Molecular Mechanisms of Long Noncoding RNA LMCD1-AS1

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Abstract

Long noncoding RNAs (LncRNAs) are one of the interesting fields in cancer researches. LncRNAs are generally dysregulated in many diseases. LMCD1 antisense RNA 1 (LMCD1-AS1) is a newly identified lncRNA with pro-tumorigenic functions on tumor cells. LMCD1-AS1 expression is increased in Hepatocellular Carcinoma (HCC). LMCD1-AS1 is a sponge of miR-106b-5p activity. LMCD1-AS1 modulates survival of osteosarcoma via targeting miR-106b-5p. LMCD1-AS1 and Sp1 are highly expressed in osteosarcoma. SP1 can bind to the promoter region of LMCD1-AS1, resulting in its overexpression in osteosarcoma. GLI2 is shown to bind to the LMCD1-AS1 promoter and is transcriptionally activated by LMCD1-AS1. LMCD1 acts as a miR-1287-5p sponge to increase GLI2 expression. LMCD1 is abundantly expressed in kidney tissue. Moreover, it is functionally involved in protein-protein interactions with transcriptional co-repressor activity, including regulation of the calcineurin-NFAT signaling cascade known to play a critical role in recovery from Acute Kidney Injury (AKI). E2F1/LMCD1-AS1/miR-345-5p/ COL6A3 axis is a newly identified regulatory mechanism, which has a function in Cholangiocarcinoma (CCA) tumorigenesis and progression and provides potential therapeutic targets for CCA. Also, LMCD1-AS1 functions in Thyroid Cancer (THCA) development. LMCD1-AS1 is overexpressed in THCA cells, and LMCD1-AS1 knockdown suppresses the malignant phenotypes of THCA cells. In THCA development, LMCD1-AS1 exerts pro-tumorigenic function through sponging miR-1287-5p to increase GLI2 expression, constituting a feedback loop of LMCD1-AS1/ miR-1287-5p/GLI2. In this review, I focus on the molecular mechanisms of newly identified long noncoding RNA LMCD1 antisense RNA 1 (LMCD1-AS1).

Keywords: Cancer; LMCD1-AS1; E2F1; Sp1

Abbreviations: LncRNA: Long noncoding RNAs

INTRODUCTION

T Cancer is a complex disease harboring a wide range of genetic mutations, chromosomal translocations, amplification and deletions, epigenetic alterations [1]. A non-coding RNA group, LncRNA, has more than 200 base pairs and does not code for proteins [2,3]. The important research field of cancer, LncRNAs, is implied in many cancers, such as breast, prostate, liver, gastric cancer, and in colon adenocarcinoma [4-7]. LMCD1 antisense RNA 1 (LMCD1-AS1) is a newly identified lncRNA [8], and is a certified oncogene in several tumor types. LMCD1-AS1 is abundantly found in the cytoplasm [9].

LITERATURE REVIEW

LIM and cysteine-rich domains-1 (LMCD1)

The novel member of the LIM domain family of proteins belongs to group 3 of the LIM proteins [10]. LIM domain proteins are defined as proteins having a double zinc fingers motif with a consensus amino acid sequence C-X2-C-X16−23-H-X2-C-X2-C-X16−23-C-X2-C, (where C represents cysteine, and X represents other amino acids). The predicted 365-amino acid LMCD1 protein contains a cysteine-rich domain in the N-terminal region and two LIM domains in the C-terminal region. LIM domain proteins are important regulators in cell growth, cell fate determination, cell differentiation, and remodeling of the cell cytoskeleton by their interaction with various structural proteins, kinases, and transcriptional regulators [11]. LIM and Cysteine-Rich Domains-1 (LMCD1) is a member of the LIM protein family [12], that initially defined by the zinc finger motifs found in Lin11, Isl-1, and Mec-3 (LIM) [13], which contains an N-terminal cysteine-rich region, two C-terminal LIM domains and a central PET. LMCD1 is a regulator of osteogenic differentiation [12], and a player in the

control of skeletal muscle mass [13]. LMCD1 regulates osteogenic differentiation of Bone Marrow Stromal Cells (BMSCs) via the BMP signaling. Some of the well-known target genes of BMP signaling are RUNX2, SP7, and DLX5. Knockdown of LMCD1 significantly inhibits the mRNA expression of COL1A1, DLX5, SP7, OCN, OPN and reduces the protein expression of COL1A1, RUNX2, SP7. LMCD1 is shown to protect RUNX2 and Smad1 protein from Smurf1-induced ubiquitination degradation thereby regulating BMP signaling [12]. The differential expression of LMCD1 causes the aberration of Notch and Hedgehog signaling pathways leading to alopecia and hair-loss [14]. Oxidative stress directly targets the Lmcd1 transcription [15]. LMCD1 acts as a co-activator for E2F1 in the modulation of thrombin-induced CDC6 expression while promoting HASMC replication [16]. Par1, Gαq/11, PLCβ3, NFATc1, E2F1, and LMCD1 are involved in thrombin-induced IL-33 expression and migration. NFATc1, E2F1, and LMCD1 bind to the NFAT site in response to thrombin [17]. Several genes, such as LMCD1, osteopontin, survivin, and PRX1 are shown to be expressed in vascular smooth muscle cells and are involved in atherosclerosis [18].

LMCD1-AS1 in acute kidney injury

Acute Kidney Injury (AKI) [19], which was previously named as acute renal failure [20] is a syndrome characterized by the rapid loss of the kidney's excretory function [21]. Researchers have identified that a 62.8kb peak region is highly associated with AKI. These are at the 3p21.6 locus, an intergenic region bounded by GRM7 (Glutamate Receptor, Metabotrophic 7) and LMCD1 (LIM and Cysteine-Rich Domains protein 1, dyxin) genes [22]. Larach et al., hypothesized that the variant in the GRM7|LMCD1-AS1 region plays a regulatory role in the expression of the LMCD1 zinc-finger protein, which is found in kidney tissue and might contribute to recovery from AKI [23]. LMCD1 antisense RNA 1 (LMCD1-AS1) facilitates tumor progression in cholangiocarcinoma and hepatocellular carcinoma. Knockdown of LMCD1-AS1 has an effect on cell proliferation, migration, and apoptosis [9].

LMCD1-AS1 in hepatocellular carcinoma

Hepatocellular Carcinoma (HCC), is a common malignancy, and is the second most common cause of death from cancer worldwide [24,25]. The mechanisms of HCC development and progression involves the inactivation of multiple tumor suppressor genes (such as p53), abnormal activation of oncogenes (K-ras, etc.,) and multiple signaling pathways (PI3K, MAPK, JAK/STAT, NF-κB, Wnt/β-catenin, etc). Abnormal regulation of epigenetic events (such as microRNAs), and exosomes that deliver a large number of protumorigenic molecules are also involved in HCC development [26]. The immune modulator transforming growth factor-β (TGF-β) is highly expressed in and associated with poor prognosis of HCC. Transcriptional expression of TGF-β is induced by the activation of β-catenin, which is one of the commonly mutated genes in HCC [27]. Wang et al. investigated a correlation among P4HA2, let7g, LMCD1-AS1, and overall survival of HCC patients [28].

LMCD1-AS1 in thyroid cancer

Thyroid Cancer (TC) is the most common endocrine tumor, accounting for 3. 4% of all cancers diagnosed every year. The molecular pathogenesis of the majority of thyroid cancer involves dysregulation of the Mitogen-Activated Protein Kinase (MAPK) and phosphatidylinositol-3 kinase (PI3K)/AKT pathways. According to

evidences of previous researches, TC cells and their precursors are responsive to insulin and Insulin-like Growth Factors (IGFs), and often overexpress the receptors for insulin (IR) and IGF-1 (IGF-1R) [29]. Activation of the Wnt/b-catenin pathway and inactivating mutations in tumor suppressor TP53 gene, but on the other hand, activating mutations in the TERT promoter are frequent seen in undifferentiated thyroid cancers [30]. Oct4 and Nanog are transcription factors required to maintain the pluripotency and self-renewal of Embryonic Stem (ES) cells [31]. Shao et al., showed that mRNA and protein levels of stemness-associated markers (OCT4 and NANOG) are all decreased following LMCD1-AS1 depletion. The transcriptional factor GLI family zinc finger 2 (GLI2) is another factor that is related to thyroid cancer. GLI2 has been shown to positively modulates LMCD1-AS1 expression, through their specific binding. There are 2 potential GLI2-binding sites, within the LMCD1-AS1 promoter. GLI2 binds to long noncoding RNA LMCD1-AS1, and contributes to LMCD1-AS1 up-regulation in THCA cells. Cytoplasmic LMCD1-AS1 sponges a shared microRNA between GLI2 and LMCD1-AS1 [9].

LMCD1-AS1 in cholangiocarcinoma

Cholangiocarcinomas (CCAs) are the most common biliary malignancy and the second most common hepatic malignancy after Hepatocellular Carcinoma (HCC). CCAs are classified anatomically as intrahepatic (iCCA), perihilar (pCCA), or distal CCA (dCCA) [32]. Cholangiocarcinoma is an epithelial malignancy originating from transformed cholangiocytes. Inflammation and cholestasis are key factors in cholangiocarcinogenesis. Proinflammatory cytokines (i.e., interleukin-6 [IL-6]) activate inducible nitric oxide synthase resulting in excess nitric oxide that mediates oxidative DNA damage, inhibition of DNA repair enzymes, and expression of Cyclooxygenase 2 (COX-2). COX-2 dysregulates CCA growth and positively regulates pro-oncogenic signaling pathways such as Hepatocyte Growth Factor (HGF), IL-6, and EGFR [33]. Myeloid Cell Leukemia Sequence 1 (MCL1) is an anti-apoptotic BCL2 family member that mediates tumor necrosis factor-related resistance to the apoptosis-inducing ligand in CCAs. IL6 increases the expression of MCL1 via constitutive activation of Signal Transducer and Activator of Transcription (STAT) Signaling and Protein Kinase B (Akt). MCL1 transcription is activated by IL6 via a p38 Mitogen-Activated Protein Kinase (MAPK)-dependent pathway. IL6 binds to the gp130 receptor, leading to its subsequent dimerization and activation of the gp130-associated Janus Kinases (JAKs), including JAK1 and JAK2, which leads to STAT3 activation. Epigenetic Silencing of Suppressor of Cytokine Signaling 3 (SOCS3) results in sustained IL6 signaling via STAT3. Activation of EGFR leads to activation of Extracellular-Signal-Regulated Kinases (ERKs) 1 and 2 (also known as p44/42 MAPK). EGFR inhibitors decrease the expression of Cyclooxygenase-2 (COX2) by CCA cells [32].

DISCUSSION

E2F1/LMCD1-AS1/ miR-345-5p/COL6A3 axis

LMCD1-AS1 can sponge miR-345-5p in CCA. Yu et al. identified the downregulation of miR-345-5p and upregulation of COL6A3 level in CCA tissues. LMCD1-AS1 expression is shown to be significantly higher in CCA tissues. E2F1 can bind directly to the promoter region of LMCD1-AS1 and activate its transcription. There has been found three potential binding sites of E2F1 on the LMCD1-AS1 promoter region. Ectopic expression of E2F1 increases LMCD1-AS1 level [34]. Alpha 3 (VI) Collagen Gene

(COL6A3), is an extracellular matrix protein, forms a microfibrillar network associated with the structural integrity [35], and is usually found in most connective tissues, including muscle, skin, tendon, and vessels [36]. COL6A3 is strongly related with many diseases [37]. COL6A3 is a downstream target of miR-345-5p. The oncogenic role of LMCD1-AS1 is partly dependent on COL6A3 expression. E2F1/LMCD1-AS1/miR-345-5p/COL6A3 might contribute to CCA development and progression. LMCD1-AS1 is upregulated in CCA cancer [34]. miR-345-5p is a tumor-suppressive miRNA in pancreatic cancer progression [38]. miR-345-5p induces inflammatory damage in hippocampal neurons [39], and has oncogenic roles [40]. miR-345-5p is also a promising blood-derived disease biomarker in Multiple Sclerosis (MS) [41].

LMCD1-AS1 and GLI2

The Glioma-Associated Oncogene (GLI) family proteins, was first identified as the downstream effector of the HH signaling pathway, highly conserved in vertebrates and invertebrates, and are critical in embryonic development and adult tissue homeostasis [42,43]. GLI family consists of GLI1, GLI2, and GLI3 in mammals [42]. That all have five C2H2 Krüppel-like zinc finger motifs [42]. The GLI transcription network up-regulates target genes, such as BCL2, FOXA2, FOXE1, FOXF1, FOXL1, FOXM1, GLI1, HHIP, PTCH1, and WNT2B, in a cellular context-dependent manner. Aberrant Hedgehog signaling in tumor cells leads to self-renewal, survival, proliferation, and invasion [44]. GLI2 is a target of multiple signaling pathways [45]. GLI proteins belong to the family of Kruppel-like factors, transcription factors with highly conserved C2H2-Zn finger DNA-binding domains [46]. miR-1287-5p has an impact on several hallmarks of cancer. miR-1287-5p has been shown to significantly downregulated in mammospheres and human Breast Cancer (BC) tissue. PIK3CB as a direct molecular interactor of miR-1287-5p [47]. GLI2 is inhibited by miR-1287-5p and disinhibited by LMCD1-AS1 [9].

LMCD1-AS1 and E2F1

E2F1 (E2 Promoter Binding Factor 1) [48], is a central player in cell cycle progression, DNA-damage response, and apoptosis [49]. E2F1 exhibits dual properties, acting as a tumor suppressor and oncogene. Cellular stress such as DNA damage or mitogenic signaling leads to the activation of E2F1 as a mediator of apoptosis in the context of a conserved cellular anti-tumorigenic safeguard mechanism [50]. The transactivation capacity of E2F1 is regulated by pRb. In its hypophosphorylated form, pRb binds and inactivates DNA binding and transactivating functions of E2F1. The growth factor stimulation of cells leads to activation of CDKs (Cyclin-Dependent Kinases), which in turn phosphorylate Rb and hyperphosphorylated Rb is released from E2F1 or E2F1/DP complex [51]. In the G1 phase, pRB interacts with E2F1 to -4 and negatively regulates the transcription of cell cycle gene promoters that contain E2F binding sites [52]. The interaction between pRB and E2F is critical for control of the cell cycle and apoptosis [53]. The Retinoblastoma protein (pRB) has the dual capability to negatively regulate both E2F-induced cell cycle entry and E2F1-induced apoptosis [54]. The Rb protein is a tumor suppressor, which plays a pivotal role in the negative control of the cell cycle and in tumor progression. The retinoblastoma family includes three members, Rb/p105, p107, and Rb2/p130, collectively referred to as 'pocket proteins' [55].

LMCD1-AS1 and osteosarcoma

LMCD1-AS1 may promote the progression of osteosarcoma via sponging miR-106b-5p.

miR-106b is an oncogenic microRNA that targets multiple KLF family members. Some functions of miR-106b include increased proliferation by miR-106b-mediated reduction of the transcription factor E2F1 and the tumor suppressor RB1. Some known miR-106b-regulated genes are RB1, IL-8, F3, YES1, FAM91A1, and ERO1L [56]. MiR-106b-5p is a member of miR-106b seed family, plays opposing functions as an oncomiR or a tumor suppressor in tumor development [57]. Expression of miR-106b-5p is significantly correlated with the number of metastatic lymph nodes. miR-106b-5p expression is also upregulated in cervical cancer [58], and is thought to be involved in the common brain neuropathological changes [59].

Osteosarcoma (OS) is the most frequent primary bone cancer in children and adolescents [60]. The most critical pathways in osteosarcoma pathogenesis are the Notch, Wnt, NF-κB, p53, PI3K/Akt, and MAPK pathways [61]. miR-106b acts in part to protect cholangiocarcinoma cells from apoptosis. KLF2, KLF6, and KLF10 are all regulated by miR-106b and are known to promote apoptosis [56]. KLF6 reduces tumorigenic features in osteosarcoma cells. He et al., found that LMCD1-AS1 is an upregulated lncRNA in osteosarcoma and therefore may be a potential therapeutic target for osteosarcoma. Knockdown of LMCD1-AS1 suppresses the expressions of mesenchymal markers, including vimentin and N-cadherin. miRNA-106b-5p is decreased in osteosarcoma and acts as a tumor suppressor. Because LMCD1-AS1 can directly bind to miR106b-5p and decrease its expressions, knockdown of miR106b-5p decreases the expression of LMCD1-AS1. The attenuation of LMCD1-AS1 can also promote miR106b-5p expression. LMCD1-AS1 and Sp1 are highly expressed in osteosarcoma. SP1 can bind to the promoter region of LMCD1-AS1, resulting in its overexpression in osteosarcoma [8].

LncRNA LMCD1-AS1 may be involved in the process of the upregulation of let-7g by aspirin

Wang et al. evaluated the anti-fibrosis effect of aspirin through targeting the NF-κB/P4HA2 axis and LMCD1-AS1/ let-7g/P4HA2 axis. TCGA database indicated that the expression of P4HA2, let-7g, and LMCD1-AS1 is closely correlated with the overall survival of HCC patients. Aspirin interferes with the interaction of lncRNA LMCD1-AS1 with miRNA let-7g to release let-7g, leading to a decrease in the expression of let-7g's target gene, P4HA2, in liver cancer cells. Aspirin significantly stifles the increase in cell proliferation induced by both TNF-α and LMCD1-AS1 [28].

CONCLUSION

Dysregulated Long noncoding RNAs (LncRNAs) have essential roles in the development of many types of disease, especially of cancer. LMCD1 antisense RNA 1 (LMCD1-AS1) is a type of long noncoding RNA and related to different molecular mechanisms. Some functions of LMCD1-AS1 include that SP1 binds to the promoter region of LMCD1-AS1 and involved in its expression. Moreover, there are three potential binding sites of E2F1 on the LMCD1-AS1 promoter region. LMCD1-AS1 is involved in thyroid cancer, osteosarcoma, cholangiocarcinomas, hepatocellular carcinoma and also in acute kidney injury. As emphasized in this

article, further investigation of this long noncoding RNA, which has important functions, will contribute more to elucidating the molecular mechanisms of diseases.

COMPETING INTERESTS

The author declares that there is no conflict of interest.

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