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Pathophysiological roles, molecular interactions and clinical implications of long non-coding RNA CCAT2 in human cancer

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Summary. Less than 2% of human genome has protein-coding ability, while over 90% is transcribed into non-coding RNA (ncRNA). NcRNA can be divided into small ncRNAs (<200 nucleotides) and long ncRNAs (>200 nucleotides). Small ncRNAs, including microRNAs (miRNAs), small interfering RNAs (siRNAs), and others have been investigated in recent years and recognized as key players in regulating cellular processes and diseases, including cancer. Long non-coding RNAs (lncRNAs) attracted increasing attention in recent years as researchers have revealed their crucial roles as regulators in embryogenesis, stem cell biology, and development. Furthermore, growing evidence indicates that dysregulation of lncRNAs is involved in cancer, and the regulatory functions and mechanisms of lncRNAs in human carcinomas have begun to emerge. Colon cancer-associated transcript 2 (CCAT2) is a lncRNA transcribed from human 8q24 gene desert, which has been recently found to be deregulated in several tumor types. In this review, we will briefly analyse the roles and mechanisms of lncRNAs CCAT2 involvement in human cancer, and we will discuss the future perspectives for research and clinical applications.

Key words: cancer, CCAT2, long non-coding RNA, carcinogenesis, oncogene, onco-suppressor

Introduction

The roughly 20.000 protein-coding genes represent nearly 2% of the human genome, while more than 90% comprises a non-coding portion that actively transcribes a massive and complex amount of RNA (1, 2). This part of the transcriptome was initially interpreted as transcriptional noise and called "dark matter" by some authors. Nevertheless, this very large amount of ncRNA was demonstrated to play numerous roles in normal cellular biology as well as in several pathological processes (3).

According to their size, ncRNAs are currently divided into two classes. The first class comprises small ncRNAs, as the recently discovered miRNAs, siRNAs and others, in addition to the well-known cellular RNAs (ribosomal, transfer, etc.). miRNAs are RNAs approximately 22 nucleotides long, which function as components of complex cellular networks involved in the regulation of numerous genes, generally by post-transcriptional silencing (4). The remaining class consists of long non-coding (lnc) RNAs, which are arbitrarily defined as non-coding RNAs greater than 200 nucleotides long (5). They have been discovered in 1990 by Brannan et al., and since then a considerable number has been described; several digital databases

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provide information about the molecular and functional features of the currently known lncRNAs (6, 7). Generally, their length reaches 100 kilobases, without significant open reading frames (ORF); they are transcribed by RNA polymerase II or III, and can be located in the nucleus or cytoplasm (8, 9). Finally, their expression levels are usually lower than those of the protein-coding genes, and a certain tissue-specificity has been described (10-12).

LncRNAs are associated with different pre- and post-transcriptional functions, including nuclear architecture and import, immunity, imprinting, epigenetic regulations, cellular trafficking, splicing, precursors of smaller RNAs, and pluripotency of the embryonic stem cells (13). They can regulate gene expression at different levels, affecting cell proliferation, differentiation and apoptosis; therefore, they have been found involved in cancer development, maintenance, and progression (3, 14). Furthermore, they have been studied as risk, diagnostic, and prognostic markers as well as indicators of responses to treatments or recurrences.

CCAT2 in human cancer

Ling et al. reported in 2013 the discovery of a novel long ncRNA, CCAT2 (Colon Cancer Associated Transcript 2), which was found to be transcribed from the 8q24 genomic region (15). The CCAT2 genomic locus is highly conserved and harbours the SNP rs6983267; after its discovery, it was shown to be involved via various mechanisms in the pathogenesis and development of several human cancers (Table 1).

Table 1. Expression levels, interactions and clinical implications of CCAT2 in human cancer as depicted in the current scientific literature.

Cancer	Regul.	Interactions described	Clinical aspects	Reference
Breast	Up	ESR1, PGR, MYC, hormone status	Response to chemotherapy	Redis et al. (18)
Breast	Up	WNT signalling pathway	Prognostic marker	Cai et al. (19)
Breast	Up	p15	Prognostic marker, therapeutic target	Deng et al (20)
Breast	Up	TGF-β signalling pathway	-	Wu et al. (21)
NSCLC	Up	-	Marker of lymph node involvement.	Qui et al. (22)
NSCLC	Up	Pokemon, p21	-	Zhao et al. (23)
SCLC	Up	-	Prognostic marker	Chen et al. (25)
Esophageal	Up	MYC amplification	Prognostic marker	Zhang et al. (26)
Esophageal	Up	-	Diagnostic marker	Wang et al. (27)
Gastric	Up	EMT	Prognostic marker	Wang et al. (28)
Gastric	Up	-	Prognostic marker	Wu et al. (29)
Gastric	Up	-	Prognostic marker.	Wang et al. (30)
Colon	Up	WNT and MYC.	Relation with CIN score, putative markers	Ling et al. (15)
Colon	Up	BubR1	Prognostic marker	Kasagi et al. (32)
Colon	Up	mir145	Therapeutic target	Yu et al. (33)
Liver	Up	-	-	Zhou et al. (34)
Liver	Up	Snail2	Prognostic marker, therapeutic target	Xu et al. (35)
Glioma	Up	WNT signalling pathway	Putative diagnostic marker	Guo et al. (36)
Glioma	Up	EMT	Prognostic marker	Zeng et al (37)
Cervical	Up	-	Prognostic marker	Chen et al. (38)
Ovarian	Up	-	Prognostic marker	Huang et al. (39)
Cervical	Up	-	-	Wu et al. (40)
Prostate	Up	EMT	Prognostic marker	Zheng et al. (41)
Bladder	Up		Control by synthetic "tetracycline-on" switch system	Li et al. (42)

Breast cancer

Past studies have demonstrated that amplification of the 8q24 genomic region occurred more frequently in solid tubular or scirrhous mammary tumors than in less aggressive histotypes, and that a correlation exists between 8q24 DNA amplification profiles and breast cancer phenotype (16, 17). In other words, alterations in genes located on 8q24 are important for the development and/or progression of a consistent subgroup of primary breast cancers (BC), particularly those characterized by invasive behaviour. This led the team of investigators that discovered CCAT2 to further investigate its role and clinical correlations in breast cancer (18). For this purpose, the authors measured the mRNA levels of CCAT2 by reverse transcription quantitative polymerase chain reaction (RT-qPCR) in a cohort of 26 non-neoplastic breast tissues and 30 breast cancer tissues, and evidenced significantly increased levels in tumor samples. Furthermore, in situ hybridization (ISH) showed a strong CCAT2 staining in epithelial cells of both neoplastic and non-neoplastic tissues; nevertheless, its expression was higher in the epithelial component of BC than in the corresponding component of healthy tissues. CCAT2 expression was detected in both invasive epithelial components and "in situ" epithelial lesions. However, in a distinct set of 15 unpaired normal breast tissues from another institute, CCAT2 expression did not vary significantly from the levels measured in 977 BC clinical specimens, despite it was significantly higher in the subgroup of tumors with aggressive pathological features (18).

As concerns the functional correlations, the authors found that increasing levels of ESR1 and PGR associated significantly with decreasing levels of CCAT2, while increasing levels of MYC (located on 8q24) were positively associated with CCAT2. Furthermore, a strong inverse association of CCAT2 expression with nodal status and hormone receptor status was demonstrated (18). Regarding prognosis, it was found that CCAT2 expression levels are informative only for patients with lymph node positive disease who have received adjuvant chemotherapy, and it was shown that it downregulates chemo-sensitivity to 5-fluorouracil in a rs6983267-independent manner (18).

In 2015, Cai et al. performed a study on human breast cancer specimens, cell lines and mice to better investigate the molecular interactions of CCAT2 and the WNT signalling pathway in breast cancer (19). The authors detected high expression levels of CCAT2 in breast cancer tissues and breast cancer cell lines, and evidenced that patients with high CCAT2 expression had a significantly poorer prognosis; the level of CCAT2 expression was correlated with overall survival rates. Experimental suppression of CCAT2 decreased cell proliferation and invasion *in vitro*, and inhibited tumorigenesis *in vivo*. The suppression of CCAT2 decreased the levels of β-catenin both in the cytoplasm and nucleus. CCAT2 knockdown reduced the expression of CCND1 and MYC in the BC cells employed, and evidenced a synergic effect of si-CCAT2 and FH535 (a WNT inhibitor) on WNT signalling activity (19).

Recent studies demonstrated that lncCCAT2 can also interact with EZH2 and inhibit the expression of p15 (20), and that its down regulation causes reduction of the protein expression levels of TGF-β, Smad2 and α-SMA in breast cancer cells (21). The pro-oncogenic role of CCAT2 in vivo was further confirmed in the latter studies.

Lung cancer

Levels of lncRNA CCAT2 in lung cancer were investigated by Qiu et al. (22). The authors examined paired non-small cell lung cancer (NSCLC) tissues and adjacent normal tissues (≥3 cm away from tumor) from 57 patients who received surgical resection for primary NSCLC and found that CCAT2 was significantly overexpressed in neoplastic tissues compared with normal tissues, with an average up-regulation fold of 7.5. It was also shown that over-expression of CCAT2 was significantly associated with the adenocarcinoma histotype. In addition, CCAT2 combined with CEA was found to be able to predict lymph node metastasis. The silencing of CCAT2 led to inhibition of proliferation and invasion in NSCLC cell lines, confirming the role of CCAT2 in promoting invasion. The authors concluded that CCAT2 is a lung adenocarcinoma-specific lncRNA providing aggressive neoplastic capabilities to NSCLC cells, which can represent a potential biomarker for lymph node metastasis (22).

The overexpression of CCAT2 in NSCLC tissues (neoplastic and adjacent non-neoplastic, 20 cases) and cell lines (Pc-9, H358, H1975 and HBE) was confirmed also in a recent article published by Zhao et al (23). Knock down of CCAT2 in NSCLC cells limited malignant growth and invasion, while artificial overexpression of CCAT2 led to opposite effects. In addition, CCAT2 knockdown significantly decreased the expression of POK erythroid myeloid ontogenic factor (Pokemon), and induced the expression of the p21 tumor suppressor. Furthermore, Pokemon overexpression could reverse the decrease of cell viability and cell invasion triggered by CCAT2 silencing (23). The authors, considering the numerous anti-neoplastic activities of p21 (24), claimed that the oncogenic potential of CCAT2 and Pokemon on NSCLC cells may depend on their inhibitory role over p21 (Figure 1).

CCAT2 was found to be overexpressed also in small cell lung cancer (SCLC) (25). Its expression was investigated in 15 pairs of primary SCLC samples, and pair-matched adjacent normal lung specimens. In addition, loss-of-function studies of CCAT2 in cell lines (DMS-53, H446 SCLC cell lines, and 16HBE normal bronchial epithelial cell line) were carried out. The results showed that CCAT2 expression is elevated in SCLC tissues and cell lines, and correlates with poor prognosis. Furthermore, knockdown of CCAT2 expression effectively suppressed SCLC cell growth and metastasis *in vitro*. These findings outline CCAT2 as an independent prognostic factor for SCLC patients, and a critical regulator of SCLC cell growth and metastasis (25).

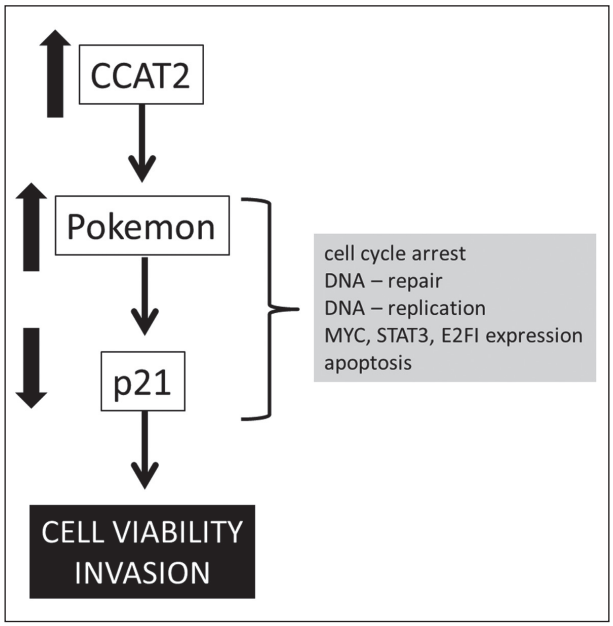

Figure 1. CCAT2 overexpression enhances the expression of Pokemon, which in turn supresses the expression of p21. The latter participates in the regulation of numerous biological processes, and its suppression leads to enhanced cell viability and neoplastic invasion.

Esophageal cancer

Two studies evaluating the expression levels and roles of lncRNA CCAT2 in esophageal cancer have been published to date. Zhang et al. evaluated CCAT2 levels in a series of surgically resected squamous cell esophageal cancer and matched para-cancerous tissues (26). They found that the tumours exhibit higher CCAT2 levels, positively correlated with advanced TNM stage, presence of lymphatic metastasis, and with the number of positive lymph nodes detected on pathological examination. An increased copy number of c-MYC correlated with increased levels of CCAT2, and the expression of CCAT2 in the c-MYC amplification group was significantly higher than that in c-MYC non-amplification group. The mean survival time was shorter in patients with high CCAT2 expression and c-MYC amplification; both CCAT2 expression and c-MYC amplification were established as independent prognostic factors by multivariate Cox regression analysis (26).

Similarly, in the study of Wang et al., CCAT2 was significantly overexpressed in squamous cell esophageal cancer tissues when compared with paired adjacent normal esophageal tissues, with an average fold of 7.18 (27). Furthermore, CCAT2 was mostly upregulated in KYSE410 cells, when normalized to a normal esophageal epithelium cell line (HEEC). A statistical correlation was found between CCAT2 expression levels and smoking status. Receiver operative curve (ROC) and the area under curve (AUC) were used to assess the diagnostic role of CCAT2, and it showed higher diagnostic performance than conventional serum biomarkers, such as alpha fetoprotein (AFP), CA153, and neuron-specific enolase (NSE).

Gastric cancer

Wang et al. investigated the biological behaviour and prognostic role of lncRNA CCAT2 in gastric cancer (GC) patients. The authors employed human gastric cancer and adjacent non-tumor tissues obtained from 85 surgical patients (28). The authors found that the expression levels of lncRNA CCAT2 were significantly higher in gastric cancer tissues than those in adjacent non-tumor tissues, and were closely correlated with higher incidence of lymph node and distant metastases. Moreover, patients with high CCAT2 expression had shorter overall survival and progression-free survival in multivariate analyses, indicating that it represents an independent factor for poor outcomes in gastric cancer patients (28). Similar results were reported also by Wu et al. in a recent study which confirms the role of CCAT2 in promoting cell proliferation and invasion in gastric cancer (29).

In a study performed in GC tissues and adjacent normal samples from 108 surgically treated individuals, as well as in normal epithelial (GES-1) and GC cell lines (SGC7901, MKN45, BGC-823 and MKN-28), CCAT2 was confirmed significantly upregulated in tumours and higher CCAT2 expression was correlated with poor survival (30). Furthermore, knockdown of CCAT2 inhibited cell migration, invasion and promoted epithelial-mesenchymal-transition (EMT) by downregulating E-cadherin expression and upregulating ZEB2, Vimentin and N-cadherin levels. Moreover, it was found that CCAT2 interacts with EZH2, LSD1, and H3k27me3, which in turn regulate E-cadherin and LATS2 expression (30). A similar mechanism of epigenetic regulation of specific genes from lncRNAs was described in an older article published by Tsai et al.; the lncRNA HOTAIR was found to serve as a scaffold for at least two distinct histone modification complexes (31). A 5′ domain of HOTAIR binds Polycomb Repressive Complex 2 (PRC2), while a 3′ domain binds to the LSD1/CoREST/REST complex, enabling the assembly of PRC2 and LSD1, and coordinating chromatin joined histone H3 lysine 27 methylation and lysine 4 demethylation (31). These studies indicate the complexity of the mechanisms of CCAT2–related oncogenesis in GC (Figure 2).

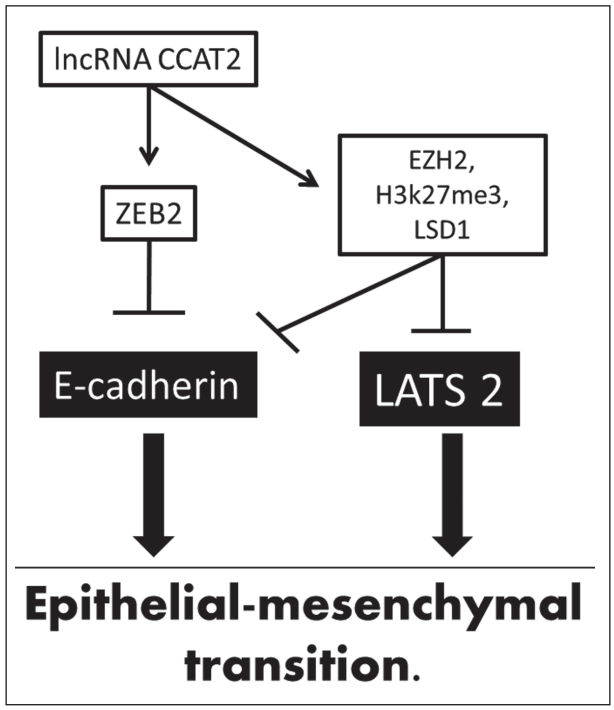

Figure 2. CCAT2 promotes epithelial-mesenchymal-transition (EMT) of gastric cancer cells by upregulating ZEB2 expression and repressing E-cadherin levels. Moreover, CCAT2 interacts with EZH2, H3k27me3, and LSD1, which negatively regulate E-cadherin and LATS2 expression, further promoting EMT.

Colorectal cancer

The discovery of lncRNA CCAT2 comes from experiments by Ling et al (15). The authors characterized CCAT2 and found that the newly identified transcript is expressed at much higher levels in colorectal tumor tissue than in the adjacent normal mucosa, and confirmed that the transcription occurs in the genomic sense orientation (15).

The authors described also an interesting model of CCAT2 locus involvement in CRC. In this model, a DNA loop brings the rs6983267 genomic region close to the c-MYC locus, and this physical association may contribute to the enhancer function of the SNP-containing region on c-MYC transcription. The enhancer region is transcribed into CCAT2, and the SNP status affects CCAT2 expression by an unknown mechanism. The CCAT2 transcript up-regulates WNT activity and increases expression of WNT

target genes (including c-MYC). This regulation by CCAT2 may lead to genomic instability and promote cell growth. Furthermore, the authors showed that c-MYC-regulated miR-17-5p and miR-20a participate in the CCAT2-enhanced cell invasion, and speculate that other mechanisms, such as c-MYC-related mechanisms or enhanced WNT signaling may coordinate the metastatic phenotype elicited by CCAT2. Finally, it was demonstrated that CCAT2 expression is regulated by the TCF7L2 transcriptional factor, indicating a positive feedback loop between CCAT2 and WNT signaling (15).

In a recent article, Kasagi et al. evaluated CCAT2 expression in 149 CRC patients and its associations with clinical and pathological characteristics, outcomes, rs6983267 genotypes, microsatellite status, DNA ploidy, and BubR1 expression (32). They confirmed that CCAT2 expression in cancer tissues was significantly greater than in healthy tissues, especially in metastases. The expression levels of the ncRNA and rs6983267 were not associated with the clinico-pathological features examined, they had not any prognostic significance, and cases with high CCAT2 expression were stable regarding microsatellite behaviour (32). Yu et al. observed that CCAT2 knockout negatively regulates the in vivo expression of miR-145 in colon cancer, impairing proliferation and differentiation. In contrast, stable up-regulation of CCAT2 decreased mature miR-145 (33). The authors also observed that CCAT2 is enriched in the nucleus and correlates with the expression of pre-miR-145 and hypothesized a novel pathogenic mechanism acting through selective block of miR-145 maturation by CCAT2 inhibition of pre-miR-145 export to cytoplasm.

Liver cancer

Zhou et al. and Xu et al. studied the involvement of CCAT2 in hepatocellular carcinoma (HCC). They observed that CCAT2 is upregulated in HCC tissues and human HCC cell lines (34, 35). Furthermore, they found that the overexpression of CCAT2 significantly promoted cell migration and proliferation, and inhibited apoptosis of HCC cells in vitro, confirming the same pattern observed in other malignancies; the suppression of CCAT2 expression resulted in opposing effects (34, 35). Furthermore, CCAT2 was shown once again to promote EMT and HCC progression by Snail2 induction (35).

Gliomas

To investigate the potential biological functions of CCAT2 in glioma, Guo et al. evaluated the CCAT2 mRNA expression in paired glioma tissues and adjacent normal tissues obtained from 134 patients with glioma. The authors found that the expression of CCAT2 is significantly higher in glioma tissues than in adjacent normal tissues, as well as in patients with advanced TNM stage in comparison to those with earlier stages (36). Additionally, the study of CCAT2 in U87-MG and U251 cells revealed that CCAT2 existed mainly in the nucleus of glioma cells. Both proliferation and colony formation assay revealed that silencing CCAT2 expression significantly inhibits its pro-neoplastic effects in cells. Moreover, knockdown of CCAT2 reduced tumor growth in nude mice. Also in this case, the authors showed that CCAT2 knockdown inhibits the transcriptional activity of WNT/β-catenin signalling pathway and the levels of downstream β-catenin target genes (c-MYC, MMP-7 and Cyclin D1), both at transcriptional and translational level (36). Thus, it is tempting to speculate that the inhibitory effect of decreased expression of CCAT2 on the malignant phenotype of glioma cells may be through a repression of the WNT/β-catenin signal pathway.

The results of Guo et al. regarding the expression levels and the impact of CCAT2 in glioma cell proliferation, migration and invasion were substantially confirmed by the findings of Zeng et al. (37). The authors studied also the interactions between CCAT2 and the expression of genes involved in the EMT in glioma cells. Of note, they found that suppressing lncRNA CCAT2 expression increased the expression of epithelial marker genes including E-cadherin and decreased the expression of mesenchymal marker genes including vimentin, N-cadherin, Twist, β-catenin, Snail. These findings suggest that knockdown of lncRNA CCAT2 could inhibit the EMT process. These results are consistent with those observed by Wang et al. in gastric cancer and those of other authors, depicting a combined mechanism which leads to EMT.

Gynecological malignancies

Regarding gynecological cancers, lncRNA CCAT2 has been studied in ovarian and cervical cancers. CCAT2 was found to be upregulated in both cancer types, and correlations between increasing expression levels and higher stages and metastasis were evidenced, as well as with poorer survival rates (38, 39). As in other types of cancer, knockdown of CCAT2 had an antineoplastic effect in cells of both ovarian and cervical cancers, in terms of cell proliferation, migration, invasion, and apoptosis (39, 40).

Urological malignancies

Zheng et al. employed human prostate cancer and paired adjacent healthy tissues and cell lines to study the implications of CCAT2 in prostate cancer onset and progression (41). The findings were very similar to those from other cancer types: the expression level of CCAT2 was higher in neoplastic tissues and cells compared to healthy tissues and normal WPMY- immortalized cells. Survival analysis revealed that patients with high CCAT2 expression had poorer overall and progression-free survivals than those with lower expression. Furthermore, multivariate analysis showed that the status of CCAT2 expression was an independent prognostic indicator for prostate cancer. Again, knockdown of CCAT2 could inhibit cell growth, migration, and invasion in vitro and stimulated EMT through abrogating N-cadherin, vimentin expression and intensifying the expression levels of E-cadherin, as observed in glioma cells (41).

CCAT2 was found to be upregulated also in bladder cancer, and its suppression caused a decrease of cell proliferation and migration as well as an induction of apoptosis in bladder cancer cells (42).

In summary, the results of the studies mentioned demonstrate the pro-oncogenic role of lncCCAT2 in numerous human cancers, both in vitro and in vivo. The main pathogenic networks involved include interactions with MYC, Pokemon, p21, p15, WNT, and other factors promoting the EMT. Therefore, lncCCAT2 may have several potential clinical applications as a biomarker of cancerogenesis and disease progression, as well as a pharmacological target and indicator of response to treatments.

CCAT2 as diagnostic and prognostic marker

CCAT2 was found to be an independent diagnostic marker in several malignancies. For instance, its value as a diagnostic marker was studied adequately and showed higher diagnostic performance than conventional serum biomarkers, like AFP, CA153, and NSE in esophageal cancer (27). Fan et al. performed recently a meta-analysis with the aim to clarify its functions as a prognosis marker in human malignancies (43). Six original studies were included with globally 725 cancer patients enrolled. The results outlined that high CCAT2 expression is significantly correlated with overall survival in cancer patients. A significant association was observed between high CCAT2 expression and poor progression-free survival in cancer patients. Furthermore, CCAT2 expression was significantly related to lymph node metastasis, distant metastasis, and tumor stage. The meta-analysis demonstrated that high CCAT2 expression may serve as a novel biomarker for poor prognosis and metastasis in cancers. Similar results were found in two further meta-analyses recently published (44,45).

Future perspectives

The discovery of CCAT2 and the mounting knowledge on its implications in human cancer represent a potential opportunity for translational applications. Nevertheless, a lot has to be done to this purpose. Firstly, it is necessary to confirm the oncogenic role of this lncRNA in other cancers not yet studied. Then, it is be useful to understand its clinical utility as s reliable diagnostic and prognostic marker. It is also necessary to determine if CCAT2 can be isolated from the blood of patients with cancer, to make its use as a disease marker even easier, with no need of tissue biopsies for determination. Moreover, it is worthy to better elucidate the functional interplay of CCAT2 with components of the WNT/b-catenin signalling pathway, p21, Pokemon, and the EMT process in order to better understand its role in cancer. Finally, it is crucial to explore the antineoplastic effectiveness of inhibitors of CCAT2 for the treatment of human malignancies.

References

1. Stein LD. Human genome: end of the beginning. Nature 2004; 431: 915-16.
2. Huang G, Wu X, Li S, Xu X, Zhu H, Chen X. Long noncoding RNA CASC2 functions as a competing endogenous RNA by sponging miR-18a in colorectal cancer. Nature Scient Rep 2016; 6: 26524.
3. Patil VS, Zhou R, Rana TM. Gene regulation by non-coding RNAs. Crit Rev Biochem Mol Biol 2014; 49: 16-32.
4. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136: 215-33.
5. Isin M, Dalay N. LncRNAs in neoplasia. Clin Chim Acta 2015; 444: 280-88.
6. Brannan CI, Dees EC, Ingram RS, Tilghman SM. The product of the H19 gene may function as an RNA. Mol Cell Biol 1990; 10: 8-36.
7. Jia H, Osak M, Bogu GK, Stanton LW, Johnson R, Lipovich L. Genome-wide computational identification and manual annotation of human long noncoding RNA genes. RNA 2010; 16: 1478-87.
8. Wu Q, Kim YC, Lu J, Xuan Z, Chen J, Zheng Y, et al. Poly A- transcripts expressed in HeLa cells. PLoS One 2008; 3: e2803.
9. Cheng J, Kapranov P, Drenkow J, Dike S, Brubaker S, Patel S, et al. Transcriptional maps of 10 human chromosomes at 5-nucleotide resolution. Science 2005; 30: 1149-54.
10. Gibb EA, Brown CJ, Wan WL. The functional role of long non-coding RNA in human carcinomas. Mol Cancer 2011; 10: 38.
11. Mercer TR, Dinger ME, Sunkin SM, Mehler MF, Mattick JS. Specific expression of long noncoding RNAs in the mouse brain. Proc Natl Acad Sci USA 2008; 105: 716-21.
12. Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. Cell 2009; 136: 629-41.
13. Palmieri G, Paliogiannis P, Sini MC, Manca A, Palomba G, Doneddu V, et al. Long non-coding RNA CASC2 in human cancer. Crit Rev Oncol Hematol 2017; 111: 31-8.
14. Khachane AN, Harrison PM. Mining mammalian transcript data for functional long non-coding RNAs. PLoS One 2010; 5; e10316.
15. Ling H, Spizzo R, Atlasi Y, Nicoloso M, Shimizu M, Redis RS, et al. CCAT2, a novel noncoding RNA mapping to 8q24, underlies metastatic progression and chromosomal instability in colon cancer. Genome Res 2013; 23: 1446-61.
16. Courjal F, Theillet C. Comparative genomic hybridization analysis of breast tumors with predetermined profiles of DNA amplification. Cancer Res 1997; 57: 4368-77.
17. Yokota T, Yoshimoto M, Akiyama F, Sakamoto G, Kasumi F, Nakamura Y, et al. Frequent multiplication of chromosomal region 8q24.1 associated with aggressive histologic types of breast cancers. Cancer Lett 1999; 139: 7-13.
18. Redis RS, Sieuwerts AM, Look MP, Tudoran O, Ivan C, Spizzo R, et al. CCAT2, a novel long non-coding RNA in breast cancer: expression study and clinical correlations. Oncotarget 2013; 10: 1748-62.
19. Cai Y, He J, Zhang D. Long noncoding RNA CCAT2 promotes breast tumor growth by regulating the Wnt signaling pathway. Onco Targets Ther 2015; 8: 2657-64.
20. Deng X, Zhao Y, Wu X, Song G. Upregulation of CCAT2 promotes cell proliferation by repressing the P15 in breast cancer. Biomed Pharmacother 2017; 91: 1160-6.
21. Wu ZJ, Li Y, Wu YZ, Wang Y, Nian WQ, Wang LL, et al. Long non-coding RNA CCAT2 promotes the breast cancer growth and metastasis by regulating TGF-β signaling pathway. Eur Rev Med Pharmacol Sci. 2017; 21: 706-14.
22. Qiu M, Xu Y, Yang X, Wang J, Hu J, Xu L, et al. CCAT2 is a lung adenocarcinoma-specific long non-coding RNA and promotes invasion of non-small cell lung cancer. Tumour Biol 2014; 35: 5375-80.
23. Zhao Z, Wang J, Wang S, Chang H, Zhang T, Qu J. LncRNA CCAT2 promotes tumorigenesis by over-expressed Pokemon in non-small cell lung cancer. Biomed Pharmacother 2017; 87; 692-97.
24. Abbas T, Dutta A. p21 in cancer: intricate networks and multiple activities. Nat Rev Cancer 2009; 9: 400-14.
25. Chen S, Wu H, Lv N, Wang H, Wang Y, Tang Q, et al. LncRNA CCAT2 predicts poor prognosis and regulates growth and metastasis in small cell lung cancer. Biomed Pharmacother 2016; 82: 583-88.
26. Zhang X, Xu Y, He C, Guo X, Zhang J, He C, et al. Elevated expression of CCAT2 is associated with poor prognosis in esophageal squamous cell carcinoma. J Surg Oncol 2015; 111; 834-39.
27. Wang J, Qiu M, Xu Y, Li M, Dong G, Mao Q. Long noncoding RNA CCAT2 correlates with smoking in esophageal squamous cell carcinoma. Tumour Biol 2015; 36: 5523-28.
28. Wang CY, Hua L, Yao KH, Chen JT, Zhang JJ, Hu JH. Long non-coding RNA CCAT2 is up-regulated in gastric cancer and associated with poor prognosis. Int J Clin Exp Pathol 2015; 8: 779-85.
29. Wu SW, Hao YP, Qiu JH, Zhang DB, Yu CG, Li WH. High expression of long non-coding RNA CCAT2 indicates poor prognosis of gastric cancer and promotes cell proliferation and invasion. Minerva Med 2017; 108: 317-23.
30. Wang YJ, Liu JZ, Lv P, Dang Y, Gao JY, Wang Y. Long non-coding RNA CCAT2 promotes gastric cancer proliferation and invasion by regulating the E-cadherin and LATS2. Am J Cancer Res 2016; 6: 2651-60.
31. Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, et al. Long noncoding RNA as modular scaffold of histone modification complexes. Science 2010; 329: 689-93.
32. Kasagi Y, Oki E, Ando K, Ito S, Iguchi T, Sugiyama M, et al. The Expression of CCAT2, a Novel Long Noncoding RNA Transcript, and rs6983267 Single-Nucleotide Polymorphism Genotypes in Colorectal Cancers. Oncology 2017: 92: 48-54.
33. Yu Y, Nangia-Makker P, Farhana L, Majumdar APN. A novel mechanism of lncRNA and miRNA interaction: CCAT2 regulates miR-145 expression by suppressing its maturation process in colon cancer cells. Mol Cancer 2017; 16: 155.

34. Zhou N, Si Z, Li T, Chen G, Zhang Z, Qi H. Long non-coding RNA CCAT2 functions as an oncogene in hepatocellular carcinoma, regulating cellular proliferation, migration and apoptosis. Oncol Lett 2016; 12: 132-38.
35. Xu Y, Wang B, Zhang F, Wang A, Du X, Hu P, et al. Long non-coding RNA CCAT2 is associated with poor prognosis in hepatocellular carcinoma and promotes tumor metastasis by regulating Snail2-mediated epithelial-mesenchymal transition. Onco Targets Ther 2017; 10: 1191-98.
36. Guo H, Hu G, Yang Q, Zhang P, Kuang W, Zhu X. Knockdown of long non-coding RNA CCAT2 suppressed proliferation and migration of glioma cells. Oncotarget 2016; 7: 81806-14.
37. Zeng J, Du T, Song Y, Gao Y, Li F, Wu R, et al. Knockdown of long noncoding RNA CCAT2 inhibits cellular proliferation, invasion, and EMT in glioma cells. Oncol Res 2016; doi:10.3727/096504016X14792098307036.
38. Chen X, Liu L, Zhu W. Up-regulation of long non-coding RNA CCAT2 correlates with tumor metastasis and poor prognosis in cervical squamous cell cancer patients. Int J Clin Exp Pathol 2015; 8: 13261-6.
39. Huang S, Qing C, Huang Z, Zhu Y. The long non-coding RNA CCAT2 is up-regulated in ovarian cancer and associated with poor prognosis. Diagn Pathol 2016; 11: 49.
40. Wu L, Jin L, Zhang W, Zhang L. Roles of Long Non-Coding RNA CCAT2 in Cervical Cancer Cell Growth and Apoptosis. Med Sci Monit 2016; 22: 875-79.
41. Zheng J, Zhao S, He X, Zheng Z, Bai W, Duan Y, et al. The up-regulation of long non-coding RNA CCAT2 indicates a poor prognosis for prostate cancer and promotes metastasis by affecting epithelial-mesenchymal transition. Biochem Biophys Res Commun 2016; 480: 508-14.
42. Li J, Zhuang C, Liu Y, Chen M, Zhou Q, Chen Z, et al. shRNA targeting long non-coding RNA CCAT2 controlled by tetracycline-inducible system inhibits progression of bladder cancer cells. Oncotarget 2016; 7: 28989-97.
43. Fan YH, Fang H, Ji CX, Xie H, Xiao B, Zhu XG. Long noncoding RNA CCAT2 can predict metastasis and poor prognosis: A meta-analysis. Clin Chim Acta 2017; 466: 120-6.
44. Wang D, Chen Z, Xu H, He A, Liu Y, Huang W. Long noncoding RNA CCAT2 as a novel biomaker of metastasis and prognosis in human cancer: a meta-analysis. Oncotarget 2017; doi: 10.18632/oncotarget.18161.
45. Tan J, Hou YC, Fu LN, Wang YQ, Liu QQ, Xiong H, et al. Long Noncoding RNA CCAT2 as a Potential Novel Biomarker to Predict the Clinical Outcome of Cancer Patients: A Meta-Analysis. J Cancer 2017; 8: 1498-506.

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