THE ENIGMATIC ROLE OF LIPOCALIN 2 IN HUMAN CANCER. THE MULTIFUNCTIONAL PROTEIN NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (NGAL) AND ITS AMBIGUOUS ROLE IN HUMAN NEOPLASIAS.

L’ENIGMATICO RUOLO DELLA LIPOCAIN A 2 NELL’INSORGENZA DEL CANCRO UMANO. LA PROTEINA MULTIFUNZIONALE LIPOCALINA ASSOCIATA ALLA GELATINASI DEI NEUTROFILI (NGAL) ED IL SUO AMBIGUO RUOLO NELLE NEOPLASIE UMANE.

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Key words: lipocalin, neutrophil gelatinase-associated lipocalin (NGAL), tumor growth

Parole chiave: lipocalina, lipocalina-associata alla gelatinasi dei neutrofili (NGAL), crescita tumorale
Abstract

**Background:** Neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin 2, has been originally identified as a 25 kDa protein covalently linked to neutrophil gelatinase B. It is an acute phase protein involved in diverse physiological processes with an important role in intracellular iron transport. Moreover, lipocalin 2 is involved in the physiopathology of neoplastic process.

**Objectives:** The aim is to review the literature concerning the role of lipocalin 2 in cancer growth and its correlation with human neoplasias.

**Methods:** A review was performed on the basis of literature search of Neutrophil gelatinase-associated lipocalin (NGAL), tumor growth, tumor invasion, tumor development and tumor biomarker in PubMed.

**Results:** Lipocalin 2 induces proliferation and angiogenesis, favors invasion and metastasis. But it can also inhibit proliferation and induce apoptosis. So far its role is contradictory and not yet completely clarified.

**Conclusions:** It is likely that the dual contradictory effects of NGAL (pro-neoplastic and anti-tumoral) are neoplasia-specific and influenced largely by the type of molecule it is complexed with.
Lipocalin protein family

The lipocalins form a large group of small, predominantly extracellular proteins previously regarded as obscure transporters of hydrophobic ligands (1).

The name of this family describes the main unifying characteristics of its members, namely their ability to bind small lipophilic molecules facilitated by a common cup-shaped tertiary structure. In addition to their ability to bind small extracellular molecules, many lipocalins bind cell surface receptors, probably to facilitate cellular internalization. Another feature shared by several lipocalins is the aggregation into macromolecular complexes.

The lipocalins are defined largely on the basis of sequences similarity, they share sufficient similarity, in the form of short characteristic conserved sequence motifs (1). The principal members of this large family are listed in Table I.

Tabella I. Members of the lipocalin protein family.

<table>
<thead>
<tr>
<th>Name</th>
<th>Oligomeric State</th>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>Retinol-binding protein</td>
<td>Monomer</td>
<td>RBP</td>
</tr>
<tr>
<td>Purpurin</td>
<td>----</td>
<td>PURP</td>
</tr>
<tr>
<td>Retinoic acid-binding protein</td>
<td>Monomer</td>
<td>RABP</td>
</tr>
<tr>
<td>α2u-Globulin</td>
<td>Dimer</td>
<td>A2U</td>
</tr>
<tr>
<td>Major urinary protein</td>
<td>Dimer</td>
<td>MUP</td>
</tr>
<tr>
<td>Bilin-binding protein</td>
<td>Tetramer</td>
<td>BBP</td>
</tr>
<tr>
<td>α-Crustacyanin</td>
<td>Octamer of heterodimers</td>
<td>---</td>
</tr>
<tr>
<td>Pregnancy protein 14</td>
<td>Homodimer</td>
<td>PP14</td>
</tr>
<tr>
<td>β-Lactoglobulin</td>
<td>Dimer/monomer</td>
<td>Blg</td>
</tr>
<tr>
<td>α1-Microglobulin</td>
<td>Monomer + complexes</td>
<td>A1M</td>
</tr>
<tr>
<td>C8γ</td>
<td>Part of complex</td>
<td>C8γ</td>
</tr>
<tr>
<td>Apolipoprotein D</td>
<td>Dimer + complexes</td>
<td>ApoD</td>
</tr>
<tr>
<td>Lazarillo</td>
<td>Monomer</td>
<td>LAZ</td>
</tr>
<tr>
<td>Prostaglandin D synthase</td>
<td>Monomer</td>
<td>PGDS</td>
</tr>
<tr>
<td>Quiescence-specific protein</td>
<td>----</td>
<td>QSP</td>
</tr>
<tr>
<td>Neutrophil lipocalin</td>
<td>Monomer/dimer + complexes</td>
<td>NGAL</td>
</tr>
<tr>
<td>Coroid plexus protein</td>
<td>Monomer</td>
<td>---</td>
</tr>
<tr>
<td>Odorant-binding protein</td>
<td>Dimer</td>
<td>OBP</td>
</tr>
<tr>
<td>von Ebner’s gland protein</td>
<td>Dimer</td>
<td>VEGP</td>
</tr>
<tr>
<td>α1-Acid glycoprotein</td>
<td>Monomer</td>
<td>AGP</td>
</tr>
<tr>
<td>Prosabin</td>
<td>----</td>
<td>PBAS</td>
</tr>
<tr>
<td>Aphrodisin</td>
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</tbody>
</table>
To date the functions of many lipocalins remain unclear, they probably are as diverse as its distribution. It is increasingly clear that many lipocalins have key physiological roles, including retinol and pheromone transport, cryptic coloration and chemoreception; they also fulfill important functions in innate immunity, cell regulation, morphogenesis and angiogenesis (1, 2). Lipocalins can also act as protease inhibitors and mediators of apoptosis and of cell homeostasis. Several lipocalin family members are acute phase reactants, such as orosomucoid, neutrophil gelatinase-associated lipocalin (NGAL), pregnant protein 14 (PP14) and alpha1-microglobulin. Both pregnant protein 14 (PP14) and alpha1-microglobulin have immunosuppressive activity. Moreover, many lipocalins are important diagnostic markers for disease and act as key mammalian aeroallergens. They have a role as mediators of defense against oxidative stress and are involved in iron metabolosim. In this article we focus on lipocalin 2 also known as neutrophil gelatinase-associated lipocalin (NGAL).

Lipocalin 2 (NGAL)

Lipocalin 2, also known as neutrophil gelatinase-associated lipocalin (NGAL), neu-related lipocalin (NRL), oncogene 24p3, siderocalin and uterocalin, was originally identified as a novel protein isolated from secondary granules of human neutrophils (3). Mature peripheral neutrophils lack NGAL mRNA expression, and NGAL protein is synthesized at the early-myelocyte stage of granulopoiesis during formation of secondary granules. The vast majority of NGAL is found in myeloperoxidase negative neutrophil granules either as monomer or homodimer. It is expressed by neutrophils and epithelial cells. Lipocalin 2 is composed of 8 β-strands that form a β-barrel-shaped tertiary structure with a hydrophobic calyx that binds small lipophilic molecules. It is a 25 kDa protein that can be covalently bound to matrix metalloproteinase-9 from human neutrophils. This complexation accounts for the about 115-135 kDa form of gelatinase B (4). The promoter region of the NGAL gene contains binding sites for a number of transcription factors including nuclear factor (NF)-kB. This could explain the constitutive, as well as inducible expression of NGAL in several non-hematopoietic tissues. NGAL mRNA is normally expressed in a variety of adult human tissues, including bone marrow, uterus, prostate, salivary gland, stomach, colon, trachea, lung, liver and kidney (5).

Several of these tissues are prone to exposure to micro-organisms, and constitutively express the NGAL protein at low levels. NGAL expression is induced in injured epithelia; for example, concentrations are raised in the serum of subjects with acute bacterial infections, in the sputum of those with asthma or chronic obstructive pulmonary disease, and bronchial fluid from the emphysematous lung. Furthermore, it is highly expressed in areas of rapid bone growth and proliferation shortly after the birth. In the estrus cycle, NGAL is found in the uterus in high amounts during proestrus and estrus, and decline during metaestrus and diestrus.

Based on these data, it is suggested that NGAL secretion is closely associated with the cell cycle proliferation and apoptosis during the remodeling of the endometrium. Finally, NGAL was found in a variety of pathological human tissues and its expression is very heterogeneous in neoplastic human tissues (6).

Functions of lipocalin 2 (NGAL)

Lipocalin 2 is an acute phase protein and it has been implicated in diverse physiological processes. It has a critical role in innate immunity to bacterial infections. A prominent characteristic of NGAL is its binding of iron and the 24p3-associates small molecular weight siderophore. Siderophores are synthesized by bacteria to acquire iron from the surroundings, and use specific transporters to recover the siderophore-iron complex, ensuring their iron supply. This siderophore-chelating property renders NGAL a bacteriostatic agent. Besides siderophores produced by eukaryotes participate in NGAL-mediated iron shuttling that is critical to various cellular responses. When NGAL is devoid of siderophore and iron, it scavenges intracellular iron causing an intra-cellular iron-depletion.

The intracellular iron depletion decreases cell’s proliferative ability and induces apoptosis. Vice versa, when NGAL is bound to siderophore and iron, the complex interacts with a NGAL receptor (NGAL-R) resulting in the transportation of this complex to the cytoplasm. After internalization the complex (siderophore-iron-NGAL) is able to release iron within the cytoplasm, leading to iron accumulation and regulating specific iron-dependent genic pathways. These events induce proliferation and epithelial transformation (7).
By forming the NGAL/MMP-9 (matrix metalloproteinase-9) complex, NGAL may protect MMP-9 from proteolytic degradation, a normal physiological mechanism of fundamental importance in controlling the activity of the protein. This would trigger an enhancement of the enzymatic activity of MMP-9. In turn, MMP-9 activity promotes cancer progression by degrading the basement membranes and extracellular matrix, liberating vascular endothelial growth factor, and thus enabling angiogenesis, invasion and metastasis (8).

**Lipocalin 2 (NGAL) in cancer**

High levels of neutrophil gelatinase-associated lipocalin (NGAL) are found in adenocarcinomas of lung, colon, and pancreas. In contrast, renal cell carcinomas of various subtypes and prostate cancers contain low NGAL levels (6). Nowadays it is recognized that NGAL is a key element in the physiopathology of neoplastic process and it is involved in the most important types of human tumors (9, 10 and references therein). The effects of this protein in cancerogenesis are influenced by multiple mechanisms of action, but the exact role of NGAL within the sphere of the neoplastic process is not yet completely clear.

For instance, NGAL has been shown to have a pro-tumoral effect in breast, stomach and esophagus cancer. In contrast, some studies show that NGAL exert an anti-tumoral and anti-metastatic effect in ovarian, and pancreatic cancers. It is well known that NGAL synthesis is induced by factors promoting the development of neoplasias and the protein is over-expressed in several types of malignancies.

Furthermore, NGAL is involved in the progression of tumors; and it has been shown as a negative prognostic factor associated with shorter overall and disease-free survival in several neoplasms. The association between NGAL expression and metastatic potential of cancer cells may be related to the ability of this protein to form complexes with the matrix metalloproteinase 9 (MMP-9).

MMP-9 (also named gelatinase B) is a member of the MMPs family, a large group of zinc-containing endopeptidases with a central role in the degradation of all types of extracellular matrix components and basement membranes, allowing cancer cells to penetrate and infiltrate the subjacent stromal matrix.

In particular, MMP-9 is involved in several processes associated with cancer development such as tumor angiogenesis and immune surveillance (11).

The over-expressed NGAL protein binds to MMP-9, thereby preventing MMP-9 degradation and increasing MMP-9 activities. In turn, MMP-9 activity promotes cancer progression.

These events may explain the pro-neoplastic activity associated with NGAL overexpression. An analysis made using zymographic technique showed that the NGAL/MMP-9 complex is enhanced in the urine from patients with high-grade and advanced-stage bladder and tumors (12). Moreover, NGAL/MMP-9 complexes are found in about 90% of the urine samples from patients with breast cancer (13).

The pro-neoplastic potential of NGAL is also supported by the capacity of NGAL to capture the intracellular iron from extracellular space within the malignant cells, a fundamental process for the maintenance of neoplastic cell multiplication. However, paradoxically, recent studies in some tumor cell lines have demonstrated that NGAL enhance the epithelial phenotype, reduce tumor growth and suppress metastasis (14).

Furthermore, other studies have demonstrated, both in vitro and in vivo, that NGAL reduces cell adhesion, invasion and angiogenesis probably through the inhibition of focal adhesion kinase phosphorylation and the suppression of vascular endothelial growth factor synthesis (15).

**Conclusions**

Neutrophil gelatinase-associated lipocalin is one of the highly interesting and enigmatic protein involved in the development and progression of human neoplasias. Actually, its role within the sphere of the neoplastic process is often contradictory and still remains to be clarified.
References


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