Enhancer RNA ADCY10P1 in Pan-Cancer

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Abstract

Background. Enhancer RNA(eRNA) ADCY10P1 is derived from enhancer regions and may have enhancer function.

Methods: Publicly available data was downloaded from The Cancer Genome Atlas (TCGA) and expression of ADCY10P1 was compared in normal tissue and pan-cancer tissue. Over survival time was analyzed. Correlations between ADCY10P1 expression and immune infiltration was analyzed. ADCY10P1-related genes were found to perform Gene Ontology (GO) analysis and Genes and Genomes (KEGG) analysis.

Results. ADCY10P1 expression was higher in most cancer groups, including KIRP, BLCA, LUAD, LUSC, STAD, and THCA. ADCY10P1 showed a protective effect on BLCA, LAML, LGG, READ, and THYM. The results of Gene Ontology (GO) analysis and Genes and Genomes (KEGG) analysis in BLCA and LGG.

Conclusion. ADCY10P1 can affect pan-cancer progress and prognosis. ADCY10P1 was an independent prognostic factor in BLCA and LGG. High expression of ADCY10P1 is beneficial to BLCA, LAML, LGG, READ, and THYM. High expression may suppress immune infiltration. ADCY10P1 may have a direct or indirect trans function. ADCY10P1 may have a dual role in the tumor, which is oncogenes and tumor suppressor genes in BLCA and READ.

Keywords. Enhancer RNA, ADCY10P1, Pan-cancer, Immune

1. Introduction

ncRNAs are potential biomarkers for diagnosing disease and using a prognostic indicator[1-3]. lncRNAs are a group of transcribed RNA molecules with a length of more than 200 nucleotides[4]. eRNAs are a specific class of lncRNAs and are derived from enhancer regions. These eRNAs are a major type of cis-regulatory elements in the genome[5] and have enhancer function. They can play a role in cis and affect the transcription of the target gene with high transcription[6]. Despite there were several publications mentioned eRNA ADCY10P1, they didn’t depict the detailed function of ADCY10P1 in pan-cancer.


2.1 Date was obtained

A list of lncRNAs was gotten and they expressed from active tissue-specific enhancers[7]. Their assigned targets were predicted by PreSTIGE[7]. We choose ADCY10P1 and found its assigned target genes C6orf130, NFYA, and UNC5CL. Home_sapiens. GRCh38.100.chr_pathpatch_hapl_scaffold.gtf was downloaded from Ensembl database (http://wwwensembl.org/index.html) for transferring gene symbol. HTseq- FPKM data, corresponding clinical data, and somatic mutation data were obtained from UCSC Xena database(http://xena.ucsc.edu/). Because these data were downloaded directly from public databases and we strictly obey the publishing policies of these databases, and there was no requirement for ethical approvals.

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2.2 Compared to the expression of ADCY10P1.

The expression of ADCY10P1 was compared in the normal specimen and corresponded cancer specimen with “wilcox.test”.

2.3 Survival Analysis

We divided samples to high expression level and low expression level of ADCY10P1 groups based on the median expression level of ADCY10P1. Kaplan-Meier analysis and Univariate Cox hazard analysis was performed by "survival" and "survminer" package to compare over survival time(OS) with pvalueFilter=0.05. R software (version 3.6.3) was utilized with the “forestplot” package.

2.4 Independent prognostic factor analysis

To verify whether ADCY10P1 was an independent prognostic factor in pan-cancer, we combined ADCY10P1 with the available clinical and pathologic data with “survival” package. Because of lacking clinical and pathologic data of Myeloid Leukemia (LAML) and thymoma (THYM), multivariate analyses weren’t performed in these two cancers.

2.5 Correlations Between Expression of ADCY10P1 and Immune infiltration

A known reference dataset provided a bunch of gene expression traits of 22 immune cells. The CIBERSORT (R script v1.03) was based on the above reference dataset to estimate the proportions of immune cells in samples. The samples were filtered with p<0.05 and with 1,00 permutations to enhance the accuracy of the estimated results. Then, the correlation between ADCY10P1 expression and Immune infiltration was calculated with Pearson Test and p<0.001.

We divided samples to high expression level and low expression level of ADCY10P1 groups based on the median expression level of ADCY10P1. Immune cells in two groups were compared with Wilcox. test and p<0.001.

2.6 Target gene and Gene Ontology (GO) analysis and Genes and Genomes (KEGG) analysis

The lncRNA and mRNA of BCLA and LGG were extracted. ADCY10P1-related lncRNA and mRNA were found based on the immune ADCY10P1’s expression with cor.test (corFilter=0.4, pvalueFilter=0.001). The tumor specimen was assigned to high-level and low-level expression groups based on the middle level expression of ADCY10P1. ADCY10P1-related lncRNA and mRNA that were compared in two groups were performed by the “Limma” package. The Entrez ID was gotten with “org.Hs.eg.db”. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of ADCY10P1-related lncRNA and mRNA was performed using the “clusterProfiler” package[8].

3. Result

3.1 ADCY10P1 Expression in pan-cancer and normal specimen

Expression levels of ADCY10P1 were analyzed to examine ADCY10P1 expression in pan-cancer and normal specimens. The results showed ADCY10P1 expression was lower in BRCA, GBM, KICH, and THCA (Figure 1). While ADCY10P1 expression was higher in BLCA, CHOL, COAD, HNSC, KIRC, LIHC, LUSC, PRAD, READ, and STAD (Figure 1).
3.2 Prognostic-related value of ADCY10P1 in pan-cancer and independent prognostic factor

We investigated the ADCY10P1-related prognostic value with Kaplan-Meier Plotter. Over Survival (OS) was shown in Figure 2. Apparently, the expression of ADCY10P1 was significantly correlated with seven cancer types from Figure 2, including bladder cancer (BLCA), acute myeloid leukemia (LAML), lower grade Glioma (LGG), rectal Cancer (READ), thymoma (THYM) and Endometrioid cancer (UCEC). Among them, only in UCEC, ADCY10P1 played a negative role. Notably, high ADCY10P1 expression levels could be used as an independent prognostic factor for a positive prognosis in BLCA and LGG (Figure 3).

![Figure 1. Expression of ADCY10P1 in normal tissue and pan-cancer tissue](image1)

![Figure 2. Over survival A. Kaplan-Meier survival curves comparing high and low expression of ADCY10P1 in different cancer types reveal the over survival. B. Univariate Cox hazard analysis.](image2)
3.3 Correlations Between ADCY10P1 Expression and Immune infiltration

As shown in Figure 4, Macrophages M1, Neutrophils, and T cells CD4 memory activated were negatively correlated with ADCY10P1 Expression, while T cells regulatory (Tregs) was positively correlated with ADCY10P1 Expression in BLCA. Macrophages M1, T cells CD4 memory resting and T cells CD8 were positively correlated with ADCY10P1 Expression in LGG, while Macrophages M2 is the opposite.

From Figure 4 A, T cells CD4 memory activated, Macrophages M1, and Neutrophils were lower expression in high-expression of ADCY10P1 group. T cells regulatory (Tregs) were higher expression in high-expression of ADCY10P1 group. From Figure 4 B, T cells CD4 memory resting and Macrophages M1 were lower expression in high-expression of ADCY10P1 group, while Macrophages M2 was high expression in low-expression group.
3.4 Target gene and Gene Ontology (GO) analysis and Genes and Genomes (KEGG) analysis

There were 207 ADCY10P1-correlated with lncRNA and 477 ADCY10P1-correlated with mRNA that included the predicted target gene NFYA in BLCA. There were 155 ADCY10P1-correlated with lncRNA and 206 ADCY10P1-correlated with mRNA in LGG.

In BLCA, the genes were most related to focal adhesion, cell−substrate adhesion junction, and cell−substrate junction. In addition, genes were highly enriched in Herpes simplex virus 1 infection, Staphylococcus aureus infection, and Phagosome signaling pathways. In LGG, the genes were most related to RNA splicing, via transesterification reactions with bulged adenosine as nucleophile, and mRNA splicing, via spliceosome. In addition, genes were highly enriched in Endocytosis, mRNA surveillance pathway, and Phospholipase D signaling pathway. (Figure 8)

Discussion

eRNAs can serve as a potential reliable marker for active enhancers in many cell types[9-17]. Moreover, eRNAs revealed to be functionally vital for gene activation, because knockdown of eRNAs expressed in various cell types always caused a reduction of transcription of specific target genes [14, 15, 18-21]. The expression levels of eRNAs are related to the cis-regulatory activity, and the expression levels of eRNAs enhance mRNA synthesis of nearby genes, which indicate an intimate correlation between enhancer function and eRNA production [22, 23]. Though these exciting researches were reported, there is no study on Enhancer RNA ADCY10P1. What’s the effect on pan-cancer?

At first, the expression level of ADCY10P1 was compared pan-cancer with normal tissue. The
results showed ADCY10P1 expression was lower in BRCA, GBM, KICH, and THCA (Figure 1). While ADCY10P1 expression was higher in BLCA, CHOL, COAD, HNSC, KIRC, LIHC, LUSC, PRAD, READ, and STAD (Figure 1). It suggested that most types of tumor tissue had a higher expression level of ADCY10P1. Over Survival (OS) were shown in Figure 2. Apparently, ADCY10P1 expression was significantly correlated with six cancer types, including BLCA, LAML, LGG, READ, THYM and UCEC. Among them, only in UCEC, ADCY10P1 played a negative role. Apparently, high ADCY10P1 expression levels could be used as an independent prognostic factor in BLCA and LGG (Figure 3). It suggested that ADCY10P1 may affect directly the prognosis in the two cancers and it was a protecting factor in these cancers. While ADCY10P1 also had an influence on the prognosis in the USEC, and READ indirectly. It suggested that the function of ADCY10P1 may vary in different contexts. From Figure 1 and Figure 2, ADCY10P1 expression was higher in BLCA and READ, but high expression of ADCY10P1 has a good survival. It suggested ADCY10P1 was protecting factor in these cancers and it is beneficial to the progress and survival of patients. It also suggested that ADCY10P1 may have a dual role in the tumor, which is oncogenes and tumor suppressor genes.

Then, understanding immune infiltration may help find the mechanisms behind tumor development. As shown in Figure 4 A, Macrophages M1, Neutrophils, and T cells CD4 memory activated were negatively correlated with ADCY10P1 Expression, and T cells CD4 memory activated, Macrophages M1, and Neutrophils were lower expression in high-expression of ADCY10P1 group. Macrophages can be trained by the T helper 2 (TH2) cells to become an immune-escape role[24]. Tumor cells can be helped by tumor-associated macrophages (TAMs) in several ways, including immune escape, tumor angiogenesis, and metastasis[25-30]. In our study, it may suppress Macrophages to result in longer survival. From Figure 4 B, T cells CD4 memory resting and Macrophages M1 were lower expression in high-expression of ADCY10P1 group, while Macrophages M2 was a high expression in the low-expression group. It indicated ADCY10P1 may mediate different immune cells in different types of cancer.

Finally, there were 207 ADCY10P1-correlated with lncRNA and 477 ADCY10P1-correlated with mRNA that included the predicted target gene NFYA in BLCA. There were 155 ADCY10P1-correlated with lncRNA and 206 ADCY10P1-correlated with mRNA in LGG. eRNAs are a major type of cis-regulatory elements in the genome[5], and several kinds of research indicated that eRNAs also are able to mediate the other genes expression in trans[31, 32]. In our study, besides its predicted target NFYA and UNC5CL, the role of ADCY10P1 may be tried to clarify by identifying other co-expressed genes. And in our study, the correlation between ADCY10P1 and a few co-expressed genes is negative. It could thus be inferred to have a direct or indirect trans function.

In BLCA, the genes were most related to focal adhesion, cell-substrate adhesion junction, and cell-substrate junction. In addition, genes were highly enriched in Herpes simplex virus 1 infection, Staphylococcus aureus infection, and Phagosome signaling pathways. In LGG, the genes were most related to RNA splicing, via transesterification reactions with bulged adenosine as nucleophile, and mRNA splicing, via spliceosome. In addition, genes were highly enriched in Endocytosis, mRNA surveillance pathway, and Phospholipase D signaling pathway.

In summary, ADCY10P1 can affect pan-cancer progress and prognosis. ADCY10P1 was an independent prognostic factor in BLCA and LGG. High expression of ADCY10P1 is beneficial to BLCA, LAML, LGG, READ, and THYM. High expression may suppress immune infiltration. ADCY10P1 may have a direct or indirect trans function. ADCY10P1 may have a dual role in the tumor, which is oncogenes and tumor suppressor genes in BLCA and READ.
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1. conception and design: Qizhan Luo and Thomas-Alexander Vögeli
2. Data analysis and interpretation: Qizhan Luo
3. Manuscript writing: Qizhan Luo
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