



LncRNA TUSC7 affects malignant tumor prognosis by regulating protein ubiquitination: a genome-wide analysis from 10,237 pan-cancer patients

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Background: Recent studies have shown that tumor suppressor candidate 7 (TUSC7) is abnormally expressed and is associated with poor prognoses in patients with cancer. However, the clinical value and biological role of TUSC7 in cancers remain unclear.

Methods: We performed a meta-analysis of 655 cancer patients from 9 selected articles in order to seek the correlation of TUSC7 expression levels and tumor size, lymph node metastasis, overall survival, and other clinical related indicators. The pan-cancer survival data in the Cancer Genome Atlas (TCGA) were extracted and meta-analyzed to validate the results from our meta-analysis. Sixty thousand public Affymetrix microarrays data were downloaded and used to mine signal pathways associated with TUSC7.

Results: Our results showed that TUSC7 is differentially expressed between cancerous and paracancerous tissues. TUSC7 had a protective effect in the prognosis of cancer, which was shown by the pan-cancer survival data meta-analysis. TUSC7 expression was not correlated with age, gender, tumor size, tumor differentiation, lymph node metastasis or TNM staging in the reported cancers. Pathway analysis revealed that TUSC7 is co-expressed with genes enriched in ubiquitin-mediated proteolysis.

Conclusions: Our study suggested that decreased TUSC7 was associated with cancer prognosis in cancer patients via regulation of ubiquitination.

Keywords: Tumor suppressor candidate 7 (TUSC7); prognosis; pan-cancer; ubiquitination; long non-coding RNA (lncRNA)

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Introduction

Cancer is the uncontrolled growth of tissue caused by various carcinogenic factors. Worldwide, cancer is the leading cause of morbidity and mortality, with about 14 million new cases in 2012. While the overall cancer mortality in the USA has declined by 25%, over the past 20 years more than 2 million Americans have died of cancer (1). Effective tumor markers assist clinicians in detecting, understanding the pathogenesis and predicting the prognosis of cancer. Emerging researches have sought to discover more effective tumor markers from the perspective of non-coding RNA (2,3), but these remain far from clinical practice.

Long non-coding RNA (lncRNA) is a genre of non-coding transcripts with a length of over 200 nt, which regulates gene expression at various biological processes, including transcriptional and post-transcriptional regulation and other epigenetic regulation. Aberrant expression of lncRNA, as oncogenic or tumor suppressor genes, is in relation to the development of cancer. Some lncRNAs have been studied in translational research, such as clinical diagnostic, prognostic evaluation (4) and treatment response prediction (5,6), but further prospective clinical validation of these reports is required.

lncRNA tumor suppressor candidate 7 (TUSC7), also known as LOC285194 and antisense RNA LSAMP3, is located at 3q13.31, and has a length of 14,347 bases. TUSC7 is expressed at low levels in tumor samples, relative to the control groups, in lung cancer, esophageal cancer, gastric cancer, liver cancer and other tumors (7-11), suggesting that it may be involved in cancer development. However, how TUSC7 epigenetically regulates downstream proteins remains unclear.

In the present translational analysis, we initially conducted meta-analyses to examine the relationship of TUSC7 expression and the clinical-pathological factors of patients with solid malignancies. The association with survival outcome was further analyzed. In addition, the Cancer Genome Atlas (TCGA) data sets were used to validate the results of the literature analysis. A broad-spectrum signal pathway analysis exhibited that TUSC7 potentially regulates the ubiquitination process. In summary, our findings imply that TUSC7 is differentially expressed in cancer and is associated with prognosis in cancer patients by its impact on protein ubiquitination.

Methods

Study selection and data extraction

The China National Knowledge Infrastructure (CNKI), Google Scholar, PubMed and Science Direct databases were searched for reports published prior to June 2017, using the key words “long non-coding RNA”, “LSAMP3”, “TUSC7”, “LOC285194” and “cancer” to find eligible studies. The included studies met the following criteria: (I) the TUSC7 expression levels in both primary tumor and paracancerous/normal tissue was examined; (II) included cancer patients with clinical data; and (III) sufficient survival data to calculate the hazard ratio (HR), odds ratios (ORs) and 95% confidence intervals (CI). In articles presenting survival curve without HR and CI, specific points from the survival curves were extracted by Engauge Digitizer version 4.1 and the survival data were obtained according to Tierney *et al.* (12). If there were duplicate data sets, the most complete or the most recent data was chosen. Exclusion criteria were as follows: (I) there were no available or insufficient data; (II) case reports; and (III) meeting abstracts. Two researchers independently extracted and reviewed relevant data from the studies, including the first author, publication year, country, cancer site, methods, number of cases, and cut-off values. If a decision could not be unanimously agreed upon, a third researcher intervened to reach a consensus.

Study quality and publication bias assessment

Literature retrieval investigators (Yusong Chen and Xiaoshun Shi) assessed the quality of all studies by reading the titles and abstracts independently and the Newcastle-Ottawa quality assessment scale was applied to evaluate the quality of literature. In the present meta-analysis, publication bias of the incorporated studies was assessed and illustrated by funnel plots. If the estimated point by the combined effect after the deletion of a study fell below the 95% CI of combined effect value, it manifested that the study had a significant impact on the combined effects.

Pan-cancer RNA-seq data and survival data from TCGA

Publicly available Level 3 RNA-seq data (HTSeq-FPKM-UQ) and the corresponding clinical data of 10,237 cancer patients were obtained from TCGA data portal website (<http://cancergenome.nih.gov>). The expression levels of

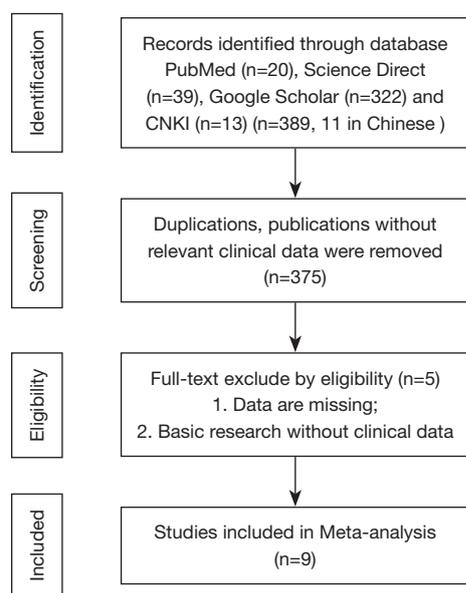


Figure 1 The flow chart of the study search and selection process.

TUSC7 and prognosis data were extracted. The HR and 95% CI were subjected to the Survival Analysis R package (version 3.0) for further meta-analysis.

Acquisition of microarray data

The 60,000 sets of microarray data were obtained from the NCBI GEO database (<https://www.ncbi.nlm.nih.gov/geo/>). The gene expression data were retrieved from the EBI ArrayExpress database by Bioconductor (13,14) and pre-processed using the Robust Multichip Average (RMA) normalization method. Datasets with low standard deviation levels were filtered out, and the remaining experimental data were used for the co-expression analysis.

Gene set enrichment analysis (GSEA)

Lung adenocarcinoma (LUAD) gene expression profiles were accessed and downloaded from the TCGA database (<https://tcga-data.nci.nih.gov/tcga/>). The GSEA was applied to mine the relevant biological processes. Briefly, the top and bottom quartiles of TUSC7 expression (high and low TUSC7 expression, respectively) were sorted, then default settings were used and the gene sets with FDR of 0.25 was regarded as cutoff for the identification of biologically relevant genes. The enriched pathways in each phenotype were sorted by the normalized enrichment score

(NES) and nominal P value.

Statistical analysis

The R (version 3.0) statistical package was used to extract survival data from the TCGA data, STATA software version 12 (Stata, Corporation, College, Station, Texas, USA) was utilized for meta-analysis and evaluation of heterogeneity among studies was performed using the Cochrane Q test and P values. If there was heterogeneity ($I^2 \geq 50\%$ or $P \leq 0.05$), the pooled OR was calculated using the random effect model. Otherwise, the fixed effect model is applied. All P values were two tailed, and the sensitivity analysis and publication bias were considered statistically significant if $P < 0.05$.

Results

Characteristics of included studies

The initial database search identified 389 articles published between 2010 and 2016 (*Figure 1*). After screening the titles and abstracts, 14 articles were further evaluated for eligibility. Of these, 5 papers were excluded because they contained either no data or incomplete data sets. Thus, 9 articles were included in the present meta-analysis. Eight different types of cancer were analyzed, including 1 study each of esophageal carcinoma, gastric cancer, hepatocellular carcinoma, non-small cell lung cancer, pancreatic ductal carcinoma and 2 studies each regarding colorectal cancer and osteosarcoma. These studies enrolled 655 participants, with a minimum sample size of 75 and a maximum sample size of 142, and the levels of TUSC7 expression in both normal or paracancerous tissues and tumor tissues were determined. The main features of the 9 studies and the Newcastle-Ottawa scale (NOS) score are summarized in *Table 1*. Since TUSC7 expression was detected by qPCR, the cut-off values differed between these studies. Not all studies reported the association between TUSC7 expression and sex, tumor size, differentiation, lymph node metastasis, distant organ metastasis and TNM staging or other indicators.

Association between TUSC7 and clinicopathological characteristics of cancers

As shown in *Figure 2A*, the differential expression of TUSC7 in cancer and paracancerous tissues and its

Table 1 Demographic characteristics of the selected studies

Lead author, year	Tumor type	Patients nationality	Detection method	Cut-off value	Newcastle-Ottawa scale	Ref
Pasic, 2010	Osteosarcoma	Canada	qRT-PCR	Mean	NA	(15)
Liu, 2013	Colon cancer	China	qRT-PCR	Average	NA	(16)
Qi, 2013	Colorectal cancer	China	qRT-PCR	Mean	8	(11)
Ding, 2014	Pancreatic ductal adenocarcinoma	China	qRT-PCR	Mean	8	(17)
Tong, 2014	Esophageal squamous cell carcinoma	China	qRT-PCR	Median	6	(10)
Qi, 2015	Gastric cancer	China	qRT-PCR	Average	4	(9)
Cong, 2016	Osteosarcoma	China	qRT-PCR	Average decreasing fold	8	(18)
Wang, 2016	Hepatocellular carcinoma	China	qRT-PCR	NA	8	(8)
Wang, 2016	NSCLC	China	qRT-PCR	Median	8	(7)

NOS contains 3 categories, including selection, comparability and outcome, with total scores ranges from 0 to 9. If the score is greater than or equal to 7, it is considered high quality. qRT-PCR, quantitative real-time polymerase chain reaction; NSCLC, non-small cell lung cancer; NA, not available; NOS, Newcastle-Ottawa scale.

association with clinicopathological features of patients were meta-analyzed. The results displayed that TUSC7 is expressed differentially between cancerous and paracancerous tissues (HR =1.833, 95% CI: 1.102–3.048). Our statistic outcome suggests that TUSC7 is differentially expressed in the existing cancer studies and sensitivity analysis showed no publication bias ($P=0.567$) in these studies ($P>0.05$) (Figure 2B). Unfortunately, the outcomes demonstrated that the expression levels of TUSC7 showed no associations with age, gender, tumor differentiation, tumor size, lymph node metastasis or TNM staging (Figure S1).

TUSC7 is associated with cancer survival

To investigate further the effect of differential expression of TUSC7 in cancer, 7 studies with OS data from 616 patients were chosen for meta-analysis (Figure 2C). We applied a random effects model due to heterogeneity ($I^2=56.7%$, $P=0.03$). Low TUSC7 expression was in association to a poorer survival rates, and TUSC7 expression had a protective role in cancer prognosis (HR =0.436, 95% CI: 0.099–0.773, $P=0.02$). According to the type of malignancy included in our studies, corresponding survival data of these tumors were portrayed (Figure S2) and extracted from the TCGA database for survival analysis (Table S1). With the results which from survival analysis were subjected

to meta-analysis (Figure 2D). The pooled survival data corresponding to the literature in the TCGA database displayed that low TUSC7 expression is associated with a poorer survival rate and is related to an adverse prognosis in cancer (HR =1.25, 95% CI: 0.88–1.63, $P=0.00$). This result implied that TUSC7 has impact on cancer survival in existing cancer studies. No clear evidence of publication bias ($P=0.83$ and $P=0.38$, respectively) in this meta-analysis were able to find (Figure 2E,F).

TUSC7 is a prognostic determinant of pan-cancer survival

TCGA program has published the genetic data for 33 cancers. To explore the broad relationship between TUSC7 and clinical outcomes, we divided the patients into high and low expression groups according to the median value of TUSC7 expression, and analyzed survival data using R (Table S1, Figure S2). After meta-analysis of the clinical and genetic data from 10,237 samples in the TCGA dataset, we confirmed that low TUSC7 expression is associated with poorer survival and is also associated with adverse outcomes in both common cancer types (HR =1.019, 95% CI: 0.88–1.16, $P=0.00$) (Figure 3A) and a pan-cancer scope (HR =1.01, 95% CI: 0.88–1.13, $P=0.00$) (Figure 3B). There was no clear evidence of publication bias ($P=0.34$ and $P=0.51$, respectively) in these meta-analyses (Figure 3C,D).

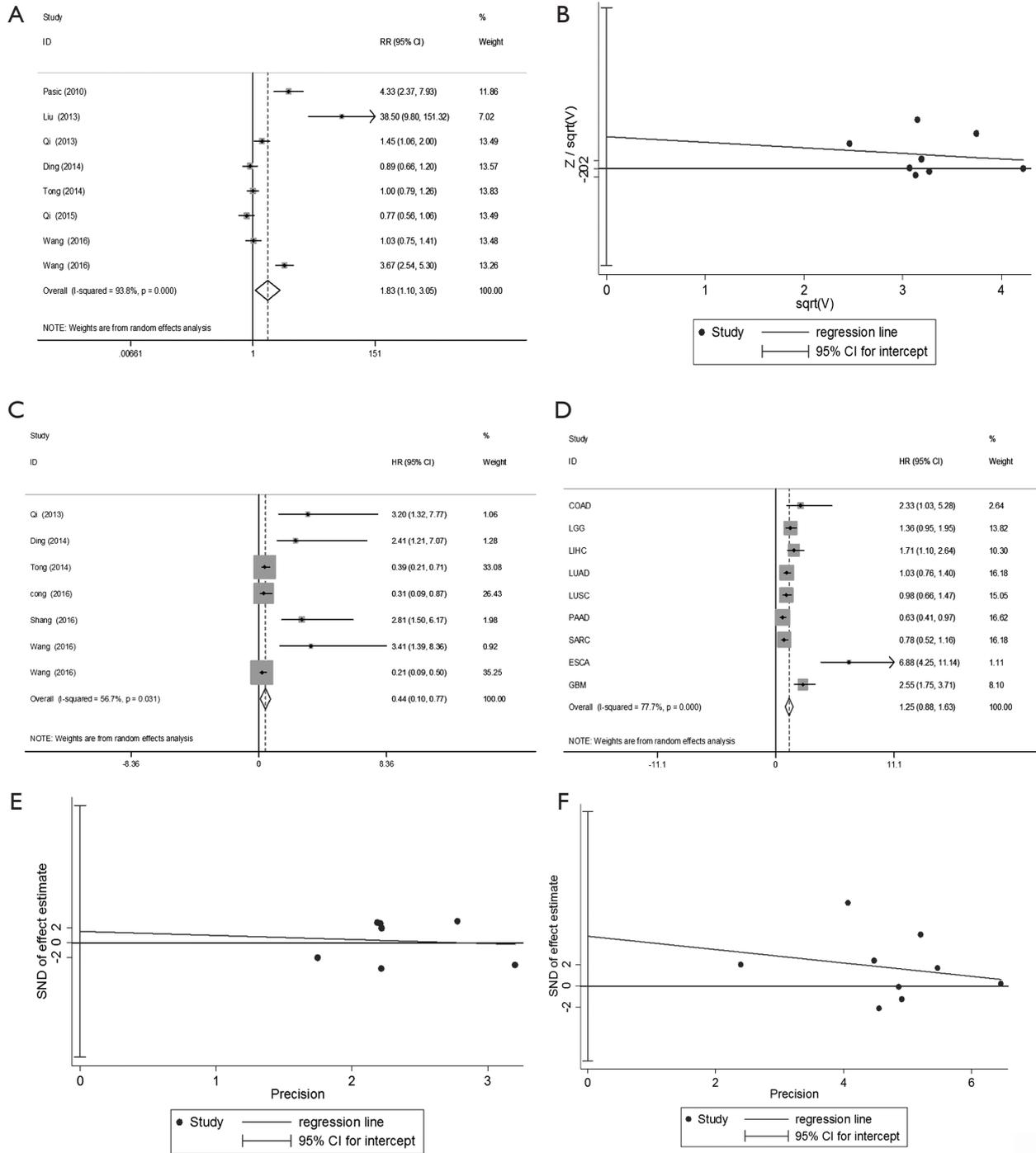


Figure 2 Meta-analysis of the difference in TUSC7 expression and the pooled HRs of OS in cancerous and paracancerous tissues. (A) Qualitative meta-analysis of studies estimating the difference of TUSC7 expression between cancerous and paracancerous tissues; (B) funnel plot for studies involved in the meta-analysis; (C) meta-analysis of HRs of OS in literature; (D) meta-analysis of HRs of OS extracted from TCGA survival data; (E) funnel plot for studies involved in the literature meta-analysis; (F) funnel plot for studies involved in the TCGA meta-analysis. COAD, colon adenocarcinoma; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PAAD, pancreatic adenocarcinoma; SARC, sarcoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; CI, confident interval.

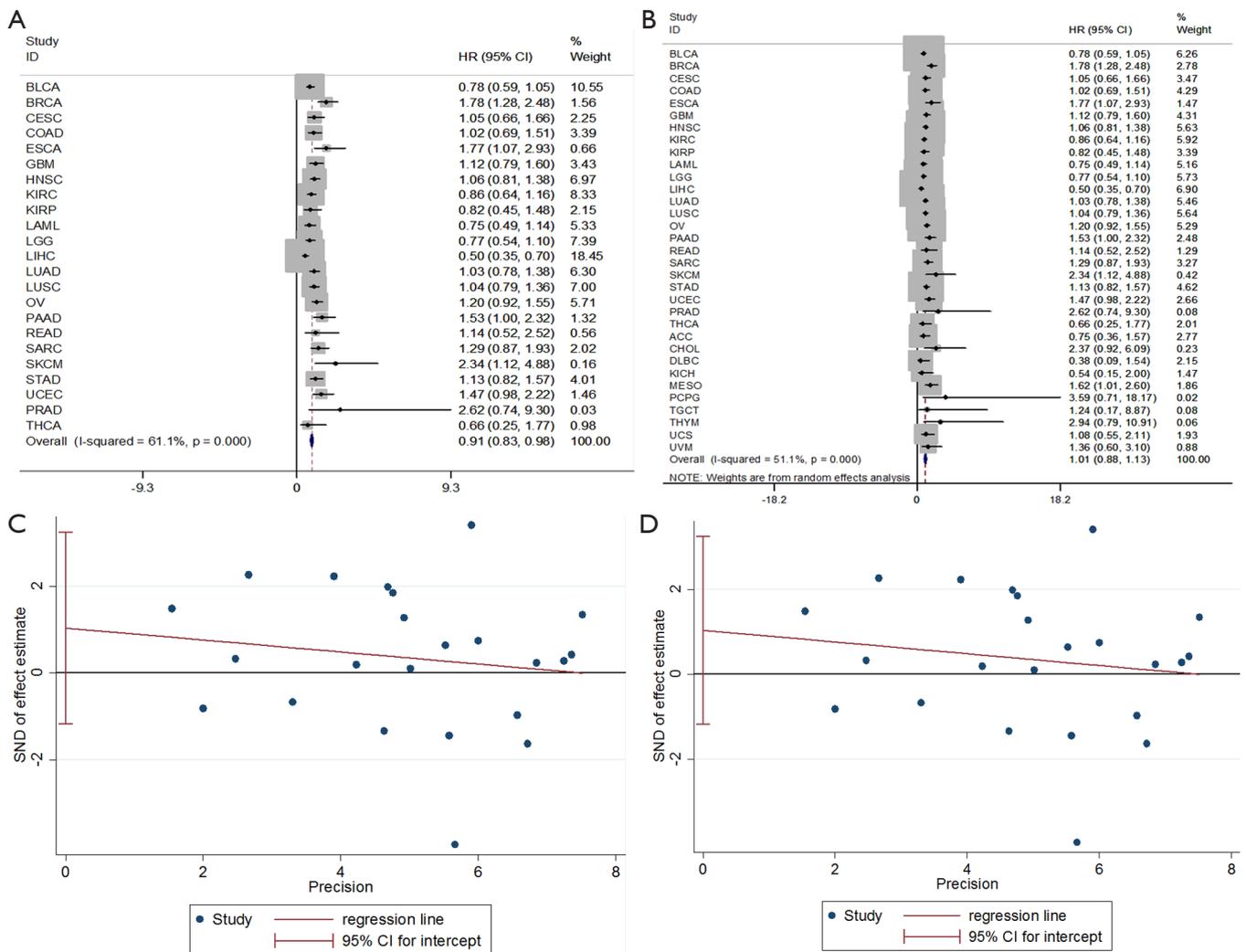


Figure 3 Meta-analysis of the pooled HRs of OS of different types of cancer with decreased TUSC7 expression in TCGA database. (A) Meta-analysis of HRs of OS extracted from TCGA survival data in common cancers; (B) meta-analysis of HRs of OS extracted from TCGA survival data at the pan-cancer level; (C) and (D) funnel plot for studies involved in the meta-analysis. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; BLBC, lymphoid neoplasm diffuse large cell carcinoma; HNSC, Head and NECK squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial Carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

The association of TUSC7 with ubiquitin mediated proteolysis pathway

Next, we aimed to reveal potential mechanisms to explain the effects of TUSC7 on cancer prognosis. The co-expression RNA transcripts of TUSC7 were identified by

analysis of its co-expression genes from over 60,000 public Affymetrix human U133-Plus 2 transcriptional profiling microarray data, including all normal and cancer tissues. The top five positive or ten negative co-expressed genes significantly enriched pathways were showed (Figure 4A,B).

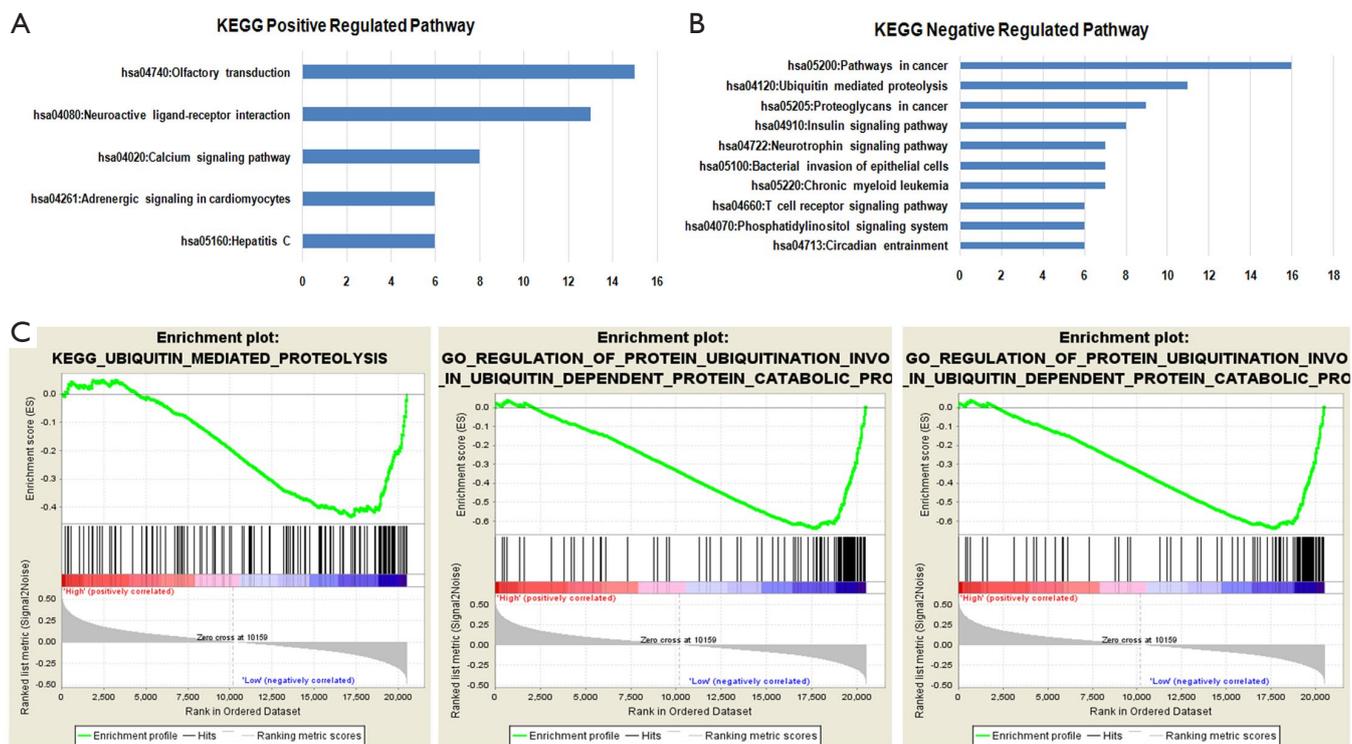


Figure 4 TUSC7 regulates protein ubiquitination in cancer cells. (A) Pathway enrichment of top 5 positive co-expression genes; (B) pathway enrichment of top 10 negative co-expression genes; (C) GSEA analysis showed that TUSC7 might have a regulatory role in protein ubiquitination.

Bioinformatics analysis externally validated that TUSC7 is downregulated in most cancer tissues, compared to normal or paracancerous tissues, and gene sets are significantly enriched in the low TUSC7 phenotype. To identify further key pathways associated with cancer prognosis, LUAD RNAseq2 data from the TCGA were subjected to the GSEA assay. We discover that ubiquitin mediated proteolysis pathway related genes were significantly enriched in the LUAD patients with low TUSC7 expression (Figure 4C). Taken together, our results indicate that TUSC7 plays a prognostic role in cancer patients by regulating ubiquitination.

Discussion

In the study of the human genome, it has been reported that most genome sequences are transcribed into non-coding RNAs (19). LncRNA is a class of non-coding RNA, having a length of more than 200 nt, which has been considered as a non-functioning genomic transcription. However, recent studies advised that lncRNAs have impacts in many cancers (20),

such as lung cancer (21), breast cancer (22), and prostate cancer (23). At present, abnormal expression of lncRNAs have been found in a variety of tumor tissues, which plays a therapeutic role in the treatment of human malignant tumors (24). These findings suggest that lncRNAs have potential roles in cancer translational medicine.

LncRNA TUSC7 was first identified as a tumor suppressor unit in osteosarcoma (15). Studies have reported that the expression of TUSC7 in cancer tissues is lower than normal tissues. However, the role of TUSC7 in cancer biology is far from fully elucidated. Pasic *et al.* (15) reported that TUSC7 is a transcriptional target of p53 and that ectopic expression of TUSC7 inhibits colorectal and breast cells growth. Deletion analysis showed that two miR-211 binding sites are associated with TUSC7-mediated growth inhibition in colorectal carcinoma (16). However, the mechanism by which TUSC7 affects gene expression modification and protein interaction has not been studied. In our findings, TUSC7 is down-regulated on the pan-cancer level and is associated with cancer prognosis. By screening TUSC7 relevant genes and mining potential

molecular pathways, we first reported that TUSC7 is widely involved in the process of protein ubiquitination.

Post-transcriptional ubiquitination can affect chromatin function and plays an important regulatory role in gene expression. Yoon *et al.* found that lncRNA HOTAIR could induce ubiquitin mediated proteolysis (25). The increased expression of HOTAIR could promote the growth and invasion of prostate cancer cells. It was found that lncRNA HOTAIR combined with androgen receptor in cancer cells, blocking the binding of E3 ubiquitin ligase MDM2 and AR, thereby preventing the ubiquitination and promoting the degradation of AR (26). Moreover, lncRNA-mediated ubiquitination contributes to the biological process of protein stability. For example, lncRNA ANCR can promote the interaction of CDK1-EZH2, increasing the phosphorylation of Thr-345 and Thr-487 in EZH2, and facilitating the ubiquitination process to degrade the targeted protein (27). By genome-wide and extensive tissue gene co-expression analysis, TUSC7 has been considered to be involved in the process of protein ubiquitination. Further biological pathway mining from the lung cancer TCGA RNA-seq data provided an independent, external validation.

However, our analysis has some limitations regarding the correlation of aberrant expression of lncRNA and clinical significance. First, the definition of low or high levels of TUSC7 was different in terms of cut-off values and detection methodology. Although real-time quantitative PCR was applied to quantify TUSC7 in all studies, the results could still have been heterogeneous due to different qPCR primer designs. Second, the follow-up schedule and differing endpoints of the TCGA studies limit the accuracy of the results. Third, most of the patients in the literature meta-analysis were Chinese, with only one study on Canadian patients. Fourth, because of the different biological characteristics of the tumors and fact that most of the literature did not contain enough clinical data, the conclusion that TUSC7 is not correlated with tumor size, regional lymph node metastasis, distant organ metastasis or TNM staging requires further validation. Finally, although data in large scale suggest that TUSC7 is associated with the ubiquitination process, it remains to be proven through molecular biology experiments.

In conclusion, though with limitations, our results revealed that the expression of lncRNA TUSC7 is under-expressed in all sorts of cancer tissues, compared to normal tissues, and is significantly correlated with OS, and is potentially a prognostic marker of cancers. Genome-wide microarray and RNA-seq data suggest that TUSC7

has a potential regulatory role in ubiquitination. More comprehensive and large-scale clinical trials are required to elucidate the prognostic value of lncRNA TUSC7 expression in various cancers.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2017.08.19>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Institutional ethical approval and informed consent were waived.

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Supplementary

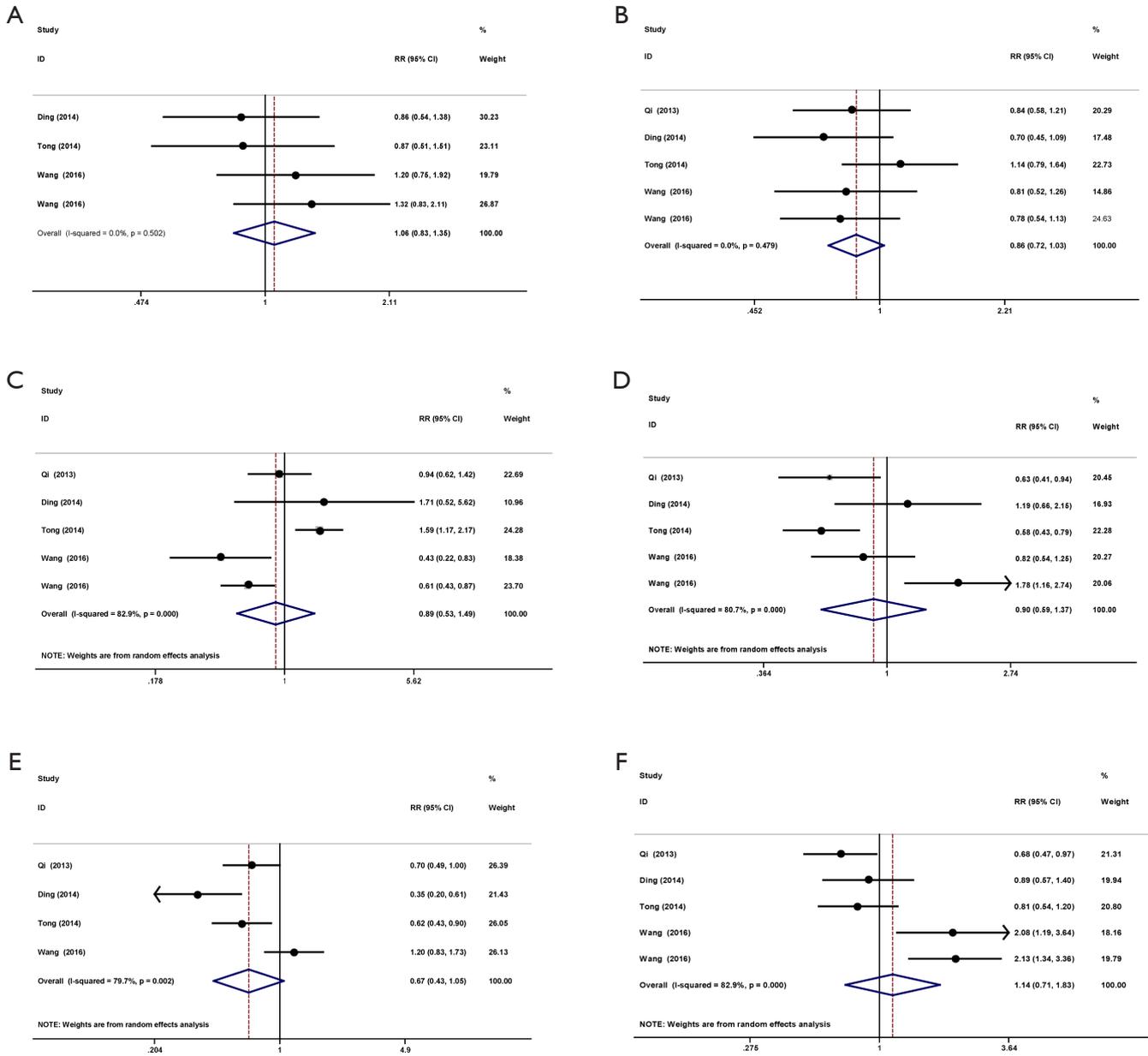


Figure S1 The expression levels of TUSC7 and clinical characteristics. TUSC7 expression is not associated with (A) age, (B) sex, (C) differentiation, (D) tumor size, (E) lymph node metastasis, and (F) TNM staging.

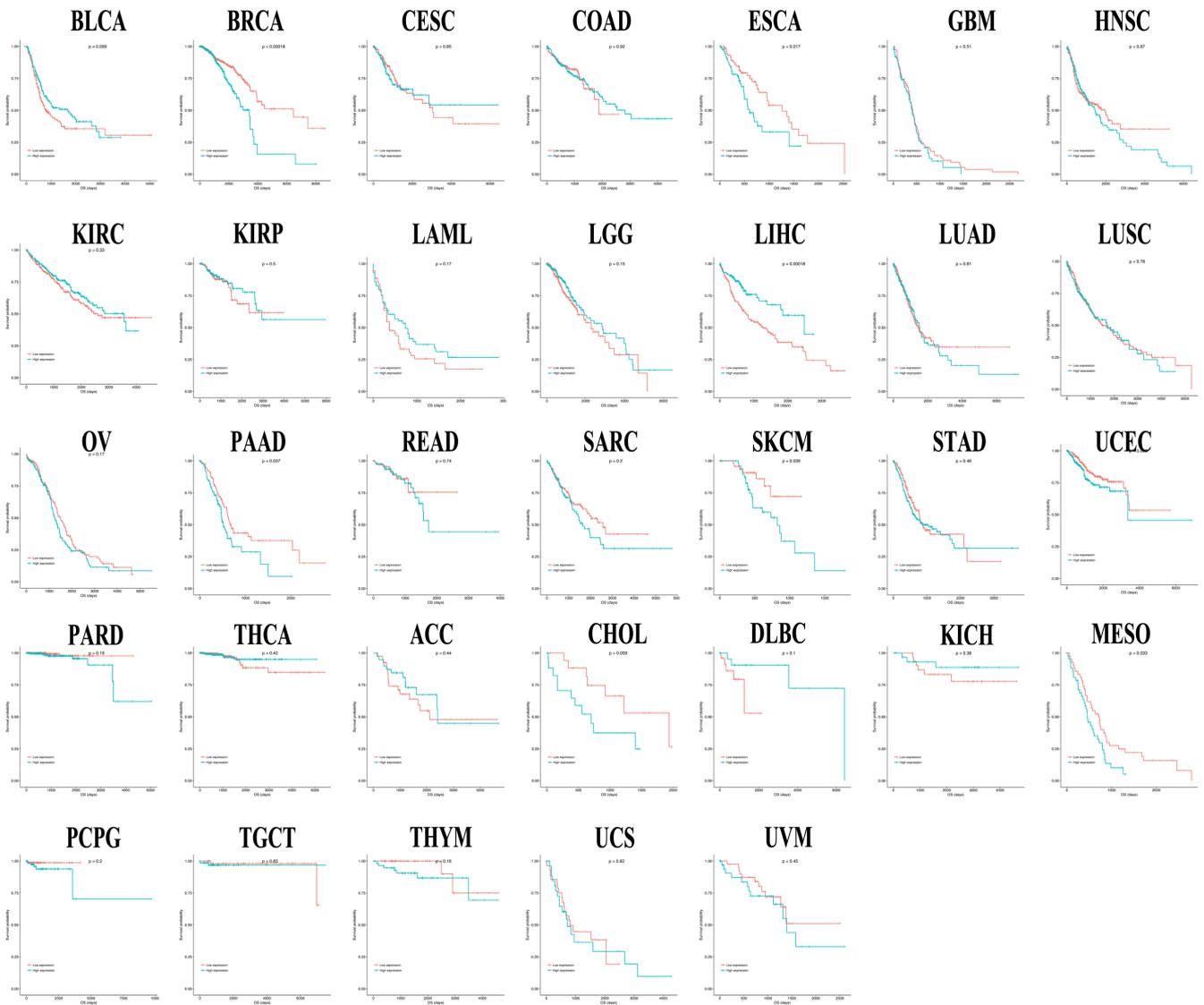


Figure S2 Kaplan-Meier analysis of TUSC7 pan-cancer survival.

Table S1 Pan-cancer survival data

Cancer types	HR	Upper 95% CI	Lower 95% CI	P value
TCGA-ACC	0.746309668	1.565467258	0.355790335	0.441012892
TCGA-BLCA	0.784099295	1.049402176	0.585868525	0.098997465
TCGA-BRCA	1.781565875	2.483294793	1.278131367	0.000179497
TCGA-CESC	1.046445392	1.663267981	0.658371334	0.846468822
TCGA-CHOL	2.370762077	6.090600958	0.922817447	0.0592904
TCGA-COAD	1.020101214	1.508160695	0.689983826	0.916877744
TCGA-DLBC	0.382187275	1.544873303	0.094549574	0.100663931
TCGA-ESCA	1.772361779	2.928825155	1.072534586	0.016954822
TCGA-GBM	1.123085924	1.601196204	0.787737312	0.509297598
TCGA-HNSC	1.059255909	1.382230511	0.811748165	0.668654386
TCGA-KICH	0.540769072	1.99947242	0.146254175	0.375397997
TCGA-KIRC	0.862077658	1.161585796	0.639795951	0.328406293
TCGA-KIRP	0.815607161	1.477276741	0.450298188	0.496095443
TCGA-LAML	0.748800848	1.14285557	0.490615547	0.168400251
TCGA-LGG	0.772608274	1.097673515	0.543807914	0.145363445
TCGA-LIHC	0.496804556	0.702130748	0.351522516	0.000180894
TCGA-LUAD	1.034698224	1.377417038	0.777252194	0.81494668
TCGA-LUSC	1.039244533	1.36196438	0.79299372	0.777732734
TCGA-MESO	1.619136129	2.604746888	1.006470846	0.032915881
TCGA-OV	1.19515983	1.551020094	0.920946817	0.173673386
TCGA-PAAD	1.525130799	2.316125255	1.004273818	0.037066273
TCGA-PCPG	3.587065754	18.16963664	0.708161698	0.202879813
TCGA-PRAD	2.618272572	9.300202968	0.737118457	0.188965935
TCGA-READ	1.141130834	2.522667758	0.516191471	0.742487515
TCGA-SARC	1.294529962	1.927955727	0.869214888	0.198820802
TCGA-SKCM	2.339378956	4.882922102	1.120782553	0.03861001
TCGA-STAD	1.130758132	1.567655195	0.815621928	0.461268987
TCGA-TGCT	1.236166055	8.87289926	0.172221781	0.827202394
TCGA-THCA	0.662940389	1.76849877	0.248510187	0.421038733
TCGA-THYM	2.938503934	10.90848091	0.791568087	0.156549361
TCGA-UCEC	1.47334208	2.224035248	0.976035288	0.057508555
TCGA-UCS	1.077718054	2.111880519	0.549972497	0.819750077
TCGA-UVM	1.360424016	3.096100334	0.597769227	0.452736665