REVIEW

Implication and role of neutrophil gelatinase-associated lipocalin in cancer: lipocalin-2 as a potential novel emerging comprehensive therapeutic target for a variety of cancer types

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Abstract

Cancer is a leading cause of mortalities worldwide. Over the past few decades, exploration of molecular mechanisms behind cancer initiation and progression has been of great interest in the viewpoint of both basic and clinical scientists. It is generally believed that identification of key molecules implicated in cancer pathology not only improves our understanding of the disease, but also could result in introduction of novel therapeutic strategies. Neutrophil gelatinase-associated lipocalin (NGAL)/lipocalin-2 (LCN2) is a member of lipocalin superfamily with a variety of functions. Although the main function of LCN2 is still unknown, many studies confirmed its significant role in the initiation, progression, and metastasis of various types of cancer. Furthermore, aberrant expression of LCN2 is also concerned with the chemo- and radio-resistant phenotypes of tumors. Here, we will review the contribution of known functions of LCN2 to the pathophysiology of cancer. We also highlight how the deregulated expression of LCN2 is associated with a variety of fatal types of cancer for which there are no effective therapeutic modalities. The unique and multiple functions of LCN2 and its widespread expression in different types of cancer prompted us to suggest LCN2 could be considered either as a valuable diagnostic and prognostic biomarker or as a potential novel therapeutic target.

Keywords LCN2/NGAL · Neoplasms · Targeting therapy · Metastasis · Chemoresistance

Introduction

Cancer is undoubtedly one of the leading causes of disease-related mortalities worldwide. Every year, millions of new cases are diagnosed with cancer and also millions of cancer-related deaths are reported. The high mortality rate of the disease has many different reasons including late diagnosis of the disease due to inefficient screening and diagnostic programs, inadequate insight on the mechanisms behind the disease, and resistance of tumors to existing treatment options. Most of these major obstacles can be lifted as our understanding of the disease and its underlying mechanisms at cellular and molecular levels is improved. A wide range of studies over the past few decades have recognized numerous genes with different expression patterns between cancerous cells and their normal origins. The altered expression profiles could be used for prognostic and diagnostic purposes. Besides, further investigations into the candidate genes, not only would expand our knowledge about the underpinning

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mechanisms of the disease, but also might introduce novel therapeutic targets [1–6].

Recently, a growing number of studies introduced lipocalin-2 (LCN2) as an important gene which is strongly associated with various aspects of malignancies including their emergence, progression and resistance to chemo- and radio-therapy strategies [6–20]. LCN2 is a small secretory protein which is known for its critical roles in iron transportation [21–23], and also, as an anti-bacterial agent of the innate immune system [24]. However, recently high levels of LCN2 expression have been reported in several types of cancer with different histological origins including breast cancer [14, 25–39] esophageal carcinoma, and prostate cancer [3, 7, 16, 40–42].

This review highlights the significant role of deregulated expression of LCN2 in a variety of fatal types of cancer for which still there are no efficient therapeutic modalities. Furthermore, different known functions of LCN2 and its contribution to the pathophysiology of various cancers will be demystified. As it will be discussed, LCN2 not only could be considered as a valuable biomarker for diagnostic and prognostic purposes, but also is a potential therapeutic target for a broad range of malignancies.

LCN2 structure

Neutrophil gelatinase-associated lipocalin (NGAL), aka LCN2, is a 178-amino acid protein that exists in three molecular forms of monomer (25 kDa), homodimer (45 kDa), and heterodimer (135 kDa), in which it is associated with matrix metalloproteinase-9 (MMP-9) [43, 44].

"Lipocalin fold" is the distinctive feature of the structure of lipocalins comprising an N-terminal 310 helix followed by eight β-sheets (A–H) in an antiparallel direction. The eighth β-sheet is followed by an α-helix (α1), which is then connected to a C-terminal β-sheet (β-sheet I). The β-sheets are consecutively connected by several loops (L1–L7). The L1, L3, L5, and L7 create open end of the molecule, i.e. its ligand-binding site. Within the lipocalin fold of various lipocalins there exist several sites with conserved amino acid sequences or secondary structures [2] (Fig. 1II). Through the open end, LCN2 binds to certain hydrophobic ligands such as siderophores (a group of iron transporter proteins of either bacterial or mammalian origin), and transports them within the body or between adjacent cells [45, 46].

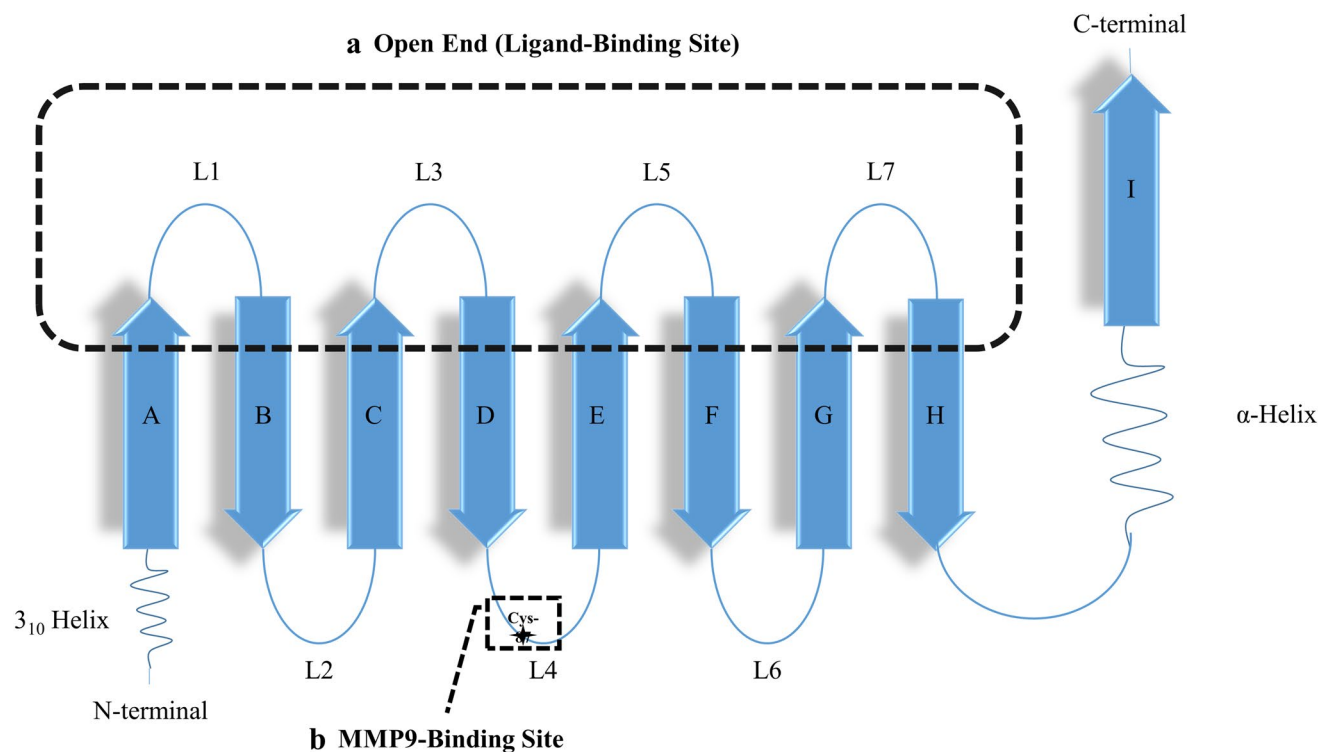LCN2 also forms a heterodimer complex by binding to matrix metalloproteinase-9 (MMP-9) which is a type of

Fig. 1 **I** Secondary structure of LCN2. The secondary structure of LCN2 includes several β-sheets (A to I), loops (L1 to L7), an α-helix, and a 310-helix. L1, L3, L5, and L7 form a site in LCN2 structure, which mediates binding of some certain hydrophobic ligands to the LCN2. Moreover, a specific cysteine residue (Cys-87) between the 4th and the 5th beta-sheets forms a disulfide bond with a certain cysteine residue of MMP-9, which leads to the formation of LCN2/MMP-9 complex. **II** 3D structure of LCN2 bound to a bacterial siderophore

human gelatinase. The Cysteine 87 (Cys-87) residue of LCN2 forms a disulfide bond with an unidentified cysteine residue in hemopexin-like C-terminal domain (PEX) of MMP9 [47, 48]. MMP-9 alone is highly unstable. However, its stability and thus its activity is significantly increased when it is associated with LCN2.This stabilizing effect is very important from both physiological and pathological points of view.

LCN2 Functions

LCN2 has a wide spectrum of functions ranging from anti-bacterial effects in the innate immune system to anti-oxidant activities in cells encountering high levels of reactive oxygen species (ROSs). This multifaceted protein is also involved in metabolic hemostasis, apoptosis modulation, regulation of inflammation and transportation of small ligands such as iron and fatty acids. LCN2 serves an important role in the innate immune system. It is secreted by immune cells and sticks to the siderophores, preventing them from delivery of sufficient iron loads to bacterial cells, thus inhibiting bacterial growth [49, 50]. Moreover, LCN2 is strongly associated with multiple inflammatory diseases such as psoriasis and periodontitis in which its expression is upregulated by neutrophils [48]. It is also secreted by various types of cells under oxidative stress. This consequently upregulates various anti-oxidants and enhances survival of the cells [4, 51–57]. LCN2 has been also implicated in cancer progression and chemoresistance. In this regard, it might act as either a pro- or an anti-tumorigenic factor depending on tumor type. This contradictory effect will be discussed in Sect. 2.2.

LCN2 and cancer

Cancer is one of the areas in which LCN2 plays many critical roles. It has significantly higher expression levels in patients suffering from cancer, and in tumor cell lines when compared to controls. This protein promotes proliferation of malignant cells and significantly enhances their motility and invasiveness. In vivo studies have shown lower survival rates and higher metastasis in mice with higher levels of LCN2. LCN2 is also involved in resistance of cancer cells to some chemo- and radio-therapeutic modalities. Each of these effects, which are exerted through different pathways in different types of tumors, will be thoroughly discussed in the following sections.

Proliferation of tumor cells

LCN2 expression is upregulated at both transcriptional and translational levels in nearly all types of cancer. Moreover, univariate and multivariate studies have demonstrated that LCN2 expression positively correlates with tumor grade and invasiveness. Hence, LCN2, alone or alongside other related molecules such as MMP-9, could be used as a strong diagnostic or prognostic biomarker in a majority of cancer types. Considering the relatively small size of LCN2, it can be easily detected, not only in tissue biopsies but also in body fluids such as blood and urine samples, which are less invasive to be collected for biomarker detection. Mechanistic studies revealed that LCN2 supports tumor cells proliferation through several pathways which are different among various types of cancer. However, it is worth mentioning that LCN2 sometimes shows anti-tumorigenic effect, as is the case with oral cancer. There are several ways through which LCN2 interferes with tumor progression, although most of them are mediated by iron-dependent fashions (Fig. 2). In the following sections, the relationship between LCN2 and tumor cell growth and proliferation in different types of cancer will be discussed.

Breast cancer

High expression level of LCN2 has been reported in both epithelium and stromal tissues, and also in urine samples of patients with breast cancer. There is a strong correlation between LCN2 expression and clinicopathological features of patients suffering from breast cancer. Therefore, it is strongly suggested to consider LCN2 as a valuable biomarker in breast cancer [31, 34, 39, 58–63].

Moreover, LCN2 expression is also correlated with human epidermal growth factor receptor 2 (HER2/neu) and estrogen receptor (ER) status, and poor overall and disease-free survival rates in breast cancer patients [34, 59, 64–66]. There are two subtypes of breast tumor cells including ER^+ and ER^- cells. The ER^+ cells express high levels of ER and grow more rapidly in response to estrogen while the ER^- cells are less estrogen responsive. Therefore, hormone-therapy is a suitable therapeutic choice for the ER^+ subtype. ER and LCN2 inhibit the expression of each other in breast tumor cells. Therefore, in the ER + cells in which expression of ER dominates LCN2, the aggressiveness of the cells is declined, while in the ER^- cells it is completely reverted [27]. It has been reported that the LCN2 overexpression reduces ER expression which renders MCF7 cells less sensitive to the hormone therapy [39]. Therefore, LCN2 silencing can therapeutically be considered as a suitable approach especially in the ER^- subtype of breast cancer; where hormone-therapy could not be effective.

In an in vivo study, the size of xenograft tumors developed by injection of LCN2-overexpressing MCF7 cells in nude mice showed to be significantly bigger than the tumors resulting by injection of parental MCF7 cells [67]. In another study on $ErBb2^+$ breast cancer xenograft mice the size of the tumors showed to be in the order of LCN2 +/+
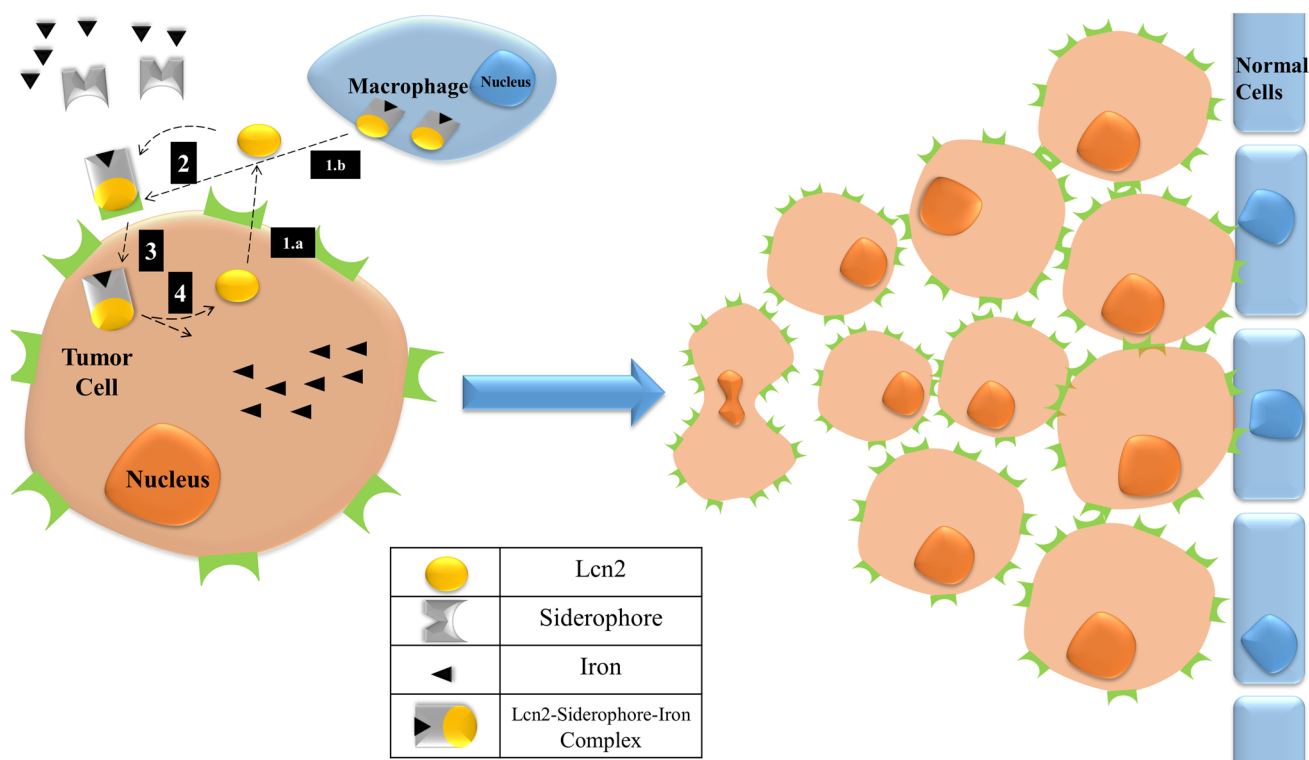
Fig. 2 LCN2 promotes tumor cells proliferation, mostly in an iron-dependent manner. *1.a* Tumor cells increases expression and secretion of LCN2 into the tumor microenvironment *1.b* Likewise, in breast cancer tissues, tumor-associated macrophages (TAMs) (which contain high amount of iron), further elevate the tumor cells intracellular levels of iron, through transfer of iron-loaded LCN2 molecules to the tumor cells. *2* In the tumor microenvironment, the LCN2 molecules (secreted by the tumor cells) are loaded with iron. *3* The iron-loaded LCN2 molecules (originated from either the TAMs or the tumor cells themselves), are internalized into the tumor cells. *4* The internalized LCN2 molecules release their iron ions inside the tumor cells which significantly accelerates the tumor cells proliferation

mice > LCN2 +/− mice > LCN2 −/− knockouts [34]. Moreover, it was revealed that dying breast tumor cells stimulate tumor-associated macrophages (TAMs) by releasing sphingosine 1-phosphate (S1P) resulting in increased LCN2 production and secretion by TAMs. This is mediated by a Janus Kinase/Signal Transducer and Activator of Transcription-3 (JAK/STAT3) pathway, and in turn promotes tumor growth in vivo [32]. Likewise, interleukin-10 (IL-10) can also induce macrophages to overexpress and secrete LCN2 into their culture medium which consequently fosters MCF7 cells proliferation [68]. Besides TAMs, it has been proposed that adipose tissue-derived LCN2 can also boost up breast cancer cells proliferation [26]. Taken all together, these studies demonstrate that LCN2, of either tumor or stromal origin, remarkably accelerate breast tumor progression.

Esophagus cancer

Several studies have shown that there is a positive correlation between the high expression of LCN2 or its receptor (LCN2R) and the poor prognosis of patients with esophageal cancer [15, 18, 69]. Likewise, multivariate studies indicated

that both LCN2 and LCN2R might serve as two independent prognostic markers in esophageal carcinoma [15, 18, 69]. However, despite remarkable effects on cell migration and invasion, several studies have declared that LCN2 overexpression/knockdown does not affect cell proliferation and tumor growth neither in vitro nor in vivo in esophageal cancer [69].

Thyroid cancer

LCN2 and MMP-9 are highly expressed in thyroid carcinoma, especially in aggressive and metastatic subtypes [70, 71]. According to a study, LCN2 silencing in thyroid cancer cells attenuates their survival against serum-deprivation (SD)-induced apoptosis. This anti-apoptotic function of LCN2 is mediated through an iron-dependent manner since following addition of holo-LCN2 or iron-loaded ferritin to the culture medium of the cells, their resistance to the SD-induced apoptosis is significantly improved, while the addition of iron chelators declines the resistance [70].

It has been also suggested that LCN2 could serve as a valuable, sensitive, and specific diagnostic biomarker, which

can distinguish papillary thyroid cancer cases from benign lesions and normal cases. However, its expression pattern was shown to be not significantly different among the three papillary thyroid cancer subtypes, i.e. papillary thyroid carcinoma-classic, papillary thyroid carcinoma-follicular variant, and papillary thyroid micro-carcinoma [11, 72].

Mouth cancer

Unlike the previously discussed cancers, some studies indicated that LCN2 is downregulated in oral cancer [2, 73]. LCN2 expression is even further declined in parallel with oral cancer progression especially in metastatic subtypes [74]. Oral cancer patients expressing high levels of LCN2 show longer survival rates. Altogether, these findings indicate that LCN2 is associated with a good prognosis and can be used as a suitable prognostic biomarker in oral cancer. One of the mechanisms through which LCN2 exerts its anticancer effects in oral cancer might be related to autophagy. According to a study, LCN2 silencing reduces autophagy in oral cancer cells through activation of the mTOR signaling pathway. The reduced autophagy contributes to oral cancer cells survival and tumor progression [2]. Nevertheless, further studies are still needed to unravel the exact mechanism(s) by which LCN2 is involved in the suppression of oral cancer.

Liver cancer

LCN2 and LCN2R are overexpressed at both mRNA and protein levels in hepatocellular carcinoma (HCC) patients and HCC tissues [75, 76]. Furthermore, according to survival analyses, the higher expression level of LCN2 is strongly associated with poor prognosis of the HCC patients, which showed by their shorter overall survival than those with lower LCN2 expression. This higher expression level revealed a positive correlation with poor clinicopathological features of the patients, including vascular invasion, TNM stage, and tumor recurrence. These findings suggest LCN2 as a valuable prognostic factor and/or a potential therapeutic target in HCC [75, 76].

Pancreatic cancer

LCN2 had shown controversial effects on tumor progression and metastasis in pancreatic cancer. In some studies it has been revealed that LCN2 expression is higher in pancreatic cancer patients or corresponding cell lines, compared to normal samples, and this higher expression level was strongly correlated with poor prognosis and shorter survival rates [77–83]. It has been reported by an in vivo study that *lcn2 −/−* obesity-driven and/or syngeneic mice bearing pancreatic ductal adenocarcinoma (PDAC), had longer survival rates compared to their *lcn2 +/+* counterparts. Furthermore, tumor volume in the *lcn2 −/−* mice bearing syngeneic PDAC was significantly lower than the *lcn2 +/+* subjects [78].

The absence of a sensitive and specific biomarker is one of the major obstacles in diagnosis/prognosis of pancreatic cancer. Therefore, despite the controversies on the correlation between LCN2 level and progression of pancreatic cancer, a number of studies proposed it as a potential biomarker [79, 84–86]. It has been found that LCN2 is substantially downregulated after treatment of PDAC cells with epidermal growth factor (EGF) [87]. E-cadherin downregulation by short hairpin RNA constructs attenuated LCN2 expression in PDAC cells. On the other hand, overexpression of E-cadherin resulted in increased LCN2 expression, and to some extent, antagonized the EGF-mediated suppression of LCN2. These mechanisms are exerted partly via activation of the epidermal growth factor-mitogen-activated protein kinase-extracellular signal-regulated kinases (EGFR-MAPK-ERK) signaling pathway, which downregulated E-cadherin with a consequent decrease in the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation (a well-known LCN2 promoter-activating transcription factor) [87].

In contrast, other studies suggested that LCN2 might be an anti-tumorigenic factor. For instance, Xu Bi and his colleagues tried to cure PDAC using oncolytic viruses genetically manipulated to express high levels of LCN2. Their in vitro and in vivo findings revealed effective suppression of the tumors through cytolysis and apoptosis [83].

Taken all together, apparently, the exact function of LCN2 in pancreatic cancer progression is still controversial and unclear, and thus requires further studies to be illuminated.

Prostate cancer

LCN2 expression in primary and advanced stages of prostate cancer is higher than normal prostate tissue [7, 16]. This higher expression level is consistently associated with the clinicopathological features of patients [42]. In a prospective study, LCN2, in combination with prostate-specific antigen (PSA), has been suggested as a valuable diagnostic biomarker to discriminate prostate cancer from benign prostatic hyperplasia [41].

Several in vitro and in vivo studies demonstrated that LCN2 overexpression promotes proliferation of prostate cancer (PCa) related cells and tumors, while LCN2 downregulation or knockout produces the opposite results [16, 42, 88]. For example, in a recent study, LCN2 knockout in a castration-resistant prostate cancer cell line, PC3, using clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas9) gene editing tool, substantially abrogated cell growth, colony-forming ability, and survival, which is partly induced by apoptosis [88]. Furthermore, in two different studies using xenograft mice, it was

revealed that the tumors resulting from injection of LCN2-knockdown PCa cells, grow slower than controls, while the tumors resulting from injection of LCN2-overexpressing PCa cells, produced opposite results [16, 42]. Although the exact mechanism underlying these effects has not been fully investigated yet, Ding and coworkers suggested that androgen receptor activities might be involved in these LCN2-mediated pro-tumorigenic effects in prostate cancer, at least in castration-resistant subtypes [16].

Increased cell proliferation has been shown following overexpression of LCN2 in normally low-LCN2-expressing prostate cancer cell lines. Conversely, inhibition of LCN2 in these cell lines, including castration-resistant subtypes, decreased tumor growth [88]. Consistently, in xenograft nude mouse models, tumors resulted by injection of LCN2-knockdown prostate cancer cells shows significantly lower growth rates than control groups [7, 16, 42].

A majority of primary prostate cancers are androgen-dependent. Androgen deprivation therapy can be an effective strategy to inhibit these types of prostate cancer. However, this strategy is not suitable for castration-resistant prostate cancer (CRPC) in which tumor cells are hormone-refractory and do not respond to androgen deprivation therapy. LCN2 is involved in the castration resistance process by increasing androgen receptor (AR) transcriptional activity. LCN2 has markedly higher expression in CRPC, compared to primary castration-sensitive prostate carcinoma. Interestingly, LCN2 upregulation in prostate tumor cells increases expression of SLC45A3, a downstream gene of AR, but does not affect AR expression [16].

LCN2 is also involved in endoplasmic reticulum (ER) stress-mediated tumorigenesis in prostate cancer [40]. Tumor cells can adapt to ER stress through a conserved set of intracellular pathways altogether known as the unfolded protein response (UPR) [89]. Of note, ER stress induces LCN2 expression in human and murine prostate tumor cells in the NF-κB- and the UPR-dependent manners [40].

Lung cancer

LCN2 is overexpressed in lung cancer and its expression increases even further in metastatic subtypes [90]. For example, LCN2 expression showed to be upregulated at both mRNA and protein levels in lung adenocarcinoma tissues or even exosomes, compared to their normal adjacent tissues [91–93]. Importantly, in another study, it was suggested that LCN2 not only can serve as an advanced-stage biomarker but also could be used as an early-stage diagnostic biomarker of lung adenocarcinoma, which is of great importance, because most of lung cancer cases are usually diagnosed late and at lethal stages [94]. In a retrospective study, it has been shown that there is a remarkable positive correlation between LCN2 level and overall survival rate of patients with lung cancer [95]. According to the results of a phase II clinical trial on patients suffering from lung cancer, those who inherently had higher serum levels of LCN2, poorly responded to vandetanib treatment and showed lower progression-free survival rates [96].

Downregulation of LCN2 in lung adenocarcinoma cell line, A549, resulted in inhibition of cell proliferation and apoptosis induction. According to this in vitro study, LCN2 exerts its anti-apoptotic effect through the nuclear factor erythroid 2–related factor 2/ heme oxygenase-1 (Nrf2/HO-1) pathway. In other words, the downregulation of LCN2 in A549 cells increased activity of reactive-oxygen species (ROSs) by disrupting the Nrf2/HO-1 signaling pathway, which serves very important anti-oxidant and anti-apoptotic roles. Consistently, preclinical experiments also showed slower lung tumor formation in nude mice following depletion of LCN2 expression (91). All these findings together, suggest that LCN2 plays pro-tumorigenic roles in lung cancer, in part by suppressing oxidative stress-mediated apoptosis and elevating survival of cancer cells.

Kidney cancer

There are three different histologic subtypes of kidney cancer: clear cell renal carcinoma (CCRC), papillary cell renal carcinoma (PRC), and chromophobe renal cell carcinoma. It has been reported that the PRC and the chromophobe subtypes have the highest levels of LCN2. Besides, there is a positive correlation between LCN2 level and tumor aggressiveness, and a negative correlation between the LCN2 level and progression-free survival (PFS) of patients with kidney cancer [10, 97]. Moreover, high concentration of LCN2/MMP-9 complex in serum is associated with shorter PFS and poor overall survival of the CCRC subtype. Intriguingly, the LCN2 is expressed by neutrophils infiltrating CCRC cells, and the density of LCN2-expressing neutrophils is strongly associated with pejorative PFS and survival [98]. Furthermore, LCN2 expression showed a positive association with a key angiogenic protein, vascular endothelial growth factor (VEGF), in renal cancer. However, in another study, it has been suggested that LCN2 is not a sensitive or specific urinary biomarker of kidney cancer, both in CCRC and PCRC subtypes [99]. These findings suggest that LCN2 can be a good candidate for targeted therapy of renal cancer.

Colon cancer

LCN2 has higher expression in tumor tissues than in normal adjacent tissues of patients with colorectal cancer (CRC). There is also a positive correlation between LCN2 and MMP-9 expression, and clinicopathological features of the patients with CRC. The LCN2 upregulation was significantly correlated with the depth of invasion, lymph

node metastasis, venous involvement, and advanced pTNM (tumor, node, metastasis) stage, which are characteristics of an aggressive phenotype [100]. The LCN2 expression is upregulated in parallel with tumor progression suggesting this factor might be a good predictor of overall survival (OS) of patients with CRC [101]. It was revealed that high serum level of LCN2 is correlated with shorter disease-free survival (DFS) and higher neoplastic tissue volume in patients with metastatic CRC. These findings have confirmed the role of LCN2 in CRC tumor progression and malignant development. Likewise, in adenomatous polyposis coli multiple intestinal neoplasia (APCmin) mice, the LCN2 level of tumor tissues showed to be higher than those of normal intestinal epithelia of control mice. However, there was no significant difference between LCN2-null APCmin mice and LCN2-normal APCmin mice in terms of tumorigenesis, multiplicity, and invasiveness. These findings suggest that LCN2 is not a strong oncogene in intestinal cancer but still could be considered as a potential biomarker of poor prognosis in colorectal cancer studies [102].

A number of studies proposed LCN2 as a proto-oncogenic factor that is upregulated in CRC cells, however, some other studies gave the opposite results.

Ovarian cancer

LCN2 is upregulated in endometrial cancer, and also is strongly associated with aggressive features of patients suffering from endometrial cancer and poor survival rates [103–110]. It has been also suggested that LCN2 can be considered as a valuable prognostic and/or diagnostic biomarker of endometrial cancer. Furthermore, several mechanistic in vitro studies reported that either forced overexpression of LCN2 in endometrial cancer cells or treatment of these cells with recombinant LCN2, significantly accelerates the proliferation of the cells and prolongs their survival through reduction of apoptosis rate [1, 111]. In case of the treatment with recombinant LCN2, it was shown that LCN2 forced the cells to express interleukin-8 (IL-8), which mediated the anti-apoptotic effect of LCN2, by inhibition of caspase-3 activation [111]. These results suggest that LCN2 can be considered as a potential target for therapeutic intervention in endometrial cancer.

Leukemia

There are four main types of leukemia including acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML). The AML1-MTG8 fusion transcription factor generated by the t (8; 21) translocation alters the expression of several genes including LCN2. These genes are essential for normal differentiation and proliferation of hematopoietic progenitors and any changes in their expression may result in AML [112].

According to a series of studies, plasma samples of patients with CML have elevated LCN2 levels compared to healthy individuals. It has been also demonstrated that LCN2 is crucial for induction of leukemia in a CML mouse model [9, 113, 114]. LCN2 contributes to the breakpoint cluster region (Bcr)-Abelson murine leukemia viral oncogene (Abl)-mediated suppression of normal hematopoiesis, which is characteristic of CML. LCN2 is secreted by BCR-ABL-transformed-hematopoietic cells, and perturbs normal hematopoiesis in bone marrow and spleen by induction of apoptosis in normal hematopoietic cells (HCs) [9, 113]. Several murine hematopoietic cell lines (mHCs) transformed by the BCR-ABL oncogene, persistently expressed and secreted LCN2 to culture medium. This conditioned medium (CM) induced apoptosis in normal mHCs. However, the BCR-ABL-transformed-mHCs were resistant to the apoptotic effects of LCN2. The BCR-ABL-transformed-mHCs also induced leukemic phenotypes including attenuating normal hematopoiesis in the mice, which was significantly reversed by decreasing the expression of LCN2 [9, 113]. The reduction of LCN2 expression also inhibited the invasion of the BCR-ABL-transformed-mHCs in marrow and spleen [9, 113]. Normal HCs and a wide range of hematopoietic cell lines need interleukin-3 (IL-3) for their proliferation and survival. Nevertheless, the transformation of HCs with BCR-ABL abrogates this requirement. Normal HCs produce low levels of LCN2 but IL-3-starvation makes them secreting high amounts of LCN2 [115]. The BCR-ABL transformation renders HCs' growth independent of IL-3 since the BCR-ABL fusion protein makes HCs resistant to apoptosis [115]. In the absence of IL-3, which is an inhibitor of LCN2 expression, normal HCs secret high levels of LCN2 into culture medium which induces apoptosis in HCs through activation of the pro-apoptotic protein Bad even after addition of IL-3 into the medium [115]. BCR-ABL-transformed HCs which are capable of constitutive expressing high amounts of LCN2 independent of IL-3, are resistant to the apoptosis induced by LCN2. The BCR-ABL prevents LCN2-induced apoptosis through inhibition of the B-cell lymphoma 2 (Bcl-2)-associated death promoter (Bad) pro-apoptotic protein, and also by activating the anti-apoptotic proteins Bcl-XL and Bcl-2 [116]. BCR-ABL oncoprotein increases LCN2 expression but decreases LCN2R expression in murine HCs. Since LCN2 performs its pro-apoptotic function in murine HCs through binding to its receptor, therefore the BCR-ABL renders BCR-ABL-transformed-mHCs (but not nearby normal mHCs), refractory to pro-apoptotic effects of LCN2, which may ultimately lead to the dominance of the BCR-ABL-transformed-mHCs population over normal mHCs in co-culture settings or bone marrow.

Metastasis

Metastasis is a multi-step process through which cancer cells are spread from their initial sites to other sites of the body. This may lead to formation of new tumors. Nearly all types of tumors have an epithelial origin. In the epithelial tissues, cells are tightly connected, which significantly inhibits their motility and also blocks their access to the blood vessels. To be able to metastasize, cancer cells need to gradually lose their epithelial characteristics and gain more mesenchymal features, through a process that is known as epithelial to mesenchymal transition (EMT) [117]. In fact, during the EMT, tumor cells increase their migratory and invasive capacities [117]. LCN2 induces the EMT and metastasis in primary tumor cells through three main ways, including:

Upregulation of EMT-activator transcription factors (EMT-ATFs)

EMT is activated and mainly governed by a group of specific transcription factors (EMT-activator transcription factors or EMT-ATFs). There are three types of EMT-ATFs, including Snails, zinc finger e-box binding homeoboxs (ZEBs), and basic helix-loop-helix (bHLHs) [118]. LCN2 induces EMT in cancer cells through activation of some of these EMT-ATFs via a variety of signaling pathways. For example, in breast and prostate cancers, LCN2 promotes EMT through the upregulation of Slug, a well-known EMT-ATF from the Snail family (Fig. 3) [7, 39].

Upregulation of gelatinases

The ability to degrade the extracellular matrix (ECM) proteins and invade the basement membrane is mainly mediated through a group of enzymes called gelatinases. There are two main types of the gelatinases in human body, gelatinase

A or MMP-2, and gelatinase B or MMP-9 [119]. LCN2 promotes the invasiveness of cancer cells by upregulating both gelatinases, however in different ways. LCN2 enhances MMP-9 activity by binding to it and preventing it from auto-degradation [120, 121]. On the other hand, LCN2 provokes overexpression of the MMP-2 at transcriptional level, which can further assist the tumor cells to invade the basement membrane [42, 122].

Promoting angiogenesis

Angiogenesis enhances tumor progression through two main pathways. First, it feeds tumor cells and removes their waste products. Second, it supports tumor cells dissemination and metastasis to distant parts of the body [123–125]. LCN2 induces angiogenesis by upregulating VEGF, an important angiogenic factor, at the transcriptional level and also increases its bioavailability in the ECM (Fig. 4) [29, 32, 105, 126, 127].

In this section, we will explore the relationship between LCN2 and tumor metastasis, and how LCN2 is involved in different steps of tumor cells migration, invasion, and angiogenesis in different types of cancer.

Breast cancer

There is a consistent relationship between LCN2 level and metastasis in breast cancer. Patients with metastatic breast cancer have higher concentrations of LCN2 in their urine and blood samples, as compared to non-metastatic and/or healthy controls, and this overexpression is strongly associated with the tumors invasiveness and aggressiveness [39, 58–61, 63]. Induction of LCN2 overexpression promotes invasive behaviors in either human or murine breast cancer cells, while its downregulation in LCN2-overexpressing breast cancer cell lines significantly attenuated the aggressive properties of the cells such as migration and invasion [26, 30, 34, 38, 39, 64,
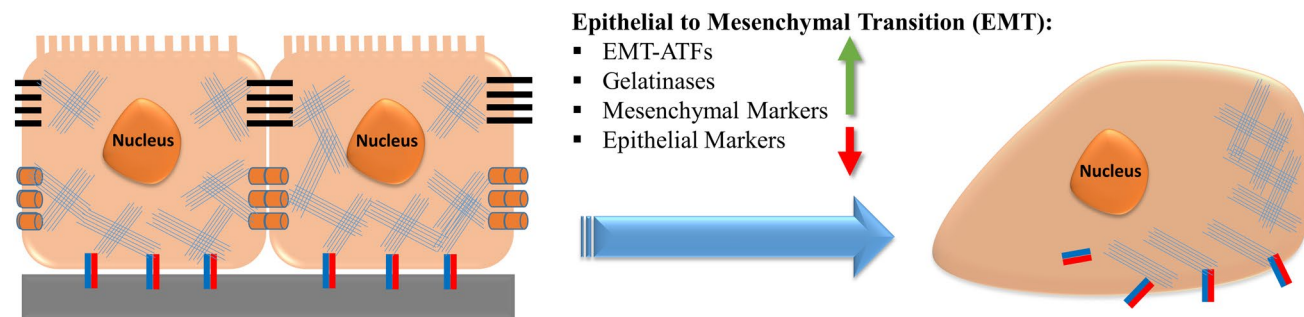

Epithelial to Mesenchymal Transition (EMT):
- EMT-ATFs
- Gelatinases
- Mesenchymal Markers
- Epithelial Markers

Fig. 3 LCN2 induces EMT through several mechanisms. For instance, in breast and prostate cancers, LCN2 stimulates expression of Slug, which is a well-known EMT-ATF. Furthermore, LCN2 also upregulates two main gelatinases i.e. MMP-2 and MMP-9. These

events change cell–cell adhesion, cell polarity, and cellular markers, which altogether alter the cells phenotype from epithelial to mesenchymal
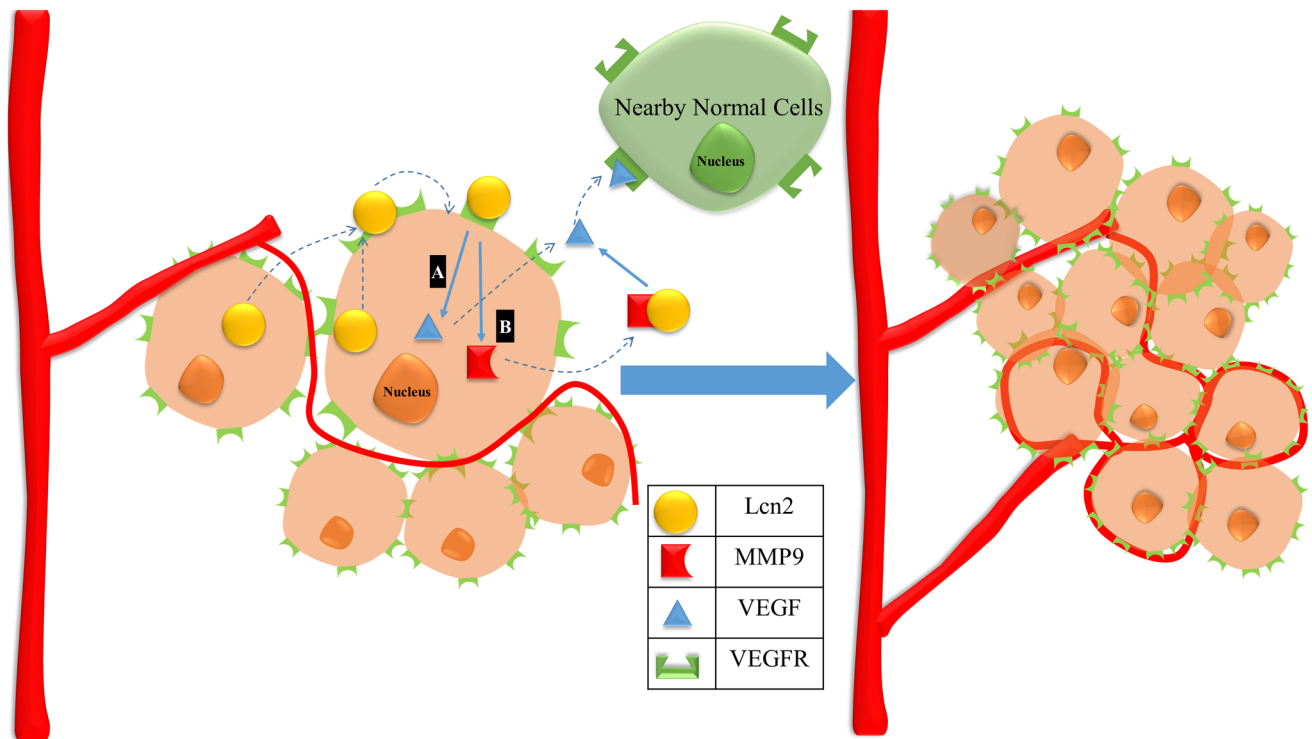
Fig. 4 LCN2 induces angiogenesis in tumor microenvironment. **a** LCN2 secreted by tumor cells forces them to over-secret VEGF. **b** LCN2 also elevates bioavailability of VEGF in the tumor microenvironment. In this regard, LCN2 induces overexpression of MMP-9, and improves its stability at the protein level as well. The stable and active MMP-9 enhances the bioavailability of VEGF through digesting its nearby ECM components

65]. Likewise, high-LCN2-expressing breast tumors exhibited a poorly differentiated phenotype and increased local tumor metastasis and lymph node invasion in orthotopic models [39]. As mentioned before, a group of transcription factors named EMT-ATFs plays a remarkable role either in the beginning or during EMT. LCN2 increases the expression of three key EMT-ATFs namely Slug, ZEB1, and Snail in breast cancer cells, which substantially enhances metastatic characteristics of the tumor cells. LCN2 also induces EMT by increasing expression of mesenchymal markers such as fibronectin and vimentin, and decreasing expression of epithelial markers such as E-cadherin [26, 37, 39].

LCN2 is also involved in modulation of breast cancer cells aggressiveness, through Hypermethylated in cancer 1 (HIC1)/LCN2 axis. HIC1 is a frequently deleted or epigenetically silenced tumor suppressor gene in human cancers, especially breast cancer, where its re-expression may suppress cancer progression. The silenced-expression of HIC1 has been reported only in triple-negative subtype of breast cancer (TNBC). The HIC1 silencing increases cell migration and invasion through upregulation of LCN2 in human breast cancer cells (only in the TNBC) [14]. Therefore, it seems that the HIC1/LCN2 axis might serve as a subtype-specific prognostic biomarker in the TNBC.

It has been shown that LCN2 increases angiogenesis in breast cancer cells in vitro and in vivo. LCN2 exerts this function mostly by increasing vascular endothelial growth factor (VEGF) level, a key angiogenic factor in many types of cancer. LCN2 knockdown in MDA-MB-231 TNBC cell line resulted in significantly decreased expression of VEGF, and thus impaired endothelial tube formation and angiogenesis [29]. Moreover, according to another study, mice injected with LCN2-overexpressing breast cancer cells developed bigger tumors that had higher angiogenetic activity as well [67]. According to a study conducted by Yang J and colleagues, the pathway (or at least one of the pathways) through which LCN2 causes these effects, begins with activation of hypoxia-inducible factor 1α (HIF-1α) through extracellular signal-regulated kinase (ERK) pathway, then, the augmentation of HIF-1α expression increases VEGF levels, which finally leads to the promotion of angiogenesis [128].

While almost all of the studies that have been discussed so far, had mainly focused on the tumor-cell-derived LCN2, it is very important to mention that there are also other sources of LCN2 that play significant roles in cancer-related processes. Tumor-associated macrophages (TAMs) reportedly are forced by breast tumor cells to increase their LCN2

yield. The TAM-derived LCN2 will provide the nearby breast tumor cells an additional source of iron, which is an essential factor for their proliferation and motility [20–22, 32, 37, 68]. The tumor stroma-derived LCN2 increases breast cancer cells proliferation and metastasis both in in vitro and in vivo settings [20, 36, 68]. For instance, in an in vitro study, the release of LCN2 from TAMs induced EMT in MCF7 cell line and promoted local migration and invasion into the ECM [37].

Nuclear factors of activated T-cells (NFATs) are a group of transcription factors comprising five members (NFAT1 to NFAT5) that participate in regulating cancer cells motility and invasiveness in several types of cancer such as breast cancer [129–132]. Recent studies have demonstrated that NFATs modulate the expression of LCN2 in breast cancer cells [27]. Different NFATs have distinct effects on the LCN2 expression. If a specific type of NFAT upregulates LCN2, it will promote cell migration and invasion, and vice versa. For example, NFAT3 which is specifically expressed in the ER + breast cancer cells suppresses LCN2 expression and inhibits their motility and invasiveness [27]. However, regarding NFAT1, LCN2 expression level is substantially increased by direct binding of NFAT1 to the LCN2 promoter. Therefore, contrary to NFAT3, NFAT1, promotes migration and invasion of breast cancer cells [28]. It has been suggested that LCN2 and NFAT1 induce their pro-migratory effects on breast cancer cells through regulating the expression level of a tumor necrosis factor (TNF)-like receptor, which is called TNF-related weak inducer of apoptosis receptor (TWEAKR) and its ligand, TNF-related weak inducer of apoptosis (TWEAK) [28]. The exact mechanism downstream of NFAT1, through which LCN2 cooperates with TWEAKR and TWEAK, is relatively complex. According to a working model, in the absence of LCN2 TWEAK binds to TWEAKR which inhibits cell migration, and in the presence of LCN2, TWEAK interacts with an unknown receptor which leads to the elevation of the breast cancer cells motility [28]. Altogether, these findings suggest that LCN2 is actively involved in breast cancer development by serving several critical roles in initiation, progression, and aggressiveness of the disease. Therefore, the approaches that target this gene in breast cancer cells might be promising.

However, in contrast to the previous studies, some studies have suggested that LCN2 is pro-metastatic in murine breast cancer. According to one of these studies, which conducted on a mouse mammary tumor virus-polyoma middle tumor-antigen (MMTV-PyMT/FVB/N) model, there is no significant difference between LCN2 knockout and the control mice in terms of breast cancer metastasis. Several reasons have been proposed regarding these controversial results such as the differences in genetic background between the mice used in this study and the previous studies [133]. In another study, using almost the same mouse model, it has been reported that disruption of LCN2 expression does not affect metastasis of mammary tumors to lung [25].

In conclusion, the majority of studies suggest that there is a significant correlation between LCN2 and metastasis of breast cancer, which can be considered as a valuable target for cancer therapy.

Esophagus cancer

LCN2 expression is upregulated in metastatic subtypes of esophageal carcinoma [134]. LCN2 promotes cell motility in esophageal carcinoma cells at least through two major mechanisms including elevation of MMP-9 activity, and rearrangement of the cytoskeleton by activation of the extracellular cell-to-kinases (MEK/ERK) signaling pathway [17].

In vitro studies have shown that LCN2 overexpression in low-LCN2-expressing esophageal squamous carcinoma cells (ESCCs) dramatically increases cell motility and invasion. Conversely, LCN2 knockdown in high-LCN2-expressing ESCCs diminished cell migration and invasion [135]. Consistently, immunohistochemistry and zymography analyses showed that ESCC tissues have high MMP-9-related gelatinase activity, and also there was a positive correlation between LCN2/MMP-9 complex level and depth of tumor invasion [69].

The overexpression of LCN2 also contributes to increased cell motility by inhibiting stress fiber formation and enhancement of filopodia extension [17]. It has been suggested that LCN2 exerts these effects through the MEK/ERK signaling pathway, which activates the expression of proteins that are implicated in filopodia formation and stress fiber suppression. The LCN2 overexpression markedly promoted pERK1/2 expression and vice versa [17]. Likewise, chemical inhibition of pERK1/2 abolished the LCN2-mediated migration and invasion in ESCCs. Interestingly, forced expression of LCN2 and/or treatment of ESCCs with recombinant LCN2 protein increased the endogenous LCN2 level. It has been suggested that the exogenous LCN2 may promote the endogenous LCN2 expression through a positive feedback manner [17].

In vivo studies have indicated that severe combined immunodeficiency (SCID) mice which received LCN2-overexpressing ESCCs had much more lung metastasis compared to the SCID mice injected with parental ESCCs. In addition, LCN2 overexpression showed to decrease the overall survival rate in vivo [17].

Thyroid cancer

It has been reported that exogenous expression of LCN2 in thyroid cancer cell lines intensifies invasiveness and metastasis in vitro and in vivo [71]. Likewise, LCN2 increases MMP-9 activity in the thyroid tumor cell lines through

promoting MMP-9 stability, without any changes at mRNA levels [71]. The knockdown of LCN2 in a thyroid cancer cell line decreased its ability to form tumors in nude mice [70]. The nuclear factor-κB (NF-κB) signaling pathway modulates LCN2 expression, thus any disruptive interference in the pathway removes the tumorigenic capability [70]. Although these results may indicate that LCN2 is a pro-metastatic protein in thyroid cancer, further mechanistic studies must be performed in this regard.

Mouth cancer

In vitro studies revealed that LCN2 silencing increases proliferation, migration, and invasion capacities of oral cancer cell lines, while forced expression of LCN2 yields reverse effects [2, 73]. Moreover, the downregulation of LCN2 in oral cancer cells enhances EMT through upregulating MMP-9 and promotes survival via upregulating Bcl-2 and Cyclin-D and downregulating caspase-9 and p53 [2]. Consistently, in vivo investigations showed that mice bearing LCN2-knockdown oral tumor cells, not only had more lung metastasis but also had bigger primary tumors, in contrast to the mice bearing the parental oral tumor cells. Further explorations suggested that LCN2 exerts its pro-migratory effects through downregulation of carbonic anhydrase IX (CAIX), therefore, the patients with weak LCN2 to CAIX ratio had lower lymph node metastasis. LCN2 downregulates CAIX expression in oral cancer cells. At the transcriptional level, LCN2 downregulates hypoxia-induced factor 1a (HIF1a), which suppresses CAIX transcription. At posttranscriptional level, LCN2 downregulates CAIX expression by activating miR-4505 [2, 73].

Liver cancer

LCN2 is overexpressed in clinical patients with hepatocellular carcinoma (HCC) and is positively correlated with thyroid hormone receptor α (TRα) levels, too. Both TRα and LCN2 showed a similar expression pattern in relation to the survival rate, tumor grade, tumor stage, and vascular invasion. The thyroid hormone, 3, 3′, 5-triiodo-L-thyronine (T3), which regulates cell growth, development, differentiation, and also cancer progression via interaction with the TRα, upregulates LCN2 expression in the hepatocellular carcinoma cell lines. The overexpression of LCN2, either as a result of T3 treatment or genetic manipulations, increased migration and invasion both in vitro and in vivo. Consistently, immunodeficient mice injected with LCN2-overexpressing hepatocellular cancer cells showed more metastatic nodes in the lung tissue in comparison with the mice injected with moderately LCN2-expressing parental cells. The same results were achieved by administration of exogenous T3 in mice injected with a T3-responsive-hepatocellular

carcinoma cell line. In order to further investigate the underlying mechanism(s) of LCN2- and/or T3-induced metastasis, involvement of focal adhesion kinase (MET/FAK) pathway, which is a key mediator of EMT, was also assessed, showing that protein levels of c-Met and FAK are upregulated in the LCN2-overexpressing hepatocellular cancer cells and the aforementioned T3-regulated liver carcinoma cell lines after exposure to T3 [75].

Surprisingly, ectopic expression of LCN2 via genetic manipulation in HCC cells remarkably diminished their growth both in vitro and in vivo. Furthermore, it attenuated the expression of matrix metalloproteinase-2 (MMP-2) and the invasive capacities of the cells. LCN2 may exert these functions at least in part via disrupting the c-Jun N-terminal kinase (JNK) and phosphatidylinositol 3′-kinase (PI3K)/Akt signaling pathways in HCC cells [136].

These controversial findings suggest that further investigations need to be performed to elucidate the exact relationship between LCN2 and liver cancer progression.

Pancreatic cancer

Overexpression of LCN2 is positively associated with low degree of EMT, high expression of E-cadherin, low level of vimentin, and diminished rate of cell migration and invasion in pancreatic cancer [82]. It is noted that LCN2 decreases invasion/adhesion in pancreatic cancer cells by blocking FAK activation, and repression of angiogenesis by reducing VEGF production [81].

However, as stated before, the relationship between LCN2 and pancreatic cancer development is relatively unclear, while in contrast to the anti-metastatic and anti-angiogenic effects of LCN2 mentioned above, some other studies reported pro-metastatic and pro-angiogenic effects for LCN2. For example, it has been reported that MMP-9 expression was markedly higher in LCN2+/+ mice than LCN2−/− mice in syngeneic PDAC models. This study has also indicated that LCN2 overexpression increases tumor cells association with the basement membrane. Overexpression of LCN2 did not affect PDAC cell lines proliferation and migration while strongly changed their invasiveness. It has been proposed that LCN2 overexpression increases the invasiveness of PDAC cells not by elevating MMP-9 expression, but via binding to MMP-9 and enhancing its gelatinase activity [127]. Further studies are required to elucidate the exact relationship between LCN2 and pancreatic cancer metastasis.

Prostate cancer

LCN2 reportedly has a strong positive correlation with prostate cancer cells (PCa) migration and invasion as well as prostatic tumors metastatic capacity. Firstly, LCN2 has

significantly higher expression level in high-grade (or advanced) PCa patients and cell lines (like PC3) in comparison to primary PCa patients and cell lines (such as LNCaP) [7, 16]. Secondly, multiple studies have shown that forced overexpression of LCN2 in PCa cells increases their motility, invasion, [7, 42, 88] and metastatic capabilities [16], while inhibiting the expression of LCN2 yields the opposite effects. Moreover, it has been found that forced LCN2 overexpression in PCa cells significantly enhances epithelial marker E-cadherin, and declines the mesenchymal markers fibronectin and vimentin, and thereby promotes EMT in PCa [7]. Further mechanistic investigations revealed that LCN2 may exert these pro-metastatic effects partly via upregulating Slug through ERK signaling pathway [7]. These findings suggest that LCN2 is strongly involved in prostate cancer invasiveness and metastasis, and thus could be considered as a valuable target for prostate cancer treatment.

Lung cancer

LCN2 expression showed a positive correlation with lung tumors metastatic capacity. Patients who have higher levels of LCN2 are more likely to develop advanced and metastatic tumors [90]. Further mechanistic investigations in this regard showed that LCN2 overexpression in cancer stem-like cells confers mesenchymal phenotype, declines E-cadherin level, and promotes mesenchymal markers like vimentin, MMP-9, and also two EMT-ATFs including Slug and twist-1 [137]. Importantly, the NF-kB signaling pathway may play an important role in all of these LCN2-mediated pro-tumorigenic processes, since when the pathway was inhibited, all of the effects were canceled. Consistently, the LCN2-overexpressing cancer stem-like cells produced more metastatic activities, in comparison to corresponding counterparts in xenograft investigations [137]. All these findings unanimously prove that LCN2 might play a pivotal role in the progression and development of metastatic lung adenocarcinoma.

Colon cancer

LCN2 overexpression in CRC cell line reduces cell–cell adhesion and changes cytoskeletal organization inside the cells, whereas LCN2 knockdown produces the opposite effects [120]. LCN2 overexpression in CRC cells also increases cell motility and invasion and cell–matrix adhesion. Limei Hu and coworkers reported that LCN2 can exert all of these effects both in iron- and MMP-9 -dependent and -independent manners. They also found that LCN2 increases cell motility by altering the subcellular localization of filamentous-actins (F-actins), microtubules, α-catenin, and E-cadherin/β-catenin complex in colorectal cancer cells, without any impact on the expression profiles of these proteins [120]. LCN2 overexpression in CRC cells, could lead to the polarized localization of Rac1 in the leading edge of migrating cells, too [120]. Therefore, Rac1 is a mediator of the pro-migratory effects performed by LCN2 in CRC.

Another important molecule that is involved in the LCN2-related tumorigenesis in CRC is miR-138. It has been reported that miR-138 decreases CRC cells migration and growth by targeting LCN2. miR-138 has lower expression levels in CRC patients and cell lines when compared to healthy controls. Besides, metastatic CRC tissues express miR-138 about two times lower than primary cells. This difference in miR-138 expression level is metastatic-site-dependent. For instance, metastatic CRC tissues with liver origin have substantially higher levels of miR-138. These data indicate that miR-138 downregulation acts as a contributing molecular mechanism to the LCN2 overexpression in CRC patients with liver metastasis [138].

Surprisingly, in other studies, it has been suggested that LCN2 may be a potential metastasis suppressor in CRC. For example, in a study LCN2 expression level was significantly lower in metastatic or advanced-stage CRC tissues than in non-metastatic or early-stage CRC tissues. Knockdown of LCN2 using small interfering RNA (siRNA) in CRC cells expressing high levels of LCN2 induced an increase in cell proliferation and a morphological switch from epithelial state to mesenchymal mode (EMT), which in turn increased tumor cells migration and invasion. Knockdown of LCN2 also induced glucose consumption and lactate production, accompanied by an increase in energy metabolism-related genes level. In another study, it was reported that LCN2 might be an important negative regulator of EMT, invasion, and metastasis in CRC. In an in vivo study, LCN2-overexpressing mesenchymal stem cells were injected into colon cancer-bearing nude mice. This strategy substantially reduced liver metastasis, in part, via transcriptionally downregulating VEGF [126].

Taken together, these results show that LCN2 function in the CRC metastasis is still unclear and controversial. Therefore, further studies need to be implemented to determine the precise role of LCN2 in this regard.

Ovarian cancer

In vitro studies have shown that forced overexpression of exogenous LCN2 in moderately-LCN2-expressing ovarian cancer cells enhances their invasiveness [103]. Also, treatment of RL95-2 cells with recombinant LCN2 promoted their migration by interleukin-8 (IL-8)-mediated manner [111]. However, LCN2 knockdown resulted in opposite effects [1, 139]. Likewise, LCN2 expression reportedly has a positive correlation with VEGF level in patients with ovarian cancer [105]. These results indicate that LCN2 might

have a pro-metastatic function in the development of endometrial cancer which requires further investigations.

Stomach cancer

Several studies indicated that patients with gastric cancer have higher levels of LCN2 in their blood serum and stomach tissues [19, 33, 140, 141]. It is suggested that overexpression of LCN2 might be considered as an independent prognostic and ancillary diagnostic factor in gastric cancer [33, 141]. On the other hand, it has been found that the LCN2/MMP-9 complex and the free-MMP-9 have significantly higher expression levels in gastric cancer tissue homogenates in comparison with adjacent healthy tissues, which leads to an increase in extracellular tumor-associated MMP-9 and enhances tumor metastasis. This is markedly associated with a poor survival rate and a bad prognosis in patients with gastric cancer [33, 142]. Therefore, it could be considered as a valuable independent prognostic and diagnostic and also pro-metastatic factor in gastric cancer, which still warrants further mechanistic investigations.

Urinary bladder cancer

Urinary LCN2, MMP-9, and LCN2/MMP-9 complex levels are significantly higher in patients with bladder cancer than normal subjects. LCN2 overexpression has also been detected in bladder cancer cells, which is likely to have resulted from hypomethylation of its promoter [143]. Furthermore, it has been proved that there is a positive correlation between LCN2, MMP-9, LCN2/MMP-9 complex, and invasiveness of cancer cells. Accordingly, their expression levels were higher in the t2-t4 subtype, which is a muscle-invasive subtype of bladder cancer. These findings may suggest that LCN2, MMP-9 and their complex could be considered as valuable diagnostic biomarkers and also as pro-metastatic factors in the bladder cancer [12].

Brain cancer

LCN2/MMP-9 complex activity is increased in gliomas. It has been found that LCN2/MMP-9 activity is remarkably higher in brain tumor tissues and preoperative urine samples. However, this activity was declined seven days after surgery and increased again in cases with tumor recurrence. The LCN2/MMP-9 level is also strongly correlated with MRI-based tumor assessments. These findings suggest that LCN2/MMP-9 activity might serve as a novel biomarker for glioma prognosis and diagnosis [144]. On the other hand, it can be also proposed that LCN2 performs like a pro-metastatic agent through promoting MMP-9 gelatinase activity in brain cancer cells. However, further mechanistic studies are required in this regard.

Therapeutic resistance

Chemotherapy and radiotherapy are of the most common therapeutic tools used in cancer treatment, usually after surgical removal of tumors. However, several barriers have attenuated the efficiencies of these tools. One of the main problems that cause failure of either chemo- or radio- therapy is resistance of tumor cells against anti-cancer drugs and X-ray irradiation. LCN2 seems to be one of those genes that are implicated in the chemo- and radio-resistance of cancer cells [1, 2, 4–6, 145]. In this section, the studies that investigated LCN2 involvement in chemo- or radio- resistance of cancer cells will be reviewed.

LCN2 expression has been associated with radioresistance of lung cancer cells. In an in vitro study, it was observed that treatment of A549 cells with X-ray irradiation remarkably upregulates LCN2 expression. In addition, LCN2 knockdown in the cells increases radiosensitivity. These findings suggest that LCN2 might play an important role in the radioresistance of lung adenocarcinoma cells and that targeting this gene could be an effective strategy to enhance radiotherapy efficiency, which warrants further investigations [146].

It has been shown that LCN2 expression promotes chemoresistance against sunitinib in renal cell carcinoma. In mice injected with LCN2-overexpressing renal cancer cells, sunitinib had weaker suppressive effects on tumor growth, as compared to those mice injected with LCN2-knockdown renal cancer cells [5].

1,3-Bis (2-chloroethyl-1-nitrosourea (BCNU) is an anti-cancer drug from the group of alkylating agents that are commonly used in glioma therapy. In a study, it was reported that BCNU-resistant mouse glioma cells have lower LCN2 levels compared to their non-resistant counterparts. Also, as expression of LCN2 was promoted in the BCNU-resistant mouse glioma cells, their chemosensitivity against BCNU markedly increased. Moreover, LCN2 knockdown in the non-resistant glioma cells, enhanced their chemoresistance against BCNU-mediated therapy [6]. In this regard, the PI3K/AKT signaling pathway which is reportedly involved in development of many types of tumors with various tissue origins has been implicated. Dephosphorylation of AKT disrupts the pathway and eliminates the mentioned effects [147]. LCN2 increases BCNU-induced AKT dephosphorylation through which it renders the mouse glioma cells more sensitive to BCNU-induced apoptosis [6].

It has been reported that LCN2 knockdown increases chemoresistance against cisplatin in oral cancer cells. Cisplatin induces apoptosis in the tumor cells via increasing oxidative stress, while LCN2, through increasing intracellular levels of iron, improves the oxidative stress and aborts the cisplatin-induced apoptosis [2].

Table 1 Summary of some roles of LCN2 in biology of different types of cancer

Types of cancer	Lcn2 expression	Mechanisms	Genes involved	References
Breast cancer	High	Migration, Invasion, and Metastasis Angiogenesis ↑	HIC1/Lcn2** Axis ↓ E-cadherin HIF-1α ERK VEGF ↑ NFAT ZEB1 Snail Fibronectin Vimentin	[14] [39] [128] [128] [128] [27, 28] [39] [39] [39] [39]
Esophagus Cancer	High	Migration, Invasion, and Metastasis Proliferation ↑	MMP-9 MAPK/ERK ↑ pERK1/2	[17] [17] [17]
Thyroid cancer	High	Invasion and Metastasis ↑	NF-κB ↑	[70]
Liver cancer	High	Migration, Invasion, and Metastasis ↑	MET/FAK c-Met ↑ FAK	[75] [75] [75]
Pancreas cancer	High and Low*	Migration, Invasion, and Angiogenesis Pancreatic Tumor Growth ⚠	VEGF NF-κB FAK EGFR-MEK-ERK MUC4 Akt MMP-9	[81] [87] [81] [87] [80] [80] [127]
Prostate cancer	High	Migration, Invasion, and Metastasis Proliferation ↑	ERK Slug ↑ UPR Grp78	[7] [7] [148] [148]
Lung cancer	High	Proliferation Invasiveness and Metastasis ↑	Nrf2/HO-1 ↑	[91]
Kidney cancer	High	Angiogenesis ↑	VEGF ↑	[99]
Colon cancer	High	Invasion and Metastasis Angiogenesis ↑	Rac1 ↑ miR-138 ↓	[120] [138]
Ovarian cancer	High	Migration Proliferation ↑	IL-8 CD44v ↑ PI3K pathway	[75] [139] [1]

Table 1 (continued)

Types of cancer	Lcn2 expression	Mechanisms		Genes involved		References
Leukemia	High	Metastasis Apoptosis	↑	IL-3 Bcl-XL	↑	[115] [116] [116]
				Bad	↓	
Mouth cancer	Low	Proliferation Migration, and Invasion Autophagy	↓	Cyclin-D miR-4505 mTOR Caspase-9 p53 CAIX HIF1a	↑ ↓	[2] [73] [73] [2] [2] [2] [2]

HIC-1 Hypermethylated in cancer 1, *LCN2* Lipocalin-2, *HIF-1a* Hypoxia inducible factor 1, *ERK* Extracellular signal regulated kinase, *VEGF* Vascular endothelial growth factor, *TAM* Tumor associated macrophage, *NFAT* Nuclear factors of activated T-cells, *MMP-9* Matrix metalloproteinase 9, *MAPK* Mitogen activated protein kinase, *NF-κB* Nuclear factor kappa-light-chain-enhancer of activated B cells, *FAK* Focal adhesion kinase, *MUC4* Mucin 4, *UPR* Unfolded protein response, *HO-1* Heme oxygenase-1, *Nrf2* Nuclear factor erythroid 2-related factor 2, *Rac1* Ras-related C3 botulinum toxin substrate 1, *IL-8* Interleukin 8, *PI3K* Phosphatidylinositol 3-kinase, *IL-3* Interleukin 3, *Bcl-XL* B-cell lymphoma-extra-large, *Bad* BCL2 associated agonist of cell death, *mTOR* Mechanistic target of rapamycin, *CAIX* Carbonic anhydrase IX

*LCN2 has controversial effects on pancreatic cancer development i.e. in some studies it performs like an oncogene while in some other studies (**) it functions as a tumor suppressor, which has been discussed in more detail in the text

The cytoprotective role of LCN2 against cisplatin has also been indicated in another recent study in which CRISPR/Cas9-mediated knockout of LCN2 expression in a prostate cancer cell line remarkably increased their sensitivity to cisplatin treatment [88].

Piecing all of these findings together, apparently, LCN2 is involved in the chemoresistance and radioresistance of cancer cells, which still warrants further investigations.

Conclusion

Considering a few exceptions, such as oral cancer in which LCN2 acts as an anti-cancer molecule, there is a positive correlation between up-regulation of LCN2 and the progression of the most types of cancer. LCN2 plays a major role in favor of cancer initiation, promotion, and metastasis by several well-known functions, including stabilization of MMP-9, promotion of angiogenesis, suppressing apoptosis, performing as an anti-oxidant agent, and activating epithelial to mesenchymal transition (Table 1). Moreover, LCN2 is implicated in the radioresistance and chemoresistance phenotypes of cancer cells. The multiple and unique functions of LCN2 in cancer initiation and metastasis and its widespread aberrant expression in many types of cancer, highlight the importance of LCN2 not only as a valuable biomarker and prognostic factor in a variety of cancer cells, but also as an emerging potential comprehensive therapeutic target. However, detailed information on how LCN2 up-regulation contributes to cancer progression, as well as more preclinical and clinical trials are still required.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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