

Review Article

FoxM1: a novel tumor biomarker of lung cancer

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Abstract: FoxM1 is a member of the Forkhead box (Fox) family of transcription factors, which is expressed in actively dividing cells and is critical for cell cycle progression. Increased expression of FoxM1 was found in many tumors including non-small cell lung cancer (NSCLC). A more recent study showed FoxM1 is associated with poor prognosis of NSCLC patients through promoting tumor metastasis; elucidated FoxM1 could exert a direct effect on the prognosis of NSCLCs patients. In this review, we summarize the role FoxM1 in lung cancer in the hope of providing insights into the utility of FoxM1 as a novel biomarker of lung cancer.

Keywords: FoxM1, biomarker, lung cancer

Introduction

Lung cancer is the leading cause of cancer death in the United States (and worldwide), causing as many deaths as next four most deadly cancers combined (breast, prostate, colon and pancreas) [1]. In 2013, lung cancer is expected to account for 26% of all female cancer deaths and 28% of all male cancer deaths [1], of which the most common variant is non-small cell lung cancer (NSCLC). To date, the 5-year overall survival rate (approximately 15%) for patients with NSCLC has not been markedly improved [2].

FoxM1 (previously called HFH-11B, Trident, FoxM1b, Win and MPP2) is a member of the Forkhead box (Fox) family of transcription factors, which is expressed in actively dividing cells and is critical for cell cycle progression [3-5]. Increased expression of FoxM1 was found in many tumors including NSCLC, hepatocellular carcinomas (HCC), basal cell carcinomas, gastric cancer and other human tumors and human neoplastic cell lines [6-13]. A more recent study showed FoxM1 is associated with poor prognosis of non-small cell lung cancer patients through promoting tumor metastasis; elucidated FoxM1 could exert a direct effect on the prognosis of NSCLCs patients [14]. In this

review, we discussed the role of FoxM1 in Lung cancer.

Structure of the FoxM1 gene and protein, and its biological functions in the lung

The human Forkhead Box M1 (FoxM1) protein belongs to a winged-helix transcription factor family [15]. The FoxM1 gene is located on the chromosomal band 12p13 [16]. It was first identified as a mitotic-phase phosphoprotein (MPP2) from a cervical cancer Hella cell line [17]. Evidence have shown FoxM1 is essential for cell cycle progression and an important cell-cycle regulator controlling transition from G1 to S phase as well as entry into and completion of mitosis [18, 19].

FoxM1 in the proliferation of lung cell was first reported in 2003 by Kalinichenko, V. V. et al [20]. Using cell line, transgene mouse and butylated hydroxytoluene (BHT) lung injury model, they clarified ubiquitous expression of the forkhead box M1B transgene accelerates proliferation of distinct pulmonary cell types (including alveolar type II epithelial cells, bronchial epithelial and smooth muscle cells, and endothelial cells of pulmonary capillaries and arteries) following lung injury. This effect was associated with the earlier expression of the cell cycle pro-

moting cyclin A2, cyclin E, cyclin B1, cyclin F, and cyclin dependent kinase-1 (Cdk1) genes and diminished protein levels of Cdk inhibitor p21Cip1.

FoxM1 in lung development was clarified by mouse embryos model [21], FoxM1^{-/-} embryonic lungs displayed diminished mesenchyme proliferation, hypertrophy of smooth muscle cells of pulmonary arteries and severe abnormalities in the development of pulmonary microvasculature. This was associated with diminished pulmonary levels of the platelet endothelial cell adhesion molecule 1 (Pecam-1), TGF receptor type II, a disintegrin and metalloprotease domain 17 (ADAM-17) protein, VEGF receptors, Plk-1, Aurora B kinase, Lama4, and the Foxf1 transcription factor. The direct transcriptional target for Foxm1 transcription factor was shown lama4 by cotransfection experiments.

The mechanism of FoxM1 in lung cancer

FoxM1 in lung cancer

Il-Man Kim first reported human Foxm1 protein is abundantly expressed in highly proliferative human non-small cell lung cancers (NSCLC) as well as in mouse lung tumors induced by urethane [10]. The expression of FoxM1 in human lung carcinomas was examined by cancer profiling array, quantitative real-time RT-PCR, immunostaining and western blot. All these experiments showed that FoxM1 expression is induced in a significant percentage in human lung adenocarcinomas and squamous cell carcinoma tissue samples compared with normal lung tissue. Immunostaining results showed FoxM1 expression correlate with PCNA expression and RNase protection assay showed FoxM1 expression associate with elevated levels of cell cycle-promoting cyclin A2 and cyclin B1. These indicated Foxm1 is involved in stimulating proliferation of lung tumor cells. In urethane-mediated lung tumor induction mouse model, results have shown conditional deletion of the Foxm1 fl/fl allele could decrease the total number and size of lung tumors. Immunohistochemical stain results shown FoxM1 is not expressed in untreated mouse lungs, but is expressed in bronchial and alveolar epithelial cells of the urethane-treated Foxm1 fl/fl mice, dsRNA-treated Mx-Cre Foxm1^{-/-} mice displayed a significant reduction of nuclear Foxm1 levels

in pulmonary epithelial cells after urethane tumor induction, indicating FoxM1 induces the growth of lung tumors. The role of FoxM1 in tumor growth was further investigated by immunohistochemical detection of BrdUrd, and the results shown increased Foxm1 expression is associated with high DNA replication rates of lung tumor cells. The role of FoxM1 in the proliferation was further investigated in A549 human lung adenocarcinoma cells; the depletion of FoxM1 levels by siFoxM1 could significant decrease in expression of S phase promoting cyclin A2 and M phase-promoting cyclin B1 genes. The role of Foxm1 in DNA replication was also investigated in A549 human lung adenocarcinoma cells, the depletion of Foxm1 could cause a 70% reduction in the number of A549 cells undergoing DNA replication and a significant reduction in the number of cells undergoing mitosis. The depletion of Foxm1 by siFoxM1 could reduce anchorage-independent growth of A549 lung adenocarcinoma cells on soft agar.

These data above indicate FoxM1 expression in tumor cells is essential for progression of chemically-induced lung cancer in vivo. But lung cancer lesions contain not only tumor cells, but also inflammatory cells, endothelial cells and stromal fibroblasts. The role of FoxM1 in lung cancer associate endothelial cells was clarified by Balli et al [22]. In the study, pulmonary tumorigenesis induced by urethane administration was compared in mice genetically deleted for FoxM1 in endothelial cells, the result shown lung tumor number and size were increased in enFoxm1^{-/-} mice, and suggest that endothelial-specific expression of FoxM1 restricting lung tumorigenesis by limits lung inflammation and canonical Wnt signaling in lung epithelial cells.

FoxM1 and lung cancer cell properties (cell proliferation, cell survival, drug resistance)

In A549 lung cancer cells radiation could induce FoxM1-mediated G2/M arrest, the inhibition of matrix metalloproteinase-2 could enhance radiosensitivity by abrogating it [23]. FoxM1 expression is significantly associate with cisplatin-based chemotherapy resistance [24]. DDP-sensitive A549 and the corresponding DDP-resistant cell subline (A549/DDP) were introduced in this study. At both mRNA and protein

levels the expression of FoxM1 was significantly higher in A549/DDP cell subline than in A549 cells. Thiostrepton, the FoxM1 inhibitor could cause cell death and proliferative arrest in the cisplatin-resistant cells by the downregulation of FoxM1 expression. Knockdown of FoxM1 by siRNA could suppress cell migration and invasion in both A549 and A549/DDP cells. And cisplatin resistance in A549/DDP cells could be partially reversed by siRNA-mediated FoxM1 inhibition.

MicroRNAs (miRNAs) have been implied to play crucial roles for epithelial-to-mesenchymal transition (EMT) of NSCLC cells, in NSCLC cells miR-149 could inhibit EMT. Yang et al found miR-149 directly targeted FoxM1, and in A549 cells FoxM1 was involved in the EMT induced by TGF-beta1 [25].

FoxM1 as a therapeutic target

Conditional deletion of FoxM1 from lung epithelial cells prior to tumor initiation could decrease the number and size of lung tumors induced by urethane or 3-methylcholanthrene (MCA)/butylated hydroxytoluene (BHT) [26]. This was associated with diminished proliferation of tumor cells and reduced expression of Topoisomerase-2a (a critical regulator of tumor cell proliferation). In cultured lung adenocarcinoma cells, depletion of FoxM1 could significantly decrease Topoisomerase-2a expression in mRNA and protein levels. Indicating FoxM1 is a promising target for anti-cancer therapy and Topoisomerase-2a is a direct target of FoxM1 in lung cancer cells [26].

Prognostic role of FoxM1 in lung cancer

Yang and colleagues firstly reported FoxM1 overexpression correlated with the poor survival of lung cancer patient [27]. In 69 cases of squamous cell carcinoma specimens, immunohistochemistry analyses results shown FoxM1 immunoreactivity in 26 (37.7%) of the 69 squamous cell carcinoma cases. FoxM1 expression correlated with poor differentiates, lymph node metastasis, cancer stage and survival. And multivariate analysis revealed FoxM1 was independent poor prognostic factor and associated with progressive pathologic features and an aggressive clinical course. Liu et al reported FoxM1 in NSCLC is an independent prognostic

factor and negatively correlated with prognosis, but they found no relationship between FoxM1 expression and other critical clinical parameters [28].

Wang et al assessed the FoxM1 expression in 162 NSCLC patients by immunohistochemistry [24]. FoxM1 expression was associated with a significantly lower response rate, poor progression-free survival and overall survival. FoxM1 positivity was an independent prognostic factor for PFS and OS by multivariate analyses.

Recently, the prognostic roles of FoxM1 overexpression in NSCLCs and the potential underlying mechanisms have been clarified by Xu et al [14]. FoxM1 expression was examined in 175 NSCLC specimens by immunohistochemistry. FoxM1 overexpression was significantly associated with poorer tissue differentiation, positive smoking status, higher TNM stage, lymph node metastasis, advanced tumor stage, and poor prognosis. FoxM1 expression increased the hazard of death by multivariable analysis. The potential underlying mechanisms of FoxM1 in the prognostic role of NSCLC was investigated by in vitro and in vivo experiments, the results shown knockdown of FoxM1 could inhibit the migratory and invasive abilities of NSCLC cells, but enforced expression of FoxM1 could increase the invasion and migration of NSCLC cells. The cellular mechanism of FoxM1 in tumor metastasis is by inducing epithelial-mesenchymal transition (EMT).

Conclusions and future perspectives

FoxM1 is expressed in lung cancer; FoxM1 takes part in lung cancer cell properties (cell proliferation, cell survival, drug resistance). It is a therapeutic target for lung cancer and its overexpression correlated with the poor survival of lung cancer patient. More research in FoxM1 and lung cancer may give us a new chance for lung cancer therapy.

Disclosure of conflict of interest

None.

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