해부 · 생물인류학 제 37 권 제 4 호
Anat Biol Anthropol Vol. 37, No. 4 (2024) pp. 303~311
https://doi.org/10.11637/aba.2024.37.4.303

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Original Article

# Clinical and Prognostic Significance of TSC2 and TSC1 Expressions in Rectal Cancer

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**Abstract** : Tuberous sclerosis complex 1 (TSC1) is a crucial component of the mTOR pathway, impacting cell growth, proliferation, and autophagy, while Tuberous sclerosis complex 2 (TSC2) is a tumor suppressor gene that regulates cell growth and is associated with the mTOR signaling pathway. In the present study, we analyzed TSC1 and TSC2 mRNA expression levels in rectal cancer, and evaluated clinicopathological and prognostic characteristics of TSC1 and TSC2. TSC1 and TSC2 mRNA expression was examined in various cancer types using The Cancer Genome Atlas (TCGA) data. The patients were split into two subgroups, each comprising 50% of the total, based on the TSC gene expression levels. This division was made to assess the clinical characteristics associated with the expression of TSC1 and TSC2 genes. Lower TSC1 expression was linked to venous invasion in rectal cancer patients, and there was also a related trend with lymphatic invasion, although it didn't reach statistical significance. Lower TSC2 expression was observed in younger patients and had some associations with sex and M stage, but these correlations were not statistically significant. When it came to survival analysis, TSC1 expression did not significantly impact overall survival, while higher TSC2 expression was associated with a poorer prognosis in rectal cancer. TSC2 may have important role in rectal cancer and its molecular mechanisms and clinical characteristics should be studied further.

**Keywords** : Colorectal cancer, Tuberous sclerosis, TSC1, TSC2, The Cancer Genome Atlas

## INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide and is a fatal malignancy, particularly in women [1,2]. In particular, rectal cancer is the second most common disease in the colon and has poor clinical

[†]These authors contributed equally to this work.
This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2022R1I1A1A01068949).
The author(s) agree to abide by the good publication practice guideline for medical journals.
The author(s) declare that there are no conflicts of interest.
**Received:** October 15, 2024; **Revised:** December 19, 2024; **Accepted:** December 23, 2024
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ISSN 2671-566X (Online)

outcomes despite chemical and molecular targeted treatments [3]. Additionally, it has high morbidity and mortality rates and poor prognosis [4]. CRC pathogenesis is based on genetic and epigenetic changes that alter the oncogene levels [5]. Therefore, identifying new biomarkers of rectal cancer to improve patient outcomes is crucial.

TSC2 is a tumor suppressor that regulates cell growth and proliferation and is related to the mammalian target of rapamycin (mTOR) signaling pathway, which participates in tumorigenesis [6]. Additionally, the TSC1 gene is an essential component of the mTOR signaling pathway and plays an important role in cell growth, proliferation, and autophagy. TSC1 is known to have an important function in cell-cell adhesion, and TSC1 loss is associated with human diseases such as psoriasis [7,8]. TSC1 has a tu-mor-suppressive effect in human cancers such as liver, lung, bladder, breast, ovarian, and pancreatic cancers [7]. AMPK can inhibit mTORC1 activation by activating the hamartin (encoded by TSC1)/tuberin (encoded by TSC2) complex [9]. According to "the two-hit hypothesis, bi-allelic TSC1 or TSC2 inactivation can activate Rheb, which stimulates mTORC1 phosphorylation and activation, which is central to tumor pathogenesis in tuberous sclerosis (TSC) [7-10]. Inhibition of mTOR signaling leads to a reduction in CRC tumor cell growth and inhibition of CRC initiation and progression. Moreover, TSC2 forms a heterodimer with TSC1, and when mutated, it is involved in regulating tumor growth in patients with tuberous sclerosis [6]. Previous studies examining the clinical or pathological characteristics of TSC1/TSC2 are absent. However, recently publicly available gene expression databases have been used for cancer research [11,12], and although their accuracy remains debatable, these databases can yield meaningful results.

Here, we analyzed the prognostic value of TSC1 and TSC2 in various cancers using data from The Cancer Genome Atlas (TCGA). TCGA has been recently used and has provided beneficial data in cancer biology. Tumor Immune Estimation Resource (TIMER) provided gene expression in various types of cancer, and it suggested that TSC1 and TSC2 may serve as an important candidate for treating cancer. Therefore, we focused clinicopathological characteristics of TSC1 and TSC2 in rectal cancer. This results potentially uncovered novel strategies for rectal cancer using TSC1 and TSC2.

## METHODS

### 1. TIMER analysis

TIMER (https://cistrome.shinyapps.io/timer/) provides a comprehensive web inter-face for exploring tumor genomics and immunology data and for visualizing systematic analyses of immune infiltrates according to different cancer types. The expression of TSC1 and TSC2 has been studied in various tumor types (Fig. 1).

### 2. TCGA data analysis

We used primary data from TCGA portal (http://cancergenome.nih.gov/) in March 2023. This provided the *P*-value ranking for TSC1 and TSC2 prognosis for each cancer type (Fig. 2). The cancer type that showed the most promising results (rectal cancer) was selected, and a detailed analysis was performed. In total, 158 rectal cancer patients were profiled for the survival analysis. Survival was defined as the time interval from surgery until the date of death.

### 3. LinkedOmics database analysis

The LinkedOmics database (http://www.linkedomics.org/admin.php) analyzed 32 TCGA cancer-related multidimensional databases. TSC2 co-expression was statistically analyzed in scatterplots or heat maps using Pearson's correlation coefficient. Function module of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, miRNA-target enrichment, kinase-target enrichment, Gene Ontology biological process (GO), transcription factor-target enrichment, and LinkedOmics analyses using gene set enrichment analysis. The rank criterion was a false discovery rate $<0.05$, and 500 simulations were analyzed.

### 4. Statistical analysis

Data were analyzed using SPSS (version 25.0; IBM SPSS, Armonk, NY, USA). The TNM stage was determined according to the seventh edition of the American Joint Committee on Cancer staging system. Clinicopathological characteristics, including age, sex, carcinoembryonic antigen level, and pathological TNM stage, were analyzed using the chi-square test. Spearman's correlation coefficient was used for the correlation analyses between

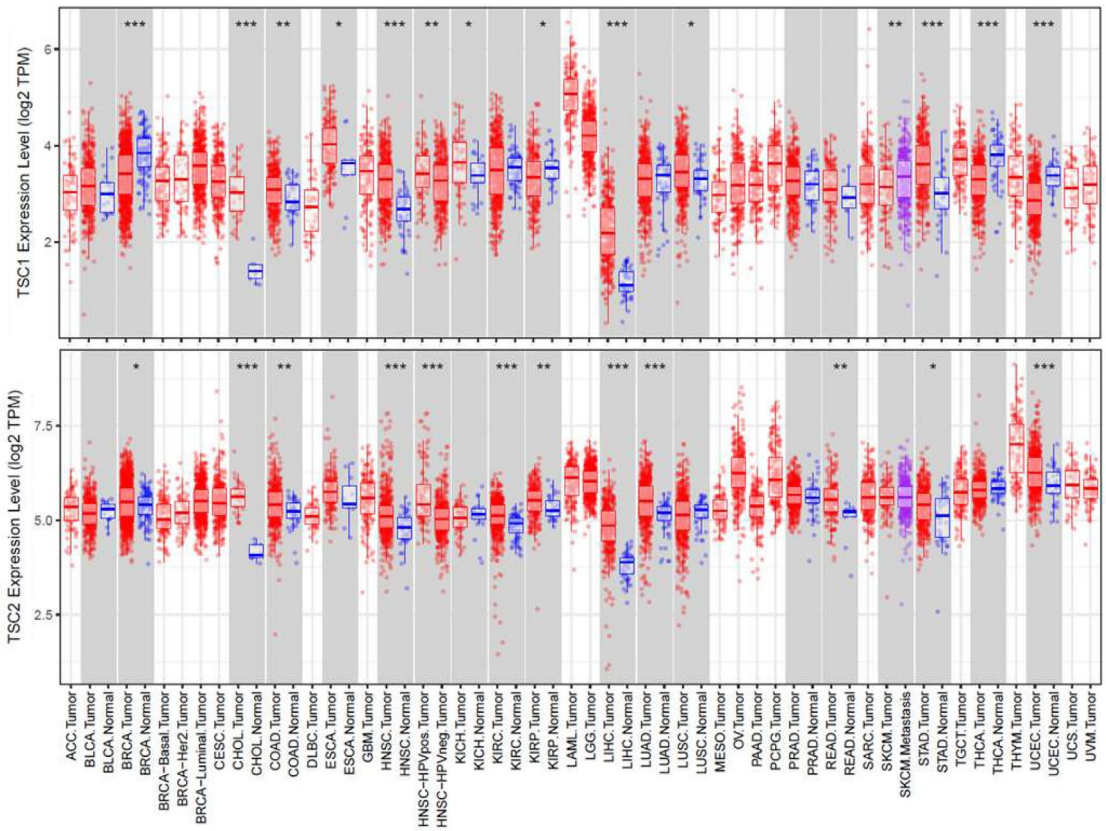


**Fig. 1.** TSC1 and TSC2 expression in different human tumor tissues compared with normal tis-sues using the TIMER database.

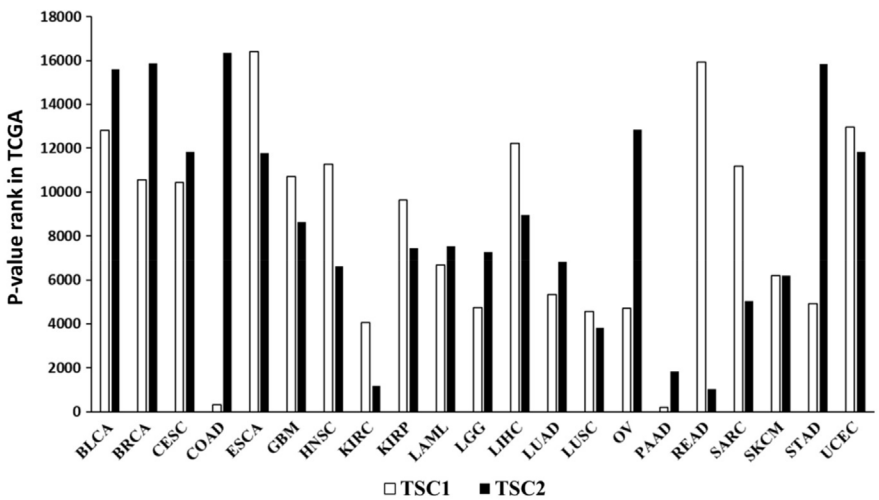


**Fig. 2.** The rank of survival value of TSC1 and TSC2 in various cancers.

the TSC genes and variables related to rectal cancer. Univariate survival analysis was performed using Kaplan-Meier curves and the log-rank test. Overall survival was defined as the time between diagnosis and mortality. Statistical significance was set at $P<0.05$.

**Table 1.** Clinical characteristics of TSC1 and TSC2 gene in rectal cancer.

| | TSC1 | | | TSC2 | | |
|---|---|---|---|---|---|---|
| | High | Low | *P*-value | High | Low | *P*-value |
| Age | | | | | | |
| <60 | 27 (55.1%) | 22 (44.9%) | 0.360 | 17 (35.4%) | 31 (64.6%) | 0.013 |
| ≥60 | 51 (47.2%) | 57 (52.8%) | | 62 (56.9%) | 47 (43.1%) | |
| Gender | | | | | | |
| Female | 35 (49.3%) | 36 (50.7%) | 0.930 | 41 (57.7%) | 30 (42.3%) | 0.091 |
| Male | 43 (50%) | 43 (50%) | | 38 (44.2%) | 48 (55.8%) | |
| Lymphatic invasion | | | | | | |
| No | 44 (53.7%) | 38 (46.3%) | 0.080 | 43 (51.8%) | 40 (48.2%) | 0.681 |
| Yes | 22 (38.6%) | 35 (61.4%) | | 31 (55.4%) | 25 (44.6%) | |
| CEA | | | | | | |
| ≤5 | 28 (45.9%) | 33 (54.1%) | 0.678 | 29 (47.5%) | 32 (52.5%) | 0.833 |
| >5 | 22 (50%) | 22 (50%) | | 20 (45.5%) | 24 (54.5%) | |
| Venous invasion | | | | | | |
| No | 55 (53.9%) | 47 (46.1%) | 0.004 | 50 (49%) | 52 (51%) | 0.157 |
| Yes | 9 (25.7%) | 26 (74.3%) | | 22 (62.9%) | 13 (37.1%) | |
| Pathologic stage | | | | | | |
| Stage I | 10 (34.5%) | 19 (65.5%) | 0.232 | 16 (53.3%) | 14 (46.7%) | 0.445 |
| Stage II | 24 (51.1%) | 23 (48.9%) | | 27 (57.4%) | 20 (42.6%) | |
| Stage III | 28 (58.3%) | 20 (41.7%) | | 23 (48.9%) | 24 (51.1%) | |
| Stage IV | 11 (45.8%) | 13 (54.2%) | | 9 (37.5%) | 15 (62.5%) | |
| M stage | | | | | | |
| M0 | 59 (50%) | 59 (50%) | 0.567 | 64 (54.2%) | 54 (45.8%) | 0.088 |
| M1 | 10 (43.5%) | 13 (56.5%) | | 8 (34.8%) | 15 (65.2%) | |
| N stage | | | | | | |
| N0 | 36 (45.6%) | 43 (54.4%) | 0.515 | 45 (56.25%) | 35 (43.75%) | 0.143 |
| N1 | 24 (55.8%) | 19 (44.2%) | | 21 (48.8%) | 22 (51.2%) | |
| N2 | 17 (53.1%) | 15 (46.9%) | | 11 (35.5%) | 20 (64.5%) | |
| T stage | | | | | | |
| T1 | 5 (55.6%) | 4 (44.4%) | 0.525 | 4 (44.4%) | 5 (55.6%) | 0.546 |
| T2 | 10 (37%) | 17 (63%) | | 17 (60.7%) | 11 (39.3%) | |
| T3 | 56 (52.3%) | 51 (47.7%) | | 52 (49.1%) | 54 (50.9%) | |
| T4 | 7 (53.8%) | 6 (46.2%) | | 5 (38.5%) | 8 (61.5%) | |

## RESULTS

To evaluate the clinical characteristics of TSC1 and TSC2 expression, patients were divided into two subgroups according to the median values of TSC and TSC2 expression (Table 1). First, lower TSC1 expression was observed in patients with venous invasion ($P=0.004$). Although no statistically significant relation was found, it was related to lymphatic invasion ($P=0.080$). Particularly, lower TSC2 expression was observed in younger patients ($P=0.013$). Sex and M stage correlated with TSC2 expression; however, the correlation was not statistically significant ($P=0.091$ and $P=0.088$, respectively). No other clinical characteristics were associated with TSC1 or TSC2 expression.

And then, a correlation analysis also showed that TSC1 and TSC2 expression had a correlation to APC, KRAS, P53, Age, and CEA level (Table 2). A positive correlation in borderline (R=0.154, $P=0.054$) was observed for Spear-

**Table 2.** Correlation analysis in rectal cancer.

| | | TSC1 | TSC2 | APC | KRAS | P53 | Age | CEA |
|---|---|---|---|---|---|---|---|---|
| TSC1 | R | 1 | −0.146 | 0.308 | 0.044 | −0.200 | −0.030 | 0.083 |
| | P | | 0.069 | <0.001 | 0.581 | 0.012 | 0.708 | 0.398 |
| TSC2 | R | −0.146 | 1 | −0.265 | −0.234 | 0.080 | 0.154 | −0.078 |
| | P | 0.069 | | 0.001 | 0.003 | 0.320 | 0.054 | 0.432 |
| APC | R | 0.308 | −0.265 | 1 | 0.261 | −0.148 | −0.222 | −0.013 |
| | P | <0.001 | 0.001 | | 0.001 | 0.064 | 0.005 | 0.893 |
| KRAS | R | 0.044 | −0.234 | 0.261 | 1 | −0.085 | 0.014 | −0.007 |
| | P | 0.581 | 0.003 | 0.001 | | 0.290 | 0.857 | 0.942 |
| P53 | R | −0.200 | 0.080 | −0.148 | −0.085 | 1 | 0.078 | −0.104 |
| | P | 0.012 | 0.320 | 0.064 | 0.290 | | 0.330 | 0.290 |
| Age | R | −0.030 | 0.154 | −0.222 | 0.014 | 0.078 | 1 | 0.008 |
| | P | 0.708 | 0.054 | 0.005 | 0.857 | 0.330 | | 0.932 |
| CEA | R | 0.083 | −0.078 | −0.013 | −0.007 | −0.104 | 0.008 | 1 |
| | P | 0.398 | 0.432 | 0.893 | 0.942 | 0.290 | 0.932 | |

man's correlation coefficient analysis between TSC2 expression and age, despite it being statistically insignificant. Additionally, correlation analysis demonstrated that TSC1 expression was positively correlated with APC expression ($R = 0.308$; $P < 0.001$) and negatively correlated with P53 expression ($R = -0.200$; $P = 0.012$). Contrastingly, TSC2 expression is negatively correlated with APC ($R = -0.265$; $P = 0.001$) and KRAS expression ($R = -0.234$; $P = 0.003$). Although the result was borderline, TSC2 expression was associated with TSC1 expression ($R = -0.146$; $P = 0.069$).

A univariate survival analysis was performed to determine the prognostic value of TSC in rectal cancer (Fig. 3). In the overall survival analysis, TSC1 expression was in-significant (2572.44 ± 313.27 vs. 1842.97 ± 146.49 d; $\chi^2 = 0.063$; $P = 0.801$). Conversely, a higher TSC2 expression showed poorer prognosis for rectal cancer (2946.26 ± 393.67 vs. 2143.18 ± 334.88 d; $\chi^2 = 4.98$; $P = 0.026$). The results were not statistically significant when stratified by other variables.

To clarify the function of TSC2 in rectal cancer, a heat map was used to identify the top 50 genes positively and negatively correlated with TSC2, respectively (Fig. 4). Then, we further analyzed the biological pathway classification of GO and found that TSC2 and its co-expressed genes were involved in semaphoring-plexin signaling pathway, neuron projection guidance, regulation of small GTPase mediated signal transduction, neural tube development, insulin-like growth factor receptor signaling pathway, and et al. (Fig. 5).

KEGG analysis was performed and results showed that co-expressed genes were enriched ABC transporters, choline metabolism in cancer, phospholipase D signaling pathway, basal cell carcinoma, cortisol synthesis and secretion, and insulin secretion (Fig. 6).

## DISCUSSION

To the best of our knowledge, this study highlighted for the first time a significant correlation between TSC genes and rectal cancer using TCGA data. TSC1 and TSC2 interact with TBC1D7 to form a heterotrimeric complex that works as a GTPase-activating protein for Rheb, which activates mTORC1, an essential cell metabolism and proliferation regulator [13,14]. The mTOR signaling pathway regulates diverse biological functions such as protein translation, cell growth, metabolism, angiogenesis, and immune response [15,16]. Abnormal mTOR activation is found in several diseases, including diabetes, and may promote oncogenic processes vital to the overall tumor development, including tumor initiation, progression, and metastasis [17]. A deregulated mTOR pathway was recently found to be related to diverse malignancies, such as breast, skin, lung, colorectal, and liver cancers, and some hereditary cancers

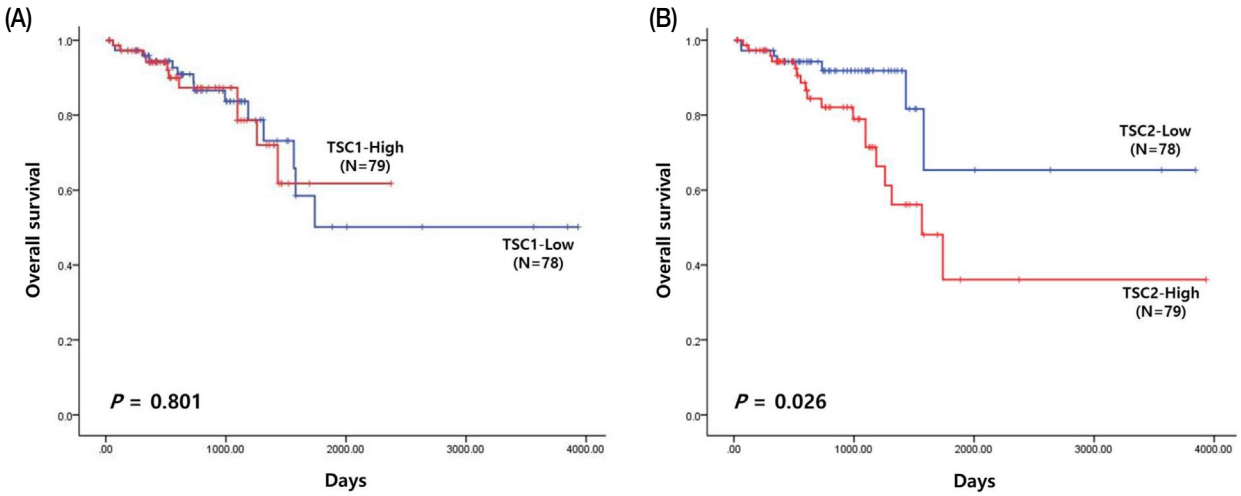


**Fig. 3.** Survival analysis in rectal cancer. (A) TSC1 expression (B) TSC2 expression.

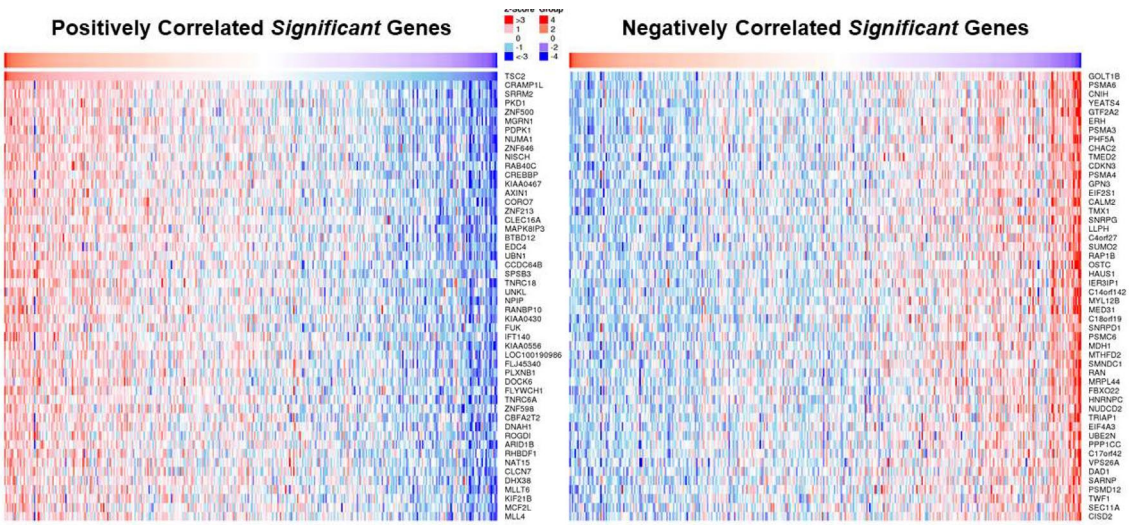


**Fig. 4.** TSC2 co-expression genes were analyzed using the LinkedOmics database. Heat maps showing the top 50 genes positively and negatively correlated with TSC2 gene in rectal cancer.

were related to mutations in genes coding for the upstream elements of the mTOR pathway [18]. Additionally, some mutated elements of the PI3K pathway, which is upstream of the mTOR signaling pathway, are often associated with diverse malignancies. Furthermore, the LKB1-AMPK-mTOR signaling pathway genes, which are involved in TSC1 and TSC2 genes, are known to change cell metabolism and play an important role in the malignant behavior of cancer [18,19]. A recent cohort study conducted in the Netherlands suggested that the mTOR-PI3K-Akt pathway may be associated with CRC development [20]. Several studies have suggested that TSC genes are associated with CRC. Previous studies have suggested that these three SNPs, including TSC1, could be used as prognostic biomarkers for CRC, and TSC1 was detected as a down-regulated differentially expressed gene between normal adenoma and adenoma-carcinoma [19,21]. Furthermore, the TSC2 gene c.3915G>A variant exhibited protective effects against sporadic CRC in a Mexican population [6].

We analyzed the correlation between TSC1 and TSC2 and rectal cancer and found that TSC1 expression was lower in patients with venous invasion, and TSC2 expres-

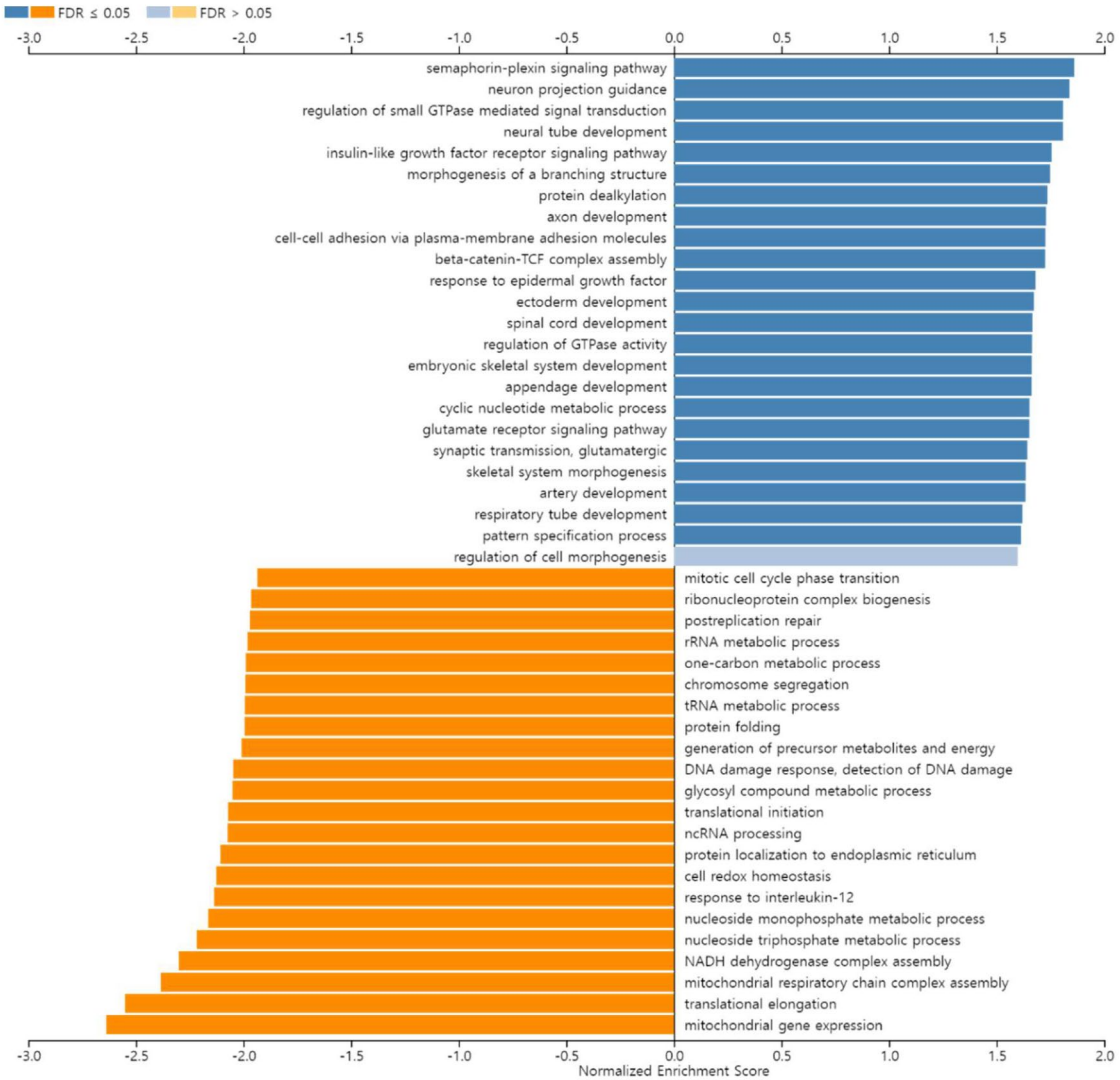


**Fig. 5.** GO functions were analyzed using the LinkedOmics database. Biological process enrichment analysis of TSC2 co-expressed genes by gene set enrichment analysis (GSEA).

sion was lower in younger patients than in older patients. Next, we evaluated survival data to confirm the prognostic value of TSC1 and TSC2 mRNA expression in rectal cancer. TSC2, but not TSC1 expression, was associated with the overall survival. TSC2 as a hub for multiple signaling pathway networks, can regulate cell cycle, autophagy, and other physiological functions, and is closely related to breast cancer pathogenesis, treatment, and prognosis. Low TSC2 expression was recently shown to be associated with poor breast cancer prognosis [22]. Although previous breast cancer results have been contradictory, our study and accumulating evidence suggest that TSC genes may be associated with rectal cancer progression and clinical characteristics. In this study, biological process enrichment analysis showed that the TSC2 was closely related to semaphoring-plexin signaling pathway, neuron projection guidance, regulation of small GTPase mediated signal transduction, neural tube development, insulin-like growth factor receptor signaling pathway in rectal cancer. And KEGG analysis showed that it was closely related to ABC transporters, choline metabolism in cancer, phospholipase D signaling pathway, basal cell carcinoma, cortisol synthesis and secretion, and insulin secretion. These result suggested that TSC2 expression may contribute to rectal cancer progression via neuro-hormonal pathways. Subsequent studies to detect the molecular mechanisms

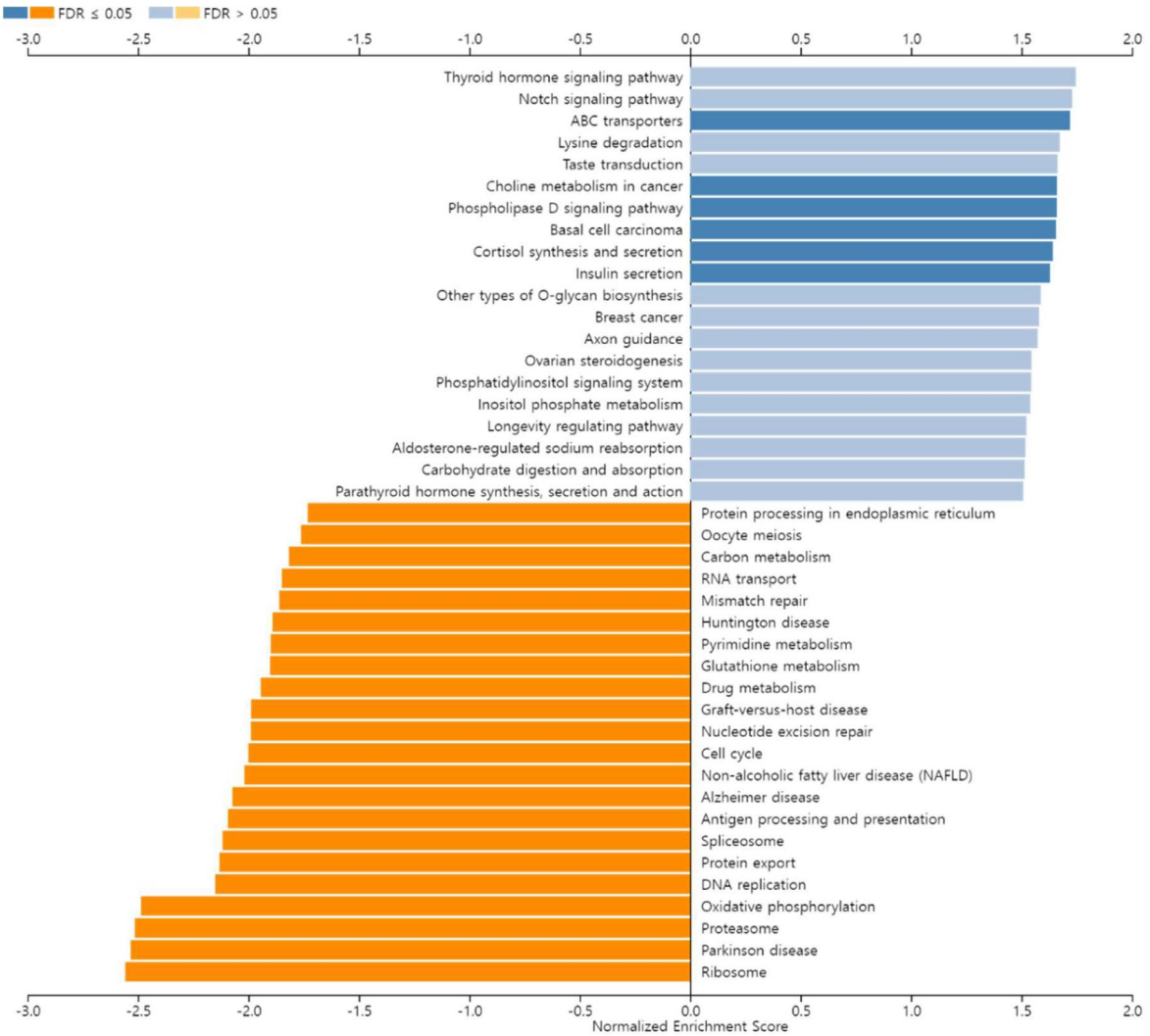


**Fig. 6.** KEGG pathway analysis of TSC2 co-expressed genes by GSEA.

and clinical characteristics of TSC genes in rectal cancer need to be conducted in larger CRC case studies.

## CONCLUSIONS

To conclude, this study is the first to establish a significant correlation between TSC genes and rectal cancer using TCGA data. It suggests that TSC2 could be a potential candidate for treating rectal cancer. Overall, we highlight the need for further exploration of the molecular mechanisms and clinical characteristics of TSC genes in rectal cancer, particularly in larger case studies. We suggest that TSC genes may play a role in rectal cancer progression and clinical features, paving the way for future investigations.

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