The role of lipocalin-2 in various cancers: a literature review

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Abstract: Lipocalin-2 (LCN2), also known as neutrophil gelatinase associated lipocalin, has been identified as a crucial iron protein that impedes bacterial growth during physiological and inflammatory states. In recent times, the oncological impact of LCN2 has been extensively studied in various cancer types, including colorectal cancer, gastric cancer, prostate cancer, breast cancer, and pancreatic cancer. Different levels of LCN2 have been linked to increased cell proliferation, angiogenesis, cell invasion, and metastasis. Consequently, LCN2 has emerged as a promising therapeutic target against various cancer types. This review consolidates the most notable findings on the expression, biological functions, and regulation of LCN2, along with the proteins with which LCN2 interacts in cancer.

Keywords: lipocalin-2; NGAL; carcinoma; EMT

1. Introduction

Lipocalin2 (LCN2), also referred to as neutrophil gelatinase-associated lipocalin (NGAL) or 24p3 is a 25 kDa soluble and secretory protein. LCN2 was originally isolated from SV40-infected mouse kidney cells(1). Belonging to the Lipocalin superfamily, LCN2 is a circulating protein that facilitates the transportation of small, hydrophobic molecules such as steroids, fatty acids, tretinoins, prostaglandins, etc(2, 3). Lipocalin 2 (LCN2), is encoded by a gene located at chromosome 9 locus 9q34.11. The LCN-2 gene produces many functional transcripts that eventually encode for a 198 amino acid-secreted protein. (Lipocalin-2: Structure, function, distribution, and role in metabolic disorders), The LCN-2 structure is comprised of a 310-helix at the N-terminal, an alpha-helix at the C-terminal, and an eight-stranded highly conserved beta-barrel that forms a closing calyx in an antiparallel direction(4). At the N-terminal of LCN-2, there is a signal transduction of 20-amino acid polypeptide, which is separated from the molecule before release. This region is adjacent to the region where Lipocalin combines with its ligand, called the Lipocalin region. Eight-segment antiparallel of LCN2 β- Folding to form a conservative barrel-like tertiary structure is an internal ligand binding site that allows the Lipocalin region to bind to its ligand. The binding cavity of LCN-2 is much larger and more polar than other Lipocalin proteins. This allows LCN-2 to bind with receptors located on the surface of the plasma membrane, and form large molecular complexes by binding with larger and less hydrophobic ligands such as mammalian proteins, which in turn enables cells to perform key functions in cell division and regulation [3]. LCN-2 was initially isolated from neutrophil particles released from human infection and inflammation sites (2, 5). LCN-2 is elevated in the serum of patients with acute bacterial infection (6). Lcn2 was subsequently found to play a role in a new iron (iron carrier) transport pathway, which is independent of transferrin (7, 8). It can combine with bacterial iron carriers to inhibit bacterial growth by depleting iron and display antibacterial effect (7, 9). Later studies clarified other functions, such as protecting matrix metalloproteinase-9 from degradation (10) and inducing apoptosis of pre-B cells(11).

LCN2 plays a significant role in a range of biological processes such as cell apoptosis (12), lipid metabolism (13), cell migration, tumor invasion (14), and metastasis (15). Activation of LCN2 has been linked to several diseases, including kidney injury, obesity, type 2 diabetes, tumorigenesis, and metastasis. The differential expression of LCN2 in various human tumors and its potential mechanisms of action have garnered attention both domestically and internationally. This review provides an overview of LCN2 expression in relevant tumors.

2. LCN2 Expression in Cancer

The LCN2 gene is a recently identified tumor-related gene that exhibits diverse biological functions depending on the cancer type with which it is associated. Extensive research has demonstrated a significant correlation between aberrant expression of LCN2 and the proliferation, invasion, and metastasis of specific types of cancer cells. However, the potential benefit of targeting LCN2 has not been applied to any cancer because of contradictory reports about the role of LCN2. A study has reported that higher levels of LCN2 expression are linked to unfavorable outcomes in patients with colorectal cancer (16), whereas other studies have yielded contradictory results (17, 18). A tumor suppressing role of LCN2 has been found for instance in gastric cancer(14) and pancreatic cancer(19), whereas LCN2 promotes tumorigenesis in breast cancer(20) or prostate cancer(21). LCN2 has become the subject of numerous studies as a potential therapeutic target in various types of cancer by scholars both domestically and internationally.

2.1 LCN2 and colorectal cancer

According to Chaudhary et al.(16), over-expression of LCN2 in colon cancer cells has been linked to tumor progression and resistance to chemotherapy. This resistance to the drug 5-fluorouracil was observed both in vitro and in vivo and was attributed to the inhibition of ferroptosis by LCN2. Specifically, LCN2 was found to decrease intracellular iron levels and promote the expression of glutathione peroxidase 4 and the cysteine glutamate antiporter xCT, which are involved in the ferroptosis pathway. The upregulation of xCT was found to be dependent on increased levels of ETS1 in cells that over-expressed. Zhang et al.(22) also observed resistance to 5-fluorouracil, which was attenuated by LCN2 through the activation of an SRC/AKT/ERK-mediated antiapoptotic program. The interaction between LCN2 and integrin β3 was found to increase the stability of the integrin, resulting in the recruitment of SRC to the cytomembrane and downstream activation of the AKT/ERK cascade. The authors also noted that increased LCN2 expression was associated with a poor prognosis in patients with colorectal cancer.

In contrast, Feng et al(17). found that LCN2 suppresses metastasis in colorectal cancer by reducing NF-κB-dependent activation of snail and epithelial-mesenchymal transition. In their study, LCN2 was found to be highly expressed in 66.5% of the specimens, and this expression was significantly associated with positive E-cadherin in the membrane and negative nuclear β-catenin. When LCN2 expression was higher and NF-κB expression was negative, there was a negative correlation with nuclear accumulation of snail and a prediction of a favorable prognosis. The study demonstrated that LCN2 blocked cell proliferation, migration, and invasion in vitro and in vivo, and inhibited the translocation of NF-κB into the nucleus. Conversely, NF-κB was found to reverse the effect of LCN2 on epithelial-mesenchymal transition and promote snail expression.

Kou et al.(18) reported that the expression of LCN2 was higher in long-duration ulcerative colitis (UC) and ulcerative colitis-associated carcinogenesis (UCAC) compared to normal tissue, but lower in UCAC than in UC. This suggests that low expression of LCN2 could potentially serve as a novel biomarker for evaluating cancer surveillance related to the progression of UC to UCAC.

2.2 LCN2 and gastric cancer

According to Kubben et al., lipocalin-2 levels are significantly enhanced in gastric cancer (GC) compared to adjacent control tissue. We found that in GC tissue lipocalin-2 levels are in general 30 times higher than corresponding MMP-9 levels, presumably leading to MMP-9/lipocalin-2 complex formation of a substantial part of the MMP-9 fraction after it has been released from the cells(23).

The expression of LCN2 is reduced in diffuse-type and epithelial-mesenchymal transition (EMT) type gastric cancer (GC). This reduction in LCN2 expression promotes the proliferation, migration, and invasion of GC cells by inducing Matrix Metalloproteinases-2 (MMP-2) activity and enhancing EMT signaling in GC cells. Gene Set Enrichment Analysis (GSEA) shows that LCN2 downregulation is associated with EMT signaling in human samples. Furthermore, low levels of LCN2 protein and mRNA are linked to poor prognosis in GC patients. LCN2 plays a crucial role in EMT signaling via MMP-2 activity during GC progression. Subgroup analysis shows that LCN2 expression is significantly decreased in EMT type GC compared to epithelial type GC. Therefore, LCN2 expression is linked to the epithelial phenotype of gastric tumors and declines as the tumor becomes

undifferentiated. Thus, LCN2 may be a promising therapeutic target for reversing EMT signaling in GC patients with poor outcomes.(14).

2.3 LCN2 and prostate cancer

LCN2 protein and mRNA expression are higher in PC3 and DU145 cells than in LNCaP and 22Rv cells, and prostate cancer tissue correlated significantly with tumor differentiation ($P < 0.017$) and Gleason's grade ($P < 0.02$). LCN2 knockdown in PC3 and DU145 cells decreased cell proliferation, colony formation, cell cycle arrest, migration, and invasion. Conversely, LCN2 overexpression in 22Rv cells produced the opposite effect. Subcutaneous xenografts in mice models showed decreased tumor growth in the LCN2-knockdown mice.

In the study by Rahimi et al.(24), LCN2 knockout in a highly aggressive and invasive cancer cell like PC3 decreased cell proliferation and increased the sensitivity of cisplatin. Conspicuously, loss of LCN 2 expression effectively enhanced cisplatin-induced apoptosis in PC3 cells. LCN2 knockout by CRISPR/Cas9 technology decreased the cell migration capacity of PC3 cells as well.

Lu et al.(21) reported a CXCL1-LCN2 paracrine network was confirmed in prostate cancer tissue samples, which was correlated with the biochemical recurrence of prostate cancer. Of note, the CXCL1-LCN2 axis activates Src signaling, triggers the EMT, and consequently promotes the migration of prostate cancer cells, leading to enhanced tumor metastasis.

2.4 LCN2 and breast cancer

Valashedi et al.(25) reported that the use of CRISPR/Cas9 to target Lcn2 resulted in a decrease in cellular proliferation and migration capability, as well as an increase in the vulnerability of MDA-MB-231 cells to cisplatin. Additionally, the loss of Lcn2 expression effectively promoted erastin-mediated ferroptosis in MDA-MB-231 cells, indicating that inhibiting Lcn2 could be a useful strategy for sensitizing MDA-MB-231 tumor cells to ferroptotic cell death.

Inflammatory breast cancer (IBC) is a primary breast cancer subtype known for its aggressive nature, characterized by a rapid onset, high risk of metastasis, and poor clinical outcomes. In a study by Villodre et al.(20), elevated expression of LCN2 is associated with poor prognosis and shorter overall survival in IBC patients. Depletion of LCN2 in IBC cell lines has been found to reduce colony formation, migration, and cancer stem cell populations in vitro, as well as inhibit tumor growth, skin invasion, and brain metastasis in mouse models of IBC. Analysis of proteomics data indicates that LCN2 silencing in IBC cells results in reduced expression of proteins involved in the cell cycle and DNA repair.

2.5 LCN2 and pancreatic cancer

In a study conducted by Hao et al.(19), it was confirmed that LCN2 is significantly upregulated in pancreatic carcinoma compared to normal tissues. The clinicopathological analysis showed that Lcn2 expression negatively correlated with the degree of tumor differentiation. Surprisingly, the knockout of LCN2, which exhibited high LCN2 expression, actually increased the stemness of cancer stem cells, resulting in enhanced sarcosphere formation and tumorigenesis. Furthermore, it was discovered that LCN2 regulates stemness in pancreatic cancer by activating the AKT and c-Jun pathways. LCN2 acts to suppress the stemness properties of pancreatic carcinoma by activating the AKT-c-Jun pathway, suggesting that it could be a promising candidate for inhibiting the stemness of pancreatic cancer.

Lemecha et al.(26) inhibited Lcn2 using an anti-Lcn2 antibody neutralization or genomic ablation in mice. The deficiency of Lcn2 significantly improved body temperature in tumor-bearing mice, as evidenced by the increased expression of Ucp1 and β3-adrenergic receptors in BAT. Moreover, inhibiting Lcn2 prevented the loss of fat and muscle in tumor-bearing mice. In contrast to tumor-bearing WT mice, Lcn2-knockout mice showed reduced ATGL expression in iWAT and decreased expression of muscle atrophy molecular markers MuRF-1 and Fbx32. Suppression of Lcn2 through therapeutic targets may offer a way to minimize the progression of cachexia.

3. Conclusion

Malignant tumors have emerged as the leading cause of human mortality. Early detection and effective treatment can significantly reduce cancer-related deaths. Previous studies have demonstrated differential expression levels of LCN2 across various human tumors, implicating LCN2 in the pathogenesis and progression of these tumors. However, the regulatory mechanisms underlying LCN2 expression and its variants remain incompletely understood. Investigating the correlation between LCN2 expression levels and its variants in different tissues, as well as exploring the release of cytokines in various tissue microenvironments and their impact on LCN2 expression through corresponding signaling pathways, may shed light on the etiology of tumor diseases and provide novel directions for cancer diagnosis and prevention.

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