TMUB1 as a diagnostic and prognostic marker for colorectal cancer

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TMUB1 as a diagnostic and prognostic marker for colorectal cancer

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Abstract

Background. Transmembrane and ubiquitin-like domain-containing protein 1 (TMUB1) is overexpressed in a large number of liver and esophageal tumors. However, only a few reports on the clinical significance of TMUB1 in colorectal cancer (CRC) exist.

Methods. Here, we evaluated the clinical significance and potential biological role of TMUB1 using bioinformatics analysis. Univariate and multivariate analyses were performed to evaluate the relationship of TMUB1 with clinicopathological features. Gene set enrichment analysis (GSEA) was performed to identify the biological function of TMUB1, while any associations between the expression of TMUB1 and the infiltration of 24 immune cells were analyzed using imple-sample GSEA.

Results. TMUB1 was significantly overexpressed in CRC tissues compared with normal controls. High expression of TMUB1 in CRC was associated with T stage, neotype, and residual tumor. Moreover, TMUB1 was identified as an independent factor of poor disease-free survival (DFS) and short overall survival (OS). GSEA demonstrated that TMUB1 was related to hypoxia, angiogenesis, adipogenesis, inflammatory response, IL6-JAK-STAT3 signaling, apoptosis, mitotic spindle, and IL2-STAT5 signaling. The expression of TMUB1

negatively correlated with the abundance of T helper cells, Tcm cells, macrophages, and Th2 cells, whereas it positively correlated with the abundance of several immune cell types, including CD56bright and CD56dim NK cells.

Conclusions. TMUB1 may be a potential diagnostic and prognosis biomarker for colorectal cancer.

Introduction

Colorectal cancer (CRC) is the third most common type of cancer and the fourth most deadly cancer worldwide (Dekker et al. 2019). The incidence of colorectal cancer has been on a rise in patients younger than 50 y old, with many patients being diagnosed at the advanced stages of the disease, thereby losing the opportunity for surgery. Early detection, interventions, and improved treatments are important to reduce morbidity and mortality associated with CRC (Thanikachalam and Khan 2019). The pathogenic mechanisms underlying CRC include chromosomal instability (CIN), microsatellite instability (MSI), and the CpG island methylator phenotype (CIMP) (Marmol et al. 2017). These alterations can be measured and used as biomarkers. Although some biomarkers are used for diagnosis, consistent and sensitivity biomarkers are still lacking (Coghlin and Murray 2015). Hence, searching for new, specific, and sensitive molecule biomarkers is required to improve prediction, diagnosis, prognosis, and therapy in CRC.

Abnormal proliferation may occur may cause tumorigenesis. Transmembrane and ubiquitin-like domain-containing protein 1 (TMUB1), first reported in 2005, is significantly increased during liver regeneration (Della Fazia et al. 2005). In particular, TMUB1 is overexpressed in a high number of human tumors. Overexpression of TMUB1 in tumor cell lines was shown to strongly reduce proliferation by arresting the cell cycle in G0/G1 (Della-Fazia et al. 2020). TMUB1 is an ubiquitin-like protein shuttling between the cytoplasm and the nucleus. Many investigations showed TMUB1 not only controls proliferation, but also play an impotant role in apoptosis, cycle regulation, and genomic stability (Castelli et al. 2014). However, the expression of TMUB1 and its potential prognostic impact on colorectal cancer (CRC) has not yet been explored.

In this study, we evaluated the differential expression of TMUB1 between specimens from patients with CRC and normal tissues, performed correlation analysis between the TMUB1 expression and clinicopathological factors to assess the prognostic role of TMUB1, and identified relevant pathways and immune cell types associated with the high level of expression of *TMUB1* observed in tumor samples from patients with colorectal cancer.

Materials & Methods

RNA-sequencing data and clinic information analysis from TCGA data

Gene expression data (level 3 HTSeq-FPKM) and clinical information were collected from a total of 619 cases of CRC and rectal cancer from TCGA（(https://portal.gdc.cancer.gov/)）. Exclusion criteria included samples without clinical information. The HTSeq-FPKM(Fregments Per Kilobase per Million) data were converted to TPM (transcripts per million reads)data for subsequent analysis. According to median value of TMUB1 expression, tumor samples were divided into high- and low-expression groups. All data used in the study were in accordance with publication guidelines stated by TCGA (http://cancergenome.nih.gov/publicaionguidelines). Characteristics of patients, including gender, race, TNM stage, and tumor location were recorded and are listed in Table 1. The expression of TMUB1 in paired tumor and adjacent samples and nonpaired samples was analyzed using the Wilcoxon signed rank test and Wilcoxon rank sum testpai, respectively.

Survival analysis

The Kaplan-Meier (KM) Plotter (http://kmplot.com) was used to calculate the overall survival (OS) and disease-free survival (DFS) to analyze the prognosis of patients with tumors. Univariate and multivariate Cox regression analyses were used to compare the prognostic value of the TMUB1 expression and other clinical features as definite factors.

Gene set enrichment analysis

We performed GSEA using the R package clusterProfiler (3.8.0) (Yu et al. 2012) to predict differential pathways and significant functions between high- and low-expression groups. Each analysis was based on 1,000-times gene set permutations. The expression level of TMUB1 was used as a phenotype label. The pathways enriched in each phenotype were analyzed based on the adjusted *P*-value of less than 0.05, FDR *q*-value of less than 0.25, and normalized enrichment score of more than 1 in the enrichment of the MSigDB Collection (h.all.v7.0.symbols.gmt [Hallmarks]).

Immune cell characteristics analyzed by ssGSEA

Simple-sample GSEA (ssGSEA) was performed using the GSVA package (Bindea et al. 2013) in R (3.6.3) to analyze the infiltration of 24 types of tumor-infiltrating immune cells in CRC tissue samples. We quantified the relative score of each immunocyte from the gene expression profile of each sample. Wilcoxon rank sum test was performed to analyze the infiltration of immune cells between low- and high-expression TMUB1 groups, while Spearman correlation was used to analyze the correlation between TMUB1 expression and these immune cells.

Results

TMUB1 Overexpression in patients with colorectal cancer

Analysis using the Wilcoxon rank sum test revealed that the expression level of TMUB1 in 619 tumor tissues was higher than that in 51 normal tissues ($P < 0.001$; Fig. 1A). Likewise, using the Wilcoxon signed rank test we observed that the expression of TMUB1 was significantly higher in 50 tumor tissues than that in the 50 paired normal tissues in the TCGA cohort ($P < 0.001$, Fig. 1B). Both results showed a significant overexpression of TMUB1 in patients with CRC. We further noticed that the ROC curves of the TMUB1 expression showed that the status of the TMUB1 expression could be serve as a biomarker for CRC, (AUC: 0.864) in the TCGA dataset (Fig. 1C)

Association between TMUB1 expression and clinicopathological variables

We detected a significant difference between TMUB1 overexpression and T stage ($P < 0.001$), pathologic stage, and residual tumor when the Kruskal-Wallis rank sum test was used (Fig. 2). We also analyzed the relationship between clinicopathological features of CRC and TMUB1 TPM values using the logistics regression method and found that TMUB1 significantly correlated with T stage (OR = 0.63, 95 % CI: 0.42-0.94, $P = 0.017$) and residual tumor (OR = 2.35, 95 % CI: 1.20-4.88, $P = 0.016$) (Table 3).

High TMUB1 expression associated with adverse outcomes in Colorectal Cancer

KM analysis revealed that the TMUB1 overexpression significantly correlated with shorter OS (HR = 1.73, 95 % CI: 1.22-2.47, $P = 0.002$), and poorer disease-specific survival (DFS) (HR: 2.00, 95 % CI: 1.26-3.17, $P = 0.003$) (Fig. 3).

More specifically, univariate analysis revealed that TMUB1 expression (HR: 1.73, 95 % CI: 1.216-2.473, $P = 0.002$) significantly associated with a poor OS. We found that other clinicopathological variables associated with poor OS included T stage, N stage, M stage, pathologic stage, age, residual tumor,and CEA level . Using multivariate analysis we observed that TMUB1 (HR: 2.077, 95 % CI: 1.101-3.921, $P = 0.024$) remained independently associated with OS along with pathologic stage (HR: 7.127, 95 % CI: 1.529-33.227, $P = 0.012$), and age (HR: 2.886, 95 % CI: 1.389-5.996, $P = 0.005$) (Table 4).

Similarly, univariate analysis revealed that TMUB1 expression (HR: 2.003, 95 % CI: 1.264-3.174, $P = 0.003$) significantly associated with a poor DFS. We also observed that other

clinicopathological variables associated with poor DFS included T stage, N stage, M stage, race, residual tumor CEA level, and pathologic stage. Using multivariate analysis, we found that TMUB1 expression (HR: 2.538, 95 % CI: 1.204-5.350, P= 0.014) remained independently associated with DFS along with M stage (HR: 4.5402.538, 95 % CI: 1.574-13.096, P= 0.005) (Table 5).

GSEA identified TMUB1-related signaling pathways

We conducted GSEA between the datasets of low and high TMUB1 expression to identify differentially activated signaling pathways in CRC (Fig. 4). Accordingly, GSEA showed that many key signaling pathways, such as hypoxia, inflammatory response, angiogenesis, adipogenesis, IL6-JAK-STAT3 signaling, apoptosis, mitotic spindle, and IL2-STAT5 signaling were differentially enriched in the TMUB1 high-expression phenotype.

Association between TMUB1 expression and immune infiltration

Using the ssGSEA, we found that the TMUB1 expression negatively correlated with the abundance of several immune cell types, including T helper cells (r= -0.451, P< 0.001), Tcm cells (r= 0.-413, P< 0.001), macrophages (r= -0.250, P< 0.001), and Th2 cells (r= -0.234, P< 0.001), whereas it positively correlated with the abundance of several immune cell types, including CD56bright (r= -0.230, P< 0.001) and CD56dim NK cells (r= -0.223, P< 0.001) (Fig. 5).

Discussion

TMUB1, as an ubiquitin-like transmembrane protein shuttling from nucleus to cytoplasm, plays a significant role in controlling proliferation and genomic stability (Della-Fazia et al. 2020). Moreover, TMUB1 is overexpressed in a large number of CNS, liver, and esophageal tumors. To the best of our knowledge, the TMUB1 expression, and its potential prognostic impact on CRC has not yet been explored. Thus, the potential role of TMUB1 in CRC was the focus of the present study.

Bioinformatic analysis of high throughput RNA-sequencing data from TCGA demonstrated that TMUB1 overexpression in CRC was associated with T stage, residual tumor, and poor prognosis, suggesting that TMUB1 may serve as a potential diagnostic and prognostic marker in CRC.

However, TMUB1 was demonstrated to be negatively correlated with HCC pathological malignancy (Chen et al. 2019) as low expression of TMUB1 correlated with poor prognosis in patients with HCC. This discrepancy may be attributed to TMUB1 playing different roles in

different tissues. To this end, any TMUB1-specific functions in specific tissues should be explored.

Enrichment analysis of target gene sets using GSEA revealed some important networks of transcription factors and target kinases. More specifically, using GSEA, we observed that the TMUB1 overexpression was associated with hypoxia, inflammatory response, angiogenesis, adipogenesis, IL6-JAK-STAT3 signaling, apoptosis, mitotic spindle, and IL2-STAT5 signaling. Collectively, these results suggested that TMUB1 may play a key role in the molecular mechanism underlying CRC tumorigenesis. Further, these findings were consistent with the fact that hypoxia and inflammation are typical characteristics of cancer (D'Ignazio, Batie and Rocha 2017). The vasculature is an important microenvironmental component and a potential therapeutic target of CRC (Andreuzzi et al. 2020). Abdominal visceral fat is a well-recognized risk factor for CRC and fat as a dietary risk factor has also been associated with an increased risk for CRC (Ocvirk et al. 2019). In priming liver regeneration, the increased level of interleukin 6 (IL-6) was shown to upregulate TMUB1 expression (Liu et al. 2012, Liu et al. 2014), with TMUB1 regulating hepatocyte proliferation via STAT3 pathway (Fu et al. 2019). Moreover, TMUB1 exerts a regulator effect in stabilizing p53 and directing p53 mitochondrial apoptosis and cytoplasmic localization (Hafner et al. 2019, Li et al. 2012). TMUB1 is a main regulator of protein stability in the cell cycle. For instance, during the S phase of centrosome duplication, TMUB1 localized in the gap between centrosomes. The presence of two centrosomes during mitosis is critical for the formation of the bipolar mitotic spindles. Accordingly, knocking down TMUB1 led to abnormal spindle formation in cells, with TMUB1-silenced cells showing a high degree of centrosome amplification during mitosis, associated with multinucleated cells and multipolar spindles [18]. However, the association between the TMUB1 expression and IL2-STAT5 signaling was first the first time, and the regulatory mechanism underlying this association needs to be further elucidated.

In the tumor microenvironment, cancer cells and immune cells exert their effects by either promoting or repressing anticancer immunity. For instance, densities of T follicular helper (Tfh) cells increase along with tumor progression(Bindea et al. 2013). In our study, the TMUB1 expression negatively correlated with the abundance of T helper cells, Tcm cells, macrophages, and Th2 cells, whereas it positively correlated with the abundance of several immune cell types, including CD56bright and CD56dim NK cells. Hence, the correlation between immune cells and TMUB1 suggested that TMUB1 plays a complex role in regulating cancer immunity because of the various roles it plays in immune cells.

The correlation between TMUB1 and CRC should be verified using both cell-based experiments and clinical samples. As the prediction of protein expression using mRNA

expression levels was far from perfect, the correlation between the mRNA and protein expression of TMUB1 is required in further studies. In addition, wet lab work on the mechanisms underlying the TMUB1 functions are required to avoid missing any important signaling pathways explaining the mechanism of TMUB1 function in CRC.

Conclusions

In conclusion, the TMUB1 expression may be a potential diagnostic and prognostic molecular marker of poor survival in CRC. Moreover, IL6-JAK-STAT3 signaling, apoptosis, angiogenesis, adipogenesis, IL2-STAT5 signaling, inflammatory response, and TNFA signaling via NF-KB may be among the key pathways regulated by TMUB1 in CRC. Further experimental validation is required to exhibit verify the biological impact of TMUB1.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Authors' contributions

TH contributed to study design, conducted the literature search, analyzed and synthesized data, and drafted the final manuscript. QL contributed to interpretation of the data and revision of the manuscript. XL and HP J were major contributors to study design, analysis and interpretation of data. JQ and HR Zh were also heavily involved in revising the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

TH - No competing interests. QL – No competing interests. XL – No competing interests. JQ – No competing interests. HE Zh – No competing interests.HP J – No competing interests.

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Figure legends

FIG. 1: (A) Expression level of TMUB1 in paired tumor and adjacent normal samples analyzed using the Wilcoxon rank sum test. (B) Expression level of TMUB1 in paired tumor and normal samples analyzed using the Wilcoxon signed rank test. (C) Receiver operating characteristic (ROC) curves of the expression of TMUB1 in the TCGA.
FPR: False Positive Rate, TPR: True Positive Rate

FIG. 2: Association between the expression level of TMUB1 and clinicopathological characteristics in patients with colorectal cancer. A: pathologic stage, B: T stage, C: residual tumor

FIG. 3: Impact of the TMUB1 expression on overall survival and disease specific survival in patients with colorectal cancer.

FIG. 4: Enrichment plot from GSEA showing several pathways differentially enriched in TMUB1-related colorectal cancer, including hypoxia, inflammatory response, angiogenesis, adipogenesis, IL6-JAK-STAT3 signaling, apoptosis, mitotic spindle, and IL2-STAT5 signaling.
ES, enrichment score; NES, normalized ES; ADJ P-val, adjusted *P*-value

FIG. 5: (A) Expression level of TMUB1 associated with immune infiltration in the tumor microenvironment. The size of dots represents the absolute value of Spearman's *r*.(B) Correlation between the relative enrichment score of the expression level of TMUB1 and cells.

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TABLE 1: Characteristics of patients with colorectal cancer based on TCGA.

TABLE 2: Association between the expression of TMUB1 and clinicopathological features.

TABLE 3: Expression of TMUB1 associated with clinicopathological characteristics (logistic regression).

TABLE 4: Survival outcomes and multivariate analysis of TCGA data.
(A) Association with overall survival and clinicopathological characteristics in patients with colorectal cancer using Cox regression. (B) Multivariate survival model after selection of variables.

TABLE 5: Disease specific survival and multivariate analysis of TCGA data.
(A) Association with disease specific survival and clinicopathological characteristics in patients with colorectal cancer using Cox regression. (B) Multivariate survival model after selection of variables.

Figures

Figure 1

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Impact of the TMUB1 expression on overall survival and disease specific survival in patients with colorectal cancer.

Figure 4

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Figure 5

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