Role of neutrophil gelatinase-associated lipocalin in renal cell carcinoma (Review)

KAI CHE[1,2], WENKAI HAN[1,2], MINGXIN ZHANG[1*] and HAITAO NIU[1*]

[1]Department of Urology, The Affiliated Hospital of Qingdao University; [2]Department of Clinical Medicine, Qingdao University, Qingdao, Shandong 266003, P.R. China

Abstract. Human neutrophil gelatinase-associated lipocalin (NGAL) is a glycoprotein present in a wide variety of tissues and cell types. It exists as a monomer of 25 kDa, a homodimer of 45 kDa or a heterodimer of 135 kDa (disulfide bound to latent matrix metalloproteinase-9). NGAL is considered the biochemical gold standard for the early diagnosis of acute kidney injury and has attracted much attention as a diagnostic biomarker. NGAL has controversial (i.e. both beneficial and detrimental) effects on cellular processes associated with tumor development, such as cell proliferation, survival, migration, invasion and drug resistance. Therefore, the present review aimed at clarifying the role of NGAL in renal cell carcinoma (RCC). Relevant studies of NGAL and RCC were searched in PubMed and relevant information about the structure, expression, function and mechanism of NGAL in RCC were summarized. Finally, the following conclusions could be drawn from the literature: i) NGAL can be detected in cancer tissues, serum and urine of patients with RCC; ii) NGAL is not a suitable diagnostic marker for early screening of RCC; iii) NGAL expression may be used to predict the prognosis of patients with RCC; and iv) Further research on NGAL may be helpful to decrease sunitinib resistance and find new treatment strategies for RCC.

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Correspondence to: Professor Haitao Niu or Dr Mingxin Zhang, Department of Urology, The Affiliated Hospital of Qingdao University, 16 Jingsu Road, Qingdao, Shandong 266003, P.R. China
E-mail: dr.zmx@foxmail.com
E-mail: niuht0532@126.com

*Contributed equally

1. Introduction

Renal cell carcinoma (RCC) is the most common type of kidney cancer, accounting for 3% of adult malignancies (1). Although the morbidity of RCC continues to increase in most countries, mortality has remained the same in numerous highly developed countries during the last decades (2). RCC can occur in both men and women, but the ratio of male to female incidence is ~1.5:1, and the morbidity was 4.5 times higher in developed countries than in developing countries in 2008 (2). Early detection of RCC is difficult, as this disease lacks early clinical manifestations (3). By the time RCC is diagnosed, 20-30% of patients are already in the advanced stages of the disease and have developed distant metastasis, which results in a poor prognosis, with a 5-year survival rate of <10% (3).

According to the 2016 World Health Organization classification (4), the most common histotypes in RCC are clear cell RCC (ccRCC), papillary RCC (pRCC) and chromophobe RCC (chRCC), which account for 75-80, 10-15 and 6-11% of RCC cases, respectively (5). The histotype of RCC is an important predictor of tumor prognosis, but it cannot be fully determined by imaging examinations such as abdominal ultrasound or computed tomography. The application of biopsies is controversial since they require experienced cytopathologists and can lead to complications, such as bleeding and tumor spreading along the biopsy route (6). Therefore, a biomarker with good sensitivity and specificity is urgently required, and further studies on the molecular mechanisms of RCC are required to improve the early diagnosis and treatment of RCC.

Neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin 2 (LCN2), belongs to the apolipoprotein superfamily. NGAL is a 25-kDa secreted glycoprotein that was first discovered by Kjeldsen *et al* (7) in 1993 while studying matrix metalloproteinase-9 (MMP-9) in the specific granules of neutrophils. NGAL has received much attention for its biological role as an early warning sign of acute and chronic kidney diseases, and it is regarded as an optional biomarker of renal tubular injury (8). In the last two decades, studies on NGAL in cardiovascular and cerebrovascular diseases, as

well as diabetes, inflammatory diseases and cancer, have been performed, and as a tumor biomarker, NGAL has attracted much attention (9,10). Previous studies on NGAL have found that the mRNA expression levels of NGAL differ in different types of cancer, and its role may vary (11,12). Additionally, NGAL is expressed in several types of RCC (13) and it may be involved in the development, proliferation, invasion and metastasis of RCC. The purpose of the present review was to discuss the advances in the understanding of the role of NGAL in RCC.

2. Literature search

Data and references from relevant articles were identified by searches of the electronic database PubMed (https://pubmed. ncbi.nlm.nih.gov/) using the search terms 'neutrophil gelatinase-associated lipocalin', 'NGAL', 'lipocalin 2', 'Lcn2', 'renal cell carcinoma', 'carcinoma', 'renal cell' and 'RCC'. The last search was run in March 2020. Studies on animal models were excluded. The eligibility criteria included the following: i) Studies on NGAL expression in RCC; ii) Studies that evaluated NGAL as a prognostic or diagnostic marker in RCC; iii) Studies on the functions of NGAL in RCC cell lines *in vitro*; and iv) Studies identified from relevant references from eligible studies, such as studies on the structure of NGAL or the association between NGAL and breast cancer, colorectal cancer (14), pancreatic cancer (15) or renal cancer. Eligible studies were screened by two authors, and no disagreement existed between authors.

3. Review of NGAL in RCC

Structure and biological function of NGAL. The human NGAL gene is a monocistronic gene located on chromosome 9q34, with a total length of 5,869 bp, including a 3,696 bp coding region (7 exons and 6 introns), a 1,695 bp 5'-terminal non-transcribed region and a 178 bp 3'-terminal non-transcribed region (11). The cDNA sequence of the NGAL gene was first identified by cloning in 1994 and consists of a coding region and a 5'-terminal untranslated region. The peptide chain encoded by the NGAL gene contains 198 amino acid residues, including a leading sequence (containing 20 amino acid residues) and the mature peptide (containing 178 amino acid residues) (11). The molecular weight of NGAL is 22.6 kDa, which increases to 25 kDa after glycosylation (16,17). NGAL belongs to the apolipoprotein superfamily and has a highly conserved tertiary calyx-shaped structure, composed of 8 anti-parallel β-strands, a C-terminal α-helix and an N-terminal 310-helix (Fig. 1). In humans, NGAL can exist in at least three forms: A monomer of 25 kDa, a homodimer of 45 kDa and a heterodimer of 135 kDa (disulfide bound with MMP-9) (18).

Vaidya *et al* (19) found that under normal physiological conditions, NGAL can be synthesized during a narrow window of granulocyte maturation in the bone marrow, and that NGAL exists in peroxidase-negative neutrophil granules. Additionally, NGAL is expressed at a low level in a variety of cell types, including fat cells, cartilage cells, endometrial carcinoma cells, epithelial cells, endothelial cells, fibroblasts, liver cells, keratinocytes, macrophages, mesangial cells, microglia, lung cells, spleen cells, thymic cells and vascular

smooth muscle cells (9). In addition, it is expressed in small amounts in the epithelium of normal human organs, such as lung, kidney, uterus and breast, as well as in the gastrointestinal tract (20). Furthermore, NGAL can be detected in different biological fluids, including blood and urine (21), bile (22), bronchoalveolar lavage fluid (23), hydrothorax (24), ascites (25) and cerebrospinal fluid (26).

The calyx-like structure of NGAL enables it to bind to low molecular weight siderophores to form a NGAL-siderophore-iron complex, which can induce the differentiation of precursor cells into epithelial cells, promote the maturation of primitive renal epithelial cells, scavenge free radicals and decrease the damage caused by oxidative stress to promote the repair of renal injury (27). In addition, NGAL can be coupled with relevant receptors on the cell surface, such as the megalin-cubilin multiscavenger complex found on the brushborder surface of renal tubular epithelial cells, and induce the cell to phagocytose siderophores (28). NGAL can strongly bind to enterobactin, and its iron-binding activity provides a highly effective antibacterial effect, which competitively inhibits the iron intake of bacteria, blocks their access to iron, which is an important nutrient for bacteria, and ultimately inhibits their growth (29). Furthermore, NGAL serves a role in the inflammatory response *in vivo*, transporting a number of lipophilic molecules that mediate inflammation, such as leukotriene B, platelet activating factor and lipopolysaccharide (30). Another important function of NGAL is to bind to MMP-9 to form heterodimers, preventing the endogenous degradation of MMP-9 and thus maintaining its ability to degrade a large number of structural molecules, such as collagen, fibronectin and laminin (31).

There is evidence indicating that NGAL can be produced by renal tubules and that its concentration is significantly increased after renal injury (13). The diagnostic and prognostic value of NGAL in acute kidney injury (AKI) has been conclusively proven in a series of clinical studies (11). Currently, NGAL is considered the biochemical gold standard for the diagnosis of AKI. When AKI occurs, NGAL concentrations increase in both urine and blood, with the increase in urine being more marked than that in blood (32). It has been confirmed that NGAL levels are positively correlated with the degree of AKI, and NGAL concentrations in urine and blood can be used as an early, sensitive and highly specific biological marker for the diagnosis of AKI (32-34).

Sequence analysis indicated that the cDNA of NGAL and its mouse homolog 24p3 have 71.3% homology, and in regards to the coding sequence, the homology is 74.2% (35). In mice, 24p3 has been proven to be an oncogene, suggesting that NGAL may also be a novel oncogene in humans (36). Increasing attention has been paid to the role of NGAL in the development and progression of cancer. Some progress has been made in uncovering the association between NGAL and various malignancies, such as breast cancer (37), colorectal cancer (14), pancreatic cancer (15), renal cancer (38) and liver cancer (10).

NGAL expression in RCC tissues. Preliminary studies have revealed that NGAL protein expression in the kidney is very limited, but NGAL protein can be detected in renal proximal tubular epithelial cells (20), whereas studies on NGAL

Table I. Studies of the gene or protein expression levels of NGAL in RCC tissues.

First author, year	Type of cancer	NGAL measuring method	No. of patients	No. of controls	Main outcome(s)	Refs.
Friedl et al, 1999	RCC	IHC	12	-	10/12 cases with RCC had very low NGAL expression, while 2 had moderate levels.	(20)
Barresi et al, 2010	Renal tumor	IHC	30	30	NGAL immunoexpression was found in 28/30 cases.	(13)
Perrin et al, 2011	ccRCC	IHC	74	-	NGAL was observed in neutrophils infiltrating ccRCC rather than tumor cells.	(39)
Zhang et al, 2015	RCC	IHC	189	-	NGAL was found in 14/105 ccRCC and 57/84 pRCC cases.	(38)
Rehwald et al, 2020	RCC	Immunofluorescence staining/quantitative PCR	41	41	There was a significant increase in NGAL protein expression in tumor tissues but no significant changes were observed in NGAL mRNA expression.	(41)
Liu et al, 2018	ccRCC	TCGA and GEO database analysis	533 samples in TCGA; 11 GEO datasets	Paired paracancerous tissues	Lower gene expression levels of NGAL in ccRCC samples were observed compared with in paired paracancerous tissues.	(40)

NGAL, neutrophil gelatinase-associated lipocalin; RCC, renal cell carcinoma; IHC, immunohistochemistry; ccRCC, clear cell RCC; pRCC, papillary RCC; TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus.
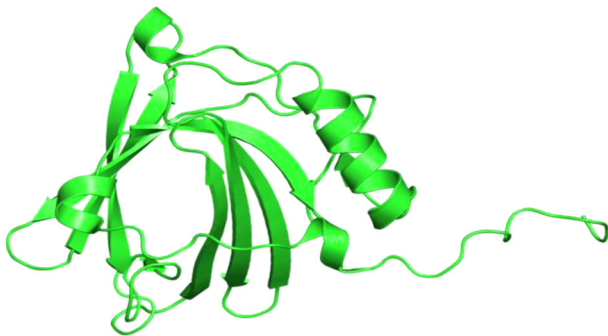

Figure 1. Regularized average nuclear magnetic resonance structure of human neutrophil gelatinase-associated lipocalin from the Protein Data Bank database (ID, 1NGL; http://pdb101.rcsb.org/).

protein expression in RCC have not yielded consistent results (Table I). Notably, according to immunohistochemistry (IHC) staining intensities, Friedl et al (20) found that 10/12 cases of RCC, which is considered to originate from proximal convoluted tubules, had very low NGAL expression, while moderate NGAL expression was found in 2 cases. This was supplemented by further research by Barresi et al (13) in 2010, where NGAL expression was examined by an intensity-distribution (ID) score (≥4 was defined as high NGAL expression) in 30 renal tumors. The results revealed that NGAL was weakly expressed in collecting duct epithelial cells and urothelial cells of the renal pelvis, as well as in normal para-carcinoma renal tubules, whereas variable levels of NGAL were detected in 28/30 patients with RCC (1 patient with ccRCC and 1 with a sarcomatoid tumor

were excluded, with negative NGAL staining) (13). Moreover, different expression patterns were found in NGAL-positive ccRCC (13). In addition to the staining in the cytoplasm of tumor cells, most ccRCCs had distinct membrane staining, which may be due to the fact that NGAL has not yet been internalized as a result of binding to membrane receptors (13). In another study, Zhang et al (38) found that 57/84 patients with pRCC had positive NGAL expression (ID score >2 was identified as high NGAL expression) in the cytoplasm of cancer cells, while only 14/105 patients with ccRCC had cells that expressed NGAL. However, Perrin et al (39) studied 74 RCC samples by IHC and found that NGAL was not expressed in renal tumor cells, but was expressed in neutrophils infiltrating ccRCC tissues (39). The aforementioned studies indicate that there are no unified results on NGAL expression and its subcellular localization in RCC, which may be associated to its differential functions in tumor cells.

The aforementioned studies were conducted by IHC in tumor tissues, but control groups were rarely included. By contrast, Liu et al (40) analyzed 12 ccRCC datasets [1 dataset from The Cancer Genome Atlas (TCGA) and 11 datasets from the Gene Expression Omnibus (GEO) database] and found that, compared with in the paired tissues adjacent to the carcinomas, the gene expression levels of NGAL in the ccRCC group were decreased. Rehwald et al (41) suggested that NGAL gene expression may be inconsistent with NGAL protein expression, since significant increases in NGAL protein expression in ccRCC samples were detected, compared with in corresponding adjacent healthy tissues, but no significant changes in mRNA levels were identified by quantitative PCR

and immunofluorescence staining when comparing tumor tissues with adjacent healthy tissues (41). Further studies are required to elucidate the differences in gene and protein expression levels of NGAL between RCC and normal tissues.

NGAL as a diagnostic and prognostic marker in RCC. Due to the insidious onset of renal cancer and the lack of early clinical symptoms, a non-invasive screening method is urgently required to improve the early diagnosis and treatment of renal cancer. Therefore, searching for a biomarker with excellent sensitivity and specificity that can be found in the blood or urine of patients with kidney cancer has become a research hotspot. NGAL has attracted considerable attention as a tumor biomarker. Previous studies have revealed that elevated NGAL expression may help predict disease-free survival (DFS) in patients with colorectal cancer (42), but the prognostic utility and diagnostic accuracy of NGAL in RCC remain uncertain (10).

NGAL is expressed in tissues, serum and urine of patients with RCC. Studies on whether NGAL can be used as a diagnostic and prognostic biomarker for RCC are currently underway (Table II). By analyzing NGAL expression in renal tumor tissues, Barresi *et al* (13) revealed that high NGAL expression is associated with the pRCC and chRCC histotypes and is significantly associated with the Fuhrman grading of ccRCC and pRCC (43). There was no significant association between NGAL expression and patient age, sex, serum iron levels, tumor size or tumor stage (13). Zhang *et al* (38) studied the association between NGAL expression and the prognosis of patients with ccRCC and pRCC, and found that high NGAL expression was associated with decreased overall survival (OS) and DFS in patients with pRCC, but it was not associated with OS and DFS in patients with ccRCC. Survival analysis of 533 patients with ccRCC in the TCGA database revealed that high NGAL expression was associated with a decreased survival rate compared with low NGAL expression (40,41). Although high NGAL expression in tumor tissues is associated with the type of tissue, it is impossible to distinguish different histotypes of RCC based on NGAL expression, but it may help to predict the OS and DFS of patients with RCC.

With current technological limitations, the only clinical test of NGAL in biofluids is used to diagnose AKI, as its levels are associated with the severity of kidney injury in adults and neonates (44-46). Whether the NGAL level in biofluids can be used as a diagnostic and prognostic indicator for patients with kidney cancer is under investigation. Previous studies have revealed that elevated levels of urinary and serum NGAL in patients with RCC are independent of histotype, stage and grade (47,48). NGAL was not a sensitive or a specific urinary biomarker for RCC. Although the concentration of NGAL in urine (uNGAL) in 67 patients with renal cancer undergoing nephrectomy (0.52 ng/mg creatinine; range, 0.28-0.73 ng/mg creatinine) was statistically different from 55 patients undergoing non-nephrectomy (typically orthopedic) surgery (0.15 ng/mg creatinine; range, 0.04-0.31 ng/mg creatinine), only 8 patients had an uNGAL level with no overlap with the control group before nephrectomy. In addition, there was no significant association between uNGAL and tumor size with stage (47). In another study, Saint *et al* (49) revealed that high NGAL expression was associated with increased tumor stage and Furhman grade in patients with renal cancer and that high

uNGAL excretion was associated with poor progression-free survival (PFS) and disease-specific survival (DSS) in patients with ccRCC. Although uNGAL may not be suitable as a specific biomarker for RCC (6,47,48), elevated levels of NGAL in serum (sNGAL) are associated with decreased PFS in patients with RCC (39,50). Although high concentrations of NGAL in biofluids do not help to diagnose RCC, these are negatively associated with the prognosis of patients with RCC. Measuring the levels of NGAL may be helpful for selecting the appropriate treatment for patients with advanced RCC. Patients with advanced RCC were treated with sunitinib, a small molecule tyrosine kinase inhibitor with targets including vascular endothelial growth factor, platelet-derived growth factor receptor-α and β, and stem cell factor receptor, which has become one of the two main first-line treatments available for RCC (51). With 177 ng/ml being the cut-off value, low sNGAL predicted a longer PFS than high sNGAL and was superior to the best available clinical factor, the Motzer score (50). Furthermore, the expression levels of NGAL in RCC cells can be used as a predictive biomarker for sensitivity to sunitinib before targeted therapy (51).

Mechanism of NGAL in RCC. Since the synthesis of NGAL is induced by cancer-promoting factors, it is considered to serve a key role in the development and progression of human tumors (52). Previous studies have highlighted how NGAL is involved in the development, proliferation and invasion of cancer. In fact, elevated levels of this protein have been detected in the serum or urine of patients with different types of tumor, such as colon, breast, brain, thyroid, esophageal and bladder cancer (10). However, depending on the type of tumor, NGAL may have distinct roles in promoting or preventing cancer (10). Bolignano *et al* (52) observed that when NGAL acts as an intracellular iron carrier and protects MMP-9 from degradation, it has a significant cancer-promoting effect, as identified in human tumors including breast, stomach, esophageal, rectal, thyroid and brain tumors. By contrast, Zhang *et al* (53) reported that NGAL inhibited the production of the cancer-promoting factor hypoxia-inducible factor 1 (HIF-1), phosphorylation of focal adhesion kinase and synthesis of vascular endothelial growth factor (VEGF), and had an antitumor and anti-metastatic effect on colon, ovarian and pancreatic tumors.

In a recent study of RCC, Rehwald *et al* (41) revealed that iron-loaded NGAL had tumor-promoting effects in RCC cell lines, while iron-free NGAL had the opposite effect. In another study, Yu *et al* (51) reported that NGAL promoted RCC cell proliferation by enhancing the activation of the Ras-GTP, Erk1/2 and STAT1a signaling pathways. Currently, a number of hypotheses have been proposed regarding the possibility of NGAL promoting tumor progression, most of which involve the binding of NGAL to MMP-9 or the involvement of NGAL in iron uptake (41,54). Based on the aforementioned literature, it is likely that NGAL may serve a role in the development, progression, invasion and metastasis of RCC through the aforementioned pathways (Fig. 2).

MMP-9 is a gelatinase that degrades a wide range of substrates, including collagen, fibronectin and laminin, to promote tumor invasion and metastasis (11). In addition, numerous experimental evidence supports that MMP-9 is

Table II. Studies of NGAL as a biomarker for the diagnosis and prognosis of RCC.

First author, year	Type of cancer	Sample	NGAL measuring method	Main outcome(s)	Refs.
Barresi et al, 2010	Renal tumor	Tissue	IHC	1. Increase in NGAL expression was parallel to the histological grade of the tumors in ccRCC and pRCC. 2. High NGAL expression was significantly associated with pRCC and chRCC histotype.	(13)
Zhang et al, 2015	RCC	Tissue	IHC	1. NGAL expression was a significant predictor of decreased OS and DFS in pRCC, but not in ccRCC. 2. In pRCC, NGAL expression was significantly associated with Fuhrman grade, tumor size, TNM stage and presence of lymph node metastases.	(38)
Perrin et al, 2011	ccRCC	Tissue/serum	IHC/ELISA	1. By IHC, NGAL was not expressed in renal tumor cells but was expressed in neutrophils infiltrating ccRCC tissue. 2. High NGAL concentrations in serum (>150 ng/ml) were associated with shorter PFS. 3. High concentrations of NGAL-MMP-9 complex (>15 ng/ml) in serum were associated with short PFS and poor OS.	(39)
Morrissey et al, 2011	RCC	Urine	ELISA	1. Levels of uNGAL were not significantly associated with tumor size or stage.	(47)
Di Carlo, 2013	16 ccRCC, 4 oncocytoma	Serum/urine	ELISA	1. Values of sNGAL and uNGAL in patients with ccRCC were not increased compared with the mean values of healthy subjects.	(48)
Shalabi et al, 2013	RCC	Urine	ELISA	1. uNGAL is not suitable as a specific biomarker for RCC.	(6)
Saint et al, 2017	Kidney tumors	Urine	ELISA	1. uNGAL was associated with tumor stage, and Furhman grade. 2. uNGAL excretion was associated with ccRCC PFS and disease-specific survival.	(49)
Porta et al, 2010	Advanced RCC	Serum	ELISA	1. sNGAL can predict a longer PFS in patients with kidney cancer treated with sunitinib malate.	(50)
Liu et al, 2018	ccRCC	TCGA database	Data analysis	1. NGAL significantly predicted the clinical outcome of patients with ccRCC.	(40)
Rehwald et al, 2020	ccRCC	TCGA database/tissue	Data analysis/ Immunofluorescence staining	1. There was a significantly decreased patient survival probability associated with higher NGAL expression. 2. A significant increase in NGAL protein expression was observed in ccRCC samples, which was associated with tumor grade and tumor stage.	(41)

NGAL, neutrophil gelatinase-associated lipocalin; RCC, renal cell carcinoma; IHC, immunohistochemistry; ccRCC, clear cell RCC; pRCC, papillary RCC; chRCC, chromophobe RCC; OS, overall survival; DFS, disease-free survival; TNM, tumor-node-metastasis; PFS, progression-free survival; MMP-9, matrix metalloproteinases-9; uNGAL, urinary NGAL; sNGAL, serum NGAL; TCGA, The Cancer Genome Atlas.
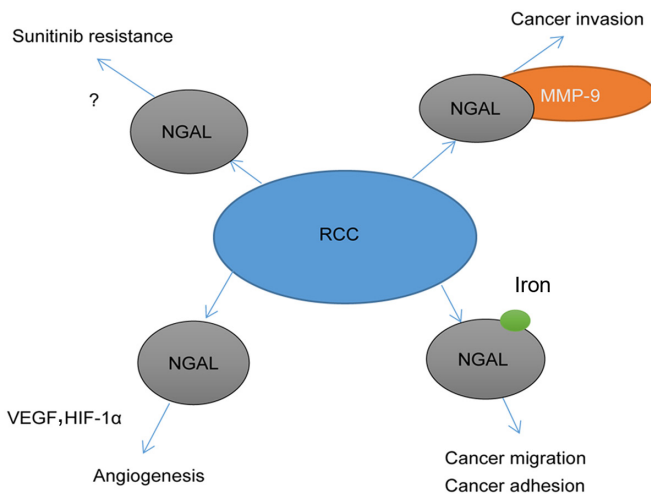
Figure 2. Potential biological mechanisms of NGAL in RCC. NGAL, neutrophil gelatinase-associated lipocalin; RCC, renal cell carcinoma; MMP-9, matrix metalloproteinases-9; VEGF, vascular endothelial growth factor; HIF-1α, hypoxia-inducible factor 1α.

directly involved in angiogenesis (55,56). NGAL can regulate the activity of MMP-9 by binding to pro-MMP-9 and forming a ternary complex to decrease the degradation of MMP-9 (57). In a ccRCC cohort, high levels of the MMP-9/NGAL complex in patient serum were associated with shorter PFS and lower OS than low levels of the complex (39), suggesting that NGAL may promote invasion and metastasis of cancer by binding with MMP-9 in ccRCC.

Due to the high proliferation rate of tumor cells, large amounts of iron are required to maintain their enhanced metabolic conversion (58). The unique iron transport capacity of NGAL is crucial to its tumor-promoting ability. Several studies have explored the mechanisms by which NGAL promotes human cancer, emphasizing how NGAL promotes iron uptake in the extracellular space of malignant cells to sustain tumor cell proliferation. In breast cancer, for example, blocking the release of iron-loaded NGAL by inhibiting tumor-associated macrophages significantly decreased tumor growth (59). Rehwald *et al* (41) found a significant increase in NGAL expression, especially iron-loaded NGAL, in tumor tissues of patients with RCC compared with that of adjacent healthy tissues. Iron-loaded NGAL accounts for ~2% of NGAL in healthy tissues and ~20% of NGAL in tumor tissues (41). Similarly, in a study with RCC cell lines, compared with iron-free NGAL and mutated NGAL (which was unable to bind to iron), iron-loaded NGAL significantly enhanced the migration and adhesion of RCC cells (786-O, RCC4, A498 and CAKI1), but cell proliferation remained unchanged after stimulation with the aforementioned three molecules (41). These results indicate that although iron-loaded NGAL cannot promote the proliferation of RCC cells, it may be able to promote the invasion and metastasis of RCC by enhancing the migration and adhesion of RCC cells.

In addition to the aforementioned hypotheses, NGAL is considered to be a cytokine that promotes angiogenesis in tumors (60). Overexpression of NGAL in a ductal adenocarcinoma cell line (PANC1) characterized by low endogenous NGAL expression significantly enhanced tumor invasion, adhesion and growth, and promoted VEGF and HIF-1α expression, which serve an important role in cancer angiogenesis (11). However, Ferreira *et al* (61) argued that in the presence of ferrous iron, NGAL is involved in iron absorption, which inhibits HIF-1α. In addition, NGAL has similar features to NF-κB, such as the potential to protect thyroid cancer cells from apoptosis induced by growth factor deprivation (11). However, to the best of our knowledge, the aforementioned findings have not been verified in RCC.

4. Discussion

To the best of our knowledge, the present review was the first to evaluate the possible role of NGAL in RCC. Previous studies on NGAL have found that NGAL expression varies among different types of cancer, and its role may be different. In breast cancer (62) or thyroid cancer (63), overexpression of NGAL enhances cancer cell motility and invasiveness, while in pancreatic cancer (64), overexpression of NGAL decreases tumor volume, along with local and distant metastasis. Therefore, the present review aimed to address the role of NGAL in RCC.

NGAL can be detected in most RCCs. Studies of the TCGA and GEO databases revealed that NGAL gene expression in RCC tissues was decreased compared with that in normal tissues (40,41), while RCC samples had variable levels of NGAL protein expression. There was no significant association between NGAL expression and patient age, sex, serum iron level, tumor type, tumor size or tumor stage (13), but it may be useful for predicting patient prognosis. In addition, different studies have not obtained consistent results on NGAL expression in RCC and on whether NGAL is located in the cytoplasm or the cell membrane, whether it is secreted by neutrophils, RCC infiltrating cells or cancer cells, and why the gene and protein expression levels of NGAL are inconsistent, which requires further investigation.

NGAL has attracted considerable attention as a tumor biomarker. Whether NGAL expression in biological fluids can be used as a diagnostic and prognostic biomarker in patients with RCC is currently being investigated. Based on the existing studies, although uNGAL may be associated with tumor stage and grade, as well as PFS and DSS in patients with RCC (49), it may not be suitable as a specific diagnostic biomarker for RCC. By contrast, sNGAL may be more promising. Increased sNGAL was associated with decreased PFS in patients with RCC (39,50). Additionally, sNGAL may be used to select patients with RCC who are suitable for sunitinib treatment (51). However, whether uNGAL or sNGAL may be utilized for the diagnosis and prognosis of RCC remain controversial, as there are numerous problems to be solved. For example, no studies have confirmed that uNGAL or sNGAL originates from RCC tissues. In addition, the cut-off value for NGAL concentration in serum or urine used to distinguish between healthy individuals and patients with RCC is not always clear. In the future, the detection of NGAL in blood and urine of patients with RCC should be further studied.

Research on the role of NGAL is helpful for the treatment of patients with RCC. NGAL is likely to promote invasion and metastasis of RCC cells by binding to MMP-9, and it may also promote cancer development by binding to iron or inducing angiogenesis via VEGF and HIF-1 (11). Experiments

in vitro have proven that iron-loaded NGAL can contribute to the migration and adhesion of RCC cells, but it has no effect on the proliferation of tumor cells (41). Blocking the binding of NGAL to MMP-9 or iron may inhibit the invasion and metastasis of RCC. Cancers characterized by high NGAL expression, such as chRCC and pRCC, may be better candidates for treatment with anticancer agents that function as iron chelators than cancers with low NGAL expression (65). In addition, a previous study has revealed that patients with RCC develop resistance to sunitinib by upregulating NGAL expression to activate the angiogenesis pathway (51). Therefore, we speculate that the inhibition of NGAL may decrease resistance to sunitinib.

In conclusion, although NGAL in biofluids cannot be used as a diagnostic marker for early screening of RCC, NGAL expression in serum, urine or tumor tissues may be used to predict the prognosis of patients with RCC. The prognostic value of NGAL in patients with RCC requires to be further investigated. Further research on NGAL may be helpful to decrease sunitinib resistance and identify new treatment strategies for RCC.

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Authors' contributions

MZ designed the study, and KC drafted the manuscript and revised the manuscript together with WH. HN reviewed the manuscript and gave final approval of the version to be published. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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