Colon cancer-associated transcript 1 (*CCAT1*): A potential novel target in cancer therapy

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To the Editor: Long non-coding RNAs (lncRNAs) are a class of RNA molecules comprising more than 200 nucleotides in length, which possess negligible ability to encode functional proteins. LncRNAs are involved in several cell events, and their dysregulation has been reported to mediate tumor development and progression. Colon cancer-associated transcript 1 (*CCAT1*) is consistently overexpressed in a range of cancer cells and tissues. Cancer treatment strategies that target *CCAT1* have great potential.

As a lncRNA involved in tumor development and progression, *CCAT1* was first observed to be overexpressed in colon cancer mapping to chromosome 8q24.21 and subsequently extensively studied.[1] This article attempts to summarize the information on *CCAT1* in terms of its expression patterns, biological functions, and mechanisms of action in cancer, as well as highlight its potential use in the diagnosis, treatment, and prognosis of different malignancies.

The *CCAT1* gene encodes two isoforms, namely, the short isoform *CCAT1*-S with a length of 2628 nucleotides and the long isoform *CCAT1*-L with a length of 5200 nucleotides. *CCAT1*-S is overexpressed in the cytoplasm of colon cancer cells, while the dysregulation of *CCAT1*-L expression in the nucleus has been reported in colorectal cancer (CRC) and gastric adenocarcinoma. Furthermore, *CCAT1*-S expression is affected by *CCAT1*-L knockdown, which suggests that the short isoform is derived from the long isoform.[2]

CCAT1 is overexpressed in a variety of cancer cells where it promotes proliferation, epithelial–mesenchymal transition, migration, invasion, and chemoresistance, as well as affects different clinicopathological parameters like tumor size, tumor node metastasis stage, lymph node metastasis, and overall survival (OS). In addition to causing wide attention in cancer, the overexpression of *CCAT1* has also been confirmed in non-alcoholic fatty liver disease, osteoporosis, and tuberculosis, which promotes the development of these diseases.

Presently, five mechanistic mechanisms of action have been reported for *CCAT1* in cancer. First, in the nucleus, *CCAT1* is situated in the vicinity of *C-Myc*, a locus that is 515 kb upstream of the well-studied oncogene within a super-enhancer region, and thus, *CCAT1* is considered an enhancer-derived RNA (eRNA), which is transcribed by RNA polymerase II from the transcription enhancer region.[3] Therefore, *CCAT1* interacts with *C-Myc* nearby and thus exerts oncogenic effects. On the one hand, *CCAT1* promotes *C-Myc* expression in two known ways. Involved in the formation of *C-Myc* gating, wingless and int-1-regulated *CCAT1* promotes the nuclear export of *C-Myc* mRNA and drives communications between the super-enhancer region and *C-Myc* in colon cancer cells. In addition, *CCAT1*-L also binds to the CCCTC-binding factor binding site, which is highly enriched in the chromosome 8q24 region, to regulate chromatin conformation and *C-Myc* expression. Similarly, *CCAT1*-5L, a newly identified isoform of *CCAT1* that was only detected in Hela cells, was found to coordinate with the promoter RNA and eRNA of *C-Myc* to regulate chromatin looping and C-Myc expression. On the other hand, *C-Myc* also binds directly to the E-box element of the *CCAT1* promoter region, increasing the promoter activity and expression of *CCAT1* in hepatocellular carcinoma (HCC) cells. These findings reveal that *CCAT1*-L as a nuclear lncRNA localized within the super-enhancer exerts some of its oncogenic properties by forming a feedback loop with the oncogene *C-Myc*. It is noteworthy that there is also a positive feedback loop between *CCAT1* and T-cell factor 4 in breast cancer stem cells, which activates the WNT signaling pathway, regulates *C-Myc* expression, and further enhances the oncogenic ability of *CCAT1*.

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Second, *CCAT1* and the stem cell marker Krüppel-like factor 5 (*KLF5*) establish a core regulatory circuitry in the three-dimensional genome structure of CRC, which regulates *CCAT1* expression and facilitates the maintenance of tumor stem cell properties. The transcription start site of *CCAT1* interacts with the promoter and enhancer region of the *KLF5* gene. When the *KLF5* promoter is deleted, the expression of *CCAT1* and the characteristics of cancer stem cells are also downregulated. This regulatory mechanism of two genes located between different chromosomes reveals one of the reasons for the formation of *CCAT1* carcinogenic properties.

Third, *CCAT1* is involved in forming DNA/RNA/protein complexes, thus regulating the expression of target genes, activating downstream signaling pathways and acting as a pro-tumor agent in squamous cell carcinoma (SCC). In SCC cells, *CCAT1* is regulated by two upstream transcription factors, *TP63* and *SOX2*, and recruits both to form complexes to bind to the super-enhancer region of *EGFR*, thereby promoting *EGFR* expression and exercising their oncogenic functions. Worth noting is that further research is needed to identify whether this regulatory complex has SCC specificity.

Fourth, in esophageal squamous cell carcinoma (ESCC), *CCAT1* acts as a scaffold for polycomb repressive complex 2 and suppressor of variegation 3–9 homolog 1, two epigenetic modification complexes, mediates the histone methylation of the promoter sequence of sprouty RTK signaling antagonist 4 in the nucleus, and exhibits an important role in tumorigenesis. This finding suggests that *CCAT1* may contain more protein binding sites and participate in the epigenetics modification of downstream genes in the form of a molecular scaffold, thus promoting the occurrence of cancer.

Lastly, *CCAT1* exhibits cancer-promoting effects by sponging microRNAs (miRNAs) and functioning as a competitive endogenous RNA (ceRNA) in the cytoplasm, thereby modulating the expression of miRNA target genes. Supplementary Figure 1, http://links.lww.com/CM9/B965 shows the upregulation of *CCAT1* in various cancers and its possible downstream sponged microRNAs and proteins. Notably for *CCAT1*, which is mainly localized in the nucleus, such more convincing experiments such as Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 are missing from the current studies of ceRNA hypothesis.[4]

The above five mechanisms suggest that, according to the subcellular localization, in the nucleus, *CCAT1* affects the nearby oncogene *C-Myc* to control its expression, regulates chromatin looping, interacts specifically with DNA, RNA, and proteins, and participates in the formation of molecular scaffolds and extranuclearly regulates the expression of target genes through the ceRNA mechanism to further exert its pro-tumor effects. The expression pattern of *CCAT1* and its downstream target genes and signaling pathways are summarized in Supplementary Table 1, http://links.lww.com/CM9/B965.

Currently, the potential of *CCAT1*-targeted cancer therapy is manifested in the following three aspects.

First, *CCAT1* is an important diagnostic and prognostic biomarker independently or in combination with other lncRNAs. In CRC, *CCAT1* and *CCAT2* overexpression is correlated to impaired recurrence free survival and OS, while *CCAT1*, when used in conjunction with the lncRNA HOX transcript antisense RNA, it possesses a good diagnostic value. Among gastric cancer (GC) patients, serum extracellular vesicle *CCAT1* levels are upregulated, indicating that it has potential as a diagnostic marker. In non-small cell lung cancer (NSCLC), serum levels of *CCAT1* and sex determining region Y-box 2 overlapping transcript show outstanding diagnostic value. A clinical trial on *CCAT1* (clinicaltrials.gov, NCT04269746) is evaluating the clinical utility of detecting *CCAT1* in the diagnosis of CRC and its relation to tumor staging. Besides, peptide nucleic acid beacons targeting *CCAT1* can be used as molecular sensors and real-time human cancer diagnostic tools. *CCAT1*-associated peptide nucleic acid-based molecular beacons are able to identify benign and malignant lesions in real-time during CRC surgery, while forced-intercalation-peptide nucleic acids applied to fresh tumor tissues can detect *CCAT1* expression. The treated tumor tissues exhibit a strong fluorescent signal within minutes, with no appreciable fluorescence in healthy tissues, allowing for direct and rapid pathological assessment. Because of the long-term stability of biomarkers and the high complexity of cancer cells, biosensing technologies are still inseparable from the comprehensive exploration of the molecular mechanism of *CCAT1*.

Second, *CCAT1*-targeted treatment may improve the response of cancer patients to chemotherapy and radiosensitivity in the future. The single-nucleotide polymorphism rs67085638 in *CCAT1* increases the chemoresistance of colon cancers to paclitaxel, and the knockdown of *CCAT1* also heightens the sensitivity to 5-fluorouracil. In addition, *CCAT1* is activated by the transcription factor myeloblast virtual oncogene homolog-like2, recruits DNA methyltransferase 1 in the promotor of suppressor of cytokine signaling 3, and reduces its expression, thus promoting chemoresistance to oxaliplatin in CRC. In HCC cells, *CCAT1* increases oxaliplatin sensitivity through the *CCAT1*/quaking-5/p38 mitogen-activated protein kinase signaling pathway. *CCAT1* also modulates the expression of polo-like kinase 1 and benzimidazole 1-related 1 *in vitro* and *in vivo* via sponging miR-143 to increase drug resistance to cisplatin. And *CCAT1* enhances the chemoresistance of lung adenocarcinoma cells to docetaxel through functioning as a ceRNA and sponging Let-7c and the chemoresistance of NSCLC cells to cisplatin. Similarly, *CCAT1* regulates the miR-24-3p/fascin1 axis to modulate the sensitivity to paclitaxel in prostate cancer. In patients with breast cancer, *CCAT1* knockdown promotes radiosensitivity by targeting miR-148b. There is also an axis *CCAT1*/miR-17-5p/PD-L1 regulated by the Let-7a/c-Myc engine in triple-negative breast cancer cells, and *CCAT1* combined with Let-7a may facilitate atezolizumab resistance. Likewise, *CCAT1* competitively targets miR-454, thereby contributing to the cisplatin resistance of tumor cells. Among patients with renal cell carcinoma, *CCAT1* enhances the chemoresistance of cancer cells to sunitinib, indicating a new direction for the treatment of resistance to sunitinib. Hence, the knockdown of *CCAT1*

may become a novel approach to improve the chemical sensitivity and radiosensitivity of cancer patients.

Third, small molecule drugs targeting *CCAT1* could be a strategy for cancer treatment. *CCAT1* is extremely sensitive to bromodomain and extra-terminal inhibition, and consequently, it can be used to identify CRC sufferers who will make a profit from bromodomain and extra-terminal inhibitor treatment. Fluorescent polymeric hybrid nanoparticles assembled by curcumin and small interfering RNA (siRNA) targeting *CCAT1* possess favorable anti-tumor efficacy. Similarly, a nanocomplex composed of siRNA-*CCAT1* and the vascular endothelial growth factor receptor inhibitor fruquintinib can silence *CCAT1* and inhibit angiogenesis, exhibiting excellent biocompatibility and significant anti-CRC effects. In addition, both CX3543, a small molecule agent targeting nucleolin/G-quadruplex complexes in the nucleolus which inhibits RNA polymerase I and selectively reduces *C-Myc* expression, and the combined use of resveratrol and BIBR1532, two selective telomerase inhibitors, can suppress *CCAT1* expression, inhibit cell proliferation, and induce apoptosis. In GC cells, dandelion root extract affects the expression of *CCAT1*, thereby promoting its anti-carcinogenic effects, while cantharidin downregulates *CCAT1* expression and suppresses the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signaling pathway, thereby inhibiting the progression of GC. These studies prove that these molecules have tremendous potential as targets of *CCAT1*. The clinical applications in CRC targeting *CCAT1* are shown in Supplementary Figure 2, http://links.lww.com/CM9/B965.

Moreover, most of the current potential clinical applications of *CCAT1* have chosen RNA interference techniques to knock down the expression of *CCAT1*. The advantages of this technique are its high specificity and selectivity for *CCAT1*, the ease of synthesis, and high activity. However, the technique still faces many problems like limitations of delivery vehicles, possible off-target effects, immune response, and toxicity.[5] First, efficient and safe delivery vehicles remain a crucial obstacle due to instability and endosomal escape. Second, the technology may lead to reduced expression of genes other than *CCAT1*, triggering off-target effects. Furthermore, the specific structure and vector of siRNA drugs may lead to the release of cytokines, thereby causing innate immune responses and toxicity. The application of nanoparticle-based vectors for specific and stable delivery, chemical modification of siRNA drugs to reduce off-target effects and immunogenicity, and combination with other effective drugs are key directions to address the current challenges. Once these difficulties are overcome, *CCAT1*-targeted RNA interference therapy will become a promising advanced therapeutic strategy for the future.

There is a large scale of single-nucleotide polymorphisms in the human genome, and among them, variations in the *CCAT1*-located 8q24.21 region are associated with increased cancer risk. The single-nucleotide polymorphism rs67085638 in *CCAT1* is correlated with increased risk of CRC and upregulated *CCAT1* expression. Furthermore, rs7013433 is related to the late clinical stage in CRC patients. Similarly, rs67085638 promotes the progression of GC by promoting the growth, metastasis, and invasion of tumor cells, rs1948915 is related to the susceptibility of lung cancer in northeast China, rs6983267 increases the risk of endometrial cancer meanwhile significantly associated with lymph node metastasis, and rs1948915 correlates with increased cancer risk of multiple myeloma. However, current research has mostly confirmed the effectiveness of genomic variants of *CCAT1* in cancer, and the specific functional mechanism of *CCAT1*-related polymorphisms still needs further clarification.

With notable advancements in the field of epigenetic mechanisms, the epigenetic dysregulation of lncRNAs has been demonstrated to associate with the development of malignancies. Among the lncRNAs, *CCAT1* is considered a promising biomarker for diagnosis and prognosis because of its non-invasive nature (i.e., liquid biopsy specimens) and close relationship with clinical parameters. Presently, lncRNA-based cancer treatment strategies have attracted significant attention. The clinical application of *CCAT1* faces many difficulties, and therefore, few *CCAT1*-based targeted therapies have entered clinical practice. Delivery systems targeting *CCAT1* remain the focus of research, with low specificity, possible immunogenicity, and off-target effects being the main issues. Overcoming these obstacles will require a more extensive screening approach at the preclinical study stage and the selection of therapies targeting *CCAT1* with minimal potential adverse reactions prior to the start of clinical studies. Regardless, these efforts will be beneficial for the future clinical application of *CCAT1* and the comprehensive implementation of personalized medicine, especially precision oncology.

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Conflicts of interest

None.

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