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TRIP13 upregulation is correlated with poor prognosis and tumor progression in esophageal squamous cell carcinoma

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ABSTRACT

Thyroid receptor-interacting protein 13 (TRIP13), a member of the AAA + ATPase super-family, has been proved to be upregulated and identified as a prognostic factor in multiple human cancers, However, the role of TRIP13 in esophageal squamous cell carcinoma (ESCC) and its clinic relevance remains unclear. In the present study, we performed database-mining and detected TRIP13 expression in 158 tissue samples (79 ESCC tissue and 79 matched adjunct non-cancerous tissues). We further investigated the correlation between TRIP13 expression and clinicopathological features and overall survival. Univariate and multivariate Cox regression analyses were applied to evaluate the potential prognostic value of TRIP13 in ESCC patients. In addition, the mechanisms involved in TRIP13 tumor-promoting effect was investigated. Data showed that TRIP13 expression was significantly increased in ESCC tissues, compared with the matched adjunct non-cancerous tissues. Expression of TRIP13 is significantly correlated with T status ($P = 0.027$), lymphatic metastasis ($P = 0.017$), and clinical stages of ESCC ($P = 0.009$). Kaplan-Meier analyses showed that patients with high TRIP13 expression had poor overall survival ($P = 0.0022$). Multivariate analysis indicated that TRIP13 expression might be an independent prognostic factor in ESCC patients (HR, 1.778, 95% confidence interval = 0.959–3.296, $P = 0.028$). Furthermore, downregulating TRIP13 in EC109 cell significantly attenuated the cell proliferation and progression, possibly by β-catenin regulated EMT pathway.

Conclusions: Our study demonstrated that TRIP13 might be a tumor promoting factor in ESCC and a promising prognostic indicator for ESCC patient.

1. Introduction

Esophageal cancer has emerged as the eighth most prevalent type of cancer and also the sixth cause of cancer-related death in the world [1,2]. Esophageal squamous cell carcinoma (ESCC) is the predominant histological form of esophageal cancer in Asian population [3]. Although tremendous progress has been made in the therapy strategies, the overall survival rate of 5 years is still less than 25% [1]. ESCC treatment needs novel diagnostic indicators and therapeutic targets, which will promote a better prediction of ESCC prognosis and development of novel chemotherapy drugs.

Thyroid receptor-interacting protein 13 (TRIP13) is a protein encoded by TRIP13 gene, which interacts with thyroid hormone receptors. TRIP13 is a member of the AAA^+ ATPase super-family which play a functional role in many cell signal pathways and cell activities, including protein degradation, chromosome repairing and DNA replication [4–6]. Recently, studies relieved that TRIP13 has been identified as an oncogene in many human cancers, because the overexpression of TRIP13 triggers cancer cell proliferation, migration and invasion both *in vitro* and *in vivo*. The overexpressed TRIP13 is associated with advanced clinical progression and poor prognosis in ovarian cancer [7], prostate cancer [8], hepatocellular carcinoma [5], lung cancer [9], colorectal cancer [10] and head and neck cancer [11].

However, to our best knowledge, the association between TRIP13 expression and ESCC clinical features and prognosis, and the possibly involved mechanisms were still poorly understood. In the present study, we first analyzed data from Oncomine database and found that TRIP13 mRNA levels was significantly upregulated in ESCC than non-cancerous tissue. Then immunohistochemistry staining was established and found that TRIP13 protein expression was significantly increased in ESCC tissues and the expression of TRIP13 was correlated with advanced clinical stage and poor prognosis. Furthermore, we established TRIP13

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knocked down EC109 cells and detected the roles of TRIP13 in EC109 cell proliferation, migration, and the possible involved mechanisms. The results of *in vivo* and *in vitro* study, combined with the clinical data and data from database, indicated that TRIP13 might be a good prognostic predictor and a potential therapy target of ESCC.

2. Materials and methods

2.1. Ethics statement

The study was approved by the Ethics Committee of the second affiliated hospital of the Fourth Military Medical University (FMMU, Xian, China). All the patients signed the informed consent prior to specimen collection. All the experiments on nude mice were approved by the Ethics Committee, and were conducted strictly in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (National Institutes of Health Publication No. 85-23, revised 1996).

2.2. Cell lines and animals

EC109 cell line was purchased from Shanghai Cell Bank at the Chinese Academy of Sciences (Shanghai, China). Nude mice were purchased from the Laboratory Animal Centre of FMMU. The mice were kept in special-pathogen-free environment with 12 h/12 h light/dark cycle. All the mice had free access to chow and tap water.

2.3. Cell culture and lentivirus infection

Human EC109 cells were cultured with RPMI 1640 medium (Gibco, NY, USA), supplemented with 10% fetal bovine serum (Gibco, NY, USA), penicillin (100 units/ml) and streptomycin (100 units/ml) at 37 °C in a humidified atmosphere containing 95% air and 5% CO_2. TRIP13 knock down (KD) and normal control (NC) lentiviruses were purchased from Genechem (Shanghai, China) and the infection was conducted strictly in accordance to the manufacturer's instructions. The TRIP13 KD EC109 cells were selected by puromycin and further confirmed by Western blot and RT-PCR.

2.4. ESCC patient tissue specimens collection and microarray immunohistochemistry

In this retrospective study, a total of 158 tissue specimens were collected from 79 patients who received ESCC surgery in Department of Thoracic surgery, Tangdu hospital between January 2012 and November 2013. The collected tissue specimens includes 79 ESCC specimens and 79 matched adjunct noncancerous tissues. The patients were not administrated radiotherapy or chemotherapy before surgery. Medical record provides patients' information regarding sex, age, TNM stage, differentiation grade and histological type. All tumors were staged according to *Cancer of the Esophagus and Esophagogastric Junction: An Eighth Edition Staging Primer* by Thomas et al.. Follow up of all the patients were updated to death or December 2018.

IHC staining was conducted as previously described after the tissue specimens were made into a microarray [12]. Briefly, tissue microarray section was deparaffinized, rehydrated and placed in 0.01 M citrate buffer (pH 6.0) and heated in a microwave for antigen retrieval. Then the section was incubated with 3% H_2O_2 for 15 min for elimination of the endogenous peroxidase. TRIP13 was detected by TRIP13 antibody (1:50, 19602-1-AP, Proteintech) at 4 °C overnight and further visualized using an immunohistochemistry staining kit(KIHC-5, Proteintech, IL, USA).

The expression of TRIP13 was scored by multiplication of the percentage of positive tumor cells and the staining intensity as previously described [13]. Staining intensity scores were used: 0 indicates no staining, 1 indicates weak staining, 2 indicates moderate staining and 3 indicates intense staining. The percentage of positive cells were scored as 0 (< 1%), 1 (1–25%), 2 (26–50%), 3 (51–75%), 4(76–100%). The average IHC score was 5.1266 (95% CI 4.4051–5.8604), and the medium IHC score was 6. The average value of total scores were used to separate the samples with high expression from samples low expression. IHC score ≥ 6 was identified as high expression (n = 42) and IHC score < 6 was identified as low expression (n = 37).

Table 1
Correlation of TRIP13 expression with clinicopathological variables of ESCC patients.

Clinicopathologic Variables	No. of case	TRIP13 expression		*p*-value*
		Low	High	
Age				0.086
< 60	29	17	12	
≥60	50	20	30	
Gender				0.383
Male	62	28	34	
Female	17	9	8	
Differentiation				0.279
Grade 1	2	2	0	
Grade 2	69	32	37	
Grade 3	8	3	5	
pT status				0.027
T_{1-2}	10	8	2	
T_{3-4}	69	29	40	
pN status				0.017
No	23	23	15	
Yes	41	14	27	
Clinical stage				0.009
I–II	39	24	15	
III–IV	40	13	27	
Site				0.551
Upper	6	3	3	
Middle	35	14	21	
Lower	38	20	18	

2.5. Patient characteristics

Clinicopathological characteristics of the ESCC patients were showed in Table 1. There were 62 male patients and 17 female patients, whose median age is 64 years old, ranging from 30 to 81 years old. Among all the enrolled patients, 6 patients were diagnosed with upper thoracic ESCC, 35 patients with middle thoracic ESCC and 38 patients with lower thoracic ESCC. Postoperative staging evaluation demonstrated stage I disease in 1 patient, stage II in 38 patients, stage III in 31 patients and stage IV in 9 patients. Follow up was performed every 3 months after surgery. Follow up of all the patients were updated to death or December 2018. The overall survival of the patients ranged from 0 to 80 months, and the median survival time is 27 months.

2.6. Western blot

18 pairs of frozen ESCC tumor tissue and matched noncancerous tissues were randomly picked among the 79 pair ESCC tissue. The protein expression of TRIP13 in each sample was detected by western blot as previous described [14]. The anti-TRIP13, anti-Vimentin (1:1000, Proteintech), anti-β-catenin (1:1000, Abcam, Cambridge, MA, UK), anti-E-cadherin (1:500, Cell signaling technology, Beverly, MA, USA), anti-β-actin, anti-GAPDH and anti-Histone H (1:1000, CMCTAG, Milwaukee, WI, USA) antibodies were used to detected the associated proteins. Goat anti-rabbit and goat anti-mouse HRP-linked secondary antibodies (Zhaongshanjinqiao, Beijing, China) were used at a concentration of 1:5000. The bands were visualized and captured utilizing Bio-Rad imaging system (Bio-Rad, Hercules, CA, USA).

2.7. Cell viability assay

5×10^3 cells were seeded in 96-well plate and cultured with complete medium. 10 μl CCK8 regent (7-sea, Shanghai, China) was added to each well and the cells were further cultured for 1 h. Absorbance at 450 nm was detected 12 h, 24 h, 36 h and 48 h after cell seeding.

2.8. Clonogenic assays

For cell colony formation assay, cells were seeded and cultured with complete medium in 6-well plate for 2 weeks. The medium was replaced every 3 days. 14 days later, cells were stained with 0.5% crystal violet. Colony with more than 50 cells were counted using Image J, and the colony numbers in the control group were normalized to 100%.

2.9. Cell transwell assay

Cells transwell assay was conducted to detect the cell migration and invasion ability as previously described [15]. 6.5 mm transwell chambers (Corning, NY, USA) were used and the pore size is 8 μm. After 24 h, the cells adhered to the underside of the chamber was stained with 0.5% (w/v) crystal violet for 20 min. After that, images were captured using an inverted microscope (×200 magnification) in 3 random fields to calculate cell numbers.

2.10. In vivo tumor formation in nude mice

The nude mice were divided into 2 groups, the TRIP13 NC group and the TRIP13 KD group, TRIP13 NC or KD cells (1×10^7) were subcutaneously injected into left rear limbs of the mice. After the tumors had reached about 100 mm^3, body weight and tumor size of each mouse were measured every 3 days. The tumor volume was calculated as (L × W2)/2, where L is the length and W is the width in mm. On the 24^{th} day, mice were sacrificed and the xenografts were harvested for further detection.

2.11. Statistical analyses

SPSS 21.0 software was used for statistical analysis. Associations between NOK expression and clinicopathological variables were evaluated using the χ2-test. Survival rates were calculated and survival curves were constructed using the Kaplan-Meier method and the log-rank test was used to evaluate the significance of difference. Correlation analyses of the survival time and various clinicopathological variables was determined using univariate and multivariate Cox proportional hazards analysis. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Analysis of TRIP13 expression data from online database

To determine the expression of TRIP13 in ESCC, we analyzed multiple data sets from Oncomine. In Su's ESCC statistics, TRIP13 mRNA level is significantly increased in ESCC tissue than adjacent noncancerous tissues (Fig. 1A). In addition, Oncomine database analysis also revealed that TRIP13 mRNA is over expressed in multiple cancers, which indicates a potential role of TRIP13 in cancer development (Fig. 1B). Then we further analyzed TRIP13 mRNA levels in Hu's (GSE20347) [16] (20955586) and Su's (GSE23400) [17] ESCC data sets. As shown in Fig. 1C, both of the data sets showed that TRIP13 mRNA is significantly increased in ESCC tissue than the non-cancerous tissue.

3.2. Expression of TRIP13 in human ESCC tissues

In our study, protein expression of TRIP13 was detected in 18 pairs of ESCC tissues and matched adjacent noncancerous tissues by western blot. TRIP13 protein expression was significantly increased in ESCC tissue ($P < 0.001$, Fig. 1D). IHC staining confirmed the over expression of TRIP13 in ESCC tissue (Fig. 2E, $P < 0.001$). To further evaluate the differential expression of TRIP13 between ESCC and adjunct noncancerous tissues, IHC was performed on the tissue microarray and found that TRIP13 scores were significantly higher in the ESCC tissue than those in adjunct noncancerous tissues (Fig. 2A–E). IHC scores in I/II stage group were significantly lower than those in III/IV stage group (Fig. 2F, $P = 0.0007$). In addition, IHC scores in T1 + T2 group were significantly lower than those in T3 + T4 group (Fig. 2G, $P = 0.0017$).

3.3. TRIP13 expression is correlated with clinicopathological variables of ESCC patients

The 79 specimens were divided into TRIP13 high expression group and TRIP13 low expression group as previously mentioned. The correlation between TRIP13 protein expression and clinicopathological variables in ESCC patients were statistically analyzed and summarized in Table 1. TRIP13 expression is significantly correlated with tumor size ($P = 0.027$), lymphatic metastasis ($P = 0.017$), and clinical stage ($P = 0.009$) (Table 1). There was no significant difference between TRIP13 expression and age, sex, differentiation, or tumor location. Due to the limited simple size in the T1 + T2 group, the prognostic role of TRIP13 in T1 + T2 group and T3 + T4 group was not determined.

3.4. TRIP13 expression is correlated with prognosis and survival in ESCC patients

To investigate the relationship between TRIP13 expression and the clinical outcomes of ESCC patients, the correlation between patient survival and TRIP13 expression status was analyzed. ESCC patient with high TRIP13 expression had a significantly worse prognosis than patients with low TRIP13 expression (Log-rank $P < 0.001$, Fig. 2F). The mean survival time of ESCC patients with high TRIP13 expression (n = 42) was 27.9 months and the mean survival time of ESCC patients with low TRIP13 expression (n = 37) was 44.4 months. Furthermore, we divided patients into I/II stage group and III/IV stage group, but failed to find the difference of prognosis between patients with high TRIP13 expression and low TRIP13 expression in each separated stage groups (Fig. 2I–J).

3.5. TRIP13 expression level was an independent prognostic factor in ESCC patients

To further evaluate whether TRIP13 represent a prognostic parameter in ESCC patients, we used the Cox's proportional hazards model. Univariate analysis showed that high TRIP13 expression was correlated to ESCC patients' survival (Table 2). In addition, clinical stage was also correlated to ESCC patients' survival in Univariate analysis. We then used multivariate survival analysis to assess the prognostic factors identified in the univariate analysis. We found that besides clinical stage, TRIP13 expression level was an independent prognostic factor (HR = 2.438, $P = 0.002$, Table 2). Multivariate analysis indicated that TRIP13 expression might be an independent prognostic factor in ESCC patients (HR, 1.778, 95% confidence interval = 0.959–3.296, $P = 0.028$).

3.6. Suppression of TRIP13 attenuated the aggressive tumor phenotype of EC109 cells in vitro

To investigate the potential tumor-promoting effect of TRIP13, we knocked down the TRIP13 protein expression in EC109 cells using lentivirus. The transfection efficiency was confirmed by western blot (Fig. 3D). Then we assessed the cell proliferative ability of EC109 cells by using cell viability detection and colony formation assays. TRIP13

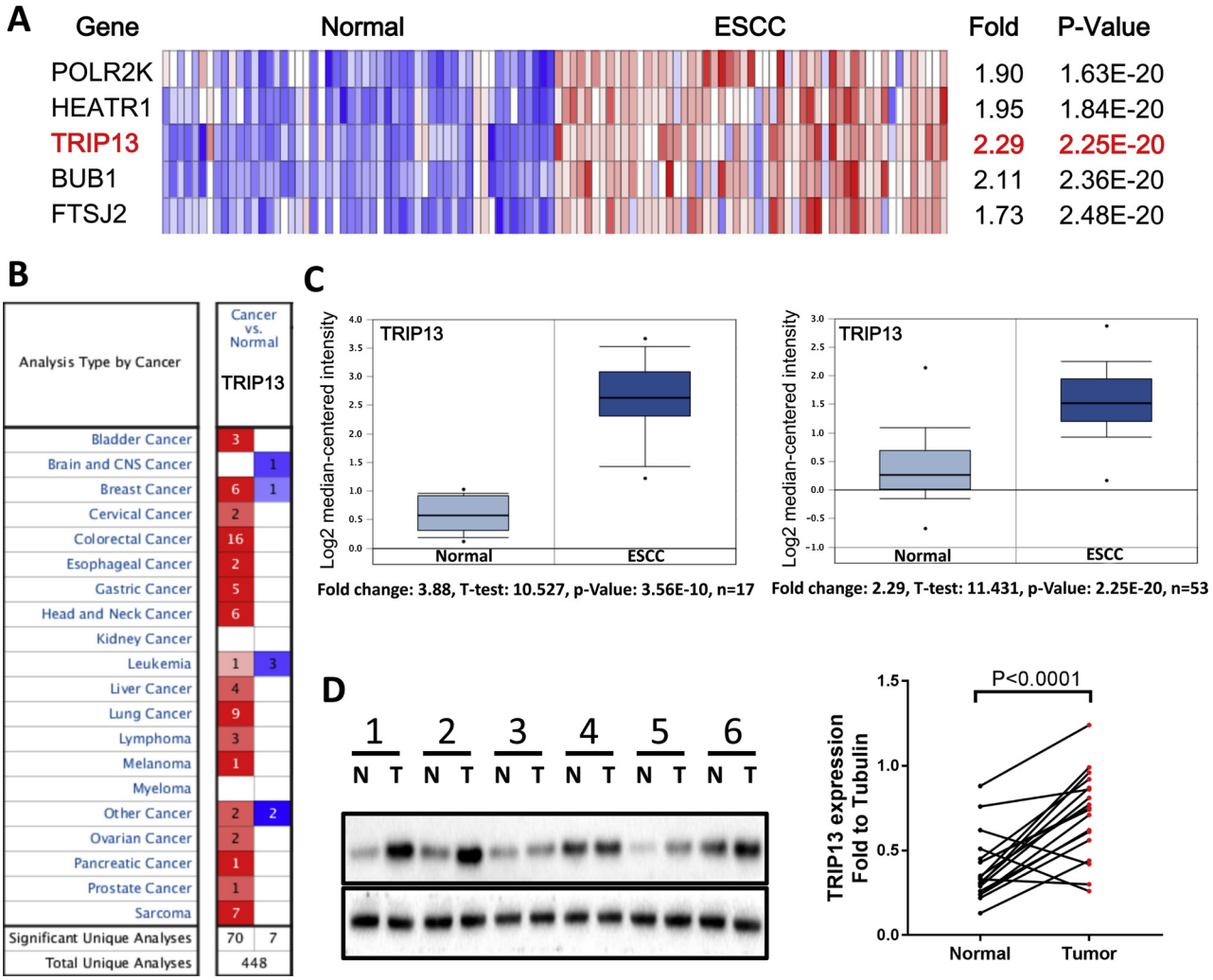

Fig. 1. TRIP13 is overexpressed in ESCC tissues. (A) Oncomine database showed that TRIP13 is one most the most significantly differently expressed genes (in the top 1%). (B) Data sets numbers from Oncomine with significant TRIP13 mRNA over expressions or down expressions. (C) TRIP13 mRNA levels in ESCC tissue and matched non-cancerous tissue in Su's and Hu's ESCC data sets. (D) Western blot analysis of TRIP13 protein expression in 18 paired ESCC tissues.

KD significantly decreased the cell growth compared to that in the TRIP13 NC group ($P < 0.01$, Fig. 3A). In addition, the colony formation assay found that knocking down TRIP13 significantly decreased EC109 cell proliferation ability (Fig. 3B).

Transwell assay was performed to investigate EC109 cell migration and invasion ability after TRIP13 knock down. As shown in Fig. 3C, TRIP13 knocking down significantly decreased the migrated and invaded cells in comparison to the NC group. These results indicated that TRIP13 might be a potential promotor of EC109 cell progression.

β-Catenin and epithelial-mesenchymal transition (EMT) play a crucial role in tumor progression. Western blot showed that, compared with TRIP13 NC group, β-catenin expression is slightly decreased in whole TRIP13 KD cells, while significantly decreased in the nucleus of the TRIP13 KD cells (Fig. 3D). These results indicated that transcriptional activity of β-catenin was decreased after TRIP13 knocking down. Expression of Vimentin was decreased and E-cadherin was increased in TRIP13 KD EC109 cells, indicating EMT was suppressed by TRIP13 knocking down.

3.7. Suppression of TRIP13 in ESCC cells attenuated in vivo xenograft growth

TRIP13 NC and TRIP13 KD EC109 cell were used to establish *in vivo* xenografts. Tumor volume was measured every 3 days after the tumors reached 100 mm^3. Tumor volume in TRIP13 KD group was significantly smaller than that of TRIP13 NC group. In conclusion, our results proved that β-catenin regulated EMT might involve in TRIP13 induced EC109 cell progression.

4. Discussion

Although great advances have been made in the therapeutic strategies of ESCC during the past decades, the overall survival remains unsatisfying. In the present study, we first applied database mining and identified that TRIP13 might play a potential role in ESCC due to its higher mRNA level in ESCC tissues than that in adjunct non-cancerous tissues. Then we discovered the correlation between TRIP13 expression and clinicopathological variables and prognosis of ESCC. Furthermore, we found that TRIP13 might promote EC109 cell progression by activating β-catenin and EMT pathway.

TRIP13 is a member of AAA + ATPase super-family. TRIP13 is first identified as a key regulator of meiotic recombination and spindle assembly checkpoint, acting on signaling proteins of the conserved HORMA domain family [18]. Carter and colleagues found that TRIP13 is upregulated in multiple cancers in 2006 [19]. From then on, a series studies further confirmed the abnormal expression of TRIP13 in human cancers and identified TRIP13 as a potential oncogene [20]. Banerjee and colleagues investigated the oncogenic role of TRIP13 in cancer first,

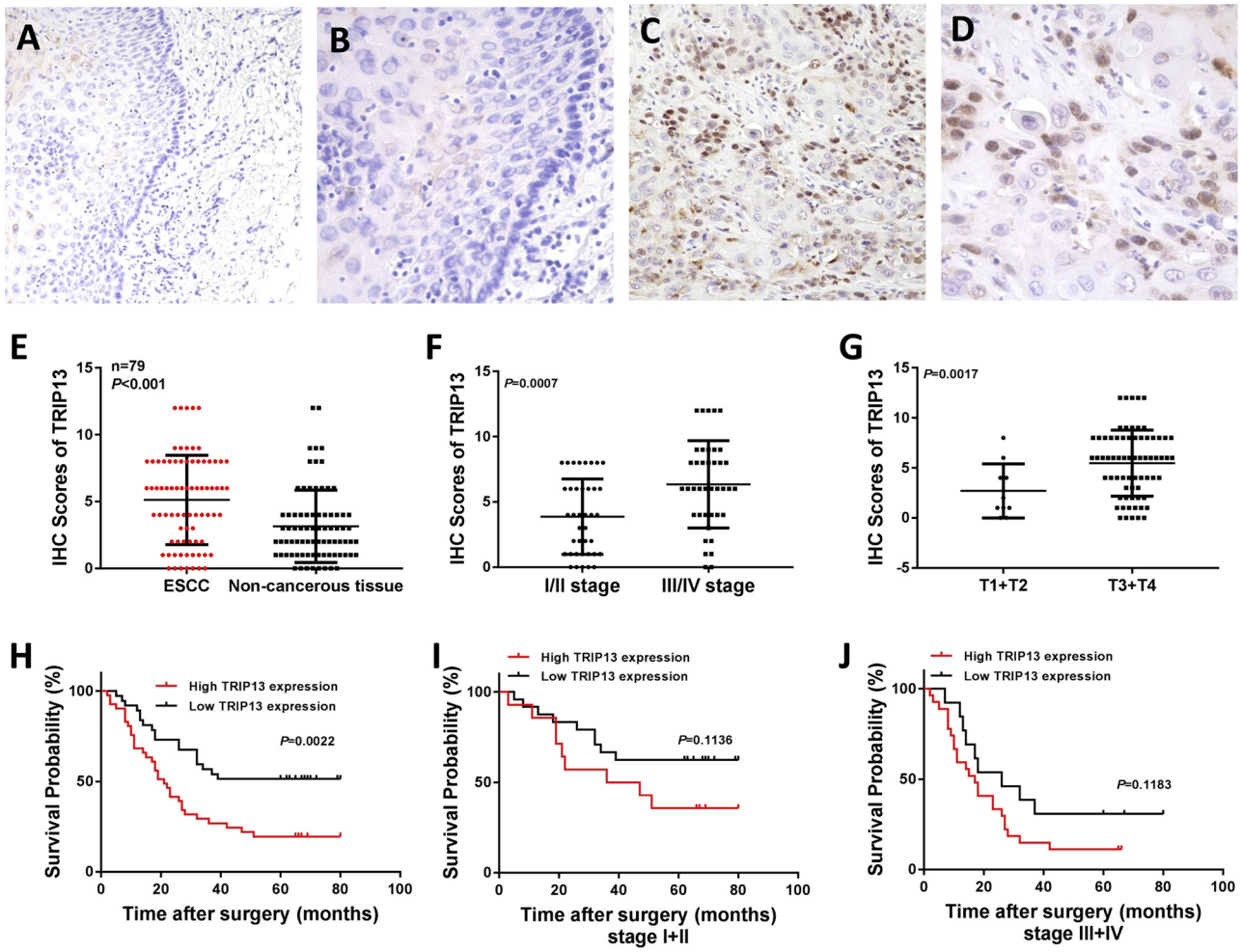

Fig. 2. TRIP13 overexpression was correlated with bad prognosis of ESCC. (A–D) Representative IHC staining images of TRIP13 (A and B: normal tissue with low TRIP13 expression, C and D: ESCC tissue with high TRIP13 expression). (E) Comparison of IHC scores in ESCC tissues and Non-cancerous tissues. (F) Comparison of IHC scores of TRIP13 between I/II stage ESCC and III/IV stage ESCC. (G) Comparison of IHC scores of TRIP13 between T1 + T2 ESCC and T3 + T4 ESCC. (H) Kaplan Meier survival test regarding TRIP13 expression in 79 ESCC specimens. (I) Kaplan Meier survival test regarding TRIP13 expression in 39 stage I and stage II ESCC specimens. (J) Kaplan Meier survival test regarding TRIP13 expression in 40 stage III and stage IV ESCC specimens.

and demonstrated that regulating TRIP13 may contribute to overcome treatment resistance in head and neck cancer [11]. Then TRIP13 has been proved to be a tumor promotor in several human cancers. Interestingly, Marks et al found that TRIP13 loss and Mad2 overexpression reduced the ability of checkpoint complexes to disassemble and significantly inhibited the proliferation of cells in culture and tumor xenografts [21]. Wang et al. suggest that premature mitotic checkpoint silencing triggered by TRIP13 overexpression may promote cancer [22]. The detailed roles of TRIP13 still needs further investigation. The tumor promoting effects of TRIP13 have never validated in ESCC, and the potentially mechanisms involved in this process remains totally unknown. In our study, we demonstrated that TRIP13 is significantly upregulated in ESCC cancer tissues than adjunct noncancerous tissues, through mining the Oncomine database, IHC staining and western blotting. These results were in consistence with previous studies of TRIP13 in other cancer types.

Studies have proved that TRIP13 might be associated with clinicopathological characteristics in serval cancer types [10,23–26]. In the present study we analyzed the correlations between TRIP13 and clinicopathological variables in 79 ESCC patients and found that TRIP13 expression is positively associated with tumor size, lymphatic metastasis, and clinical stages. These results indicated that increase of TRIP13

Table 2
Univariate and multivariate analysis of overall survival in ESCC patients.

Variables	Categories	Univariate analysis		Multivariate analysis	
		HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age (years)	> = 60/ < 60	0.772(0.432–1.379)	0.382	0.809(0.449–1.458)	0.480
Gender	Male/Female	1.270(0.666–2.422)	0.469	1.322(0.667–2.619)	0.424
Grade of Differentiation	Grade 1 + 2/Grade 3	0.635(0.271–1.490)	0.297	0.746(0.313–1.869)	0.556
Site	Upper + Middle/Lower	1.700(0.975–2.965)	0.061	1.652(0.894–3.154)	0.109
Clinical stage	I + II/III + IV	0.370(0.209–0.656)	0.001	0.412(0.232–0.813)	0.005
TRIP13 expression	High/Low	2.438(1.371–4.336)	0.002	1.778(0.959–3.296)	0.028

HR, Hazard Ratio; CI, confidence interval.

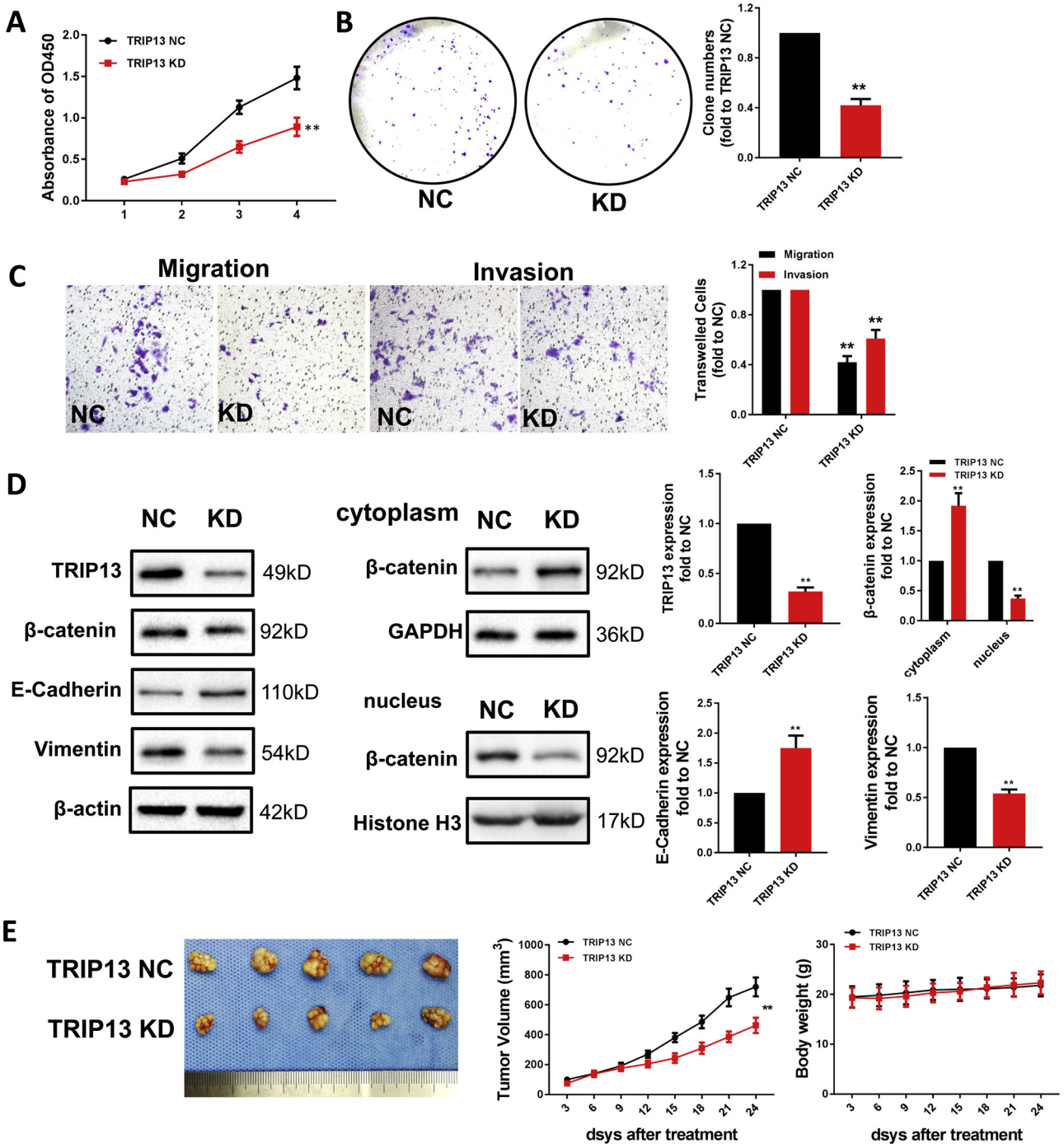

Fig. 3. TRIP13 down regulation attenuated EC109 cell proliferation and progression both *in vitro* and *in vivo*. (A) CCK-8 analysis of cell growth of TRIP13 NC cells and TRIP13 KD cells. (B) Colony formation assays of EC109 cells. (C) Transwell assay of EC109 cells (for migration and invasion). (D) Representative western blot images of TRIP13, β-catenin and EMT markers. (E) Images and analysis of the *in vivo* xenografts. $^{**}P < 0.01$ *vs* TRIP 13 NC group.

expression was associated with ESCC progression. Furthermore, Log-rank test showed that TRIP13 expression in ESCC tissue is negatively associated with prognosis. Patients with low TRIP13 expression had significantly better outcome than patients with high TRIP13 expression. However, when patients were divided in to I + II group and III + IV group, TRIP13 expression was significantly higher in III + IV group, but TRIP13 expression was uncorrelated with ESCC prognosis in each separated group. These results might attributes to the limited sample size. Whether TRIP13 is a good predictor of ESCC prognosis in specific clinical stage still needs further investigation.

The mechanisms of the tumor-promoting effect of TRIP13 haven't been fully explained, and the pathways involved might differ in different cancer types. For example, in ovarian cancer, the oncogenic role of TRIP13 might be mediated by Notch signaling [7] and in hepatocellular carcinoma by TGF-β1/smad3 pathway [5]. In the present study, we demonstrated that TRIP13 knock down decreased the tumor phenotype of EC109, indicated by colony formation assay *in vitro* and xenografts growth detection *in vivo*.

EMT takes place in epithelial cancer cells and triggers cancer progression and metastasis [27]. β-Catenin signaling plays key roles in tissue homeostasis and cell fate decisions in embryonic and post-embryonic development across the animal kingdom [28]. As a result,

pathway abnormal changes are associated with developmental disorders and many human cancers [28]. Studies have reported that for analyzing EMT and malignant cancer development, β-catenin is depicted as a relevant target [29]. β-catenin can be activated by a series of protein kinases and then assembles in the nucleus to enhance transcription of multiple EMT-related genes [29–33]. Numerous of studies has proved that β-catenin and it regulated EMT paly key role in cancer progression and treatment, including ESCC [34,35]. In the present study, we found transcriptional activity of β-catenin was inhibited when TRIP13 was knocked down, and the EMT level was attenuated. However, whether β-catenin and EMT is the dominating pathway in this process still needs further investigation and more evidence.

Taken together, for the first time we demonstrated that TRIP13 is a promising oncogene and a good prognostic indicator in ESCC. TRIP13 was significantly increased in ESCC tissues, and overexpression of TRIP13 is correlated with advanced clinical stages and bad prognosis of ESCC. *In vitro* and nude-mice based *in vivo* study confirmed the cancer promoting effect of TRIP13, and this effect might be associated with β-catenin and EMT.

Statement of author contributions

Xiaofei Li and Xiaolong Yan designed the study. Shouyin Di and Mingyang Li performed the expreiments and analyzed the data. Zhiqiang Ma and Kai Guo carried out the database mining. Shouyin Di wrote the manuscript. All authors have read and approved the final manuscript.

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Declarations of interest

The authors declare no conflict of interest.

Acknowledgments

Not applicable.

References

[1] F. Islam, V. Gopalan, S. Law, J.C. Tang, A.K. Lam, FAM134B promotes esophageal squamous cell carcinoma in vitro and its correlations with clinicopathologic features, Hum. Pathol. 87 (2019) 1–10.
[2] K.Y. Lam, L. Ma, Pathology of esophageal cancers: local experience and current insights, Chin. Med. J. 110 (1997) 459–464.
[3] I.Y. Kuo, Y.L. Huang, C.Y. Lin, C.H. Lin, W.L. Chang, W.W. Lai, Y.C. Wang, SOX17 overexpression sensitizes chemoradiation response in esophageal cancer by transcriptional down-regulation of DNA repair and damage response genes, J. Biomed. Sci. 26 (2019) 20.
[4] C. Alfieri, L. Chang, D. Barford, Mechanism for remodelling of the cell cycle checkpoint protein MAD2 by the ATPase TRIP13, Nature 559 (2018) 274–278.
[5] J. Yao, X. Zhang, J. Li, D. Zhao, B. Gao, H. Zhou, S. Gao, L. Zhang, Silencing TRIP13 inhibits cell growth and metastasis of hepatocellular carcinoma by activating of TGF-beta1/smad3, Cancer Cell Int. 18 (2018) 208.
[6] A.R. Tipton, K. Wang, P. Oladimeji, S. Sufi, Z. Gu, S.T. Liu, Identification of novel mitosis regulators through data mining with human centromere/kinetochore proteins as group queries, BMC Cell Biol. 13 (2012) 15.
[7] X.Y. Zhou, X.M. Shu, TRIP13 promotes proliferation and invasion of epithelial ovarian cancer cells through Notch signaling pathway, Eur. Rev. Med. Pharmacol. Sci. 23 (2019) 522–529.
[8] L. Dong, H. Ding, Y. Li, D. Xue, Z. Li, Y. Liu, T. Zhang, J. Zhou, P. Wang, TRIP13 is a predictor for poor prognosis and regulates cell proliferation, migration and invasion in prostate cancer, Int. J. Biol. Macromol. 121 (2019) 200–206.
[9] W. Li, G. Zhang, X. Li, X. Wang, Q. Li, L. Hong, Y. Shen, C. Zhao, X. Gong, Y. Chen, J. Zhou, Thyroid hormone receptor interactor 13 (TRIP13) overexpression associated with tumor progression and poor prognosis in lung adenocarcinoma, Biochem. Biophys. Res. Commun. 499 (2018) 416–424.
[10] N. Sheng, L. Yan, K. Wu, W. You, J. Gong, L. Hu, G. Tan, H. Chen, Z. Wang, TRIP13 promotes tumor growth and is associated with poor prognosis in colorectal cancer, Cell Death Dis. 9 (2018) 402.
[11] R. Banerjee, N. Russo, M. Liu, V. Basrur, E. Bellile, N. Palanisamy, C.S. Scanlon, E. van Tubergen, R.C. Inglehart, T. Metwally, R.S. Mani, A. Yocum, M.K. Nyati, R.M. Castilho, S. Varambally, A.M. Chinnaiyan, N.J. D'Silva, TRIP13 promotes error-prone nonhomologous end joining and induces chemoresistance in head and neck cancer, Nat. Commun. 5 (2014) 4527.
[12] P. Chen, W.M. Li, Q. Lu, J. Wang, X.L. Yan, Z.P. Zhang, X.F. Li, Clinicopathologic features and prognostic implications of NOK/STYK1 protein expression in non-small cell lung cancer, BMC Cancer 14 (2014) 402.
[13] F. Han, S. Zhang, J. Liang, W. Qiu, Clinicopathological and predictive significance of SIRT1 and peroxisome proliferator-activated receptor gamma in esophageal squamous cell carcinoma: the correlation with EGFR and Survivin, Pathol. Res. Pract. 214 (2018) 686–690.
[14] S. Di, C. Fan, Y. Yang, S. Jiang, M. Liang, G. Wu, B. Wang, Z. Xin, W. Hu, Y. Zhu, W. Li, Y. Zhou, X. Li, X. Yan, Activation of endoplasmic reticulum stress is involved in the activity of icariin against human lung adenocarcinoma cells, Apoptosis 20 (2015) 1229–1241.
[15] T.S. Liang, Y.J. Zheng, J. Wang, J.Y. Zhao, D.K. Yang, Z.S. Liu, MicroRNA-506 inhibits tumor growth and metastasis in nasopharyngeal carcinoma through the inactivation of the Wnt/beta-catenin signaling pathway by down-regulating LHX2, J. Exp. Clin. Cancer Res. 38 (2019) 97.
[16] N. Hu, R.J. Clifford, H.H. Yang, C. Wang, A.M. Goldstein, T. Ding, P.R. Taylor, M.P. Lee, Genome wide analysis of DNA copy number neutral loss of heterozygosity (CNNLOH) and its relation to gene expression in esophageal squamous cell carcinoma, BMC Genomics 11 (2010) 576.
[17] H. Su, N. Hu, H.H. Yang, C. Wang, M. Takikita, Q.H. Wang, C. Giffen, R. Clifford, S.M. Hewitt, J.Z. Shou, A.M. Goldstein, M.P. Lee, P.R. Taylor, Global gene expression profiling and validation in esophageal squamous cell carcinoma and its association with clinical phenotypes, Clin. Cancer Res. 17 (2011) 2955–2966.
[18] Q. Ye, S.C. Rosenberg, A. Moeller, J.A. Speir, T.Y. Su, K.D. Corbett, TRIP13 is a protein-remodeling AAA+ ATPase that catalyzes MAD2 conformation switching, eLife 4 (2015).
[19] S.L. Carter, A.C. Eklund, I.S. Kohane, L.N. Harris, Z. Szallasi, A signature of chromosomal instability inferred from gene expression profiles predicts clinical outcome in multiple human cancers, Nat. Genet. 38 (2006) 1043–1048.
[20] D.R. Rhodes, J. Yu, K. Shanker, N. Deshpande, R. Varambally, D. Ghosh, T. Barrette, A. Pandey, A.M. Chinnaiyan, Large-scale meta-analysis of cancer microarray data identifies common transcriptional profiles of neoplastic transformation and progression, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 9309–9314.
[21] D.H. Marks, R. Thomas, Y. Chin, R. Shah, C. Khoo, R. Benezra, Mad2 overexpression uncovers a critical role for TRIP13 in mitotic exit, Cell Rep. 19 (2017) 1832–1845.
[22] K. Wang, B. Sturt-Gillespie, J.C. Hittle, D. Macdonald, G.K. Chan, T.J. Yen, S.T. Liu, Thyroid hormone receptor interacting protein 13 (TRIP13) AAA-ATPase is a novel mitotic checkpoint-silencing protein, J. Biol. Chem. 289 (2014) 23928–23937.
[23] W. Dazhi, Z. Mengxi, C. Fufeng, Y. Meixing, Elevated expression of thyroid hormone receptor-interacting protein 13 drives tumorigenesis and affects clinical outcome, Biomark. Med. 11 (2017) 19–31.
[24] S.E. Larkin, S. Holmes, I.A. Cree, T. Walker, V. Basketter, B. Bickers, S. Harris, S.D. Garbis, P.A. Townsend, C. Aukim-Hastie, Identification of markers of prostate cancer progression using candidate gene expression, Br. J. Cancer 106 (2012) 157–165.
[25] E. Maurizio, J.R. Wisniewski, Y. Ciani, A. Amato, L. Arnoldo, C. Penzo, S. Pegoraro, V. Giancotti, A. Zambelli, S. Piazza, G. Manfioletti, R. Sgarra, Translating proteomic into functional data: an high mobility group A1 (HMGA1) proteomic signature has prognostic value in breast cancer, Mol. Cell. Proteom. 15 (2016) 109–123.
[26] Y. Tao, G. Yang, H. Yang, D. Song, L. Hu, B. Xie, H. Wang, L. Gao, M. Gao, H. Xu, Z. Xu, X. Wu, Y. Zhang, W. Zhu, F. Zhan, J. Shi, TRIP13 impairs mitotic checkpoint surveillance and is associated with poor prognosis in multiple myeloma, Oncotarget 8 (2017) 26718–26731.
[27] T. Blick, E. Widodo, H. Hugo, M. Waltham, M.E. Lenburg, R.M. Neve, E.W. Thompson, Epithelial mesenchymal transition traits in human breast cancer cell lines, Clin. Exp. Metastasis 25 (2008) 629–642.
[28] K.N. Schaefer, M. Peifer, Wnt/beta-catenin signaling regulation and a role for biomolecular condensates, Dev. Cell 48 (2019) 429–444.
[29] V. Das, S. Bhattacharya, C. Chikkaputtaiah, S. Hazra, M. Pal, The basics of epithelial-mesenchymal transition (EMT): a study from a structure, dynamics, and functional perspective, J. Cell. Physiol. (2019) (Epub ahead of print).
[30] J.I. Yook, X.Y. Li, I. Ota, C. Hu, H.S. Kim, N.H. Kim, S.Y. Cha, J.K. Ryu, Y.J. Choi, J. Kim, E.R. Fearon, S.J. Weiss, A Wnt-Axin2-GSK3beta cascade regulates Snail1 activity in breast cancer cells, Nat. Cell Biol. 8 (2006) 1398–1406.
[31] A.A. Farooqi, M. de la Roche, M.B.A. Djamgoz, Z.H. Siddik, Overview of the oncogenic signaling pathways in colorectal cancer: mechanistic insights, Semin. Cancer Biol. (2019) (Epub ahead of print).
[32] I. El Ayachi, I. Fatima, P. Wend, J.A. Alva-Ornelas, S. Runke, W.L. Kuenzinger, J. Silva, W. Silva, J.K. Gray, S. Lehr, H.C. Barch, R.I. Krutilina, A.C. White, R. Cardiff, L.D. Yee, L. Yang, R.M. O'Regan, W.E. Lowry, T.N. Seagroves, V. Seewaldt, S.A. Krum, G.A. Miranda-Carboni, The WNT10B network is associated with survival and metastases in chemoresistant triple-negative breast cancer, Cancer Res. 79 (5) (2018) 982–993.
[33] N. Ferrari, R. Ranftl, I. Chicherova, N.D. Slaven, E. Moeendarbary, A.J. Farrugia, M. Lam, M. Semiannikova, M.C.W. Westergaard, J. Tchou, L. Magnani, F. Calvo, Dickkopf-3 links HSF1 and YAP/TAZ signalling to control aggressive behaviours in cancer-associated fibroblasts, Nat. Commun. 10 (2019) 130.
[34] L. Li, J. Xu, G. Qiu, J. Ying, Z. Du, T. Xiang, K.Y. Wong, G. Srivastava, X.F. Zhu, T.S. Mok, A.T. Chan, F.K. Chan, R.F. Ambinder, Q. Tao, Epigenomic characterization of a p53-regulated 3p22.2 tumor suppressor that inhibits STAT3 phosphorylation via protein docking and is frequently methylated in esophageal and other carcinomas, Theranostics 8 (2018) 61–77.
[35] Z. Li, H. Jin, G. Mao, L. Wu, Q. Guo, Msi2 plays a carcinogenic role in esophageal squamous cell carcinoma via regulation of the Wnt/beta-catenin and Hedgehog signaling pathways, Exp. Cell Res. 361 (2017) 170–177.