**Co-upregulation of c-MYC oncogene and its adjacent lncRNAs PVT1 and CCAT1 in esophageal squamous cell carcinoma**

**Abstract**

**Background**: Whether the expression levels of long noncoding RNAs; CCAT1 and PVT1 influence the vicinity gene expression of c-MYC oncogene in ESCC patients has not been thoroughly elucidated to date. Additionally, the solidarity between these lncRNAs and the clinicopathological parameters among human ESCC cancer remains unclear. Where is the objective?

**Methods**: Eighty ESCC tumor tissues and the margin normal tissues were collected from the Tumor Bank of Cancer Institute, Imam Khomeini Hospital. The quantitative real-time PCR was performed to evaluate the expression level of CCAT1, PVT1 and c-MYC genes. Also, demographic information and the clinical pathologic characteristics including tumor grade, tumor stage, lymph node and metastasis were considered.

**Results**: PVT1, CCAT1 and MYC were significantly up-regulated in ESCC tissues as compared to the non-tumor tissues. Up-regulation of PVT1 was positively associated with the advance stage and distance metastasis, while CCAT1 up-regulation was just correlated with the advance stage. CCAT1 was identified as a significant discriminate factor in predicting ESCC (*p*<0.05). Kaplan-Meier analysis revealed that high expression level of MYC and PVT1 decrease the overall survival (*p*<0.001).

**Conclusion**: This is the first report on simultaneous up-regulation of CCAT1, PVT1 and MYC in ESCC. These results may indicate the potential role of these genes in EC and their possible regulatory role. Thus, further studies are required to identify the possible oncogenic role of these lncRNAs and their regulatory mechanism.

**Key words:** Esophageal squamous cell carcinoma, PVT1, CCAT1, LncRNA, MYC

**Introduction**

Esophageal cancer (EC) due to the lethal condition is fast becoming a serious worldwide public health concern. Evidence suggests that EC is the sixth leading cause of death and eighth most common cancer in the world [[1](#_ENREF_1)]. Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAD) are the two primary histological forms of EC. More than 90% of the EC are related to the ESCC which often occurs in countries located on the EC geographic belt that includes parts of north central China, central Asia, and north of Iran [[2](#_ENREF_2)]. The issue of few targeted diagnostic and therapeutic ways has received considerable critical attention, to find an appropriate solution for rapid diagnosis and targeted treatment.

Recently, researchers have shown a growing trend toward noncoding RNAs which are categorized according to differences in length: transcripts less than 200 nucleotides and those longer than 200 nucleotides are classified as short/small RNAs (e.g., miRNAs) and long non-coding RNAs (lncRNA), respectively [[3](#_ENREF_3), [4](#_ENREF_4)]. They act as crucial elements in many physiological processes such as embryogenesis, allelic expression, cell cycle, growth and differentiations. Existing researches report their critical role in tumorigenesis and cancer progression through abnormal proliferation, metastasis, apoptosis and drug resistance [[5](#_ENREF_5)]. Recently, researchers have shown an increased interest in oncogenic and tumor suppressing roles of ncRNAs through the regulation of vital genes in normal physiological processes [[6](#_ENREF_6)]. Evidence strongly support that the aberrant expression of lncRNAs are associated with various stages of carcinogenesis, malignancy and metastasis [[7](#_ENREF_7)].

Investigating human 8q24 chromosomal locus is a continuing concern within oncogenesis due to the presence of c-MYC gene [[8](#_ENREF_8)]. MYC plays an important role in the maintenance of cellular processes such as cell proliferation, differentiation, apoptosis, and angiogenesis [[9](#_ENREF_9)]. Recent developments in cancer have highlighted the frequently amplification and chromosomal translocation of this locus. Regulatory elements also found to be strongly influencing the expression of MYC [[10](#_ENREF_10)]. The existence of some lncRNAs in this locus could control the function of MYC through its expression. Their lack of proper function have been associated with cancer development [[11](#_ENREF_11)], therefore, they were implicated as promising markers for early cancer diagnosis.

Plasmacytoma variant translocation 1 gene (PVT1) and colon cancer-associated transcript-1 (CCAT1) are the two lncRNAs located on the right (100-200 kb downstream) and left (515 kb upstream) side of MYC gene on human chromosome 8, respectively.

Human lncRNA PVT1 contains nine exons and produces a number of noncoding transcribes with different lengths [[12](#_ENREF_12)]. Experimental studies showed the high expression level of PVT1 in various cancer types such as breast [[12](#_ENREF_12)], colon [[13](#_ENREF_13)], gastric [[14](#_ENREF_14)], and ovary [[15](#_ENREF_15)]. PVT1 could increase proliferation and metastasis of the cancer cells, suggesting its potential oncogenic role.

Human lncRNA CCAT1 with 2795 nucleotides in length comprises two exons. Recent evidence demonstrated that knockdown of CCAT1 significantly recused the MYC expression; while up-regulation of MYC increased CCAT1 expression. Consequently, CCAT1 promotes tumorigenesis and metastasis by different mechanism in several cancers [[16](#_ENREF_16), [17](#_ENREF_17)].

Based upon The Cancer Genome Atlas database (TCGA) extracted from GEPIA, Myc gene and two adjacent lncRNAs, PVT1 and CCAT1, were highly expressed in esophageal carcinoma (ESCA) specimens than the neighboring normal tissue (Figure 1A and B). The expression levels of Myc, PVT1 and CCAT1 were increased in ESCA tissues than paired normal tissues, respectively. In contrast to CCAT1, the up-regulation of PVT1 in ESCA is significantly correlated with increased MYC gene expression level (Figure 1C).

To understand the molecular mechanism underlying the control of c-MYC, PVT1 and CCAT1, some bioinformatics databases were used.

**Materials and Methods**

**Samples collection**

The Research Ethics committee of the IR.Goums.REC.1395.256 approved this study. The EC tumor tissues and adjacent normal tissues were collected from 40 patients who underwent surgery without local or systematic treatment between 2016 and 2017 from the Imam Khomeini Hospital, Tehran, Iran. However, two samples were lost during the preparation. The fresh tissue specimens were immediately frozen in liquid nitrogen and stored at -80°C for further experiments.

The mean age of patients was about 48 years (range: 29 to 81). Further, the association between c-Myc and its neighboring lncRNAs expression with clinicopathological features including tumor grade, tumor stage, lymph node, and metastasis were evaluated.

**Isolation of total RNA**

Total RNA was extracted from the frozen specimens by trizol reagent (Name of company?) according to manufactures instructions. The quality and quantity of RNA was evaluated by Nano drop and only samples with an A260/A280 ratio between 1.8 and 2.1 was considered for future analysis. To remove DNA contamination, RNA was treated with DNase enzyme in RNase-free condition. The first strand complementary DNA (cDNA) was generated using random hexamer primers by the prime script RT Reagent kit (ABI).

**Real-Time PCR**

The quantitative real-time PCR was carried out using standard SYBER green premix EX tag2 (TAKARA) in 20 μl to detect the mRNA level of MYC, PVT1, and CCAT1 genes. All qRT–PCR reactions were performed for 40 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, on ABI 7300 Real Time PCR system (Applied Biosystems, USA). All experiments were performed in duplicate and the specificity of PCR product was confirmed by melt curve analysis. GAPDH was chosen as an endogenous control to normalize the expression level of candidate genes. The characteristics of primers were indicated in Table 1. Subsequently, the expression level of target genes was calculated using 2–△△Ct method [[18](#_ENREF_18)].

**lncRNA-miRNA interaction analysis**

In order to identify and predict the interaction of lncRNAs; CCAT1 and PVT1 with miRNAs, the mirDIP 4.1 database was applied (ophid.utoronto.ca/mirDIP/). This database predicts which MiRNAs are able to be target c-MYC, PVT1, and CCAT1 with very high, high, and medium score numbers. Very high and high score numbers indicated stronger binding ability.

**Interaction analysis of c-MYC, PVT1 and CCAT1**

In order to investigate, based upon the experimental evidence, which genes to target with lncRNAs PVT1 and CCAT1 in different cancer types, a bioinformatics EVlncRNAs database was applied (biophy.dzu.edu.cn/EVLncRNAs/). Moreover, RAID v.2 database was used to identify some protein interactions with PVT1 and CCAT1.

According to the lncRNA Disease database, the regulatory relationships of these genes in cancer was examined to identify the potential mRNA target genes of CCAT1 and PVT1.

**Expression profile analysis of c-MYC, PVT1 and CCAT1 in cancer**

In order to investigate the biological role of aberrant expression of PVT1 and CCAT1 in different cancers, lncRNA Disease and lnc2cancer were used. Also, the probability of these lncRNAs role in other type of cancer was predicted with target prediction tools such as LRLSLDA-LNCSIM1//LRLSLDA-LNCSIM2.

**Statistical analysis**

Statistical analysis was performed using SPSS version 18 for windows. A value of *p*<0.05 was considered significant. To evaluate the correlation between lncRNAs, PVT1, CCAT1 and MYC gene expression and clinicopathological features, the independent two-tailed t-test were used. To evaluate the diagnostic value of targeted genes, the receive operating characteristic (ROC) curve were plotted by GraphPad prism. Kaplan-Meier analysis was performed to calculate overall survival and survival analysis compared by log rank test.

**Results**

**Up-regulation of MYC oncogene in ESCC patient**

The expression level of MYC oncogene was significantly higher in clinical ESCC specimens compared to the adjacent non-tumor specimens *(p<*0.05, Figure 2). Furthermore, there was no significant correlation between MYC expression and the mentioned clinicopathological factures (Data was not shown). If you don’t want to show the results, please merge this paragraph with the next…

**Up-regulation of lncRNA PVT1 and lncRNA CCAT1 in ESCC patient**

The expression of both lncRNAs PVT1 and CCAT1 were significantly increased in ESCC samples than adjacent normal tissues (p<0.05, Figure 2). A correlation between these lncRNAs expression level with clinicopathological features of ESCC sample were identified (Table 1). It demonstrated that ESCC patients with high expression of PVT1 were in advance stage and showed metastasis (*p*<0.05). The same result was observed for CCAT1 expression. However, no significant association was observed between the elevated expression of CCAT1 and metastasis (*p*>0.05). These results suggest that these lncRNAs might play a critical role in the development and prognosis of ESCC.

**Diagnostic value of CCAT1 as marker of ESCC**

We performed ROC curve analysis to evaluate the diagnostic value of both lncRNAs, PVT1 and CCAT1 (Figure 3). The area under the ROC curve (AUC) of PVT1 and CCAT1 were 0.61 and 0.63, respectively. This result revealed that CCAT1 was a significant discriminate factor in predicting ESCC (p=0.03) with 95% confidence interval (0.51 to 0.75). Indeed, CCAT1 showed a good suitability to classify tumor and non-tumor samples of esophageal tissues. Therefore, lncRNA CCAT1 may be a novel biomarker of poor prognosis in patients with ESCC.

**MYC and PVT1 associated with overall survival in ESCC patient**

Kaplan-Meier analysis was performed to demonstrate the correlation of MYC gene and lncRNAs PVT1 and CCAT1 with survival rate in ESCC patients. As shown in Figure 4, the overall survival of patients with the high level of PVT1 (*p*=0.007) and MYC (*p*=0.00) expression was markedly reduced compared to those with low expression level of these genes.

**Interaction analysis of MYC and two vicinity lncRNAs, PVT1 and CCAT1 in human tumors**

According to the mirIDP4.1 database, the numbers of 158 and 139 miRNAs were predicted that are able to be potential target of PVT1 and CCAT1, respectively. All of these miRNA could have interaction with MYC oncogene. Among these miRNAs, 23 of them with high and medium score class could interact with PVT1, CCAT1, and MYC. While, 24 of these miRNAs interact with PVT1 and MYC, and 11 of them have potential to be target of CCAT1 and MYC (Supplementary Table1).

The obtained information from EVlncRNA database was shown some experimental interaction of PVT1 and CCAT1 (Supplementary Table 2). This data suggested that these lncRNAs play significant role in various cancers. Also, PVT1 and CCAT1 lncRNAs-protein interaction with ‘weak evidence’ method was extracted from RAID v.2 resource (Supplementary Table3). However, these data require more extensive research to prove these interactions.

All experimentally proved interaction of PVT1, CCAT1, and MYC with microRNAs, lncRNAs, and proteins are summarized and illustrated in Figure 5.

**Expression profile of PVT1 and CCAT1 in different cancer types**

Data extracted from both Lnc2Cancer and LncRNADisease databases indicated the role of PVT1 and CCAT1 lncRNAs in the pathogenesis of some cancers. This information has been proven by various molecular experiments such as FISH, RIP, RT-PCR, Western Blot, Dual luciferase reporter gene assay, qRT-PCR, RNAi, and MTT. Also, the role of both lncRNAs are predicted in some cancers with the LRLSLDA-LNCSIM1 and LRLSLDA-LNCSIM2 methods. A list of cancers have been shown in Table 4, that experimentally interfere with both lncRNAs, PVT1 and CCAT1. This table was categorized into experimental and expected evidence. In fact, these two lncRNAs can play the role in the pathogenesis of predicted cancers.

The expression pattern comparison of target lncRNAs across tumor and non-tumor samples was conducted in different cancers (Supplementary Figure 1). PVT1 lncRNA was significantly increased in some cancers including ESCA, DLBC, COAD, CHOL, BRCA, READ, SARC, LUSC, LUAD, KIRC, GBM, and STAD. While the expression level of PVT1 in LAMC, OV, ACC, THCA, and TGCT tumor tissues was significantly reduced as compared to the non-tumor tissues. Also, the CCAT1 was extremely up regulated in READ, COAD, ESCA, STAD, LUSC, and CHOL cancerous samples. It is only in two cancer types; HNSC and LIHC that the expression of CCAT1 was reduced compared to the normal samples.

**Discussion**

Esophagus cancer is considered as a major public health problem due to the 6-year its survival is still pretty low???. It is, therefore, necessary to seek and identify new potential biomarkers for early diagnosis and targeted treatment of EC. Prior studies have demonstrated the regulatory function of lncRNAs in pathological processes and cancer biology [[3](#_ENREF_3)]. For instance, PVT1 in cervical cancer promotes progression by silencing of mir-200b [[19](#_ENREF_19)] and inhibits apoptosis in colorectal cancer [[13](#_ENREF_13)]. CCAT1 enhances hepatocellular carcinoma and gallbladder cancer progression by functioning as let-7 sponge and regulating of miRNA-218, respectively [[20](#_ENREF_20), [21](#_ENREF_21)]. It also promotes proliferation and migration of pancreatic cancer cells by MYC [[17](#_ENREF_17)]. The same function was observed in esophageal carcinoma by regulating SPRY4 and HOXB13 expression [[22](#_ENREF_22)].

Thus, the attention of research is shifting to the roles of dysregulated lncRNA expression in various cancers [[7](#_ENREF_7), [23](#_ENREF_23)]. Moreover, achieving a comprehensive knowledge of the co-expression network between lncRNAs and coding genes might have a great impact on cancer recognition.

The 8q24 chromosomal locus is a considerable site for some translocations and amplifications, which involved in various diseases. This reign??? also contains a number of important regulatory elements that any disturbances in them can result in cancer development. The oncogene MYC, located at this locus, is often activated in tumorigenesis and aberrantly expressed in different cancer types [[24](#_ENREF_24), [25](#_ENREF_25)].

This research set out with the aim of assessing the expression of MYC gene along with two vicinity lncRNAs, PVT1 and CCAT1 in ESCC patients. PVT1 as an oncogenic lncRNA has a critical role in cancer development and pathophysiology. Our result revealed that the expression level of PVT1 in ESCC samples were significantly higher than pair adjacent normal tissues. Numerous studies have reported that the over-expression of PVT1 has an important role in development of different cancer types such as gastric cancer [[14](#_ENREF_14)], colorectal cancer [[13](#_ENREF_13)], non-small cell lung cancer [[26](#_ENREF_26)], ovarian and breast cancer [[27](#_ENREF_27)]. The over-expression of PVT1 was also reported in hepatocellular carcinoma [[28](#_ENREF_28)]; the results of gain and loss of function experiments indicated that PVT1 plays a key role in cancer cells proliferation, metastasis, and cell cycle. The effect of *in vitro* knocking down PVT1 lncRNA in lung and colorectal cancers led to remarkable loss of cancer cells proliferation and invasion [[13](#_ENREF_13), [26](#_ENREF_26)].

LncRNA CCAT1, in this study, was up-regulated in ESCC tissues in comparison with margin normal tissues. This finding supports previous research into colorectal cancer tissues and peripheral blood samples of the same patients, but not in healthy control [[29](#_ENREF_29)]. The extremely expression of this lncRNA in stomach adenocarcinoma was reported [[14](#_ENREF_14)]. Another research reports that expression of CCAT1 were markedly increased in breast cancer tissues [[30](#_ENREF_30)]. LncRNA CCAT1 levels similarly in gastric cancer tissues were extremely higher than in adjacent normal tissues [[31](#_ENREF_31)]. They also demonstrated that the over-expression of CCAT1 enhances cancer cell proliferation and migration, while its silencing results in reduction of cell proliferation and migration.

The up-regulation of PVT1 in multiple cancers may be outcome of the amplification and translocation of 8q24 region. A cluster of miRNAs including miRNA1204, miRNA1205, miRNA1206, miRNA1207, and miRNA1208 are located within PVT1 locus. PVT1 controls the MYC protein stability which acts as a transcription factor and lead to an increased PVT1 transcript. The simultaneous expression of MYC and PVT1 promotes cancer cell proliferation. It is demonstrated that the co-expression of MYC and PVT1 in HER2-positive breast cancer patients plays a key role in driving tumor [[32](#_ENREF_32)]. However, PVT1 with the function of anti-apoptotic, in breast and ovarian cancers, acts independently of MYC [[33](#_ENREF_33)]. Since the inhibition of MYC alone failed to induce apoptosis [[27](#_ENREF_27)]. Although, it was suggested that the up-regulation of PVT1 locus-located miRNAs independent of c-MYC possess anti-proliferation and apoptotic functions [[33](#_ENREF_33), [34](#_ENREF_34)]. Therefore, the oncogenic role of PVT1 may be attributed to these miRNAs.

Moreover, CCAT1 silencing led to reduce transcription of MYC and chromatin loops interaction in this locus. Because MYC can directly binds to E-box element of CCAT1 promoter which results in the transcriptional activating and expression of CCAT1 [[31](#_ENREF_31)]. Since, CCAT1 expression is associated with MYC and its deregulation has an important effect on proliferation and migration of various cancers, it therefore can be introduced as a tumor oncogene.

Furthermore, to achieve more convincing conclusion, the second objective was designed to explore the association of these genes expression with clinicopathological features. The current study found that the up-regulation of PVT1 was associated with advance clinical stage and distance metastasis. These results match those observed in earlier studies. Prior study have noted the increased expression level of this lncRNA associated with lymph node and invasion of gastric cancer [[14](#_ENREF_14)]; they suggested PVT1 as a new biomarker and therapeutic target for gastric cancer. In accordance with the present finding, the up-regulation of PVT1 in lung cancer was significantly correlated with clinical tumor stage, lymph nodes and distance metastasis [[26](#_ENREF_26)].

In this study, the high expression level of CCAT1 was correlated with clinical tumor stage. So far, several findings reveal that the up-regulation of CCAT1 was associated with different clinical parameters such as tumor grade, lymph node metastasis, and clinical tumor stage. For instance, the increased level of CCAT1 in gastric cancer tissues was correlated with the tumor size and lymph node metastasis [[31](#_ENREF_31)]. The same observations was reported in breast cancer which correlated with differentiation grade, stage, and lymph nodes metastasis [[30](#_ENREF_30)].

Moreover, merging the results of published studies and conducting online bioinformatics resources performed the molecular mechanism of targeted lncRNAs, PVT1 and CCAT1, in tumors. Comprehensive networks of lncRNA-miRNA-mRNA can regulate several important cell functions. LncRNAs, in this network, play a vital role in regulating gene expression by participating in the competing endogenous RNAs (ceRNA), RNA-RNA and RNA-proteins interactions and with cis/trans regulatory function. Thus, the aberrant expression of this network has an important role in cancer pathogenesis.

For instance, miR-1204 increases the expression of p53 by sharing promoter and regulatory elements with PVT1. This miRNA potentially promotes apoptosis and cell cycle arrest [[35](#_ENREF_35)]. Mir-1207 leads to breast cancer cell progression by inhibiting STAT6 that acts as an activator of the cell cycle dependent kinase inhibitors [[36](#_ENREF_36)].

The interaction of PVT1 with mir-200 family is another example. It has been shown that miR200a and miR-200b are direct target of PVT1 in non-small cell lung cancer patients. Also, increased methylation of miR200b promoter through binding of PVT1 with EZH2 inhibits this miRNA function [[19](#_ENREF_19)]. Abnormal expression of this miRNA caused to increased cell proliferation and migration. Likewise, direct interaction of PVT1 and MYC oncogene with miR-200c promoter induces its expression. The up-regulation of miR-200c results in activation of AKT signaling pathway by inhibition of PTEN [[37](#_ENREF_37)].

Moreover, PVT1 induces HIF1a and RUNX2 expression by suppressing miR-186 [[38](#_ENREF_38)] and miR-455 [[39](#_ENREF_39)], respectively. It was also reported that PVT1 regulates CD151 and FGF2 expression through inhibiting expression of miR-152 in gastric cancer [[40](#_ENREF_40)].

The up-regulation of lncRNA CCAT1 induces cell proliferation, migration, and inhibition of apoptosis by enhancement of MYC and HMGA2 expression while reduces the Let7 expression [[20](#_ENREF_20), [41](#_ENREF_41)]. The CCAT1 has a potential role in regulating ADAM17 and WNT1 via targeting miR-152 and mediate STAT3 and ZEB1 expression level by targeting miR-130a [[42](#_ENREF_42)]. This lncRNA also promotes malignancy by inhibiting of miR-218 in gallbladder cancer [[21](#_ENREF_21)].

**Conclusion**

This is the first study reporting the expression profile of PVT1, CCAT1 and MYC oncogene in ESCC patients simultaneously. The up-regulation of PVT1 and CCAT1 were correlated with some clinicopathological parameters such as advance stage and metastasis. Therefore, they could be introduced as potential biomarkers in ESCC, especially CCAT1. Molecular mechanisms behind their interaction increase awareness in scientists to design diagnostic biomarkers and target a specific treatment in esophageal cancer. The functional mechanism underlying predicted data for these target genes with other regulatory elements are required further *in vivo* and *in vitro* investigations.

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**Figure 1: Comparison of expression profile of MYC gene and two neighboring lncRNAs, PVT1 and CCAT1 across esophageal carcinoma samples and adjacent normal tissues. (A)** The expression of MYC, PVT1, and CCAT1 are increased in ESCA tissues in comparison to normal tissues, respectively. (**B**) In comparison to MYC and CCAT1, PVT1 shows a remarkable change in ESCA tissues than normal tissues.

(**C**) Pearson correlation of MYC and PVT1, MYC and CCAT1, as well as CCAT1 and PVT1 genes retrieved from Gene Expression Profiling Interactive Analysis (GEPIA) resource. Log2 (TPM +1) was used for log-scale. TPM: Transcripts Per Million; ESCA: esophageal carcinoma.



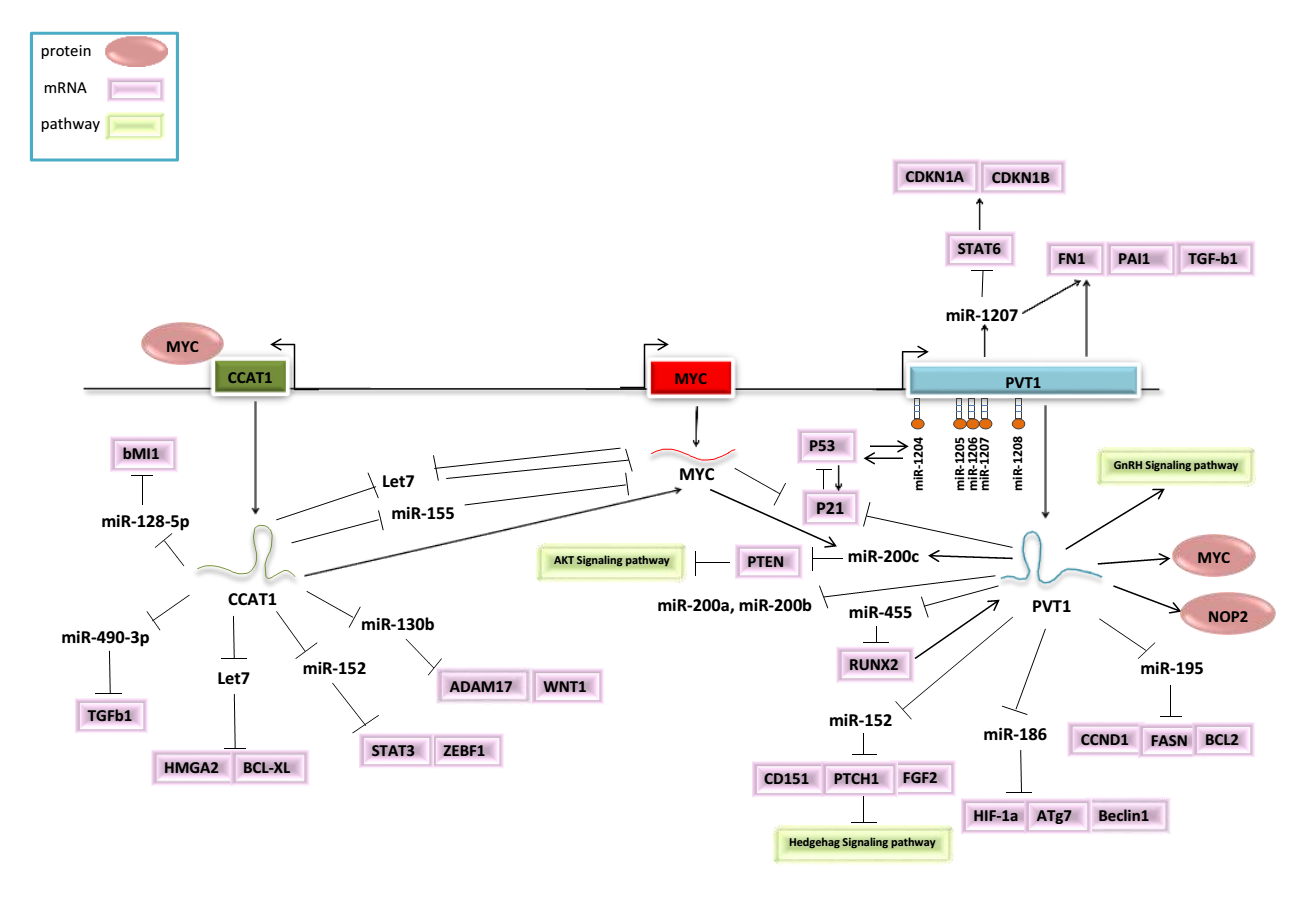
**Figure 2: The gene expression profile of MYC gene and two vicinity lncRNAs, PVT1 and CCAT1 across esophagus tumor and paired normal tissues.**



**Figure 3: ROC curve analysis to explore the discriminant ability of MYC gene and two vicinity lncRNAs, PVT1 and CCAT1 in predicting esophagus tumor.**

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**Figure 4: Association of MYC, PVT1, and CCAT1 with survival rate in ESCC patients.** Overall survival of patients with high vs low expression levels of MYC, PVT1, and CCAT1 are illustrated. Patients with high MYC and PVT1 expression have poorer survival.



**Figure 5: Prognostic significance of human 8q24 chromosomal locus and target gene network in cancers**.The molecular mechanisms of MYC and adjacent lncRNAs are illustrated.

The interaction network of lncRNA-miRNA-mRNA in different cancer types are identified. Green and purple rectangular represent pathway and mRNA, respectively. Circles represent proteins. Arrow and arrested-line signs represent the possible associations between lncRNAs, miRNAs and mRNA. lncRNA: long non-coding RNA; miRNA: microRNA.

**Table1: The primers specifications applied for the expression analysis in q-RT-PCR**

|  |  |  |
| --- | --- | --- |
| **Target** | **Sequences** | **Amplicon length (bp)** |
| **Myc**  (NM\_002467.5) | Forward :  5´-CACAGCAAACCTCCTCACAG-3´  Reverse:  5´-GGTGCATTTTCGGTTGTTGC-3´ | 187 |
| **CCAT1**  ([NR\_108049.1](https://www.ncbi.nlm.nih.gov/nucleotide/NR_108049.1?report=genbank&log$=nucltop&blast_rank=1&RID=1Y9SZZ5D015" \t "lnk1Y9SZZ5D015" \o "Show report for NR_108049.1)) | Forward:  5´-GGCACTACTCTGTCCCAACA-3´  Reverse:  5´-AGCCATACAGAGCCAACCTG-3´ | 187 |
| **PVT1**  (NR\_003367.3) | Forward:  5´-TGAGAACTGTCCTTACGTGACC-3´  Reverse:  5´-AGAGCACCAAGACTGGCTCT-3´ | 191 |
| **GAPDH**  (NM\_002046.7) | Forward:  5´-GGTGGTCTCCTCTGACTTCAACA-3´  Reverse:  5´-GTTGCTGTAGCCAAATTCGTTGT-3´ | 127 |

**Table 2: Association of PVT1 expression with clinicopathological factors in EC patients**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Clinical specify** | **samples** | **PVT1 expression** | | **P value** |
| **High Low** | |
| Age  60<  60≥  Gender  Male  Female  Tumor size(cm)  5 <  5 ≥  Tumor stage  I-II  III-IV  Tumor Grade  I-II  III-IV  Metastasis  Unknown  Yes  No  Lymph node  Yes  No | 22  16  20  18  21  17  11  27  8  30  9  19  10  16  22 | 12  7  11  8  13  6  4  15  4  15  12  6  7  12 | 10  9  9  10  8  11  7  12  4  15  7  4  9  10 | .468  .273  .624  .009  .064  .025  .33 |

**Table 3: Association of CCAT1 expression with clinicopathological factors in EC patients**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Clinical specify** | **samples** | **CCAT1 expression** | | **P value** |
| **High Low** | |
| **Age**  60<  60≥  **Gender**  Male  Female  **Tumor size (cm)**  5 <  5 ≥  **Tumor stage**  I-II  III-IV  **Tumor Grade**  I-II  III-IV  **Metastasis**  Unknown  Yes  No  **Lymph node**  Yes  No | 22  16  20  18  21  17  11  27  8  30  9  19  10  16  22 | 12  11  13  10  12  11  7  16  5  18  11  7  7  16 | 10  5  7  8  9  6  4  11  3  12  8  3  9  6 | .823  .268  .771  .038  .638  .345  .409 |

**Table 4: Experimental validated and predicted cancers for PVT1 and CCAT1 interference**

|  |  |  |
| --- | --- | --- |
| **Cancers Name** | **Detection Method** | **lncRNA** |
| esophageal squamous cell carcinoma, breast cancer, colon cancer, gallbladder cancer, Glioma, hepatocellular carcinoma, lung cancer, ovarian cancer, pancreatic cancer, retinoblastoma, Nasopharyngeal carcinoma, stomach cancer, colorectal cancer, cervical cancer, osteosarcoma, renal cell carcinoma, colorectal cancer | **Experimental** | **CCAT1** |
| Lymphoma, thyroid cancer, urinary bladder cancer | **Predicted** |
| esophageal squamous cell carcinoma , thyroid cancer, lung cancer, renal carcinoma, lung squamous cell carcinoma, bladder urothelial carcinoma, B-cell lymphoma, lymph node metastasis, plasmacytoma, osteosarcoma, multiple myeloma, colon cancer, nasopharyngeal cancer, cervical cancer, pancreatic cancer, colorectal cancer, breast cancer, gastric cancer, non-small cell lung cancer | **Experimental** | **PVT1** |
| endometrial cancer, germ cell cancer, leukemia, liver cancer, lung cancer, testicular cancer, tongue cancer, acute lymphocytic leukemia, adrenocortical carcinoma, basal cell carcinoma, glioblastoma, lymphoblastic leukemia, parotid gland cancer, rhabdomyosarcoma | **Predicted** |

Note: Data obtained from LncRNADisease v2.0 database.