165

Krawczeski CD, Woo JG, Wang Y, Bennett MR, Ma Q, Devarajan P (2011) Neutrophil Gelatinase-Associated Lipocalin Concentrations Predict Development of Acute Kidney Injury in Neonates and Children after Cardiopulmonary Bypass. *J Pediatr*,

Landro L, Damas JK, Flo TH, Heggelund L, Ueland T, Tjonnfjord GE, Espevik T, Aukrust P, Froland SS (2008) Decreased serum lipocalin-2 levels in human immunodeficiency virus-infected patients: increase during highly active anti-retroviral therapy. *Clin Exp Immunol* 152, 57-63

Lau WK, Blute ML, Weaver AL, Torres VE, Zincke H (2000) Matched comparison of radical nephrectomy vs nephron-sparing surgery in patients with unilateral renal cell carcinoma and a normal contralateral kidney. *Mayo Clin Proc* 75, 1236-1242

Le C, V, Calafat J, Borregaard N (1997) Sorting of the specific granule protein, NGAL, during granulocytic maturation of HL-60 cells. *Blood* 89, 2113-2121

Lee EK, Kim HJ, Lee KJ, Lee HJ, Lee JS, Kim DG, Hong SW, Yoon Y, Kim JS (2011) Inhibition of the proliferation and invasion of hepatocellular carcinoma cells by lipocalin 2 through blockade of JNK and PI3K/Akt signaling. *Int J Oncol* 38, 325-333

Lee HJ, Lee EK, Lee KJ, Hong SW, Yoon Y, Kim JS (2006) Ectopic expression of neutrophil gelatinase-associated lipocalin suppresses the invasion and liver metastasis of colon cancer cells. *Int J Cancer* 118, 2490-2497

Leng X, Lin H, Ding T, Wang Y, Wu Y, Klumpp S, Sun T, Zhou Y, Monaco P, Belmont J, Aderem A, Akira S, Strong R, Arlinghaus R (2008) Lipocalin 2 is required for BCR-ABL-induced tumorigenesis. *Oncogene* 27, 6110-6119

Lim R, Ahmed N, Borregaard N, Riley C, Wafai R, Thompson EW, Quinn MA, Rice GE (2007) Neutrophil gelatinase-associated lipocalin (NGAL) an early-screening biomarker for ovarian cancer: NGAL is associated with epidermal growth factor-induced epithelio-mesenchymal transition. *Int J Cancer* 120, 2426-2434

Lin H, Monaco G, Sun T, Ling X, Stephens C, Xie S, Belmont J, Arlinghaus R (2005) Bcr-Abl-mediated suppression of normal hematopoiesis in leukemia. *Oncogene* 24, 3246-3256

Mallbris L, O'Brien KP, Hulthen A, Sandstedt B, Cowland JB, Borregaard N, Stahle-Backdahl M (2002) Neutrophil gelatinase-associated lipocalin is a marker for dysregulated keratinocyte differentiation in human skin. *Exp Dermatol* 11, 584-591

Marques F, Rodrigues AJ, Sousa JC, Coppola G, Geschwind DH, Sousa N, Correia-Neves M, Palha JA (2008) Lipocalin 2 is a choroid plexus acute-phase protein. *J Cereb Blood Flow Metab* 28, 450-455

Mishra J, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, Barasch J, Devarajan P (2003) Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. *J Am Soc Nephrol* 14, 2534-2543

Miyamoto T, Kashima H, Suzuki A, Kikuchi N, Konishi I, Seki N, Shiozawa T (2011) Laser-captured microdissection-microarray analysis of the genes involved in endometrial carcinogenesis: stepwise up-regulation of lipocalin2 expression in normal and neoplastic endometria and its functional relevance. *Hum Pathol, in press*

Moniaux N, Chakraborty S, Yalniz M, Gonzalez J, Shostrom VK, Standop J, Lele SM, Ouellette M, Pour PM, Sasson AR, Brand RE, Hollingsworth MA, Jain M, Batra SK (2008) Early diagnosis of pancreatic cancer: neutrophil gelatinase-associated lipocalin as a marker of pancreatic intraepithelial neoplasia. *Br J Cancer* 98, 1540-1547

Moreno-Navarrete JM, Manco M, Ibanez J, Garcia-Fuentes E, Ortega F, Gorostiaga E, Vendrell J, Izquierdo M, Martinez C, Nolfe G, Ricart W, Mingrone G, Tinahones F, Fernandez-Real JM (2010) Metabolic endotoxemia and saturated fat contribute to circulating NGAL concentrations in subjects with insulin resistance. *Int J Obes (Lond)* 34, 240-249

Nairz M, Theurl I, Schroll A, Theurl M, Fritsche G, Lindner E, Seifert M, Crouch ML, Hantke K, Akira S, Fang FC, Weiss G (2009) Absence of functional Hfe protects mice from invasive Salmonella enterica serovar Typhimurium infection via induction of lipocalin-2. *Blood* 114, 3642-3651

Nickolas TL, O'Rourke MJ, Yang J, Sise ME, Canetta PA, Barasch N, Buchen C, Khan F, Mori K, Giglio J, Devarajan P, Barasch J (2008) Sensitivity and specificity of a single emergency department measurement of urinary neutrophil gelatinase-associated lipocalin for diagnosing acute kidney injury. *Ann Intern Med* 148, 810-819

Nomura I, Gao B, Boguniewicz M, Darst MA, Travers JB, Leung DY (2003) Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: a gene microarray analysis. *J Allergy Clin Immunol* 112, 1195-1202

Owen HC, Roberts SJ, Ahmed SF, Farquharson C (2008) Dexamethasone-induced expression of the glucocorticoid response gene lipocalin 2 in chondrocytes. *Am J Physiol Endocrinol Metab* 294, E1023-E1034

Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, Wang Y, Hood L, Zhu Z, Tian Q, Dong C (2005) A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 6, 1133-1141

Pi-Sunyer FX (2002) The obesity epidemic: pathophysiology and consequences of obesity. *Obes Res* 10 Suppl 2, 97S-104S

Pukstad BS, Ryan L, Flo TH, Stenvik J, Moseley R, Harding K, Thomas DW, Espevik T (2010) Non-healing is associated with persistent stimulation of the innate immune response in chronic venous leg ulcers. *J Dermatol Sci* 59, 115-122

Roudkenar MH, Halabian R, Ghasemipour Z, Roushandeh AM, Rouhbakhsh M, Nekogoftar M, Kuwahara Y, Fukumoto M, Shokrgozar MA (2008a) Neutrophil gelatinase-associated lipocalin acts as a protective factor against H(2)O(2) toxicity. *Arch Med Res* 39, 560-566

Roudkenar MH, Halabian R, Ghasemipour Z, Roushandeh AM, Rouhbakhsh M, Nekogoftar M, Kuwahara Y, Fukumoto M, Shokrgozar MA (2008b) Neutrophil gelatinase-associated lipocalin acts as a protective factor against H(2)O(2) toxicity. *Arch Med Res* 39, 560-566

Roudkenar MH, Halabian R, Oodi A, Roushandeh AM, Yaghmai P, Najar MR, Amirizadeh N, Shokrgozar MA (2008c) Upregulation of neutrophil gelatinase-associated lipocalin, NGAL/Lcn2, in beta-thalassemia patients. *Arch Med Res* 39, 402-407

Roudkenar MH, Kuwahara Y, Baba T, Roushandeh AM, Ebishima S, Abe S, Ohkubo Y, Fukumoto M (2007) Oxidative stress induced lipocalin 2 gene expression: addressing its expression under the harmful conditions. *J Radiat Res (Tokyo)* 48, 39-44

Seth P, Porter D, Lahti-Domenici J, Geng Y, Richardson A, Polyak K (2002) Cellular and molecular targets of estrogen in normal human breast tissue. *Cancer Res* 62, 4540-4544

Shapiro NI, Trzeciak S, Hollander JE, Birkhahn R, Otero R, Osborn TM, Moretti E, Nguyen HB, Gunnerson K, Milzman D, Gaieski DF, Goyal M, Cairns CB, Kupfer K, Lee SW, Rivers EP (2010) The diagnostic accuracy of plasma neutrophil gelatinase-associated lipocalin in the prediction of acute kidney injury in emergency department patients with suspected sepsis. *Ann Emerg Med* 56, 52-59

Shapiro NI, Trzeciak S, Hollander JE, Birkhahn R, Otero R, Osborn TM, Moretti E, Nguyen HB, Gunnerson KJ, Milzman D, Gaieski DF, Goyal M, Cairns CB, Ngo L, Rivers EP (2009) A prospective, multicenter derivation of a biomarker panel to assess risk of organ dysfunction, shock, and death in emergency department patients with suspected sepsis. *Crit Care Med* 37, 96-104

Singer E, Elger A, Elitok S, Kettritz R, Nickolas TL, Barasch J, Luft FC, Schmidt-Ott KM (2011) Urinary neutrophil gelatinase-associated lipocalin distinguishes pre-renal from intrinsic renal failure and predicts outcomes. *Kidney Int*,

Stoesz SP, Gould MN (1995) Overexpression of neu-related lipocalin (NRL) in neu-initiated but not ras or chemically initiated rat mammary carcinomas. *Oncogene* 11, 2233-2241

Tan BK, Adya R, Shan X, Syed F, Lewandowski KC, O'Hare JP, Randeva HS (2009) Ex vivo and in vivo regulation of lipocalin-2, a novel adipokine, by insulin. *Diabetes Care* 32, 129-131

te Boekhorst BC, Bovens SM, Hellings WE, van der Kraak PH, van de Kolk KW, Vink A, Moll FL, van Oosterhout MF, de Vries JP, Doevendans PA, Goumans MJ, de Kleijn DP, van Echteld CJ, Pasterkamp G, Sluijter JP (2011) Molecular MRI of murine atherosclerotic plaque targeting NGAL: a protein associated with unstable human plaque characteristics. *Cardiovasc Res* 89, 680-688

Tong Z, Chakraborty S, Sung B, Koolwal P, Kaur S, Aggarwal BB, Mani SA, Bresalier RS, Batra SK, Guha S (2011) Epidermal growth factor down-regulates the expression of neutrophil gelatinase-associated lipocalin (NGAL) through E-cadherin in pancreatic cancer cells. *Cancer*, 117, 2408-2418

Tong Z, Kunnumakkara AB, Wang H, Matsuo Y, Diagaradjane P, Harikumar KB, Ramachandran V, Sung B, Chakraborty A, Bresalier RS, Logsdon C, Aggarwal BB, Krishnan S, Guha S (2008) Neutrophil gelatinase-associated lipocalin: a novel suppressor of invasion and angiogenesis in pancreatic cancer. *Cancer Res* 68, 6100-6108

Triebel S, Blaser J, Reinke H, Tschesche H (1992) A 25 kDa alpha 2-microglobulin-related protein is a component of the 125 kDa form of human gelatinase. *FEBS Lett* 314, 386-388

Van Dyke TE, Schweinebraten M, Cianciola LJ, Offenbacher S, Genco RJ (1985) Neutrophil chemotaxis in families with localized juvenile periodontitis. *J Periodontal Res* 20, 503-514

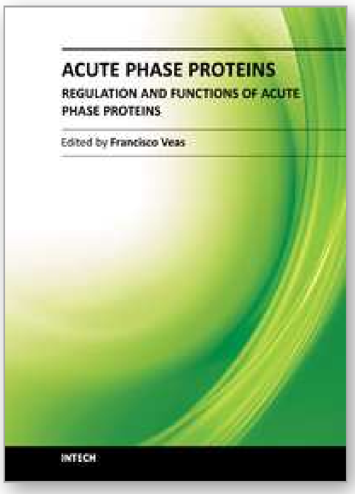
Viau A, El KK, Laouari D, Burtin M, Nguyen C, Mori K, Pillebout E, Berger T, Mak TW, Knebelmann B, Friedlander G, Barasch J, Terzi F (2010) Lipocalin 2 is essential for chronic kidney disease progression in mice and humans. *J Clin Invest* 120, 4065-4076

Villalva C, Sorel N, Bonnet ML, Guilhot J, Mayeur-Rousse C, Guilhot F, Chomel JC, Turhan AG (2008) Neutrophil gelatinase-associated lipocalin expression in chronic myeloid leukemia. *Leuk Lymphoma* 49, 984-988

Wang HJ, He XJ, Ma YY, Jiang XT, Xia YJ, Ye ZY, Zhao ZS, Tao HQ (2010) Expressions of neutrophil gelatinase-associated lipocalin in gastric cancer: a potential biomarker for prognosis and an ancillary diagnostic test. *Anat Rec (Hoboken)* 293, 1855-1863

Wang Y, Lam KS, Kraegen EW, Sweeney G, Zhang J, Tso AW, Chow WS, Wat NM, Xu JY, Hoo RL, Xu A (2007) Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance, and hyperglycemia in humans. *Clin Chem* 53, 34-41

Yang XO, Chang SH, Park H, Nurieva R, Shah B, Acero L, Wang YH, Schluns KS, Broaddus RR, Zhu Z, Dong C (2008) Regulation of inflammatory responses by IL-17F. *J Exp Med* 205, 1063-1075



Acute Phase Proteins - Regulation and Functions of Acute Phase Proteins
Edited by Prof. Francisco Veas

ISBN 978-953-307-252-4
Hard cover, 368 pages
Publisher InTech

Published in print edition October, 2011

The two volumes of Acute Phase Proteins book consist of chapters that give a large panel of fundamental and applied knowledge on one of the major elements of the inflammatory process during the acute phase response, i.e., the acute phase proteins expression and functions that regulate homeostasis. We have organized this book in two volumes - the first volume, mainly containing chapters on structure, biology and functions of APP, the second volume discussing different uses of APP as diagnostic tools in human and veterinary medicine.

How to reference

Subhankar Chakraborty, Sukhwinder Kaur, Zhimin Tong, Surinder K. Batra and Sushovan Guha (2011). Neutrophil Gelatinase Associated Lipocalin: Structure, Function and Role in Human Pathogenesis, Acute Phase Proteins - Regulation and Functions of Acute Phase Proteins, Prof. Francisco Veas (Ed.), ISBN: 978-953-307-252-4, InTech, Available from: http://www.intechopen.com/books/acute-phase-proteins-regulation-and-functions-of-acute-phase-proteins/neutrophil-gelatinase-associated-lipocalin-structure-function-and-role-in-human-pathogenesis

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821