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Review

E26 transformation-specific variant 4 as a tumor promotor in human cancers through specific molecular mechanisms

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E26 transformation-specific (ETS) variant 4 (ETV4) is an important transcription factor that belongs to the ETS transcription factor family and is essential for much cellular physiology. Recent evidence has revealed that ETV4 is aberrantly expressed in many types of tumors, and its overexpression is related to poor prognosis of cancer patients. Additionally, increasing studies have identified that ETV4 promotes cancer growth, invasion, metastasis, and drug resistance. Mechanistically, the level of ETV4 is regulated by some post-translation modulations in a broad spectrum of cancers. However, little progress has been made to comprehensively summarize the critical roles of ETV4 in different human cancers. Hence, this review mainly focuses on the physiological functions of ETV4 in various human tumors. In addition, the molecular mechanisms of ETV4-mediated cancer progression were elucidated, including how ETV4 modulates its downstream signaling pathways and how ETV4 is regulated by some factors. On this basis, the present review may provide a valuable therapeutics strategy for future cancer treatment by targeting ETV4-related pathways.

INTRODUCTION

The E26 transformation-specific (ETS) transcription factor family is a large and important evolutionarily conserved transcription factor family, consisting of 28 genes in humans and 27 genes in mice.[1] The family members recognize the GGAA core motif in the enhancers and promoters of target genes by DNA-binding ETS domains[2] and they also interact with other transcription factors. Importantly, ETS family members are involved in various aspects of cellular biology, including cell proliferation, apoptosis, differentiation, angiogenesis, and transformation, and they also play vital roles in tumor initiation, progression, and metastasis.[3]

ETS variant 4 (ETV4), also known as polyomavirus enhancer activator 3 protein (Pea3), is one important member of ETS transcription factor family, and, together with ETV1 and ETV5, comprises the Pea3 subfamily. Pea3 members possess a highly similar sequence and similar subdomain organization, with an α helix located in the C-terminal inhibitory domain that disturbs the conformation of its DNA-recognition helix by conflicting with the ETS domain.[4] ETV4 gene is located on 17q21[5] and clones by binding the adenovirus E1A enhancer element. Some important post-translation modulations, such as acetylation, sumoylation, ubiquitylation, and phosphorylation, have actions on ETV4, and meanwhile ETV4 also can target related genes, consequently governing many cancer cell progressions such as cell proliferation and motility.[6] For example, ETV4 is modulated by sex-determining region Y-box 2 (SOX2) and p63, and thus targeting ETV4 could influence SOX2-induced squamous cell carcinomas survival.[7] Furthermore, acetyl-coenzyme A (CoA) carboxylase or ATP citrate lyase can regulate ETV4 expression under hypoxia through upregulation of α-ketoglutarate, and correspondingly deletion of ETV4 protects against hypoxia-induced apoptosis.[8] In addition, ETV4 can induce the activity of leukocyte-associated immunoglobulin-like receptor-1 promoter, an immunoinhibitory receptor related to some autoimmune diseases and cancers.[9] Furthermore, recent evidence has shown aberrant expression of ETV4 in numerous human cancers such as prostate cancer,[10] breast cancer,[11] and ovarian cancer,[12] and confirmed its important role in promoting tumor cell proliferation, motility, and invasion.

Hence, our study reviews the contribution of ETV4 to the development and progress of various tumors. In addition, the underlying molecular mechanisms of ETV4 activation and ETV4-mediated tumorigenesis is discussed (Table 1).

UROLOGICAL TUMORS

Prostate cancer

Prostate cancer is one of the most frequent tumors and is the second leading cause of cancer death for males. More than 50% of patients with prostate cancer have been reported to have a chromosomal rearrangement.[13] Even though ERG (ETS-related gene) rearrangements account for the large majority of rearrangements in prostate cancer, almost 10% of rearrangements contain Pea3 subfamily members[14]. Among these, ETV4 rearrangements are noted in 6% (5/85) of men with primary prostate cancer, in 6% (4/72) with lymph node metastasis, and in 4% (1/25) with distant metastasis,[15] suggesting that ETV4 rearrangements is important for prostate cancer but may not

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Table 1. The function and related mechanisms of ETV4 in human cancers

Cancer	Expression	Function	Mechanism	References
Prostate cancer	discrepancy	induce invasion, migration, and metastasis; with poor prognosis	activate PI3K and Ras signaling; induce EMT	10,24,25,28–31,90
Bladder cancer	↑	induce cell proliferation and invasion	activate P3H4 transcription	34
Gastric cancer	↑	induce cell proliferation, metastasis, tumor growth; inhibit apoptosis	decrease KDM5D and ME1; increase KIF2A via the AKT signaling pathway; regulate MMP-1	35–37,39,40
Late-stage gastric adenocarcinoma	↑	with poor survival	co-association with ERK signaling	35
Gastrointestinal stromal tumor	↑	induce cell proliferation, invasion, and tumor growth	induce CCND1 expression and Wnt/β-catenin signaling	41
Colorectal cancer	↑	related to invasion, metastasis, and recurrence; with poor survival	ERK kinase activation blocks ETV4 binding to COP1; regulate MMP-1 and matrilysin	42,43,47
Capicua-deficient colorectal cancer	↑	induce cell proliferation and invasion	negatively related to Capicua	46
Colorectal mucinous adenocarcinoma	↑	induce cell invasiveness	induce MMP-7 expression and EMT via the ERK pathway	45
Colon cancer	↑	induce cell proliferation and invasion	activate α5β1-integrin	48,49
Hepatocellular carcinoma	↑	induce cell proliferation, migration, and invasion; reduce apoptosis	regulate MMP-1, target uPAR	50–52
		resistance to sorafenib and cisplatin	modulate IER3 mRNA level	50
Cholangiocarcinoma	↑	induce tumor progression	induce cell responsiveness to E2	53
Pancreatic cancer	↑	induce cell growth and cell cycle progression	regulate cyclin D1	54
TNBC	↑	induce advanced stage, metastasis, and invasion; with poor survival outcomes	N/S	57
Breast cancer	↑	induce cell proliferation, invasion, migration, and anchorage-independent growth; induce cancer stem cell frequency, self-renewal capability, and breast cancer formation via CIC deficiency	target MMP-13, positively correlated with Ki67 and EGFR2, but inversely related to ERα level; target cyclin D2	11,56,58,63
Capicua-rearranged undifferentiated round-cell sarcoma	↑	induce metastasis, with poor clinical outcomes	Capicua-DUX4 fusion	64,65,67–69
Ewing sarcoma family of tumors	↑	N/S	N/S	66
Small blue round-cell tumors	↑	N/S	N/S	70
Ewing sarcoma	↑	induce cell proliferation	decrease cyclin-dependent kinase inhibitors and increase stem cell-related genes	75
Endometrial cancer	N/S	induce cell growth	induce ER activity; activated by NANOG	77,78
Ovarian cancer	N/S	upregulated after chemotherapy, related to chemosensitivity to paclitaxel	miR-1307/Capicua signaling pathway	12
LUAD	↑	induce cell proliferation and invasion	increase MMP-9 and MSI2	79,81
NSCLC	↑	induce cell growth and metastasis; with advanced stage, lymph node metastasis, and poor prognosis	regulate PXN and MMP-1	80

(*Continued on next page*)

Table 1. *Continued*

Cancer	Expression	Function	Mechanism	References
Lung cancer	N/S	induce metastasis	upregulated by Capicua deficiency; regulate MMP-24	82
Papillary thyroid cancer	N/S	regulate cancer pathogenesis	activate the BRAFV600E/MAPK pathway	83,84
Melanoma	N/S	induce cell metastasis and progression	regulated by the ERK/p90(RSK)/14-3-3 pathway via Capicua, and increase MMP-25	85,86
Esophageal squamous cell carcinoma	N/S	induce tumorigenesis and metastasis	induce MMP-2 and MMP-9 and inhibit E-cadherin	87
Esophageal adenocarcinoma	N/S	induce cell proliferation, invasion, and metastasis	regulate MMP-1; activated by the ERK/MAPK pathway	88
T cell acute lymphoblastic lymphoma	N/S	with oncogenic phenotype	induced by Capicua inactivation	89

EMT, epithelial-mesenchymal transition; P3H4, prolyl 3-hydroxylase family member 4; MMP, matrix metalloproteinase; E2, 17β-estradiol; TNBC, triple-negative breast cancer; MAPK, mitogen-activated protein kinase; KDM5D, lysine demethylase 5D; ME1, malic enzyme 1; KIF2A, kinesin family protein 2A; AKT, protein kinase B; MED25, mediator subunit 25; ERK, extracellular signal-regulated kinase; IER3, immediate early response 3; COP1, constitutive photomorphogenesis 1; DUX4, double homeobox 4; EGFR2, epidermal growth factor receptor 2; ERα: estrogen receptor alpha; CSC, cancer stem cell; EE2: ethinyl estradiol; Cav, caveolin; LUAD, lung adenocarcinoma; NSCLC, non-small cell lung cancer; uPA/uPAR, urokinase plasminogen activator/urokinase plasminogen activator receptor. ↑ represents upregulation; N/S represents unknown.

be different between primary and metastatic tumors. ETV4 fusion genes in prostate tumor included TMPRSS2-ETV4, EWS-ETV4, KLK2-ETV4, CANT1-ETV4, DDX5-ETV4, UBTF-ETV4, SLC45 A3-ETV4, and HERVK17-ETV4.[13,16–20] Especially, TMPRSS2-ET V4 fusion is found in 1%–1.6% of prostate cancer patients with fusion genes.[13,18] However, by collecting paraffin blocks from 91 cases of needle biopsy and 18 cases of radical prostatectomy from Eastern Chinese prostate cancer patients, it was discovered that Eastern Chinese prostate cancer patients lack TMPRSS2-ETV4 fusion,[21] implying that TMPRSS2-ETV4 fusion is displayed diversely and is dependent on race. In addition, Ewing's sarcoma (EwS) breakpoint protein also can be fused to ETV4 by chromosome translocations, resulting in oncogenic ETS function such as promotion of cell migration and tumor transformation of prostate cancer and EwS.[19] By performing a model predicting the microarray-based ETS status, one group identified 1% positive ETV4, 6.3% positive ETV1, 0.4% positive ETV5, and 41.5% positive ERG among 4,036 prostate cancer men, which were in accordance with prostate tumor biopsy samples from 509 patients with 0.4% positive ETV4, 8.6% positive ETV1, 0% positive ETV5, and 41.2% positive ERG, suggesting that ETS status prediction models based on microarrays can be used as molecular classification for prostate cancer.[22] Significantly, another group included 146 patients in a study and grouped them into dutasteride treatment and placebo treatment averagely. They then studied the biopsy specimen and identified that ETV4 rearrangements only took place in 1 case (0.7%) in the placebo group, suggesting that ETS fusion status is not related to disease progression.[23] Hence, ETV4 status could be expected to predict molecular classification but not the progression of prostate cancer.

Clinically, a high level of ETV4 has been found to be associated with Gleason score, pathological tumor stage, and poor prognosis of patients with prostate cancer.[24] Furthermore, ETV4 was found to have a more increased expression in prostate cancer samples than in benign samples,[25] and it was expressed in cell nuclear extracts and cancer tissue protein lysates.[26] However, another group revealed that ETV4 upregulation was rarely found in prostate cancer (3%), and it was particularly more infrequent in higher-grade prostate cancer.[27] Therefore, more research is needed to confirm the expression of ETV4 in prostate cancer. Nevertheless, numerous investigations have revealed a tumor-promoting function of ETV4 in prostate cancer. For example, ETV4 is reported to promote cell invasion, transformation, motility, and metastasis of prostate cancer cells through promotion of Ras and phosphatidylinositol 3-kinase (PI3K) signaling.[10,24,28–30] Surprisingly, ETV4 has been shown to be more relevant for anchorage-independent growth compared with ETV1, which is more relevant for cell invasion.[28] In contrast, a diminished level of ETV4 greatly represses cell proliferation, invasion, mobility, and anchorage-independent growth of prostate cancer *in vitro* and inhibits cancer growth *in vivo*.[24,31] However, knocking down ETV4 in a metastatic cell line derived from a mouse model of prostate cancer affects the metastatic phenotype but does not change cancer growth,[30] suggesting that the level of ETV4 in metastatic tumors has no effect on primary tumors. Mechanistically, ETV4 deletion in prostate cancer cells partially reverses epithelial-mesenchymal transition (EMT) through the suppression of urokinase plasminogen activator/urokinase plasminogen activator receptor (uPA/uPAR), matrix metalloproteinase (MMP)-2, and MMP-9 expression levels.[24,31] Conversely, ETV4 overexpression induces the increase of EMT-specific transcription factors such as TWIST1, SLUG1, zinc finger E-box-binding homeobox (ZEB)1 and ZEB2, and influenced the levels of E-cadherin and N-cadherin.[31] Furthermore, ETV4 is observed to regulate the level of MYC and other cell proliferation genes by targeting the 5′ and 3′ MYC enhancers.[10] Of note, ETV4 is also necessary for PDZ-binding motif (TAZ) gene transcription in prostate cancer, which has the ability of promoting prostate cancer cell invasion and migration.

ETV4 not only modulates the expression of its downstream but also can be regulated by other molecular proteins to lead to the tumorigenesis of prostate cancer. For instance, caveolin (Cav)-1 and Cav-2 have

a positive correlation with the induction of castration-resistant prostate tumor cell migration via the modulation of some factors such as ETV4.[32] In addition, ETV4 has high-affinity interaction with mediator subunit 25 (MED25), attributed to its DNA-binding domain (DBD); the latter is specific for the ETV4, ETV1 and ETV5 due to its weak binding to other ETS factors.[33]

Taken together, ETV4 facilitates the EMT process and is as well regulated by multiple factors, thus promoting prostate cancer carcinogenesis and malignancy.

Bladder cancer

Bladder cancer is a fearful threat to human health globally, and ETV4 has been demonstrated to be involved in its development. It is reported that ETV4 is overexpressed in bladder cancer, and its overexpression induces cancer cell proliferation and invasion. Moreover, ETV4 can activate the transcription of prolyl 3-hydroxylase family member 4 (P3H4) via direct binding with P3H4's promoter region, and the upregulation of ETV4 relieves the repression of invasion induced by P3H4 knockdown.[34] This suggests that ETV4 is an oncogene in bladder cancer and exerts its function perhaps by regulating P3H4.

GASTROINTESTINAL CANCERS

Gastric cancer (GCa)

GCa is the third leading cause of cancer deaths worldwide, threatening the health of nearly one million people every year. ETV4 has been reported to modulate the progression of GCa. In this respect, it is noted that ETV4 is upregulated in GCa samples at the mRNA and protein levels, and it is associated with poor prognosis of GCa patients.[35–37] Consistently, ETV4 is found to be overexpressed in gastrointestinal stromal tumors and to be related to a high mitotic rate. Interestingly, in gastric adenocarcinomas with an advanced stage, the co-association of ETV4 and extracellular signal-regulated kinase (ERK) signaling was discovered to be correlated with poor survival of patients.[35] Furthermore, another group used next-generation resequencing to study 412 cancer-associated genes in 37 GCa patients with liver metastasis and 37 with primary GCa, discovering the somatic mutations of ETV4 in patients with metastatic tumors.[38] Hence, ETV4 upregulation could induce cancer progression and predict poor survival in GCa.

Mechanistically, ETV4 could target and decrease lysine demethylase 5D (KDM5D) to accelerate GCa cell metastasis.[37] In addition, ETV4 increases kinesin family protein 2A (KIF2A) expression, which is an M-type nonmotile microtubule depolymerase and is overexpressed in GCa tissue, thus inducing cell proliferation and inhibiting apoptosis of GCa MKN-45 and AGS cells through the protein kinase B (AKT) pathway.[39] Furthermore, ETV4 participates in reactive oxygen species (ROS)-induced upregulation of malic enzyme 1 (ME1), which promotes GCa growth, lung metastasis, and peritoneal dissemination.[40] Notably, the mechanism of the promotion of gastrointestinal stromal tumor cell proliferation and invasion by ETV4 is that ETV4 overexpression induces cyclin D1 (CCND1) expression and Wnt/β-catenin signaling. Similarly, Zeng et al.[41] created patient-derived xenografts by using metastatic gastrointestinal stromal tumor cells that are resistant to tyrosine kinase inhibitors, and they identified abnormal increases of ETV4 and nuclear β-catenin. Therefore, ETV4-mediated carcinogenesis may possibly be attributed to the activation of some protein kinases, as well as its downstream pathways.

Colorectal cancer (CRC)

CRC is a common cancer with about 6% of individuals developing this disease within their lifetime. Research has provided evidence for the important role of ETV4 in the development of CRC. In this way, ETV4 is reported to be more highly expressed in CRC tissues than in normal colorectal tissues.[42,43] For example, positive ETV4 staining in 62 of 100 CRC tissues and no or weak staining in normal colorectal tissues are observed by using immunohistochemistry. Similarly, using RT-PCR found that ETV4 mRNA is expressed in all of the 10 liver metastases from CRC tissues.[44]

Mechanistically, the phosphorylation of ETV4 at Ser73 occurs under ERK activation and blocks the binding of ETV4 to constitutive photomorphogenesis 1 (COP1), thus resulting in the increase of ETV4 in patients, mouse models, or cell lines of CRC.[42] Additionally, some MMPs are also identified to be involved in the aberrant expression of ETV4, such as MMP-1 and matrilysin in CRC,[44] and MMP-7 in colorectal mucinous adenocarcinoma. In details, ETV4 increases MMP-7 expression and induces EMT via the ERK pathway to enhance cell invasiveness of colorectal mucinous adenocarcinoma.[45] Notably, ETV4 expression in CRC samples is negatively related to the level of Capicua, which is located on 19q13 and is a transcriptional repressor related to tumor progression and metastasis in many cancers. This is proven by the fact that downregulation of ETV4 inhibits the enhanced cell proliferation in Capicua-deficient CRC cells.[46]

In addition, ETV4 is notably related to the depth of invasion, metastasis, and recurrence of CRC,[43,44,47] and, correspondingly, patients with ETV4-positive CRC possess worse overall survival (OS) and disease-free survival (DFS) in comparison with those with ETV4-negative cancers,[44] demonstrating that ETV4 functions as a tumor-promoting factor in CRC and predicts poor survival.

Colon cancer

Similarly, ETV4 expression is also found to be higher in colon tumor tissues than in normal colon tissues.[48] A functional study demonstrated that ETV4 deficiency leads to 90% inhibition in cell proliferation and 67% inhibition in cell invasion,[48] indicating that ETV4 is involved in the tumorigenesis of colon cancer. Interestingly, ETV4 can be upregulated by the deletion of Rab25, a tumor suppressor for colon cancer; alternatively, ETV4 overexpression in Rab25-knockdown cells can activate α5β1-integrin, leading to tumorigenesis,[49] implying that ETV4-mediated tumorigenesis can be regulated by Rab25 in colon cancer.

Hepatocellular carcinoma (HCC)

HCC has been identified as a global health problem and is the main cause for the death of patients with cirrhosis. Emerging evidence has clarified the significant role of ETV4 in HCC. One study revealed that ETV4 is expressed in a higher level in HCC tissues compared with those

in paired normal hepatocellular tissues.[50] Moreover, among the Pea3 group genes, ETV4 is reported to be the most clearly increased gene in Capicua-deficient HCC cells.[51] Mechanistically, ETV4 promotes MMP-1 expression to influence the Capicua/ETV4/MMP-1 axis, consequently promoting HCC cell progression and inhibiting cell apoptosis.[51] In contrast, deletion of ETV4 notably induces HCC cell apoptosis and reduces cell proliferation.[50] Another group found that ETV4 also can target the uPAR promoter, and therefore lead to the enhancement of cell invasion and migration of HCC.[52] Notably, ETV4 is identified to be related to the chemoresistance in HCC. In an *in vitro* study, it was demonstrated that ETV4 accelerates the resistance of HCC cells to cisplatin and sorafenib by modulating the immediate early response 3 (IER3) mRNA level.[50] Thus, ETV4 overexpression induces HCC development and chemoresistance.

Cholangiocarcinoma

To date, very few studies have been carried out to explore the role of ETV4 in cholangiocarcinoma. In one study, Singsuksawat et al.[53] xenografted KKU-213 and KKU-139 cholangiocarcinoma cell lines to nude mice and then divided them into a control group with no treatment, a 17β-estradiol (E2)-treated group, a tamoxifen-treated group, and an E2 combined with tamoxifen-treated group. They then observed that E2 enhanced the growth of xenograft tumors while tamoxifen reduced the tumor growth. For seeking E2-regulated genes, they showed that 14 genes including ETV4 were downregulated among 84 analyzed genes, and the level of ETV4 was increased in the E2-treated group and decreased in the tamoxifen-treated group in comparison with the untreated group by using real-time PCR. This research further knocked down ETV4 in cholangiocarcinoma cells and detected the decrease of cell responsiveness to E2, as well as inhibition of cholangiocarcinoma progression, suggesting that ETV4 may promote E2-induced cholangiocarcinoma development and is a significant target of tamoxifen-mediated killing of cancer.

Pancreatic cancer

Similarly, ETV4 is reported to be overexpressed in pancreatic tumor samples in comparison with that in normal pancreas. It also enhanced cell growth and induced cell-cycle progression by regulating CCND1 expression in pancreatic cancer.[54]

Collectively, the above results indicate that ETV4 plays as a cancer promoter in the diversity of gastrointestinal tumors by mediating different targets such as MMPs.

BREAST CANCER

Breast cancer is the commonest leading cause of cancer deaths among females globally, and according to molecular markers for estrogen receptor (ER) or progesterone receptor (PR) and human epidermal growth factor 2 (ERBB2/HER2), it is classified as hormone receptor-positive/ERBB2-negative breast cancer, ERBB2-positive breast cancer, and triple-negative breast cancer (TNBC). One group created a mammary-targeted dominant negative ETV4 transgene DeltaNPEA3En, showing that ETV4 is overexpressed in Wnt1 breast cancers, and DeltaNPEA3En inhibits early onset tumor formation in mouse mammary tumor (MMT) virus (MMTV)/Wnt1 virgin patients, implying a requirement for ETV4 in Wnt1-induced tumorigenesis.[55] Strikingly, the levels of ETV4 mRNA expression in breast cancer tissues are reported with large discrepancies. For example, ETV4 mRNA in 23.1% (30/130) of cancer tissues is downregulated and in 13.8% (18/130) is upregulated compared with normal breast tissues.[56] More importantly, high expression of ETV4 mRNA in breast cancer is related to Scarff-Bloom-Richardson histopathological grade III while not for the worse prognosis, implying that ETV4 could predict breast cancer aggressiveness but not the prognosis of patients with breast cancer.[56] As for TNBC, however, several studies identified a high level of ETV4 expression and a positive relationship between the ETV4 level and poor survival of patients. Especially, the ETV4 mRNA level in TNBC tissues is detected to be more than 5-fold higher than that in corresponding normal tissues, and the ETV4 protein level is shown to be upregulated in 57.0% of 135 TNBC tissues.[57] Furthermore, another study found that TNBC patients with ETV4 overexpression are more likely to be in an advanced stage, develop distant metastasis, and have a higher positive lymph node rate, lymphovascular invasion, and shorter OS and DFS, indicating that ETV4 may be an unfavorable prognostic factor in TNBC.[57]

Furthermore, with utility of *in vivo* and *in vitro* assays, Pea3 subfamily members are validated to regulate mammary epithelial cell morphology, proliferation, and migration, thus advancing their ability for morphogenesis and tumorigenesis.[58] Consistently, a functional study showed that ETV4 enhanced cell proliferation, invasion, migration, and anchorage-independent growth in MMT cell lines.[11] Mechanistically, diminished levels of ETV4 suppressed breast cancer cell invasion and cancer growth by suppressing EMT via downregulation of Snail and ZEB1.[58–60] The significantly positive correlation between ETV4 and Snail expression is also confirmed in the Gene Expression Omnibus (GEO) database.[60] Furthermore, repression of MMP-13 could inhibit ETV4-induced cell proliferation and migration in breast cancer cells, and meanwhile co-overexpression of ETV4 and MMP-13 is observed to be related to poor prognosis of breast cancer patients.[11] In addition, emerging evidence revealed that ER is also involved in the regulation of ETV4. One group exposed pregnant Sprague-Dawley mice to ethinyl estradiol (EE2) to build an ER-positive breast cancer model and compared the different genes between breast cancer mice exposed to EE2 *in utero* and control mice, observing that ETV4 expression level is higher in the EE2-exposed breast cancer group than in the control group, and it is also higher in breast cancer LCC9 cells with resistance to anti-estrogen compared with that in breast cancer LCC1 cells with sensitivity to anti-estrogen.[61] Similarly, the ETV4 level is reported to be inversely associated with ER alpha (ERα) level in breast cancer but positively related to Ki67 and epidermal growth factor receptor 2 (EGFR2) levels.[56] In a prospective and randomized phase II study, Euhus et al.[62] included 73 females with risks for breast cancer whose age was $\geq$35 years and who had a 5-year Gail risk $\geq$1.67% or a history of lobular carcinoma *in situ*, and then grouped them randomly to tamoxifen treatment and placebo treatment for 3 months. Analyzing their breast and blood samples at baseline and posttreatment, the researchers found that tamoxifen treatment decreased ETV4 and ETV5 expression, confirming the role of ER in the modulation of ETV4

expression. Moreover, *in vivo* and *in vitro* studies revealed that Capicua deficiency could accelerate ETV4 expression level, thereby leading to the increase of cancer stem cell (CSC) frequency, self-renewal capability, and breast cancer formation.[63] Additionally, ETV4 also can target CCND2 to regulate mammary tumorigenic cellular proliferation and migration.[58]

Collectively, these results validate that ETV4 is an oncogene factor in breast cancer, and it exerts tumor-promoting functions by regulating EMT and meanwhile being modulated by ER and Capicua.

ROUND-CELL SARCOMAS AND EwS

Round-cell sarcomas

Round-cell sarcomas are a heterogeneous group of cancers predominantly affecting children and young adults. ETV4 has been reported to be correlated with the carcinogenesis of round-cell sarcomas. For instance, overexpression of ETV4 is found in Capicua-rearranged undifferentiated round-cell sarcomas, which are characterized with Capicua-rearrangement and small round-cell morphology. One group studied the different expression levels of ETV4 between 17 patients with Capicua-rearranged undifferentiated round-cell sarcomas and 110 patients whose cancers were similar to Capicua-rearranged undifferentiated round-cell sarcomas in morphology, and they showed that ETV4 was positively expressed with strong nuclear staining among all patients with Capicua-rearranged undifferentiated round-cell sarcomas but negatively expressed in cancers similar to Capicua-rearranged undifferentiated round-cell sarcomas in morphology except for 6 cases with focal and very weak nuclear immunoreactivity, suggesting that ETV4 may be a valuable factor in diagnosing Capicua-rearranged undifferentiated round-cell sarcomas.[64] Similarly, Hung et al.[65] found that 90% of Capicua-rearranged sarcomas and 5% of other cancers covering five unclassified round-cell sarcomas, two Wilms cancers, one desmoplastic small round-cell cancer, one melanoma, and one small cell carcinoma displayed moderate-to-strong nuclear staining of ETV4 in more than 50% cells.

Additionally, one study used immunohistochemistry to diagnose a number of the EwS family and detected positive ETV4 but negative NKX2.2 and BCOR immunoreactivity in two small round-cell cancers, implying that ETV4 combined with NKX2.2 and BCOR immunohistochemistry significantly contributed to identifying the EwS family of tumors from Capicua or BCOR-associated sarcomas.[66] Similarly, another study addressed positive ETV4 and WT1 but negative NKX2.2 immunoreactivity in four round-cell sarcomas with characteristic histology of Capicua-rearranged sarcomas and further detected Capicua-double homeobox 4 (DUX4) fusion breakpoint by using high-throughput RNA sequencing (RNA-seq).[67] It is well known that Capicua-rearranged undifferentiated round-cell sarcoma always has Capicua-DUX4 fusion, and this fusion oncoprotein commonly predicts high metastatic trend and poor clinical outcomes of undifferentiated round-cell sarcoma.[68] Importantly, among six confirmed Capicua-DUX4 sarcomas, by scoring the expression with a validated scale, one group observed that ETV1 and ETV4 were positively expressed in five cases.[69] Additionally, Capicua-DUX4 fusion could induce the upregulation of ETV4 and CCNE1, thereby promoting cancer metastasis. Hence, this suggests that ETV4 may be a therapeutic strategy for tumors with Capicua-DUX4 expression.[68] At the same time, Kao et al.[70] revealed that the mRNA of Pea3 members (ETV4, ETV1, and ETV5) was upregulated in all samples form 14 small blue round-cell tumors lacking fusion candidates. They then observed Capicua reads in RNA-seq data and identified 7 of 14 cases with Capicua-DUX4 fusion reads, 2 of 14 with Capicua-DUX4 reads, and 5 of 14 with negative reads; at the same time, they performed fluorescence *in situ* hybridization (FISH) and found 7 of 14 cases with Capicua break-apart and used immunohistochemistry to find 7 of 11 samples with positive ETV4 immunostaining. These results suggested that overexpression of ETV4, ETV1, and ETV5 may be more reliable for diagnosing Capicua fusion-positive small blue round-cell tumors than RNA-seq algorithms and FISH.

Hence, these findings demonstrate that ETV4 is a potential biomarker for diagnosing round-cell sarcomas and as well is a critical target for treating round-cell sarcomas.

EwS

EwS is an aggressive osteolytic tumor mostly in children and adolescence, and more than 30% of these cases have metastatic disease.[71] EwS is also involved in the heterogeneous group of small round-cell cancers, and together with a diverse group of primitive neuroectodermal tumors forms the Ewing family of tumors.[72] EwS genetically has a chromosomal translocation including the EwS gene and ETS transcription factors including ETV4 (1%), ETV1 (1%), FLI1 (90%), ERG1 (5%), and FEV (1%).[73] For instance, one group observed an EWSR1-ETV4 chimeric transcript in EwS in a female child aged 7 months due to the translocation of t(17;22)(q21;q12) by using RT-PCR, cytogenetic analysis, and FISH techniques.[74] In another study, with double knockout of ETV4 and ETV5, EwS cells turned to be in an epithelial cell-like morphology, and genes related to stem cell-like Tcf15, Gbx2, Zic3, Lrh1, and Baf60c were inhibited. At the same time, overexpression of ETV5 or ETV4 leads to the emergence of Tcf15 and Gbx2, implying that ETV4 and ETV5 are of importance in embryonic stem cell proliferation via modulation of Gbx2 and Tcf15.[75] Therefore, ETV4 may be an important oncogenic fusion protein in EwS and is related to stem cell proliferation.

GYNECOLOGICAL CANCERS

Gynecological cancers, mainly including ovarian cancer, endometrial cancer, and cervical cancer, are the common cause of deaths for women with cancer. Many studies have found the functions of ETV4 in ovarian cancer and endometrial cancer. One group included six epithelial ovarian cancer patients in advanced stage and with high grade and then used DNA microarray screening to study the expression of 21,000 genes in paired cancer specimens obtained before and after chemotherapy, discovering that the chemoresistance-related genes including ETV4 were upregulated after chemotherapy, suggesting that ETV4 may be a critical target for overcoming ovarian cancer chemoresistance.[76] Mechanistically, ETV4 and ETV5 expression levels in ovarian cancer can be regulated by the miR-1307/Capicua signaling pathway, which inhibited the chemosensitivity to paclitaxel.[12] Thus,

ETV4 overexpression enhances chemoresistance in ovarian cancer, which could be regulated by Capicua. As for endometrial cancer, ETV4 is reported to influence cancer cell growth by regulating ER activity. Specifically, ETV4 knockout inhibits its binding to ER and the transcriptional reaction to estradiol, and at the same time affects chromatin accessibility at some ER binding site and impairs nuclear translocation of ER, resulting in suppression of endometrial cancer cell growth.[77] In embryonic carcinoma NCCIT cells, the pluripotency factor NANOG gene can be activated by ETV4 at the transcriptional level in a dose-dependent manner.[78] These results indicate that ETV4 has a tumor-promoting effect on some gynecological cancers, including ovarian cancer and endometrial cancer.

LUNG CANCER

Lung cancer is an important global health issue and is classified into different histopathological subtypes, including lung adenocarcinoma (LUAD) and non-small cell lung cancer (NSCLC). ETV4 has been validated to promote tumorigenesis of lung cancer. In this way, ETV4 is found to be overexpressed in LUAD in comparison with normal lung tissues in online data and in the results of qPCR, western blotting, or immunohistochemistry.[79] At the same time, ETV4 is reported to be correlated with lymph node metastasis and the late stage of NSCLC, and it predicts the poor prognosis of patients.[80] Furthermore, a functional study found that downregulation of ETV4 suppresses cell invasion and proliferation of LUAD, and it restrains cell growth and metastasis of NSCLC.[79,80] Mechanistically, it is indicated that ETV4 directly modulates the activity of PXN and MMP-1 in NSCLC, and Kaplan-Meier analysis found poor prognosis of patients with NSCLC when there is co-overexpression of ETV4 and PXN or ETV4 and MMP-1.[80] Meanwhile, ETV4 could increase MMP-9 expression[81], or target and increase MSI2 expression,[79] consequently participating in the tumorigenesis of LUAD. Moreover, knockout of Capicua recovered the expression of ETV4, resulting in the upregulation of MMP-24 and causing lung cancer metastasis.[82]

Taken together, ETV4 acts as a tumor promotor in lung cancer and predicts worse survival by targeting MMPs and being regulated by Capicua.

OTHER CANCERS

Papillary thyroid cancer (PTC)

Using weighted gene co-expression network analysis (WGCNA) to identify functional genes associated with PTC, one group found 1,062 differentially expressed genes (DEGs) between PTC and normal thyroid tissues. In the following established module containing 118 selected highly related genes, 61 genes were confirmed to be associated with PTC development, and importantly ETV4 as well as ETV5 were involved in the potential genes controlling PTC pathogenesis.[83] Similarly, another group analyzed RNA-seq data on 266 PTC samples from The Cancer Genome Atlas (TCGA) and 65 PTC samples from their institute and found that ETV4 was overexpressed in PTC tissues via the promotion of the BRAFV600E/mitogen-activated protein kinase (MAPK) pathway.[84] Thus, ETV4 is significantly responsible for PTC occurrence and development.

Melanoma

ETV4 has been demonstrated to be involved in the malignancy of melanoma. Mechanistically, ETV4 is regulated by ERK/p90^{RSK}/14-3-3 signaling via Capicua and hence is involved in the progression of melanoma.[85] Moreover, ETV4 can be activated by mitogen-activated protein kinase kinase kinase 8 (MAP3K8) via the binding of S100A8/A9 to the melanoma cell adhesion molecule, leading to the enhancement of MMP-25 and consequently inducing lung metastasis of melanoma.[86]

Esophageal cell carcinoma

In esophageal squamous cell carcinoma, neuropilin-2 can activate MAPK/ERK and ETV4, and then increase MMP-2 and MMP-9 expression levels and inhibit E-cadherin, consequently inducing tumorigenesis and metastasis.[87] In esophageal adenocarcinoma, ETV4 contributes to cell proliferation and invasion by mediating MMP-1, which is related to the MAPK/ERK pathway, thus inducing metastatic progression of esophageal adenocarcinomas.[88] Hence, ETV4 may play a tumor-promoting role in esophageal cell carcinoma through MMP-mediated pathways.

Lymphoma

Recent evidence has illustrated a close relationship between ETV4 expression and lymphoma development. It is reported that Capicua inactivation can promote ETV4 activation and then induce the progression of T cell acute lymphoblastic lymphoma in mice. Similarly, human T cell acute lymphoblastic lymphoma maintains its oncogenic phenotype dependent on ETV4 expression.[89]

CONCLUSIONS

In major types of cancer, ETV4 upregulation is a frequently occurring event and is related to advanced stages, lymphovascular invasion, metastasis, shorter survival times, and chemoresistance of patients, exhibiting oncogenic functions. The aberrant expression of ETV4 in human cancers can be regulated by many related genes including Capicua, and meanwhile ETV4 has interactions with its downstream signaling pathways such as MMPs and also can induce EMT to participate in the regulation of the tumorigenesis processes (Figure 1). In this regard, ETV4 is identified as a well-validated cancer target for the treatment of various human malignancies.

Even though the functions of ETV4 in cancers has been validated, many future challenges and open questions still need to be addressed. For example, where are the acting sites of ETV4 when ETV4 has interactions with its downstream moleculars? Also, what are the detailed molecular mechanisms of ETV4-governed EMT and other pathways? Future studies will resolve these questions and supply more evidence for therapeutic strategies that involve targeting ETV4 in human malignancies.

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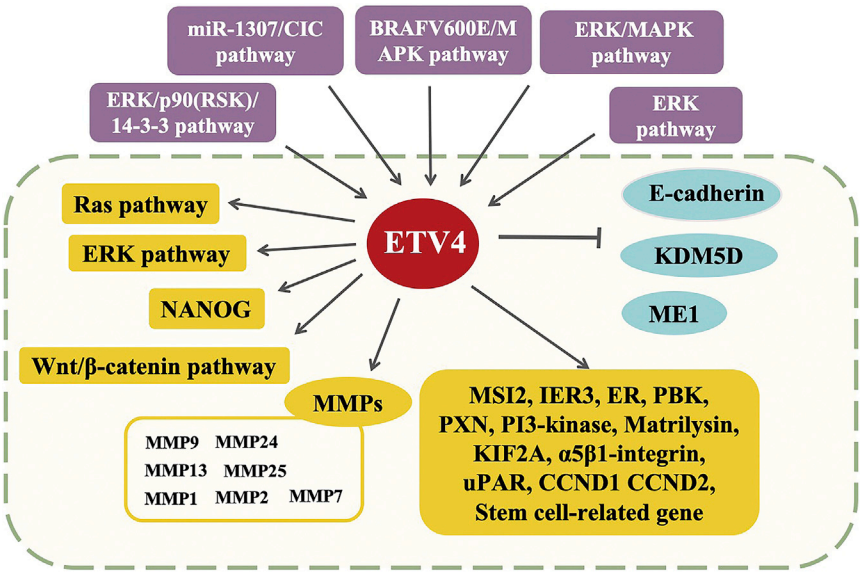

Figure 1. The signaling pathways of ETV4 in human cancer

Arrows (→) indicate "activating targets or ETV4"; the blockade from ETV4 to targets indicates "inhibiting targets."

AUTHOR CONTRIBUTIONS

W.J. and X.Z. conceptualized and design the manuscript. W.J., Y.X., and X.C. searched the literature and wrote the manuscript. X.C., S.P., and X.Z. critically viewed, and edited the manuscript. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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