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Forkhead box O proteins: Crucial regulators of cancer EMT

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ABSTRACT

The epithelial-mesenchymal transition (EMT) is an acknowledged cellular transition process in which epithelial cells acquire mesenchymal-like properties that endow cancer cells with increased migratory and invasive behavior. Forkhead box O (FOXO) proteins have been shown to orchestrate multiple EMT-associated pathways and EMT-related transcription factors (EMT-TFs), thereby modulating the EMT process. The focus of the current review is to evaluate the latest research progress regarding the roles of FOXO proteins in cancer EMT. First, a brief overview of the EMT process in cancer and a general background on the FOXO family are provided. Next, we present the interactions between FOXO proteins and multiple EMT-associated pathways during malignancy development. Finally, we propose several novel potential directions for future research. Collectively, the information compiled herein should serve as a comprehensive repository of information on this topic and should aid in the design of additional studies and the future development of FOXO proteins as therapeutic targets.

1. Introduction

The epithelial-mesenchymal transition (EMT) is an acknowledged cellular transition process in which epithelial cells acquire mesenchymal, fibroblast-like properties that involves alterations in intercellular adhesion, cytoskeleton, morphology and motility [1]. The concept of EMT was proposed over 30 years [2], and EMT has since become a hot research topic. EMT has been revealed to be involved in embryonic development [3], fibrosis [4], wound healing [3] and cancer metastasis [5,6]. The transition from the epithelial to mesenchymal phenotype endows cells with migratory and invasive behavior that is characteristic of malignant cells. The relationship between EMT and cancer metastasis has been widely investigated. Most studies have supported the concept that EMT promotes cancer metastasis [7]. EMT can trigger the dissociation of carcinoma cells from primary carcinomas, and these dissociated cells subsequently migrate and disseminate to distant sites [8]. Therefore, the modulation of EMT in cancer is a promising therapeutic strategy. However, an operational target to modulate EMT is still absent. Among the many molecules involved in EMT, Forkhead box O (FOXO) proteins may be promising operational targets.

Forkhead transcription factors (TFs) are named after the Drosophila melanogaster forkhead genes, which include a subfamily that encodes FOXO. Mammalian cells express four FOXO isoforms (FOXO1, FOXO3, FOXO4, and FOXO6) [9,10]; these different isoforms exert redundant effects and divergent functions in many diseases, including cardiovascular diseases [11], diabetes [9,12], cancer [13,14], fibrosis [15], aging [16], and stem cell activity [3]. FOXO proteins are controlled by several upstream molecules and regulate many downstream proteins [17]. Notably, FOXO proteins orchestrate EMT-associated pathways and EMT-related TFs (EMT-TFs), thereby modulating the EMT process [18,19].

The focus of the current review is to evaluate the latest research progress regarding the roles of FOXO proteins in cancer EMT. First, a brief overview of the EMT process in cancer and general background on FOXO family are provided. Next, we present the interactions between FOXO proteins and multiple EMT-associated pathways. Finally, we

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Table 1
Cancer EMT, metastasis and FOXO expression in cancer cell lines and clinical cancer specimens. (Abbreviations: ccRCC, clear cell renal cell carcinoma; EMT, epithelial-mesenchymal transition; FOXO, Forkhead box O proteins; HCC, hepatocellular carcinoma; NSCLC, non-small cell lung cancer).

Cancer type	FOXO expression	Outcome	Reference
Cancer cell lines			
Five NSCLC cell lines (95C, 95D, H1299, H460 and A549)	FOXO4↓	FOXO4 expression was lower in 95D cells (high metastatic potential) than in 95C cells (low metastatic potential).	Li et al. [108]
Five HCC cell lines (SMMC-7721, Huh7, HCCLM3, MHCC97H and SK-HEP-1)	FOXO1↓	FOXO1 expression was lower in HCC cell lines with high metastatic potential (HCCLM3, MHCC97H and SK-HEP-1) than in those with low metastatic potential (SMMC-7721 and Huh7).	Dong et al. [83]
Three metastatic ccRCC cell lines (Caki-1, ACHN, and SN12-PM6), and 4 nonmetastatic cell lines (786-O, 769-P, A-498, and Caki-2)	FOXO3↓	FOXO3 expression was lower in ccRCC cell lines with high metastatic potential (Caki-1, ACHN, and SN12-PM6) than in those with low metastatic potential (786-O, 769-P, A-498, and Caki-2 cells).	Ni et al. [50]
Clinical cancer specimens			
150 NSCLC samples	FOXO4↓	Immunoreactivity of FOXO4 was low in NSCLC compared with paired normal lung tissues. Low FOXO4 expression was correlated with decreased E-cadherin expression and elevated vimentin expression. Moreover, FOXO4 expression level was significantly correlated with TNM stage, histological differentiation and lymph node metastasis and was an independent prognostic factor in NSCLC.	Xu et al. [43]
21 patients with primary metastatic ccRCC and 114 patients with primary nonmetastatic ccRCC	FOXO3↓	FOXO3 expression was decreased in primary metastatic ccRCC samples. Patients with low FOXO3 levels had a poorer metastasis-free survival than those with high FOXO3 levels.	Ni et al. [50]
80 ccRCC patients	FOXO1↓	FOXO1 expression levels were significantly lower in patients with metastases and higher grade disease. Moreover, patients with lower *FOXO1* mRNA levels in their tumor samples had a poorer cause-specific survival outcome than those with higher expression levels.	Kojima et al. [169]
40 primary gastric cancer and corresponding lymph node metastasis specimens	FOXO4↓	Gastric cancer had lower FOXO4 expression in metastatic lesions than in corresponding primary tumor samples.	Su et al. [62]
174 gastric cancer tissue paraffin sections	FOXO3↓	Chi square analysis showed that the expression level of FOXO3 in tumor tissues was significantly correlated with the histological grade, depth of invasion, local lymph node metastasis, distant metastasis and American Association of Cancer staging.	Yang et al. [63]
226 gastric cancer patients	FOXO4↓	The FOXO4 mRNA expression level was inversely correlated with T stage and N stage. Low FOXO4 expression was associated with poor prognosis.	Li et al. [64]

propose several novel potential directions for future research. Collectively, the information compiled herein should serve as a comprehensive repository of information that is available in this area and should aid in the design of further studies and the future development of FOXO proteins as therapeutic targets.

2. EMT in cancer

EMT confers mesenchymal properties on epithelial cells and has been closely associated with the acquisition of aggressive traits by carcinoma cells [20]. Epithelial cells are characterized by obvious apico-basal polarization [21] and the sequential expression of diverse junctional complexes necessary for cell-cell adhesion that are disassembled during EMT [22–24]. The adherens junction is a major component of intercellular adhesion that includes transmembrane cadherins that form homotypic interactions between adjacent cells [25]. Among various transmembrane cadherins, E-cadherin is typically expressed in epithelial cells [26], and alteration in its expression serves as a marker of EMT. The intracytoplasmic tail of E-cadherin binds to β-catenin, which mediates the link to the actin cytoskeleton [25]. Therefore, when the adherens junction is disassembled during EMT, β-catenin and the actin cytoskeleton are also influenced, further modulating EMT. The released β-catenin translocates to the nucleus [27], binds the TF T cell factor (TCF) and regulates the expression of EMT-TFs [28]. Alteration of the actin cytoskeleton has been found to participate in the changes in epithelial cell morphology and invasiveness, which are significant features of EMT in cancer [29].

Moreover, cancer cells with a mesenchymal phenotype are more resistant to adverse circumstances. Epithelioid cancer cells tend to undergo EMT in response to harmful stimuli such as hypoxia [30], inflammation [31], and virus infection [32]. EMT is mediated by multiple pathways, including the Notch [33], Wnt [34], transforming growth factor β (TGF-β) [35], epidermal growth factor (EGF) [36], vascular endothelial growth factor (VEGF) [37], fibroblast growth factor (FGF) [38], and hypoxia inducible factor 1 (HIF-1) [39] pathways. EMT-TFs {i.e., Zinc-finger E-box binding homeobox 1/2 (ZEB1/2) [40], Twist-related protein 1 (TWIST1) [41] and Snail1 [42]} are ultimately activated by the above pathways. These EMT-TFs regulate the expression of EMT markers (i.e., E-cadherin) and finally promote EMT [43,44]. Thus, the EMT process in cancer can be summarized into four phases: (1) harmful stimulation; (2) activation of EMT-associated pathways; (3) involvement of EMT-TFs (the drivers of EMT); and (4) changes in EMT markers (i.e., E-cadherin). Notably, recent studies have reported that FOXO proteins can orchestrate EMT-associated pathways, EMT-TFs and EMT markers to repress cancer EMT, which makes them promising targets to reverse cancer EMT [18,19,45–54].

3. General background on the FOXO family

The FOXO family is the O-subfamily of proteins that belong to the larger family of Forkhead TFs, which play important roles in regulating cell proliferation and differentiation [55,56]. Four isoforms of FOXO proteins, including FOXO1/3/4/6, have been validated as being expressed in mammalian cells. The FOXO1/3/4 isoforms exhibit a high degree of homology in their sequence, that differs from that of FOXO6, and all FOXO isoforms share the same DNA binding specificity [57]. As important TFs, FOXO proteins translocate from the cytoplasm to the nucleus and modulate the transcription of multiple genes. Apart from transcriptional regulation, FOXO proteins also bind target proteins to regulate their activity and function [51]. *FOXO* mRNA can modulate target gene expression by functioning as competitive endogenous RNA [54]. Multiple pathways have been shown to regulate FOXO activity, mainly through posttranslational modifications, such as phosphorylation, acylation, ubiquitination and glycosylation. Notably,