Review article

ELF3 as an important factor in carcinogenesis – a brief review of the recent studies

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ABSTRACT

Introduction and objective

E74-like transcription factor 3 (ELF3) is mainly expressed in epithelial tissue, being responsible for differentiation and regeneration. Furthermore, it plays a role in inflammation, remodeling, allergy regulation and apoptosis. Various studies on ELF3 conducted since 1997 have also proved its connection with carcinogenesis and metastasis. This review summarizes recent advances in understanding the role of ELF3 in the following cancers: ampullary, bladder, breast, gastric, hepatocellular, nasopharyngeal, thyroid, lung and ovarian ones.

State of knowledge

There are still many unresolved and undiscovered issues regarding ELF3 mutations, however, based on research since 2016, a link to many signaling pathways important for carcinogenesis has been shown. There is no simple correlation between a specific ELF3 mutation and effect on cancer cells. In various types of cancers, ELF3 is associated with other pathways, and modifications exerted by silencing or amplifying its or associated genes, cause different effects in patient prediction. An example of the effect of ELF3 on tumor progression is achieved by negatively regulating the ZEB1 transcription factor responsible for metastasis. WNT, RAS, Akt, mTOR, HER2, Cyclin D, IRF6 are other ELF3-related factors that affects pathways crucial for tumorigenesis.

Conclusions

Further research and attempts to use ELF3 in the treatment and prognosis of cancer appear to be beneficial.

Key words: ELF3, carcinogenesis, molecular biology

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INTRODUCTION

E74-like transcription factor 3 – ELF3, a member of the ETS (E26 transformation-specific or E-twenty-six) family, also specifies as ERT, ESE-1, ESX, JEN, was first described in 1997 [1]. It is mainly expressed in epithelial tissue, however its expression in non-epithelial tissues is also possible after prior induction with TNF-α and IL-1β cytokines. ELF3 plays an important role in the differentiation and regeneration of epithelium, the correct formation of enterocytes (correct polarity and microvilli count), inflammation and remodeling (vessels, bones, cartilage), allergy regulation and apoptosis [2, 3]. It was also proved to play an important role in the pathophysiology of various cancers. ELF3 has been shown to both activate the TGFβ type II receptor gene (TβR-II) in epithelial cells and bind the TβR-II promoter *in vivo* [4]. ELF3 has been proven to have a role in epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET) – processes important in carcinogenesis and metastasis. ELF3 is a negative regulator of ZEB1 – the transcription factor responsible for EMT [5], while the ELF3 knockout causes the absence of E-cadherin and Grhl3 (grainyhead like transcription factor 3), which are key to MET initiation [2, 6]. According to our the best knowledge, studies results concerning ELF3 role in carcinogenesis are still inconsistent, thus this paper is an oncological attempt for gathering new information concerning this promising transcription factor in different malignancies. The summary of the collected data is presented in the table 1.

AMPULLARY CANCER

Vater's wart is a complex cellular environment from which adenocarcinomas arise, forming a group of histopathologically differentiated cancers. Genomic analyzes revealed that these tumors are characterized by a high frequency of ELF3 mutations, especially inactivating ones. ELF3 silencing mutations in human epithelial cells increase their motility and invasiveness [7]. In the study conducted in 2016 by Yachida et al. [7] with usage of microarrays on HBDEC2-3H10 cell lines, the ELF3 knockout stimulated the WNT and RTK-RAS signaling pathways, the activation of which is typical in cancers. In the course of aforementioned study, the most common mutations playing significant role in this cancer were identified by genome sequencing in 60 patients. The sixth most common mutation found in the analyzed group was in the ELF3 gene (after KRAS, TP53, CTNNB1, SMAD4, APC). Further study results showed significant ELF3 mutations in 21 out of 172 patients (12.2%; $p < 0.0001$). In another genomic sequencing study [8], 17 out of 152 (10.6%) patients with ampullary cancer had also a significant ELF3 mutation, most of which were inactivating frame shifts and nonsense mutations. These results,

Table 1. Influence of ELF3-related mutations on various types of cancers.

Type of the cancer	Type of ELF3-related mutation	Effect of the mutation
Ampullary cancer	Inactivation	Stimulation of the WNT and RTK-RAS signaling pathways essential in carcinogenesis [7]
Bladder cancer	Inactivation	Increased EMT, poorer diagnosis and lower overall survival [9]
	Amplification	Reduced frequency of invasion and decreased expression of mesenchymal markers [9]
Breast cancer	Inactivation	Increased estrogen-dependent cell proliferation [11] Inhibition of mTOR, HER2, Akt and Cyclin D signaling in HER2(+) cancer cells leading to reduced cell proliferation [12]
	Amplification	Reduction of estrogen-dependent cell proliferation [11]
Gastric cancer	Inactivation	Decreased level of cancers suppressor – IRF6 [13]
Hepatocellular cancer	Inactivation	Suppression of the proliferation, migration and invasion [14]
	Amplification	Promotion of the proliferation, migration and invasion [14]
Lung cancer	Inactivation	Inhibition of cells proliferation as well as metastasis in NSCLC [17]
	Amplification	Common mutation in NSCLC and LUAD with unknown effect on carcinogenesis [17, 18]
Nasopharyngeal cancer	Amplification of miR-4288 leading to increased level of ELF3	Increased malignancy of cancer cells [15]
Ovarian cancer	Inactivation	Promotion of cell proliferation and growth [19]
	Amplification	Inhibition of cell proliferation and growth [19]
Thyroid cancer	Inactivation	Inhibition of the growth, clone formation, migration and invasion [16]
	Amplification	Stimulation of the MAPK signaling pathway essential in carcinogenesis [16]

combined with previous studies, led to the conclusion that ELF3 mutations are three times more common in this specific tumor than in other ones. The ELF3 mutation co-occurred in 71% of cases with mutations in the WNT pathway (p = 0.02) [8].

BLADDER CANCER

The research conducted in 2019 by Gondkar et al. [9] was aimed at determining the pattern of ELF3 expression in bladder cancer cell lines, specifically in the mesenchymal UMUC3 cell line (human bladder transitional cell carcinoma), using the western-blot method. Attempts have been made to investigate the association between ELF3 expression and epithelial-mesenchymal transition, and thus the potential effect of ELF3 expression on cancer cell invasion. In the study group, ELF3 overexpression reduced the frequency of invasion and decreased expression of mesenchymal markers. Low ELF3 expression was shown to be associated with poorer prognosis and low overall survival. The conclusions of the aforementioned study suggest the possibility of inhibiting EMT with ELF3 in bladder cancer therapy.

BREAST CANCER

Breast cancer is the second most diagnosed cancer in the world and the fourth most fatal [10]. ERα is an estrogen receptor and transcription factor necessary for the development of the mammary gland. ELF3 connects to ERα as a transcription repressor. Estrogen displaces ELF3 from its connection with ERα contributing to the development of breast cancer [11]. According to the study by Gajulapalli et al. [11] conducted on the estrogen-dependent MCF7 breast cancer cell line (Michigan Cancer Foundation-7), ectopic ELF3 expression reduces estrogen-dependent cell proliferation, while ELF3 knockdown increases it. Further study of the interaction of ELF3 transcription factor with ERα may provide a target for new drugs in the treatment of estrogen-dependent breast cancer [11].

The exact role of ELF3 in HER2 (+) breast cancer remains unknown. However, several facts were established. Akt kinase is involved in cell survival and proliferation, which is why it plays an important role in stimulating cancer. It is activated by mTOR and HER2 proteins [12]. In the study by Kar et al. [12], ELF3 knockdown was induced in HER2(+) B BT474 luminal cells and HER2 subtype SKBR3 cells, resulting in reduced cell proliferation. HER2-dependent signaling was inhibited in BT474 cells, and ELF3 knockdown led to inhibition of mTOR activation in SKBR3 cells. In both cell lines, Akt signaling decreased. In contrast, when cells with constitutively active Akt (Myr-Akt) were tested, the antiproliferative effect of ELF3 inactivation was partially suppressed, indicating that the effect of ELF3 on Akt is indirect and is likely due to its impact on Akt activators (i.e. mTOR and HER2). It is also worth mentioning that ELF3 knockdown leads to inhibition of cyclin D1 in both cell lines, which delays the cell transition from G1 to S, and thus, proliferation.

GASTRIC CANCER

Gastric cancer is one of the most common and deadliest cancers in the world. Based on The Global Cancer Observatory statistics from 2018 [10], gastric cancer is the fifth most common cancer and the second most at risk of death. The role of ELF3 in gastric cancer is related to the regulatory factor interferon 6 (IRF6). This factor is a tumor suppressor which controls ectodermal tissue differentiation [13]. The study by Li et al. [13] provided evidence of reduced IRF6 expression in gastric cancer. Reduced IRF6 expression was associated with worse prognosis. Studies have shown that IRF6 is able to be regulated positively with ELF3 and negatively through ZEB1. The ELF3 transcription factor can directly bind to the IRF6 gene promoter and stimulate its transcription.

HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC) causes nearly 800,000 deaths per year, making it 3 with the highest mortality [10]. Epithelial-mesenchymal transition plays an important role in HCC progression. In the study described in 2018 by Zheng et al. [14], it was found that ELF3 expression is often significantly increased in HCC cells, that is associated with a worse prognosis for patients. Increased ELF3 expression in HCC promotes the proliferation, migration and invasion of HCC cells, and analogously – ELF3 inactivation leads to inhibition of these processes. In addition, ELF3 expression has been found to correlate with decreased E-cadherin expression, but increased N-cadherin and fibronectin expression, suggesting that ELF3 promotes EMT. This was proven by knockdown ELF3, which caused positive regulation of miR-141-3p, thus repressing ZEB1 expression and finally reversing EMT. Thanks to these observations, it is known that ELF3 promotes EMT by just activating ZEB1 secondary to 1miR-141-3p reduction. The consequence of studies on the role of ELF3 in HCC may be the use of this transcription factor as a prognostic biomarker or as a therapeutic target.

NASOPHARYNGEAL CANCER

Study by Ke et al. [15] discovered the potential use of ELF3 transcription factor as a target in the treatment of nasopharyngeal

cancer (NPC) by studying the role of round RNA (circRNA) in the pathogenesis of NPC. Analysis initially showed that protein kinase 3 acting on the circRNA homeodomain showed significant activity in NPC tissues and cell lines. The association between circHIPK3 expression and prognosis for NPC patients has been demonstrated. During *in vitro* studies, circHIPK3 was silenced and as a result proliferation, migration and invasion were suppressed in NPC cells. *In vivo*, however, it was observed that inhibition of circHIPK3 significantly reduced tumor growth as well as its metastasis. CircRNA was shown to be a competitive endogenous microRNA RNA (miR) – 4288 that inhibited the ELF3 pro-tumor effect in NPC cells. By increasing ELF3 expression, which resulted from the suppression of miR-4288 levels, circHIPK3 promoted the malignancy of NPC cells. Inhibition of miR-4288 reversed the anti-tumor effect of prior silencing of circHIPK3 on NPC cells. The circHIPK3-miR-4288-ELF3 feedback loop can serve as a target for NPC restriction attempts.

THYROID CANCER

Research in 2019 by Chen et al. [16] uncovered ELF3 protein role in thyroid cancer assessed after determining its overexpression in thyroid cancer tissues with a BRAF protooncogene mutation. Overexpression of ELF3 is correlated with prediction of poor diagnosis in patients with papillary thyroid cancer. The MAPK pathway is responsible for mitogen-dependent differentiation, proliferation, gene expression, movement and apoptosis in cells. Therefore, it plays an important role in carcinogenesis. In BRAF-mutant thyroid cancer cell lines ELF3 protein level was attenuated by PLX4032 (MAPK signaling pathway inhibitor). Additionally, ELF3 knockdown in mentioned cell lines inhibited the growth, clone formation, migration and invasion. Practical use of the above study would be the use of ELF3 as a prognostic marker in thyroid cancer.

LUNG CANCER

Role of ELF3 in non-small cell lung cancer (NSCLC) remains poorly understood however its upregulation was observed at mRNA and protein level. ELF3 gene silencing *in vitro* and *in vivo* led to significant inhibition of cells proliferation as well as metastasis. Conversely, its overexpression *in vitro* in NSCLC cells promoted metastasis and growth. Thus, ELF3 expression level is strongly correlated with survival rate of patients with NSCLC. ELF3 regulates cell cycle and EMT by activation of PI3K/AKT and ERK signaling pathways. Therefore, mentioned ELF3 activity can be endured by Ly294002 (inhibitor of PI3K) and U0126 (inhibitor of MEK1/2). Inhibition of ELF3 activity by suppressing PI3K/AKT signaling pathway can also be achieved by miR-320a-3p expression and there are evidences that miR-320a-3p might work as a tumor suppressor in NSCLC both *in vivo* and *in vitro*. miR-320a-3p overexpression inhibits NSCLC cells proliferation, migration and invasion and is strongly correlated with smaller tumor size and lighter weight. miR-320a-3p binds directly on the 3'UTR region of ELF3 mRNA, which could lead to decrease of ELF3 transcription. Regulation of ELF3 expression can be a promising target for further research as a potential treatment in NSCLC [17].

Due to research conducted in 2019 by Enfield et al. [18], in lung adenocarcinoma (LUAD) ELF3 plays the role as an oncogene with subtype specificity. Characteristic amplification of region 1q32.1, comprising ELF3 gene, develops in LUAD cells but not in squamous cell lung cancer (LUSC). In 80% of LUAD analyzed the ELF3 locus was affected by gene dosage or hypomethylation of promoter. The study shows that ELF3 has an important prognostic value and may also be a potential molecular target in LUAD therapy. However, it does not show similar values in LUSC.

OVARIAN CANCER

New hopes for the therapy in ovarian cancer have been approximated in the study by Yeung et al. [19]. ELF3 expression was detected by immunohistochemistry in epithelial ovarian cancer cells. The subject of the study was 112 samples from patients and from Cancer Genome Atlas (TCGA) data. It was established that the decrease in ELF3 expression in ovarian cancer cells was correlated with reduced survival. Furthermore, overexpression of ELF3 inhibited cell proliferation and growth, while silencing of ELF3 had the opposite effect. Increasing ELF3 regulation resulted in increased expression of epithelial markers, decreased expression of mesenchymal markers, and influenced the translocation of molecules signaling EMT in ovarian cancer cells. In summary, ELF3 appears to be a positive prognostic marker for ovarian cancer and what is more, by negative regulation of EMT can be a molecular target in ovarian cancer treatment.

SUMMARY

The above studies prove that the effect of ELF3 on the process of tumorigenesis and metastasis is significant and depends on the type and location of the tumor. Current genetic engineering methods, based on presented knowledge, give hope for finding new methods for diagnosing and treating malignancies. The ELF3 gene product and other related proteins may become the molecular targets for future personalized therapies.

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