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Tenascin-C is involved in promotion of cancer stemness via the Akt/HIF1α axis in esophageal squamous cell carcinoma

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ABSTRACT

Although tenascin-C (TNC), an extracellular matrix protein, has been shown to be widely expressed in stromal fibroblasts in various cancers, the role of its expression in esophageal squamous cell carcinoma (ESCC) cells remains unclear. Using immunohistochemistry, we investigated the expression of cancer stem-like cell (CSC) markers, epithelial-to-mesenchymal transition (EMT)-related genes, and the Akt/hypoxia-inducible factor-1α (HIF1α) signal pathway in ESCC tissue specimens from 154 patients. We further addressed the effects of TNC on the Akt/HIF1α axis and its putative association with cancer stemness in several ESCC cell lines by immunofluorescence imaging and western blot analysis. Our data suggest that TNC expression was positively correlated with the expression of the CSC marker SOX2 ($p = .002$), and TNC-expressing cancer cells expressed SOX2 in ESCC tissues. Moreover, TNC expression was strongly associated with EMT-related gene Snail ($p = .022$) and positively correlated with pAkt-Ser473 ($p = .004$) and HIF1α ($p = .003$). Furthermore, TNC-silencing down-regulated the expression of CSC marker SOX2 ($p < .001$) and EMT-related marker Snail ($p < .001$). The Akt inhibitor Perifosine inhibited the protein expression of pAkt-Ser473, Akt, HIF1α, and TNC in TE10 (an ESCC cell line) cells. Short-term exposure of TE10 cells to cobalt chloride caused an increase in protein expression of HIF1α, TNC, and SOX2 in a time-dependent manner. Taken together, these results suggest that TNC may enhance the cancer stem-like properties and promote EMT-like changes via the Akt/HIF1α axis.

1. Introduction

Esophageal cancer ranks seventh in terms of incidence and sixth in terms of mortality worldwide, meaning that esophageal cancer would be responsible for an estimated 1 in every 20 cancer-linked deaths in 2018. Esophageal cancer is common in several eastern and southern African countries. Although the incidence rates in eastern Africa rank third by region in men, the highest rates are reported in eastern Asia, with rates in Mongolia and China in the top five worldwide (Bray et al., 2018). The majority of esophageal cancer can be subdivided into two main histological subtypes: adenocarcinomas (AC) and squamous cell carcinomas (SCC) (Arnold et al., 2015). Esophageal squamous cell carcinoma (ESCC) is one of the major histological subtypes of esophageal cancer (Tang et al., 2013). ESCC accounts for about 90% of the 456,000 incident esophageal cancers each year (Abnet et al., 2018). Therefore, the elucidation of the molecular biology of ESCC could help the prognosis of patients with esophageal cancer, improving the ability to detect, classify, and treat the cancer (Chow et al., 2001; Lam et al., 2000, 1997; Dick, 2008; Chan et al., 2013; Lam et al., 1999).

Cancer stem cells (CSCs) are associated with the survival of residual viable cancer cells and may, thus, be important for prognosis and selection of therapeutic strategy. Increasing evidence demonstrates that CSCs cause tumor initiation and metastasis and contribute to therapeutic resistance. Therefore, CSCs are considered to be the most likely cause of cancer relapse (Bao et al., 2006; Ortensi et al., 2013; Liu et al., 2006) and have become the focus of intensive research as a potential target for cancer therapy. In particular, conventional therapies often fail to eradicate carcinoma cells that have entered the CSC-like state via activation of the epithelial-to-mesenchymal transition (EMT) program, thereby permitting CSC-mediated clinical relapse (Shibue and Weinberg, 2017).

Tenascin-C (TNC) is a large extracellular matrix protein that assembles into a hexameric structure via disulfide-linkages between the N-termini of the monomers. TNC contains several functional domains, including heptad repeats, EGF-like repeats, fibronectin type III domains, and a C-terminal globular domain (Yang et al., 2018). The fibronectin type III domain of TNC interacts with other extracellular matrix (ECM) molecules and cell surface receptors, such as fibronectin and integrins (Yang et al., 2018). TNC is transiently expressed during organogenesis, and re-expressed during pathological conditions, such as inflammation and cancer (Nong et al., 2015), and it can influence cell behavior both directly and indirectly. Nie et al. (2015) reported that TNC is not only a

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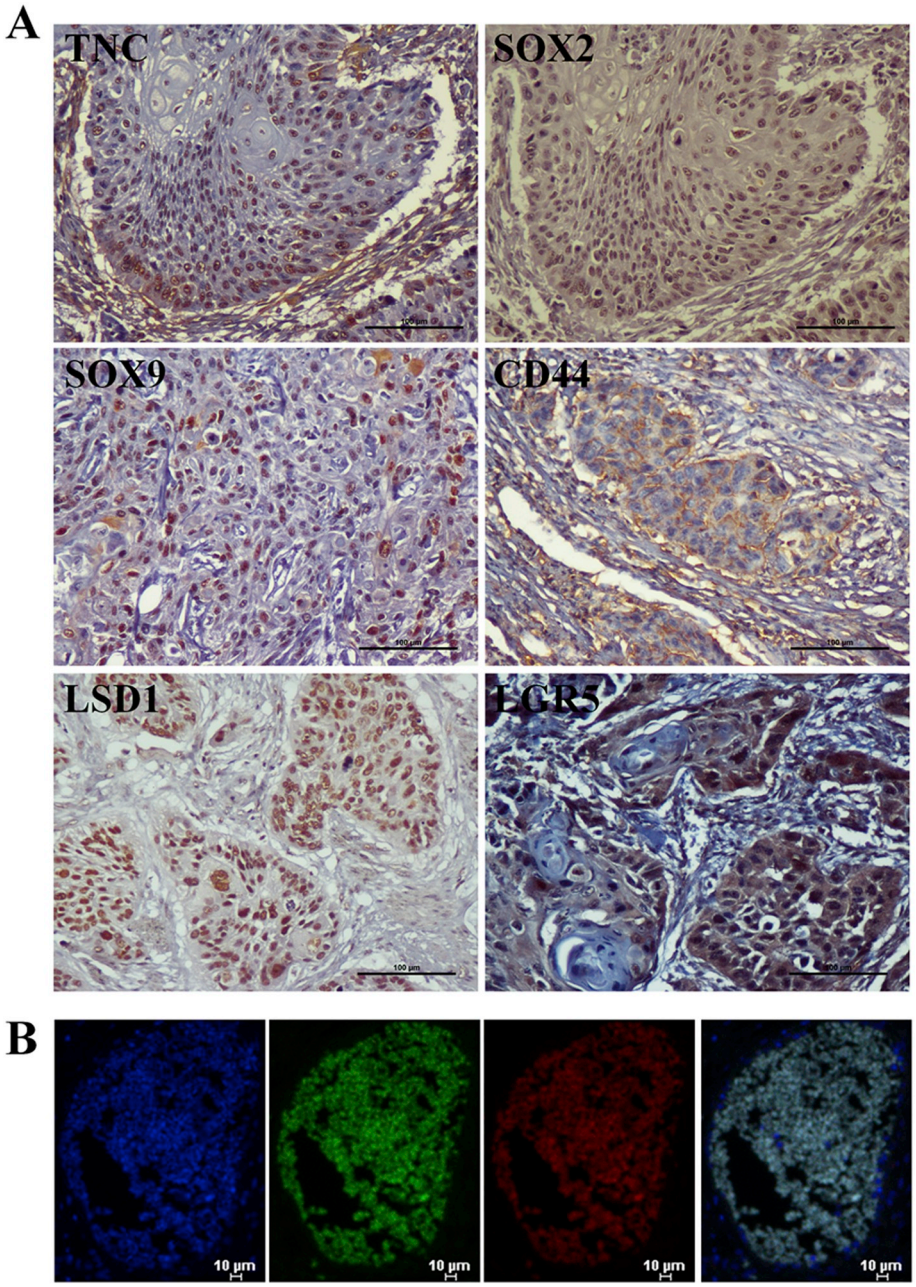

Fig. 1. Tenascin-C (TNC) induces potential cancer stemness in esophageal squamous cell carcinoma (ESCC). (A) Immunohistochemistry staining for TNC, SOX2, SOX9, CD44, LSD1, and LGR5 in ESCC (original magnification 200 ×). Scale bar = 100 μm. (B) Immunofluorescence staining for TNC and SOX2 in ESCC tissues. Blue stains for DAPI; green stains for SOX2; red stains for TNC; double labeling represents the merge. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

potential prognostic marker for glioblastoma multiforme but also a potential marker for glioma cancer stem cells.

Our previous study has proposed TNC to be a prognostic determinant (Yang et al., 2016a,b), but its role in promoting CSC-like features in ESCC has not been explored yet. Here, we show that TNC is associated with the acquisition of CSC characteristics and appears to promote EMT in ESCC epithelial cells, via Akt/hypoxia-inducible factor-1α (HIF1α), which subsequently, may result in high malignancy and poor prognosis in ESCC.

2. Materials and methods

2.1. Ethical approval

This research complied with the Helsinki Declaration and was approved by the Human Ethics Committee and the Research Ethics Committee of Samsung Medical Center (Seoul, Korea). All patients provided written informed consent according to the institutional guidelines. Patients were informed that the resected specimens were stored by the hospital and could be potentially used for scientific research, and that their privacy would be maintained. Follow-up survival data were collected retrospectively, through medical record analyses.

Table 1
The association between protein expression of TNC and cancer stem-like cell makers in esophageal squamous cancer tissues.

Variable	n	TNC(−) n(%)	TNC(+) n(%)	χ^2	R	P
Sox2				9.195	0.268	0.002
Negative	95	78(82.1)	17(17.9)			
Positive	39	21(53.8)	18(46.2)			
Sox9				0.127	0.031	0.722
Negative	69	52(75.4)	17(24.6)			
Positive	65	47 (71.9)	18(28.1)			
CD44				0.015	0.011	0.904
Negative	93	69(74.7)	24(25.3)			
Positive	41	30(73.7)	11(26.3.)			
LSD1				0.160	0.035	0.690
Negative	87	63(72.3)	24(27.7)			
Positive	47	36(75.6)	11(24.4)			
LGR5				1.766	0.122	0.184
Negative	70	56(80.0)	14(20.0)			
Positive	64	43(67.2)	21(32.8)			

2.2. Tissue specimens

A total of 154 formalin-fixed and paraffin-embedded tumor tissue samples, specifically 134 ESCC and 20 adjacent non-tumor esophageal mucosa samples, were obtained from the Department of Pathology at the Samsung Medical Center in accordance with the protocols approved by the Institutional Review Board (No. 2014-09-060-001). None of the patients included in the study received any preoperative chemotherapy or radiotherapy (Yang et al., 2016a,b).

2.3. Cell lines

The ESCC cell lines TE1, TE8, ECG10, TE11, and TE10 were purchased from the RIEKN BRC Cell Bank (Tsukuba, Japan). All cell lines were maintained in RPMI-1640 (Life Technologies, Grand Island, NY) medium supplemented with 10% FBS (Life Technologies). Cells were treated (for 48 h unless otherwise specified) with Perifosine (Per, Abcam, Cambridge, UK) according to the manufacturer's instructions.

2.4. Immunohistochemistry staining

Immunohistochemistry (IHC) staining on tissue microarray samples was performed as previously described (Yang et al., 2017). The primary antibodies anti-TNC (1:100, Abcam, UK), anti-SOX2 (1:100, R&D, USA), anti-SOX9 (1:100, Abnova, USA), anti-CD44 (1:100, ZSGB-BIO, China), anti-LSD1 (1:250, Sigma, USA), anti-LGR5 (1:100, Millipore, USA), anti-E-cadherin (1:100, Abcam, UK), anti-Snail (1:100, Abcam, UK), anti-vimentin (1:100, Abcam, UK), anti-pPI3K p85 (1:80, Abcam, UK), anti-pAkt-Thr308 (1:100, Abcam, UK), anti-pAkt-Ser473 (1:100, Abcam, UK), anti-NFκB p65 (1:100, CST, USA), and HIF1α (1:100, BD, USA) were prepared in PBS containing 3 mg/ml goat globulin (Sigma, St. Louis, MO, USA) and incubated for 60 min at room temperature, followed by three successive washes with PBST buffer. The secondary antibodies HRP-conjugated goat anti-rat IgG and goat anti-rabbit IgG (Zsbio, Beijing, China) were used, and the ImmPACT AEC Peroxidase Substrate (VECTOR Laboratories) chromogen was used for the detection.

2.5. Evaluation of the IHC analysis

The specimens were independently scored by two pathologists (ZTY & YHX) who were blinded to the clinicopathological data. The staining results were scored semi-quantitatively, as described in detail previously (Yang et al., 2016a,b). Briefly, according to the staining intensity and to the area, the tissue samples were scored from 0 to 3. Cases with scores between 1 and 3 were considered positive for the expression of each protein. In case of discrepancies, a final score was established by a reassessment by both pathologists using a double-headed microscope (Yang et al., 2016a,b).

2.6. Chemically-induced hypoxia

Hypoxia was induced by exposing normoxic-cultured cells to 100 μmol/l cobalt chloride ($CoCl_2$) (Sigma-Aldrich, St. Louis, MO, USA) for various durations (0 h, 6 h, 12 h, and 24 h).

2.7. Western blot analysis

Cell lysates were produced in RIPA lysis buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton x-100, 1% Na-Doc, 0.1% SDS) supplemented with protease inhibitor cocktail (Roche). Cell extracts were quantitated using a BCA protein assay kit (Thermo). Western blot was carried out as previously described (Yang et al., 2017) and was performed using anti-TNC (1:1000), anti-SOX2 (1:500), anti-Snail (1:1000), anti-HIF1α (1:1000), and anti-β-actin (1:1000, Bioss, China). The protein bands were detected using an ECL system (Merck) according to the manufacturer's instructions.

2.8. Immunofluorescence (IF) analysis

For tissue staining, sections on microslides were deparaffinized with xylene, hydrated using a diluted alcohol series, and immersed in 3% H_2O_2 in methanol to quench endogenous peroxidase activity. Sections were then treated with TE buffer (10 mM Tris and 1 mM EDTA, pH 9.3) at 98 °C for 30 min. ESCC tissues were fixed with 4% paraformaldehyde for 10 min, permeabilized with 0.2% Triton X-100 for 5 min at room temperature, and blocked with 1% BSA in PBS for 30 min. The mixture of polyclonal anti-rabbit TNC and monoclonal anti-mouse SOX2 antibodies (1:50) was incubated overnight at 4 °C. The next day, after washing with PBS, cells were incubated with secondary Alexa Fluor 568 goat anti-mouse IgG (Invitrogen, A12380) and Alexa Fluor 488 goat anti-rabbit IgG (Invitrogen, A11008) antibodies (1:150 dilution) for 60 min at room temperature. Slides were mounted in VectorShield mounting media with DAPI (Vector lab, H-1200), and the fluorescence was detected with the Axiovert200II (Carl-Zeiss) confocal microscope.

2.9. Cell transfection

Three different TNC siRNAs (s7068, s7069 and s7070) targeting TE10 was designed and synthesized by Ambion (Life Technologies) (Carlsbad, USA). The sequence of each TNC siRNA was listed in Supplementary Table1. For the drug treatments, we dissolved TNC siRNAs in Nuclease-free Water and diluted the solutions to the 50 μM. 2×10^5 cells were plated into 6-well plates with 2 ml of culture medium. After 24 h, the cells were transfected with 100 pmol TNC siRNA using 5 μl/well Lipofectamine 2000 (Invitrogen, Life Technologies, Grand Island, NY) according to the manufacturer's instructions. After 48 h, total protein were collected to determine the TNC knockdown efficiency by Western blot.

2.10. Statistical analysis

Correlations were examined using Pearson's chi-square test or Fisher's exact test, as appropriate. All tests were two sided, and $p < .05$ was considered significant. The statistical analysis was performed using GraphPad Prism 5.01 (GraphPad Software, Inc., La Jolla, CA, USA) and SPSS 19.0 statistical software (SPSS Inc., Chicago, IL, USA).

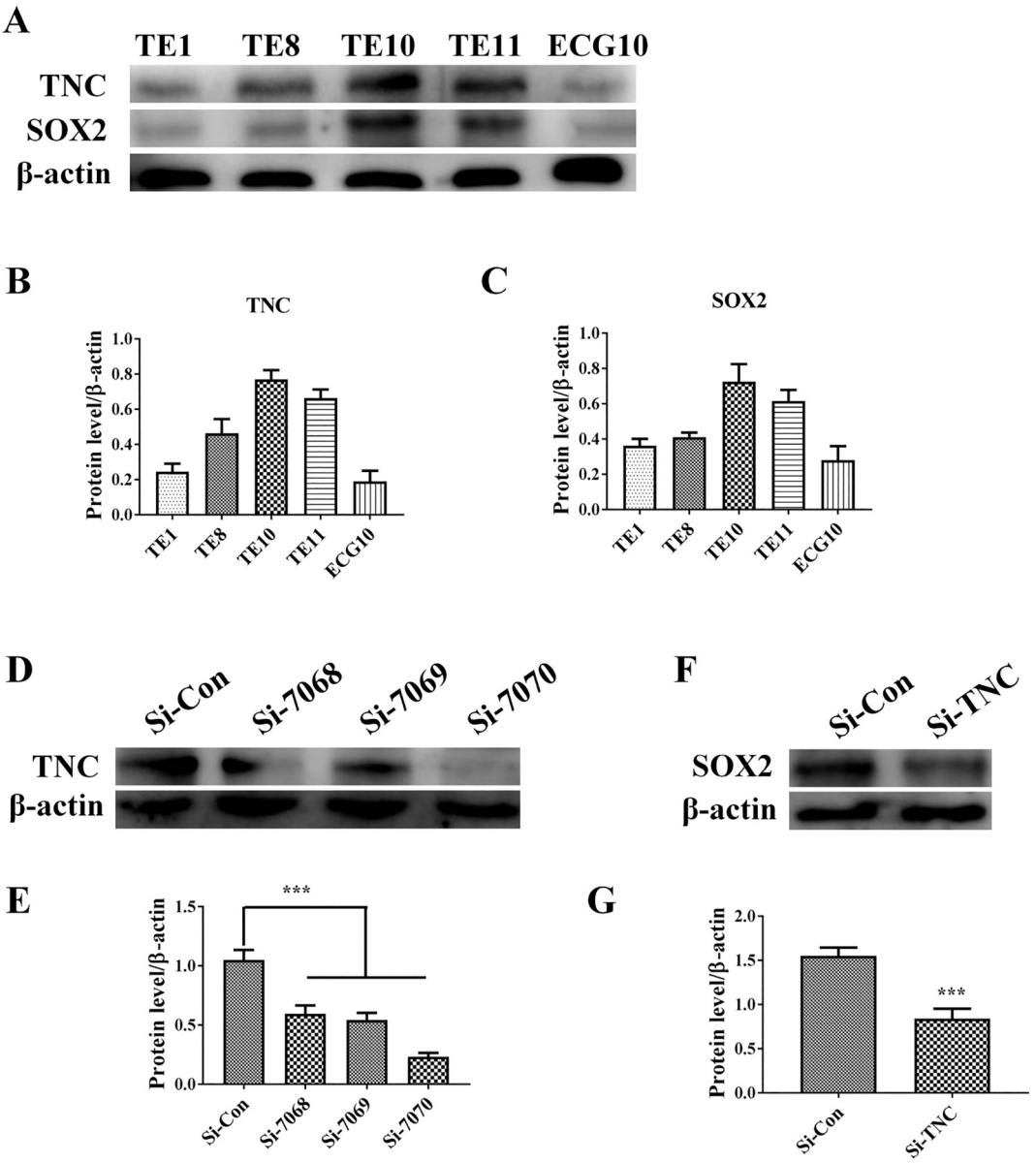

Fig. 2. (A–C) Western blot analysis for the protein expression of Tenascin-C (TNC) and the cancer stemness gene SOX2 in esophageal squamous cell carcinoma cell lines. β-actin was used as a loading control. (D, E) Protein expression of TNC in the TE10 cells after treatment with control siRNA and TNC siRNA (s7068, s7069 and s7070) for 48 h was confirmed by Western blot analysis. (F, G) Protein expression of SOX2 in the TE10 cells after treatment with TNC siRNA compared with control siRNA. Western blot data were normalized to those for β-actin and expressed as fold changes relative to levels in the control group. $^{***}P < .001$ versus control.

3. Results

3.1. TNC expression is associated with the expression of cancer stemness marker SOX2

The association of TNC with other cancer stemness-related genes, including SOX2, SOX9, CD44, LSD1, and LGR5 was investigated in 134 ESCC human tissues (Fig. 1A). We observed that TNC expression was positively correlated with the expression of the stemness-related gene SOX2 ($p = .002$), but not with the other CSC markers, such as CD44, LSD1, SOX9, and LGR5 (Table 1). The co-expression of TNC and SOX2 was examined in ESCC tissues (Fig. 1B) using confocal microscopy. Positive signals for TNC and SOX2 expression were mainly localized in the nuclei and cytoplasm of ESCC cells. To verify the IHC results, we examined the protein levels of TNC and SOX2 in ESCC cell lines using western blot. Expression of both TNC and SOX2 were higher in TE10 and TE11 cells than in the other ESCC cell lines (Fig. 2A–C). The high expression of TNC may positively regulate the stemness of ESCC cells.

We also performed loss of function studies to further explain the association between TNC and cancer stemness marker SOX2. TNC siRNAs such as s7068, s7069 and s7070 (all $p < .001$) effectively knocked down, and s7070 was the most obvious knockdown in TE10 cells (Fig. 2D, E). s7070 was next used in the following knockdown experiments. The results of Western blot analysis also showed that the TNC knockdown group exhibited dramatically decreased SOX2 expression compared to that in control group in the TE10 ($p < .001$) cells (Fig. 2F, G).

3.2. TNC expression correlates with cancer epithelial cell EMT-like change in ESCC

In the present study, a strongly positive TNC expression was detected in ESCC invasion front only (Fig. 3A). Moreover, TNC expression was significantly correlated with the expression of the EMT-related

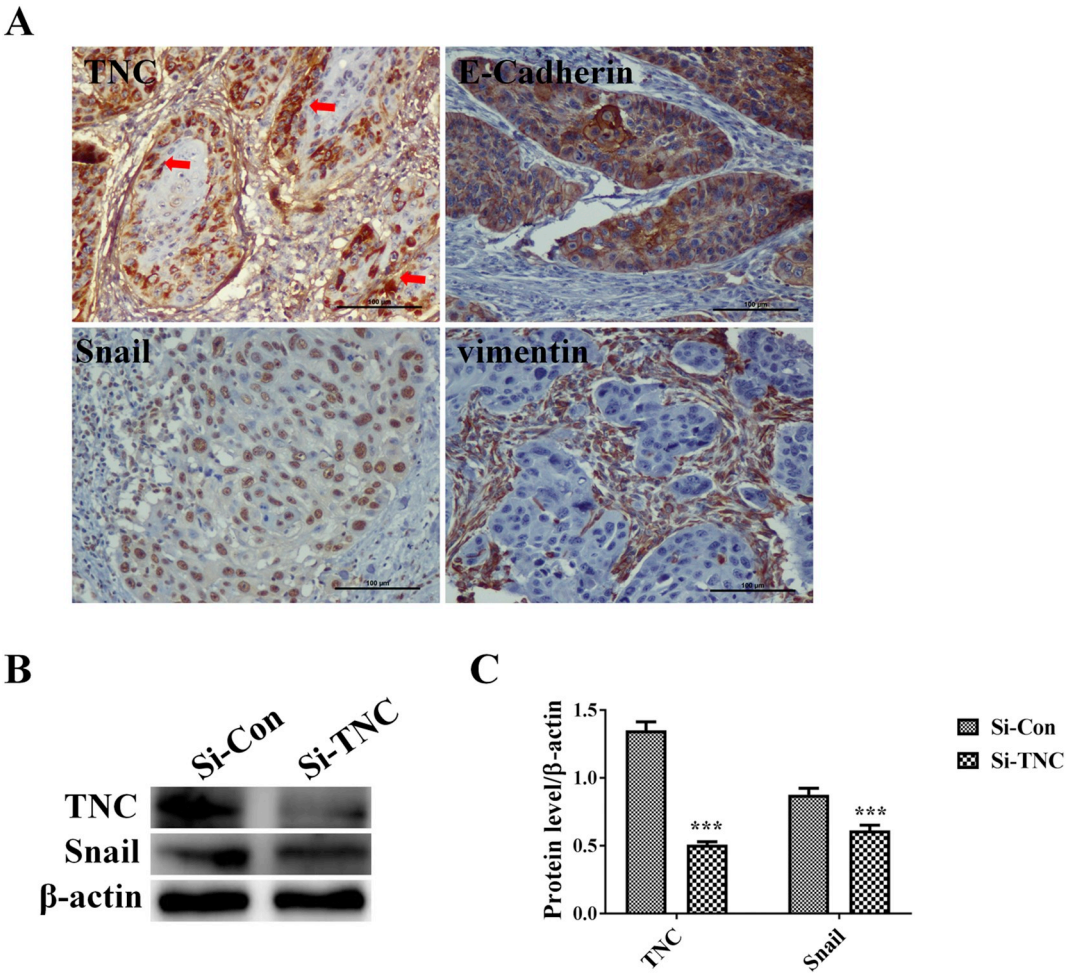

Fig. 3. Tenascin-C (TNC) expression is correlated with epithelial cell epithelial-to-mesenchymal transition in esophageal squamous cell carcinoma (ESCC). (A) Immunohistochemistry staining of TNC in ESCC invasion front (arrows) and the expression of E-Cadherin, Snail, and vimentin in tissues of ESCC patients (original magnification 200×). Scale bar = 100 μm. (B, C) Protein expression of TNC and Snail after treatment with TNC siRNA compared with control siRNA in TE10 cells. Western blot data were normalized to those for β-actin and expressed as fold changes relative to levels in the control group. $^{***}P < .001$ versus control.

Table 2
The association between protein expression of TNC and that of epithelial-mesenchymal-transition related genes in esophageal squamous cancer tissues.

Variable	n	TNC(−)n(%)	TNC(+)n(%)	$\chi 2$	R	P
E-Cadherin				0.002	0.004	0.967
Negative	35	26(74.2)	9(25.7)			
Positive	99	73(73.7)	26(26.3)			
Snail				5.278	0.233	0.022
Negative	39	23(59.0)	16(41.0)			
Positive	95	76(80.0)	19(20.0)			
Vimentin				0.833	0.087	0.361
Negative	68	52(76.5)	16(23.5)			
Positive	66	47(71.2)	19(28.8)			

gene Snail ($p = .022$), but not of E-cadherin ($p = .967$) and vimentin ($p = .361$) (Table 2). Specifically, Snail was localized in the cytoplasm and in the nuclei, E-cadherin was present in the membrane of cancer cells, whereas vimentin was detected in the cytoplasm of stromal fibroblasts (Fig. 3A). This indicated that TNC expression was present along with EMT-like change in ESCC cells. Furthermore, TNC knockdown group exhibited significantly decreased Snail ($p < .001$) in TE10 cells compared to the control group (Fig. 3B, C).

Table 3
The association between protein expression of TNC and signaling pathway in esophageal squamous cancer tissues.

Variable	n	TNC(−) n(%)	TNC(+) n(%)	χ^2	R	P
pPI3K p85				0.001	0.002	0.982
Negative	107	79(73.8)	28(26.2)			
Positive	27	20(74.1)	7(25.9)			
pAkt-Ser473				8.106	0.262	0.004
Negative	85	71(83.5)	14(16.5)			
Positive	49	28(57.1)	21(42.9)			
pAkt-Thr308				0.526	0.080	0.468
Negative	66	51(77.3)	15(22.7)			
Positive	68	48(70.6)	20(29.4)			
NFκB p65				0.003	0.005	0.956
Negative	106	78(73.6)	28(26.4)			
Positive	28	21(75.0)	7(25.0)			
HIF1α				9.052	0.268	0.003
Negative	60	49(81.7)	11(18.3)			
Positive	74	50(67.6)	24(32.4)			

3.3. TNC expression is positively correlated with promotion of cancer stemness through intracellular signaling pathway Akt/HIF1α in ESCC

In addition, TNC expression was positively correlated with pAkt-Ser473 ($p = .004$) and HIF1α ($p = .003$), but not with pPI3K p85, pAkt-Thr308, and NFκB p65 (Table 3; Fig. 4). We applied the Akt inhibitor

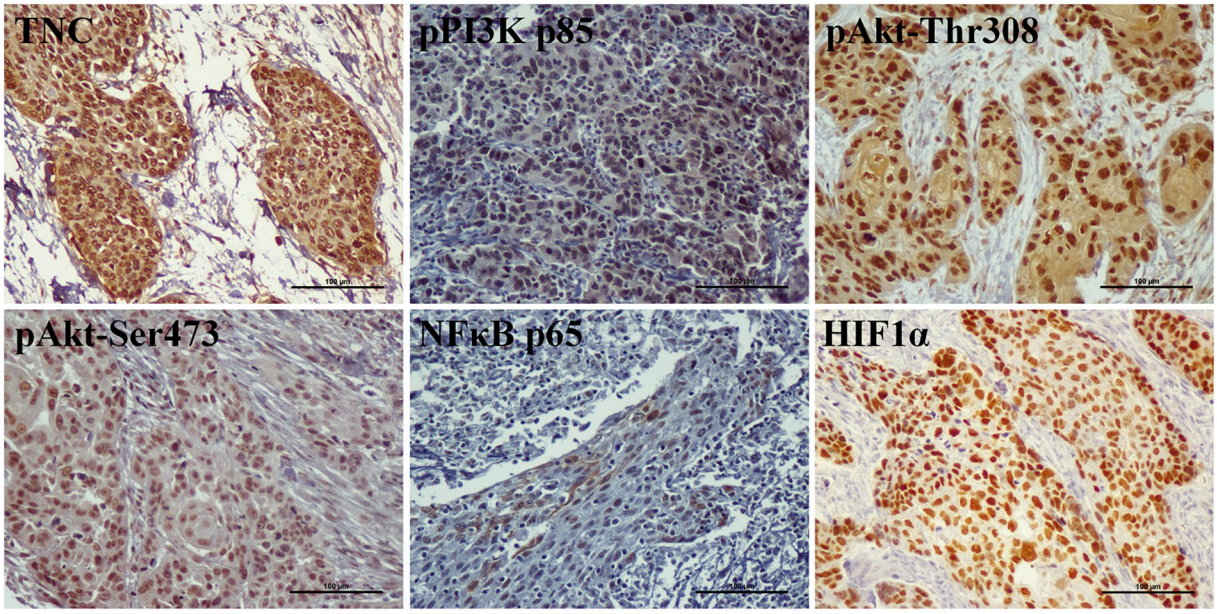

Fig. 4. Immunohistochemistry staining for tenascin-C (TNC), pPI3K p85, pAkt-Thr308, pAkt-Ser473, NFκB p65, and hypoxia-inducible factor 1α (HIF1α) in esophageal squamous cell carcinoma tissues (original magnification 200×). Scale bar = 100 μm.

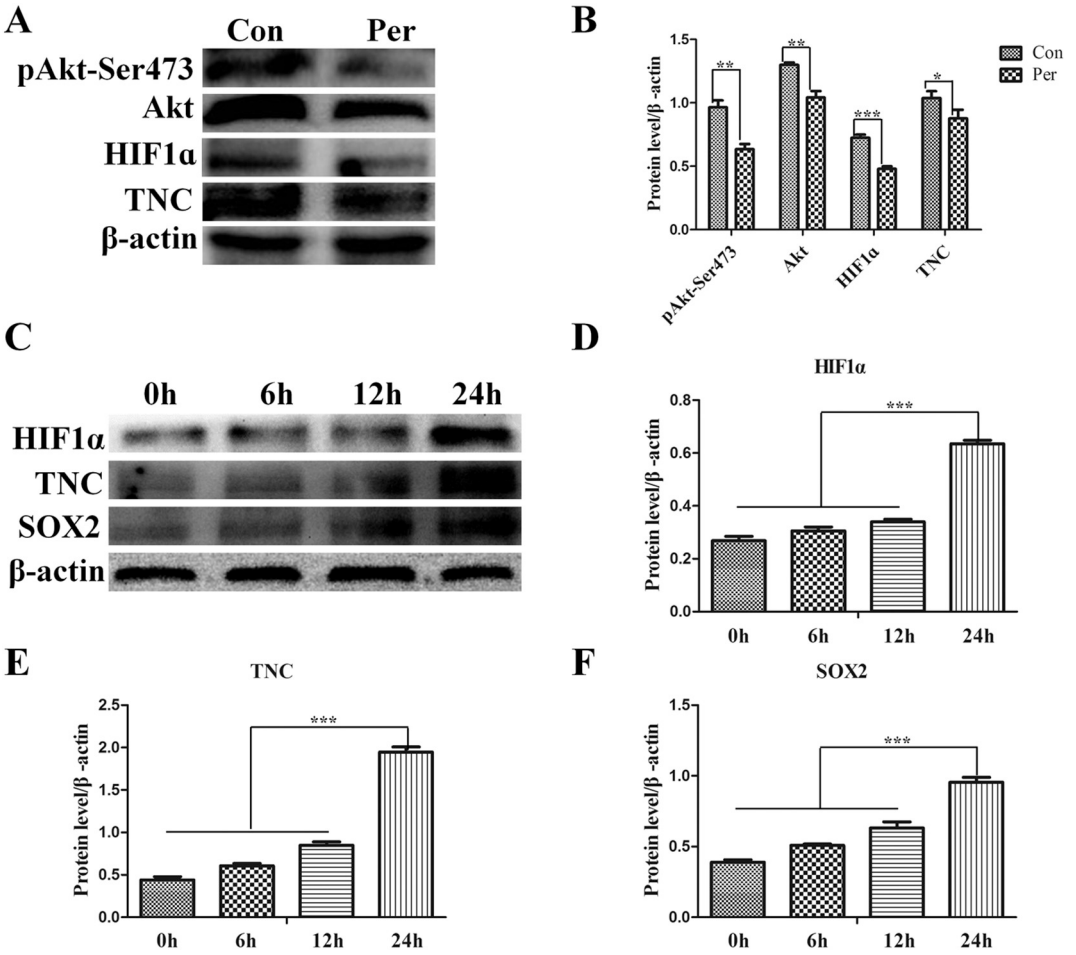

Fig. 5. (A, B) Representative western blot showing the protein expression of pAkt-Ser473, Akt, HIF1α, and tenascin-C (TNC) in cells treated with Perifosine (Per), compared with untreated cells used as control group; β-actin was used as a loading control. (C–F) Western blot analysis of hypoxia-inducible factor 1α (HIF1α), TNC, and SOX2 protein levels in TE10 cells after treatment with $CoCl_2$ (100 μmol/l) for 0 h, 6 h, 12 h, and 24 h. β-actin was used as a loading control. $^{*}p < .05$, $^{**}p < .01$, and $^{***}p < .001$ versus control.

Per (8.9 μM) in the ESCC cell line TE10. Per not only inhibited pAk t-Ser473 ($p = .001$), Akt ($p = .001$), and HIF1α ($p < .001$), but also significantly inhibited TNC ($p = .034$) in TE10 cells (Fig. 5A, B).

Furthermore, the hypoxic-response marker HIF1α, TNC, and SOX2 were detected in TE1 cells in normoxia and hypoxia. The effects of the hypoxia-induced $CoCl_2$ treatment on TE1 cells and stemness were examined. When exposed to $CoCl_2$ (6 h, 12 h, and 24 h), the protein expression of HIF1α, TNC and SOX2 was higher than that in normoxia group, and it was dependent on the exposure time. The proteins, HIF1α ($p < .001$), TNC ($p < .001$), and SOX2 ($p < .001$) increased more sharply upon treatment with $CoCl_2$ for 24 h than what was observed in normoxia (Fig. 5C–F). These results suggest that TNC and SOX2 expression may be controlled in response to hypoxia like HIF1α.

4. Discussion

Increasing evidence has suggested that CSCs are capable of inducing malignant transformation, leading to cancer progression and metastasis (Haraguchi et al., 2006; Hermann et al., 2010; Salama and Platell, 2009; Todaro et al., 2010). Thus, the identification of inducing factors and molecular mechanisms associated with CSCs in ESCC could help assess the prognosis of the ESCC patients. In a previous study (Yang et al., 2016a,b), elevated expression of TNC in tumor tissues has been positively correlated with poor prognosis in ESCC patients. The current study revealed that TNC may play a crucial role in CSC characteristics associated with aggressiveness and metastatic spread of ESCC cells, via the Akt/HIF1α axis, leading to reduced survival of ESCC patients.

In the breast cancer oncosphere model, TNC-induced survival and increased lung metastasis were linked to an enhanced expression of the pluripotency markers SOX2, Oct4, and Nanog, as well as that of the adult stem cell markers Musahsi (Msh1) and Lgr5 (Oskarsson et al., 2011). In our study, we found that only SOX2, but not the other makers, were co-upregulated with TNC, because of many of the therapies labeled as “no statistical different” in trials using inadequate samples have not received a fair test. Moreover, TNC not only co-localized with SOX2 in the ESCC cells and tissues, but also that it knockdown significantly down-regulated SOX2 expression, which indicating that TNC may positively regulate the stemness of ESCC cells, resulting in poor prognosis.

EMT is a crucial process for the generation and maintenance of CSCs, and the invasive front of ESCC where EMT occurs, might form a CSC niche in ESCC (Sato et al., 2015). In our study, TNC was overexpressed in the invasive front of ESCC, and positively correlated with the EMT process. Nagaharu et al. (2011) suggested that TNC directly induced EMT, and that TNC and TGF-ß are jointly responsible for EMT-like events in breast cancer cells. Consistent with previous findings, our results indicated that TNC may be critical in the process of EMT on invasive fronts of ESCC and may contribute to the critical process of generation and maintenance of CSCs.

Recently, a study reported that the activation of the miR21/PTEN/AKT axis is associated with the development of a CSC phenotype, which results in chemoradioresistance, EMT, and a malignant phenotype in patients with poor prognosis (Yang et al., 2016a,b). Besides, intratumor hypoxia, along with a poor prognosis, might be related to the increase in immature, highly aggressive, and therapy-resistant malignant cells with stem cell-like characteristics (Mimeault and Batra, 2013; Li and Rich, 2010; Mazumdar et al., 2009; Heddleston et al., 2010; Keith and Simon, 2007). Importantly, HIF1α activity is regulated by protein kinase phosphorylation, potentially via the universal phosphorylation signal transduction pathway of the PI3K/Akt (Zhang et al., 2018). In the present study, TNC positively correlated with pAkt-Ser473 and HIF1α. Moreover, the Akt inhibitor Per inhibited the expression of HIF1α and TNC at the protein level in TE10 cells. Furthermore, the expression of HIF1α, TNC, and SOX2 was upregulated in hypoxic environment in a time-dependent manner, compared to the control group. These observations suggested that the TNC expression level may be an indicator of the aggressiveness of ESCC via the Akt/HIF1α axis. Nevertheless, further study is essential to elucidate the regulatory mechanism for TNC to promote potential CSC features in ESCC cells, via the activated Akt/ HIF1α axis.

In conclusion, the overexpression of TNC could induce cancer stemness via the activated Akt/HIF1α axis, leading to poor prognosis of ESCC patients. Moreover, the targeted therapy of TNC in the tumor microenvironment could achieve a better clinical outcome for ESCC patients.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yexmp.2019.03.007.

References

Abnet, C.C., Arnold, M., Wei, W.Q., 2018. Epidemiology of esophageal squamous cell carcinoma. Gastroenterology 154 (2), 360–373.
Arnold, M., Soerjomataram, I., Ferlay, J., et al., 2015. Global incidence of oesophageal cancer by histological subtype in 2012. Gut 64, 381–387.
Bao, S., Wu, Q., McLendon, R.E., et al., 2006. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 444 (7120), 756–760.
Bray, F., Ferlay, J., Soerjomataram, I., et al., 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin.
Chan, D., Tsoi, M.Y.T., Di Liu, C., et al., 2013. Oncogene GAEC1 regulates CAPN10 expression which predicts survival in esophageal squamous cell carcinoma. World J. Gastroenterol. 19 (18), 2772–2780.
Chow, V., Law, S., Lam, K.Y., et al., 2001. Telomerase activity in small cell esophageal carcinoma. Dis. Esophagus 14 (2), 139–142.
Dick, J.E., 2008. Stem cell concepts renew cancer research. Blood 112 (13), 4793–4807.
Haraguchi, N., Inoue, H., Tanaka, F., et al., 2006. Cancer stem cells in human gastrointestinal cancers. Hum. Cell 19 (1), 24–29.
Heddleston, J.M., Li, Z., Lathia, J.D., et al., 2010. Hypoxia inducible factors in cancer stem cells. Br. J. Cancer 102, 789–795.
Hermann, P.C., Bhaskar, S., Cioffi, M., et al., 2010. Cancer stem cells in solid tumors. Semin. Cancer Biol. 20 (2), 77–84.
Keith, B., Simon, M.C., 2007. Hypoxia-inducible factors, stem cells, and cancer. Cell 129 (3), 465–472.
Lam, K.Y., Tsao, S.W., Zhang, D., et al., 1997. Prevalence and predictive value of p53 mutation in patients with oesophageal squamous cell carcinomas: a prospective clinico-pathological study and survival analysis of 70 patients. Int. J. Cancer 74 (2), 212–219.
Lam, K.Y., Law, S., Tin, L., et al., 1999. The clinicopathological significance of p21 and p53 expression in esophageal squamous cell carcinoma: an analysis of 153 patients. Am. J. Gastroenterol. 94 (8), 2060–2068.
Lam, K.Y., Law, S., Tung, P.H., et al., 2000. Esophageal small cell carcinomas: clinicopathologic parameters, p53 overexpression, proliferation marker, and their impact on pathogenesis. Arch. Pathol. Lab. Med. 124 (2), 228–233.
Li, Z., Rich, J.N., 2010. Hypoxia and hypoxia inducible factors in cancer stem cell maintenance. Curr. Top. Microbiol. Immunol. 345, 21–30.
Liu, G., Yuan, X., Zeng, Z., et al., 2006. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. Mol. Cancer 5, 67–79.
Mazumdar, J., Dondeti, V., Simon, M.C., 2009. Hypoxia-inducible factors in stem cells and cancer. J. Cell. Mol. Med. 13 (11−12), 4319–4328.
Mimeault, M., Batra, S.K., 2013. Hypoxia-inducing factors as master regulators of stemness properties and altered metabolism of cancer- and metastasis-initiating cells. J. Cell. Mol. Med. 17 (1), 30–54.
Nagaharu, K., Zhang, X., Yoshida, T., et al., 2011. Tenascin C induces epithelial-mesenchymal transition-like change accompanied by SRC activation and focal adhesion kinase phosphorylation in human breast cancer cells. Am. J. Pathol. 178 (2), 754–763.
Nie, S., Gurrea, M., Zhu, J., et al., 2015. Tenascin-C: a novel candidate marker for cancer stem cells in glioblastoma identified by tissue microarrays. J. Proteome Res. 14 (2), 814–822.
Nong, Y., Wu, D., Lin, Y., et al., 2015. Tenascin-C expression is associated with poor prognosis in hepatocellular carcinoma (HCC) patients and the inflammatory cytokine TNF-α-induced TNC expression promotes migration in HCC cells. Am. J. Cancer Res. 5 (2), 782–791.
Ortensi, B., Setti, M., Osti, D., et al., 2013. Cancer stem cell contribution to glioblastoma invasiveness. Stem Cell Res Ther 4 (1), 1–11.
Oskarsson, T., Acharyya, S., Zhang, X.H., et al., 2011. Breast cancer cells produce tenascin C as a metastatic niche component to colonize the lungs. Nat. Med. 17 (7), 867–874.

Salama, P., Platell, C., 2009. Colorectal cancer stem cells. ANZ J. Surg. 79 (10), 697–702.
Sato, F., Kubota, Y., Natsuizaka, M., et al., 2015. EGFR inhibitors prevent induction of cancer stem-like cells in esophageal squamous cell carcinoma by suppressing epithelial-mesenchymal transition. Cancer Biol. Ther. 16 (6), 933–940.
Shibue, T., Weinberg, R.A., 2017. EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. Nat. Rev. Clin. Oncol. 14 (10), 611–629.
Tang, K.H., Dai, Y.D., Tong, M., et al., 2013. A CD90(+) tumor-initiating cell population with an aggressive signature and metastatic capacity in esophageal cancer. Cancer Res. 73, 2322–2332.
Todaro, M., Francipane, M.G., Medema, J.P., et al., 2010. Colon cancer stem cells: promise of targeted therapy. Gastroenterology 138 (6), 2151–2162.
Yang, C.F., Yang, G.D., Huang, T.J., et al., 2016a. EB-virus latent membrane protein 1 potentiates the stemness of nasopharyngeal carcinoma via preferential activation of PI3K/AKT pathway by a positive feedback loop. Oncogene 35 (26), 3419–3431.
Yang, Z.T., Yeo, S.Y., Yin, Y.X., et al., 2016b. Tenascin-C, a prognostic determinant of esophageal squamous cell carcinoma. PLoS One 11 (1), e0145807–e0145823.
Yang, Z.T., Cui, Y., Ni, W.D., et al., 2017. Gli1, a potential regulator of esophageal cancer stem cell, is identified as an independent adverse prognostic factor in esophageal squamous cell carcinoma. J. Cancer Res. Clin. Oncol. 143, 243–254.
Yang, Z.T., Zhang, C.Y., Qi, W.B., et al., 2018. Tenascin-C as a prognostic determinant of colorectal cancer through induction of epithelial-to-mesenchymal transition and proliferation. Exp. Mol. Pathol. 105, 216–222.
Zhang, Z., Yao, L., Yang, J., et al., 2018. PI3K/Akt and HIF-1 signaling pathway in hypoxia-ischemia (review). Mol. Med. Rep. 18 (4), 3547–3554.