

# Comprehensive Analysis of Potential Key Genes in Progression of Large Cell Neuroendocrine Lung Carcinoma

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## Research article

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# Abstract

## Background

Pulmonary LCNEC (large-cell neuroendocrine carcinoma) is a kind of high-grade lung neuroendocrine tumors (NET). There's an increase in the pathologically diagnosis of LCNECs during recent years. However, the underlying mechanisms of the progression of LCNEC still remains unclear.

## Methods

In this article, we utilized three GEO datasets to elucidate the significant candidate genes and pathways in LCNEC: GSE1037, GSE11969, and GSE44447, including 17 LCNEC samples and 31 normal lung tissues, were analyzed using several bioinformatics methods to explore the key genes involved in the development of LCNEC. We identified ten hub genes which might be important in the pathogenesis of LCNEC. Then we performed western blot on specimens from four patients diagnosed with LCNEC in our hospital to validate these genes.

## Results

Results showed that three genes (CCT4, CDC20, KNTC1) expressed highly in LCNEC.

## Conclusion

The results of our study might provide new insights for the development of new biomarkers and therapeutic targets of LCNEC.

## Background:

Large cell neuroendocrine carcinoma (LCNEC) accounts for nearly 3% of all lung cancers. It is a rare form of aggressive lung cancer with an extremely high proliferation rate [1]. This type of lung cancer occurs more frequently in male smokers who smokes heavily, whereas uncommon in females who do not smoke [2]. LCNEC is often categorized in the NSCLC group. However, it is also a member of the lung neuroendocrine tumor (NET) group [3] because it arises from lung cells of neuroendocrine system. The WHO 2015 classification system is commonly used for nomenclature [4].

LCNEC is classified into three subsets according to molecular characteristics as well as clinical features [5]. SCLC-like, NSCLC-like are major subsets and minor subset is carcinoid-like [6]. The different classification of subsets lead to different management strategies [7].

Patients diagnosed of LCNEC have dismal prognosis [8]. Compared with other types of lung cancers, LCNEC also has different metastatic patterns [9]. Surgery is the primary treatment for early stage disease. Researches based on the SEER database showed that primary surgical treatment also has significant survival benefits, even for late stage patients [10] [11]. Chemotherapy is an important treatment as well [12] [13]. Derks enucleated LCNEC patients who carry a wild-type RB1 gene perform better with NSCLC-GEM/TAX treatment than with SCLC-PE chemotherapy [14]. PD-L1 expression was found in 10.4% of all LCNEC patients [15]. Hermans found that PD-1 was positive in 16% of stage IV LCNEC [16]. Wang's experience showed that immunotherapy benefited PD-L1 negative patients as well [17]. DLL3 inhibitors and immunotherapy could be additional methods for patient-LCNEC patients [18]. Research carried out by Zheng elucidated that ALK inhibitor could also benefit [19]. Other researches have shown that CD146 [20], Stathmin-1 [21], YAP1 [22], Topoisomerase II, Somatostatin, and ERCC1 [23], CSLC [24] Napsin A [25], BAI3, CDX2 and VIL1 [26] might be biomarkers for LCNEC. Demes research also revealed that elevated miR-21 and miR-34a could be exploited as supportive markers [27]. Patients with KRAS mutations are more prone to have brain metastasis [28].

However, the cause and underlying molecular events of lung large cell neuroendocrine carcinoma are still far from clear. In our study, we applied integrated bioinformatics to identify key pathogenic genes involved in LCNEC and then validated those genes in LCNEC specimen tissues.

## Methods:

### 1 Bioinformatics Analysis

In our research, we downloaded three microarray datasets from NCBI-GEO (NCBI-Gene Expression Omnibus database <https://www.ncbi.nlm.nih.gov/geo>) : GSE1037, GSE11969, GSE44447. The platform for GSE1037 is GPL962, which includes 19 normal lung samples and 8 LCNEC specimens. The platform for GSE11969 is GPL7015, which consists of 5 normal lung samples and 2 LCNEC samples. The platform for GSE44447 is GPL14550, which includes 7 normal lung samples and 6 LCNEC samples.

There are 17 LCNEC samples and 31 normal samples in total. We filtered differentially expressed genes using the limma software package. Samples with a corrected P-value of 0.05 and log fold change FC of 2 were considered DEGs.

Venn diagram was used to find the common differentially expressed genes in the three datasets.

In order to get hub genes, Cytoscape ([www.cytoscape.org](http://www.cytoscape.org)) was used. Enrichment analysis was performed using Metascape (<http://metascape.org>).  $P < 0.05$  was considered statistically significant.

### 2 Specimen validations

This study was approved by Fujian Provincial Hospital Ethics Committee. The investigations conform to the principles in the declaration of Helsinki. Written consent of the patients were obtained.

Specimens of four patients diagnosed with LCNEC from Jan 2019 to Oct 2019 in our department was surgically resected. We only included specimens of histologically pure LCNEC patients. All cases met the WHO 2015 criteria for LCNEC. Western blot was performed on the four specimens. Procedures were carried out according to protocols. ImageJ was used to analyze band intensity. All analyses were performed using SPSS version 26.0 for Windows (SPSS Inc. Chicago. IL). p-values < 0.05 were considered statistically significant.

## Results:

The LCNEC expression microarray datasets GSE1037, GSE11969, and GSE44447 were standardized, and the heatmap results are shown in Fig. 1, Fig. 2 and Fig. 3. When the GSE1037 dataset was screened by the limma package, 219 DEGs were obtained (Fig. 1). When GSE11969 was screened, 9465 DEGs were obtained (Fig. 2). As for GSE44447, 8493 DEGs were obtained (Fig. 3). Venn Diagram (Fig. 4) was drawn to get the common genes. We got 39 genes (Table 1, Table 2). Then we used Cytoscape to identify hub genes. Top 10 genes in network string interactions ranked by closeness method were CDC20, KIF11, CDC7, KNTC1, CCT4, UHRF1, RANBP1, RPS6, LMO7, HLA-DMB. Metascape was used to perform enrichment analysis. Results (Fig. 5, Fig. 6) showed that the hubs genes were enriched in regulations of mitotic cell cycle, mitotic cell cycle checkpoint, HTLV-1 infection, adaptive immune system.

Western blot results showed that three genes: CCT4(Fig. 7), CDC20(Fig. 9), KNTC1(Fig. 11) were overly expressed in LCNEC specimens. Compared with adjacent lung tissues, the expression levels of CCT4(Fig. 8), CDC20(Fig. 10), KNTC1(Fig. 12) in LCNEC were statistically significant ( $p < 0.05$ ).

## Discussion:

LCNEC is an aggressive tumor[29].It has unique biological and molecular features. Patients diagnosed with LCNEC have dismal prognosis. Since this tumor is extremely rare, it is very difficult to be clinically diagnosed. It is classified differently due to pathological features[30] [31]. LCNEC has similar features with small cell lung cancer, including aggressiveness, relations with smoking, extremely high rates of progression, certain gene expressions. Standard treatments of LCNEC and are still not well established. Since current treatment results are not satisfactory. There's emerging need for better clarification of the underlying features of LCNEC and discovery of new options for treatments.

In our study, we used bioinformatics method to reveal key genes and pathways involved in pathogenesis of LCNEC. Results showed that CDC20(Cell Division Cycle 20), KIF11(Kinesin Family Member 11), CDC7(Cell Division Cycle 7), KNTC1 (Kinetochore Associated 1), CCT4(Chaperonin Containing TCP1 Subunit 4), UHRF1(Ubiquitin Like With PHD And Ring Finger Domains 1), RANBP1(Ran-specific binding protein 1), RPS6(Ribosomal protein S6), LMO7(LIM Domain 7), HLA-DMB(HLA class II histocompatibility antigen, DM beta chain) were hub genes involved. Pathways enriched were regulations of mitotic cell cycle, mitotic cell cycle checkpoint, HTLV-1 infection, adaptive immune system. we validated data using sample tissues dissected from LCNEC patients. Further we got three significant important genes: CCT4,

CDC20 and KNTC1. They are all protein coding genes. CCT4 (Chaperonin Containing TCP1 Subunit 4) is related to pathways including cooperation of prefoldin and TriC/CCT in actin and tubulin folding ,and cargo trafficking to the periciliary membrane. Gene ontology annotations related to this gene include unfolded protein binding. CDC20 (Cell Division Cycle 20) is related to pathways like CDK-mediated phosphorylation and removal of Cdc6, as well as mitotic roles of polo like kinases. Gene ontology annotations related to this gene include enzyme binding and protein C-terminus binding. KNTC1's (Kinetochore Associated 1) related pathways are cell cycle, mitotic and mitotic prometaphase.

However, there are limitations to our study. Since LCNEC is an extremely rare disease, the collection of large data set is very difficult. For further studies, we would like to collaborate with other hospitals to gather more data on LCNEC.

## **Conclusion:**

In conclusion, our study identified key genes in pulmonary large cell neuroendocrine carcinoma development, results showed that CCT4, CDC20 and KNTC1 are upregulated in lung large cell neuroendocrine carcinomas and they have the potential to become valuable therapeutic targets for LCNEC in the future.

## **List Of Abbreviations**

LCNEC: Large cell neuroendocrine carcinoma

SCLC: Small cell lung cancer

NSCLC: Non-small cell lung cancer

NET: lung neuroendocrine tumor

CDC20: Cell division cycle 20

KIF11: Kinesin family member 11

CDC7: Cell division cycle 7

KNTC1: Kinetochore associated 1

CCT4: Chaperonin containing TCP1 subunit 4

UHRF1: Ubiquitin like with PHD and ring finger domains 1

RANBP1: Ran-specific binding protein 1

RPS6: Ribosomal protein S6

## Declarations

**Ethical Approval and Consent to participate:** This study was approved by Fujian Provincial Hospital Ethics Committee. The investigations conform to the principles in the declaration of Helsinki. Written consent of the patients were obtained.

**Consent for publication:** All authors consent for publication.

**Availability of supporting data:** Supporting data is available .

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**Contributions to Authorship:** Conceptualization, Xiaojie Pan; Data curation, Xiaojie Pan; Formal analysis, Xiaojie Pan; Investigation, Tianxing Guo; Methodology, Wenshu Chen; Project administration, Xiaojie Pan; Writing – original draft, Xiaojie Pan and Zhaoxian Lin; Writing – review & editing, Zhaoxian Lin and Mingfan Ye. All authors have read and approved the manuscript.

**Conflicts of Interests:** Authors declare no conflicts of interests.

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## Tables

Due to technical limitations, Tables 1-2 are provided in the Supplementary Files section.

## Figures

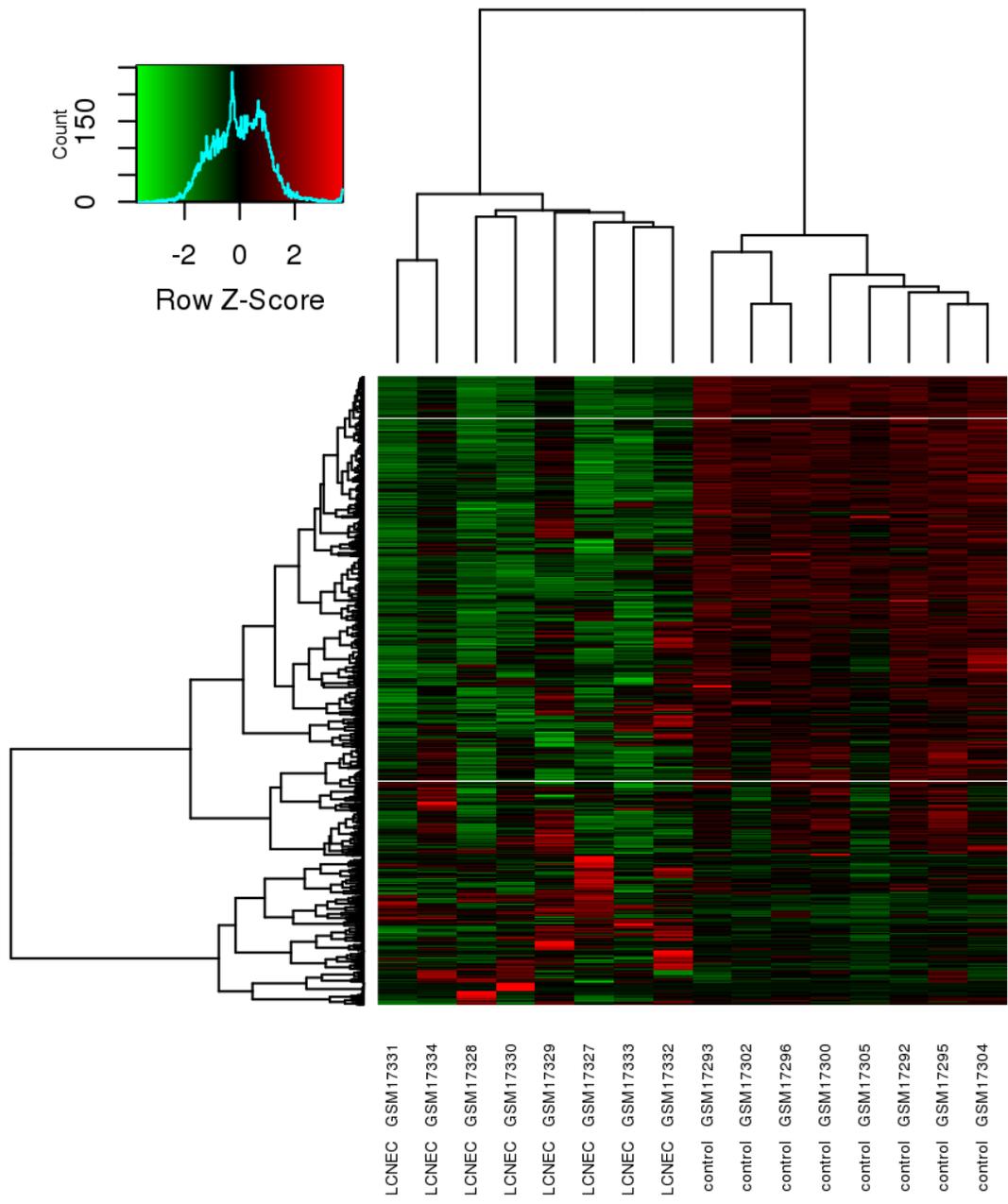
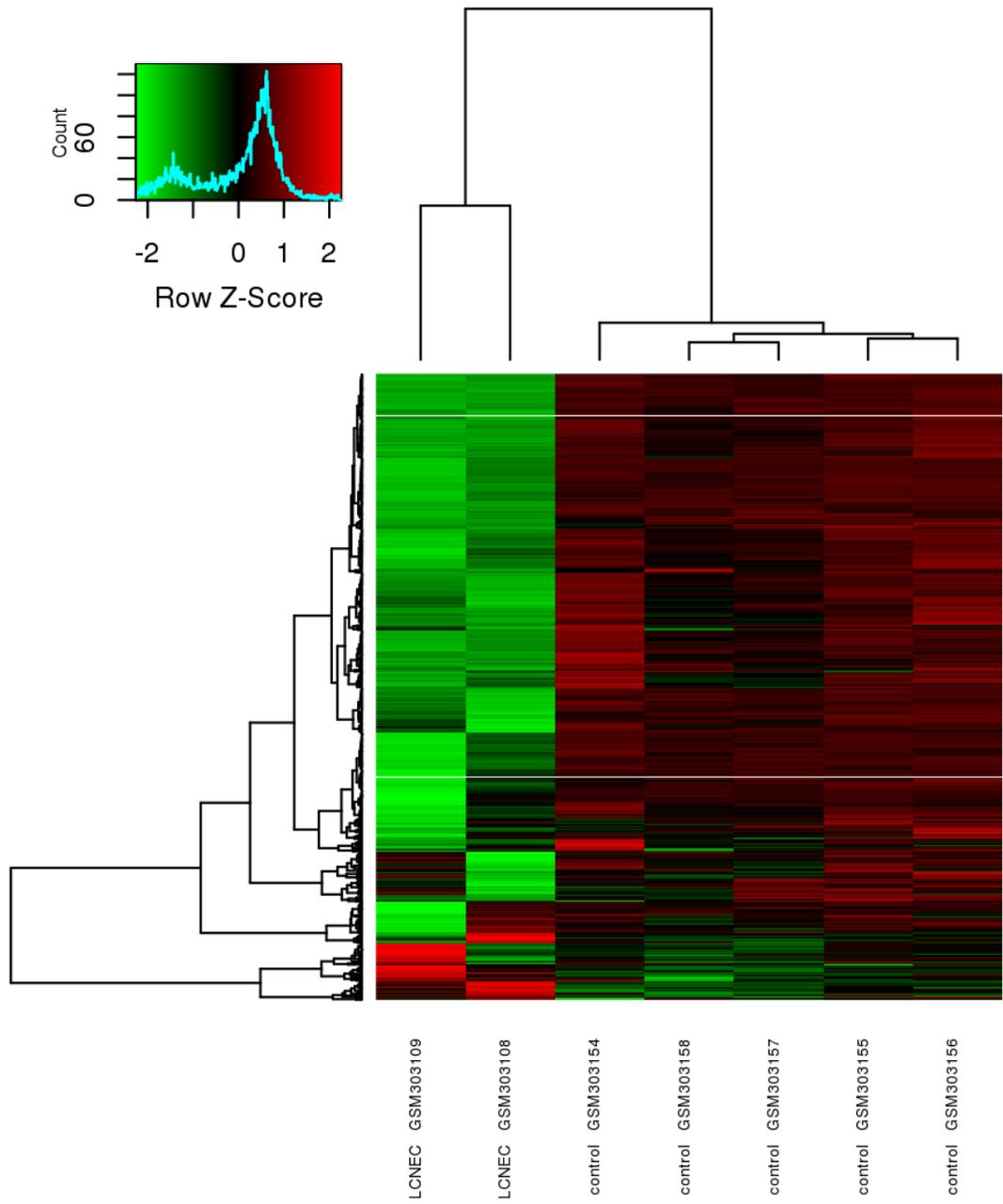


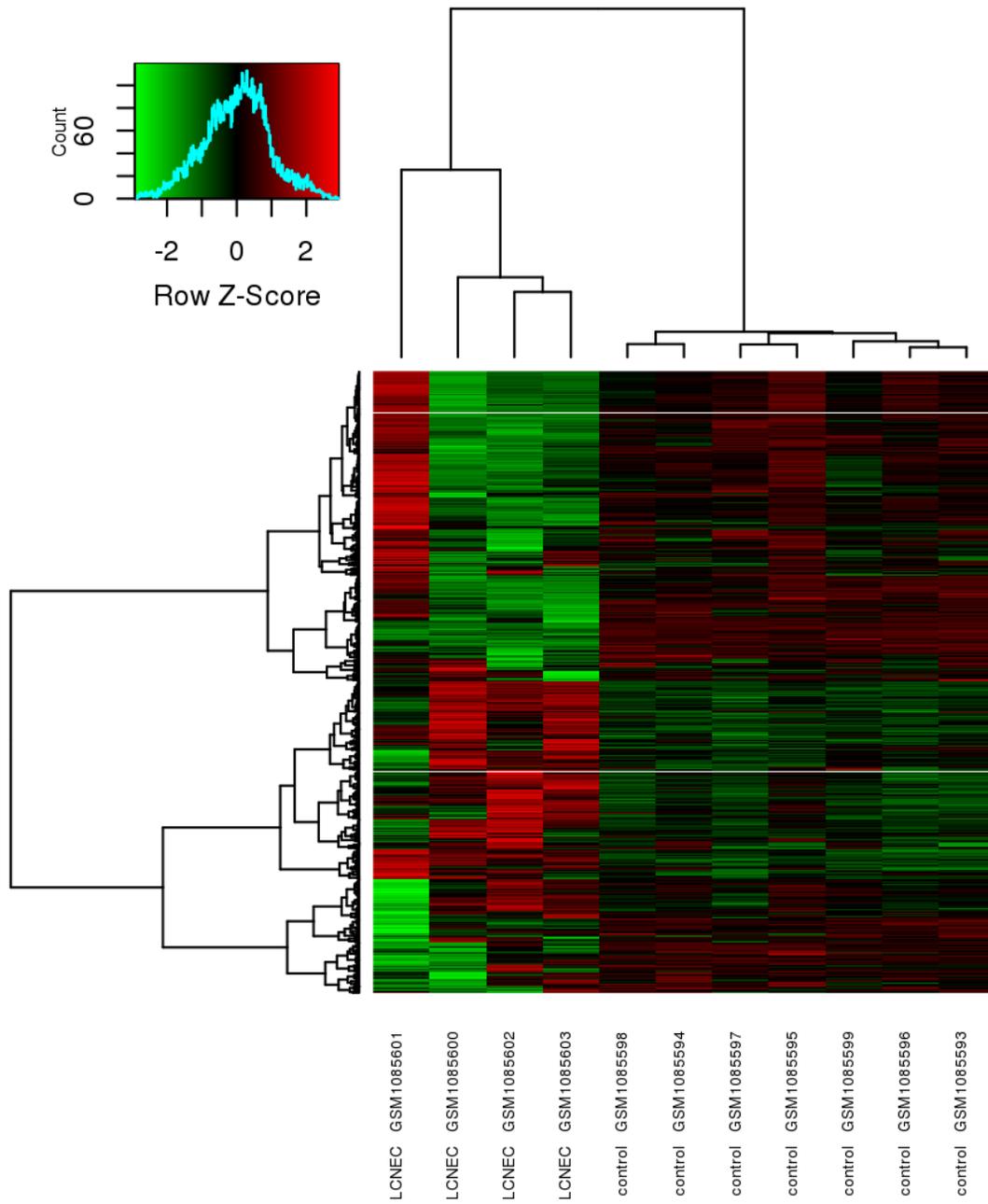
Figure 2

GSE1037 heatmap



**Figure 4**

GSE11969 heatmap



**Figure 6**

GSE44447 heatmap

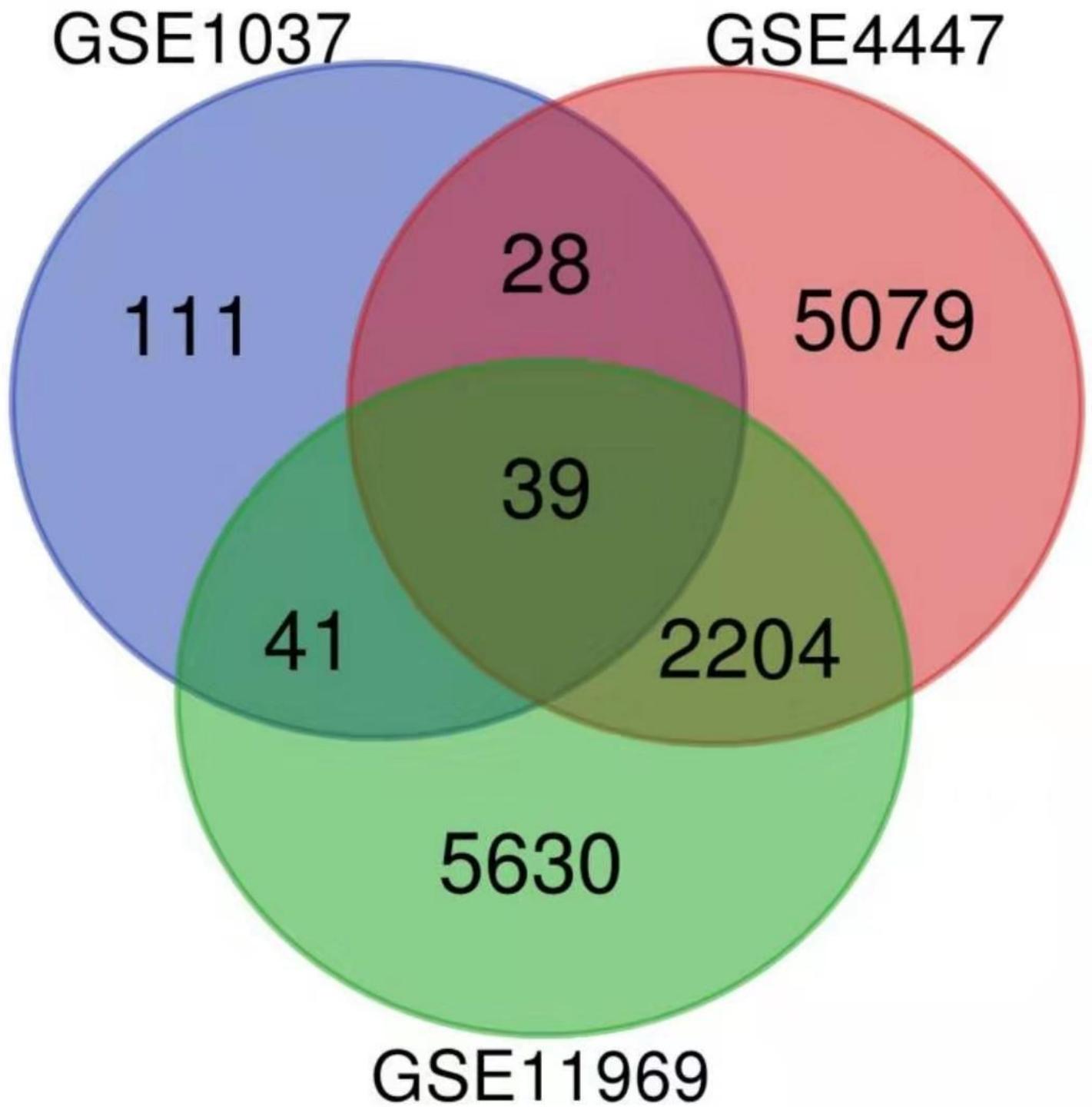


Figure 8

VENN Diagram

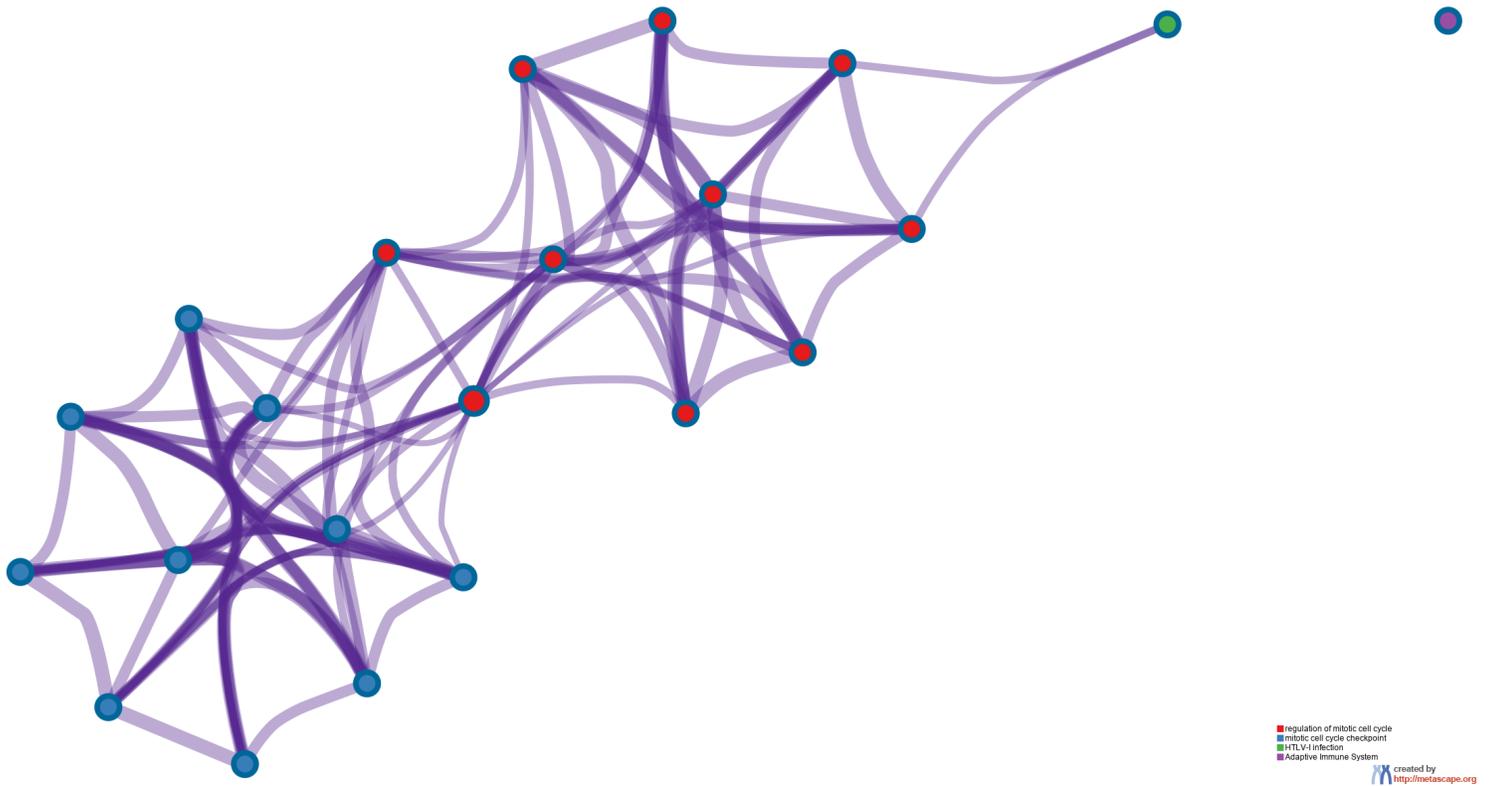


Figure 10

Enrichment analysis

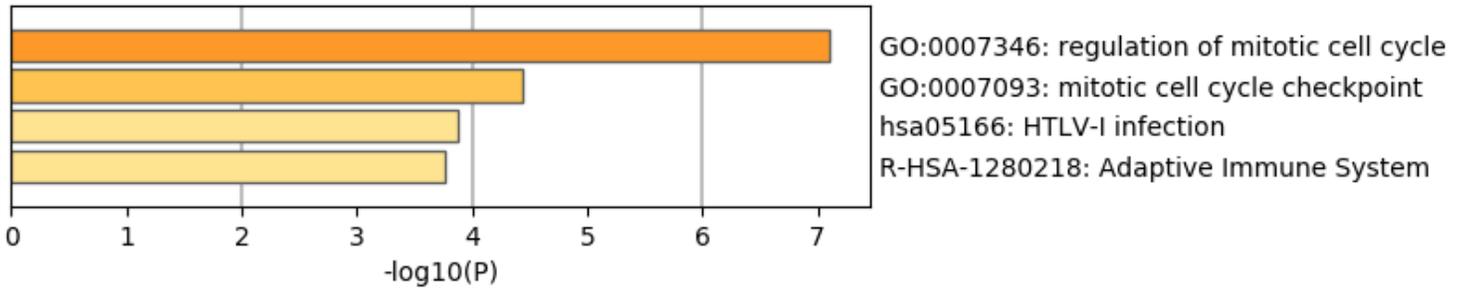
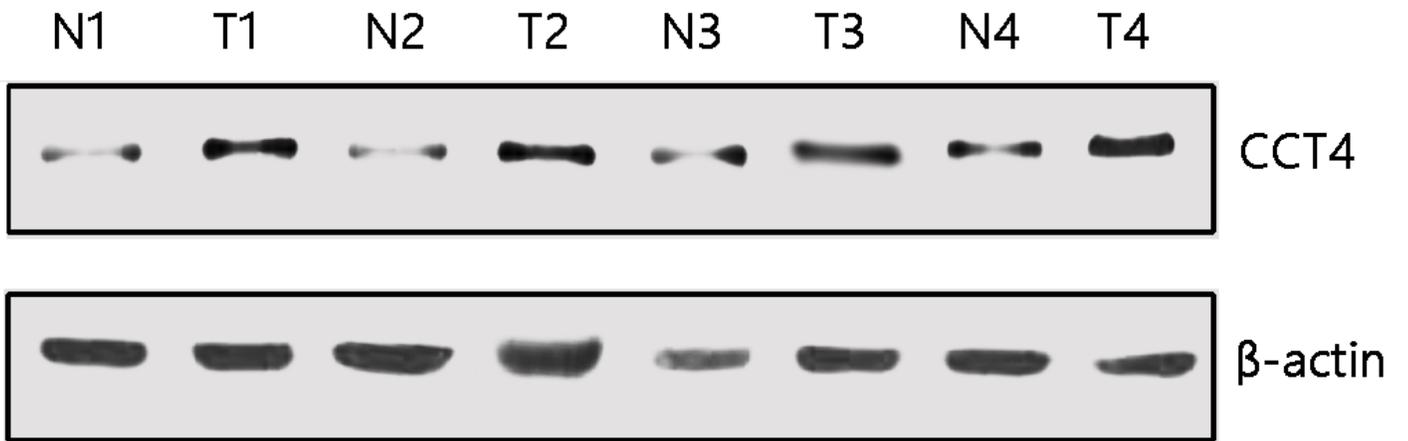


Figure 12

GO enrichment analysis



**Figure 14**

Western blot result of CCT4.

# CCT4

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$p < 0.0001$

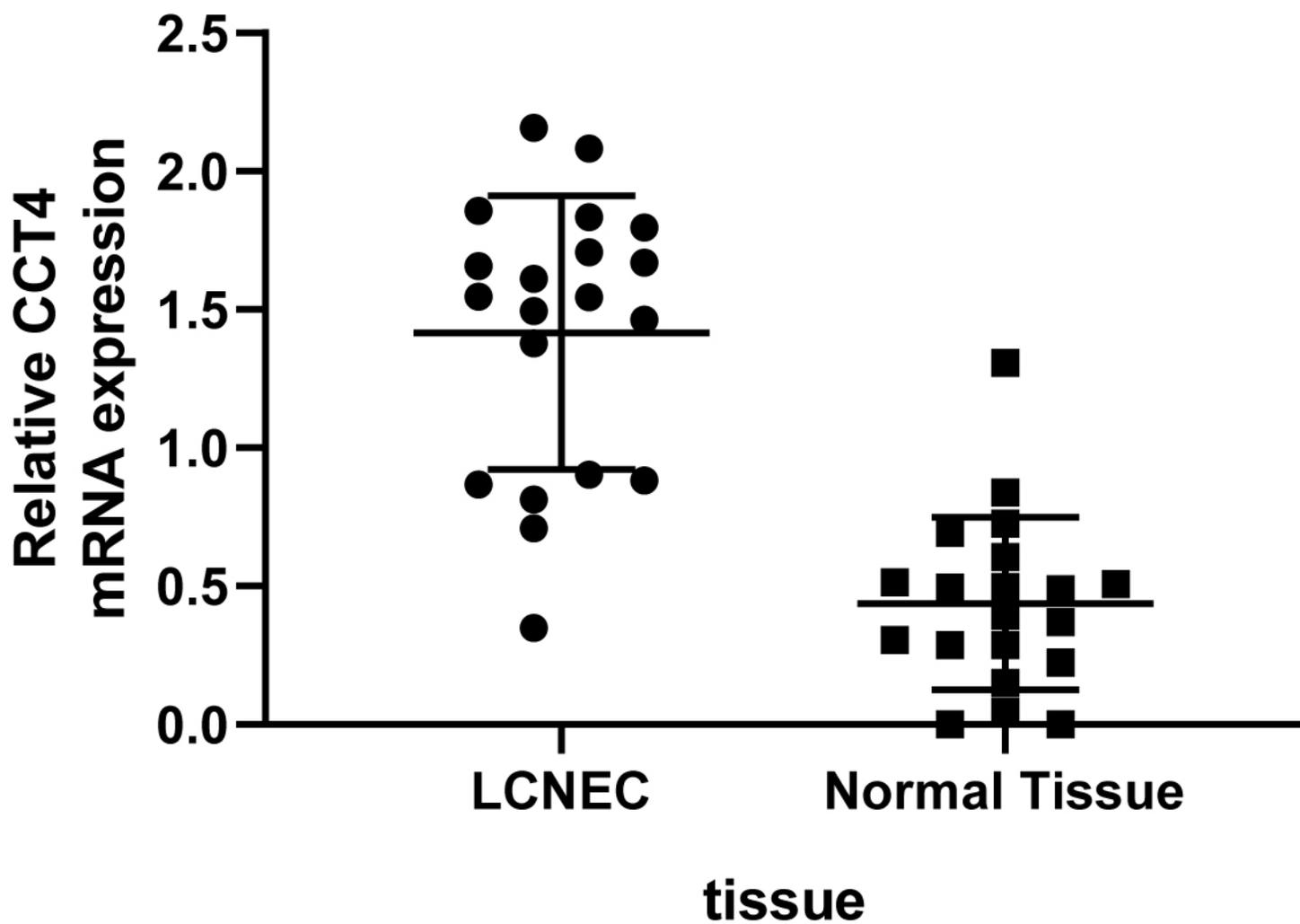
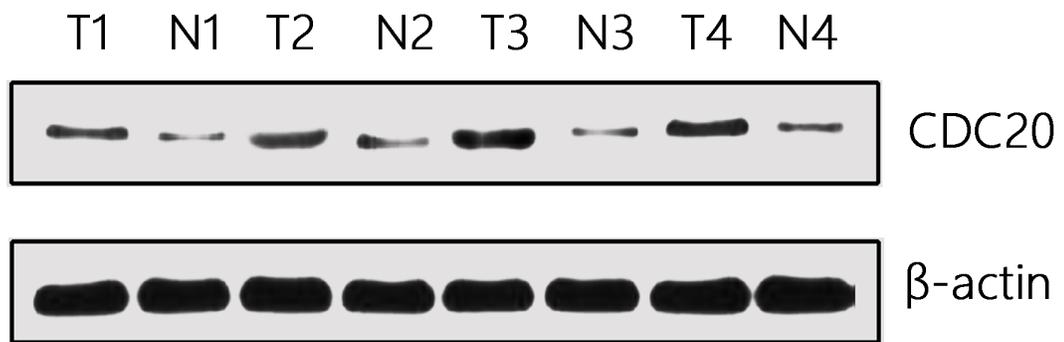


Figure 16

The difference in CCT4 expression between LCNEC specimens and adjacent normal tissues.



**Figure 18**

Western blot results of CDC20.

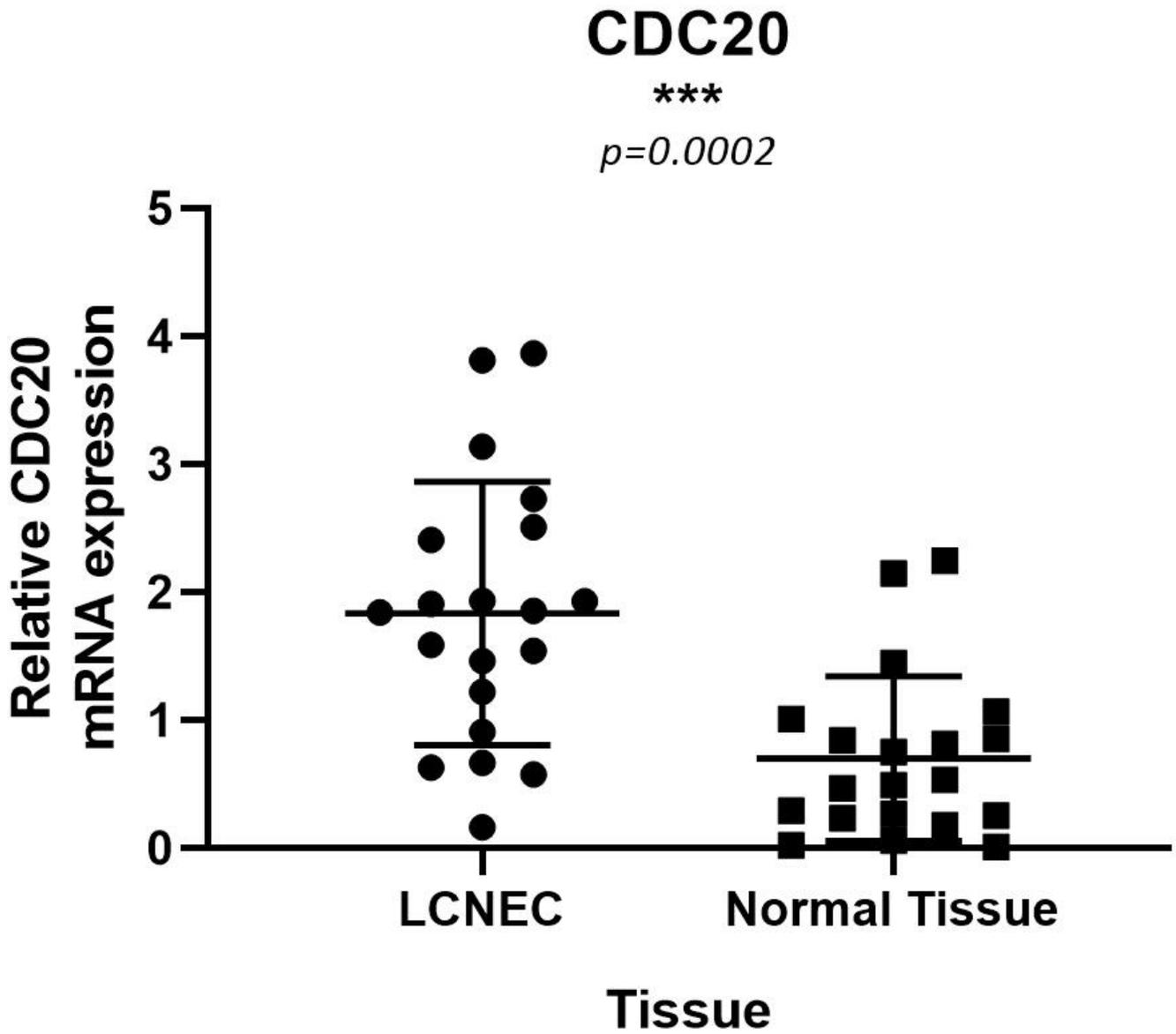
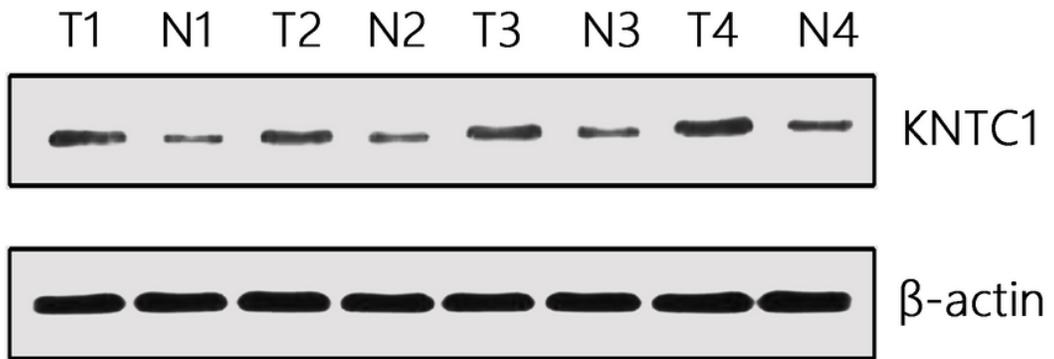


Figure 20

The difference in CDC20 expression between LCNEC specimens and adjacent normal tissues.



**Figure 22**

Western blot result of KNTC1.

# KNTC1

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$p=0.0004$

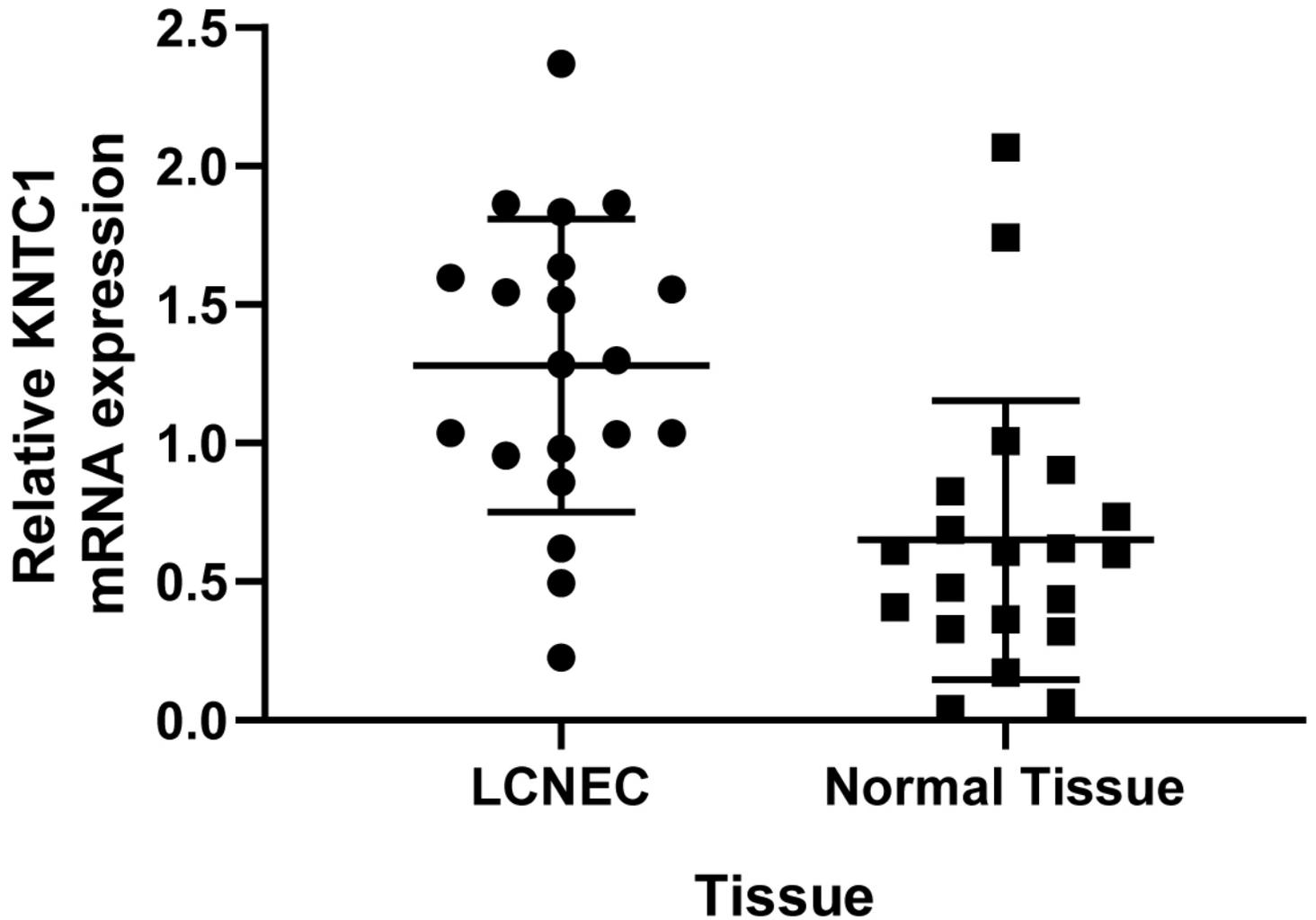


Figure 23

The difference in KNTC1 expression between LCNEC specimens and adjacent normal tissues.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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