RESEARCH ARTICLE

Involvement of FoxM1 in Non-Small Cell Lung Cancer Recurrence

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Abstract

Background: Predictive biomarkers for lung cancer recurrence after curative tumor resection remain unclear. This study set out to assess the role of FoxM1 in the recurrence of non-small cell lung cancer. Methods: Immunohistochemistry for FoxM1 expression was performed on paraffin-embedded tumor tissues from 165 NSCLC patients. Association of FoxM1 expression with clinicopathological parameters and disease free survival were evaluated. Results: Our results indicated FoxM1 expression to be significantly associated with poorer tissue differentiation (P =0.03), higher TNM stage (P <0.01), lymph node metastasis (P <0.01), advanced tumor stage (P <0.01), and poorer disease free survival (P <0.01). Multivariable analysis showed that FoxM1 expression increased the hazard of recurrence (hazard ratio= 1.96, 95% CI, 1.04-3.17, P <0.05), indicating that FoxM1 is an independent and significant predictor of lung cancer recurrence. Conclusion: Therefore, FoxM1 is an independent risk factor for recurrence of NSCLC. Elevated FoxM1 expression could be used as an indicator of poor disease free survival.

Keywords: Lung cancer - non-small cell lung cancer - FoxM1 - recurrence - predictive factor

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Introduction

Surgery is regarded as the standard of care for early stage non-small cell lung cancer (NSCLC) (Rami-Porta et al., 2007). Patients who are medically inoperable and receiving either no treatment or conventional radiotherapy are significantly less likely to survive than are those who receive surgery (Rowell & Williams, 2001; Lagerwaard et al., 2002). Local recurrences at the primary tumor site in up to 50% of patients might be responsible for this low survival rate. However, data on lung cancer recurrence after curative tumor resection is sparse.

FoxM1 is a transcriptional factor, known for its function as a regulator in cell cycle. It is reported that FoxM1 plays an essential role in development of hepatocellular carcinoma (Park et al., 2011), prostate carcinoma (Kalin et al., 2006), and colorectal cancer in mice (Yoshida et al., 2007). Moreover, FoxM1 is an essential molecule in the regulation of oxidative stress, which contributes to malignant transformation and tumor cell survival (Park et al., 2009).

Increasing evidence revealed that FoxM1 is also important in lung cancer development. FoxM1 could induce lung cancer transformation and stimulate tumor cell proliferation (Kim et al., 2006; Wang et al., 2008). It conferred to acquired cisplatin resistance in breast cancer (Kwok, 2010) and primary resistance of gefitinib in non-small cell lung cancer (NSCLC) (Xu et al., 2012). Our previous work also demonstrated that FoxM1 could mediate NSCLC tumor cell metastasis. But whether FoxM1 could predict recurrence risk in NSCLC patients still needs to be determined.

In this study, we set out to analyze NSCLC patients in Zhongshan Hospital, Fudan University, Shanghai, China, during the given period of time. The correlation between clinicopathological parameters and FoxM1 expression was investigated, then we sought to find out whether FoxM1 is the key factor for recurrence in NSCLC patients.

Materials and Methods

Patients and tumor samples

A total of 201 histologically confirmed NSCLC patients who underwent curative surgical resection were consecutively recruited between January 2005 and February 2008 at Zhongshan Hospital, Fudan University, Shanghai, China. Four patients were excluded as they previously received radiotherapy and/or chemotherapy. Furthermore, another 32 patients with poor quality and/or quantity of tissue samples (n=11), incomplete clinical data (n=9), died of other causes originally (n=4) and those who had low-grade malignant tumors including mucoepidermoid carcinomas or carcinoids (n = 8) were also excluded. The remaining 165 patients comprised the subjects of this study. Most of these patients were received post-surgical adjuvant chemotherapy with a cisplatin-based regime, according to the treatment guideline for NSCLC at the surgical time. Participants involved in

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this research, provided their written informed consent according to the principles expressed in the Declaration of Helsinki. Medical Ethics and Human Clinical Trial Committee at Zhongshan Hospital, Fudan University has approved this study as well as the consent procedure.

Epidemiologic and clinical data collection

Patients' clinicopathological information, including gender, age, smoking status, tumor stage, nodal status, TNM stage, histological grade and extent of resection (single-lobe lobectomy versus more extensive resection; bilobectomy or pneumonectomy) was obtained retrospectively from in-person interview at the time of initial visit, follow-up in the clinics, clinical records and pathological reports. An individual who smoked more than 100 cigarettes in history was defined as an ever smoker, otherwise as a never smoker (Travis, 2004).

Patients' follow-up

Preoperative evaluation included physical examination, chest x-array, computed tomography (CT) of the chest and abdomen, bone scintigraphy, brain magnetic resonance imaging (MRI) and blood test. Most patients were postoperatively evaluated by physical examination, chest x-array, CT of the chest and abdomen, bone scintigraphy and brain MRI to confirm relapse. In some patients, we used PET-CT to detect recurrence. In our study, the TNM status was redetermined according to the 7th edition staging system for NSCLC (Sobin et al., 2009).

Recurrence was differentiated from second primary lung tumors by a multidisciplinary tumour board (MDT) review of available imaging and pathology results. Several aspects were used to differentiate second primary tumors from local recurrences, including the interval between the occurrence of the first and second primary tumors and the location of the new lesion (Martini et al., 1995). The first evidence of recurrence on imaging was used to define the sites and time of initial recurrence.

Follow-up information on patient recurrence was updated at 3-month intervals through in-patient visit, direct calling, or medical chart review. The latest follow-up in this study was carried out on May 2012.

Immunohistochemical staining for FoxM1

An antibody against FoxM1 (Sigma Aldrich Inc., MO, USA) and a standard immunohistochemical technique were used for detecting FoxM1 expression as previously described (Liu et al., 2011). The percentage area stained positive was categorized into four groups: less than 25% tumor cells positive=0; 25% to 50% tumor cells positive= 1; 50% to 75% tumor cells positive =2; more than 75% tumor cells positive=3. The staining score was categorized into four groups as negative = 0, weak =1, moderate = 2 and intense =3 (Liu et al., 2011). Labeling score was determined by multiplying intensity score by the percentage area stained positive, which scores as 0, 1, 2, 3, 4, 6 and 9.

The staining score was categorized into two groups as weak/negative staining (score < 4) and strong staining (score ≥ 4)(Liu et al., 2011; Xia et al., 2012). The highest labeling score among the three tissue sections was entered for statistical analyses. The pathologists who performed the immunohistochemical assessment of FoxM1 were blinded to the patients' histopathologic and follow-up data.

Statistical analysis

Fisher's exact test was used to evaluate the correlation between the clinicopathological variables and the expression of FoxM1. Disease free survival (DFS) was defined as the duration from the date of surgery to the date of recurrence or the end of the follow-up. DFS analysis based on the clinicopathological variables and FoxM1 expression were plotted by Kaplan-Meier method. Multivariable analysis was performed with Cox proportional hazards regression model to examine the independent prognostic effect on DFS by adjusting for confounding factors. All tests were two-sided and P-value<0.05 was considered to be significant in all analyses. SPSS 17.0 was used to do the statistical analyses in this paper.

Results

Patients' clinical characteristics

The characteristics of the patients are summarized in Table 1. The overall follow-up durations ranged from 1 to 84 months (median, 42 months).

Relationships between FoxM1 expression and clinicopathological characteristics

A total of 84 patients had no recurrence at the end of

Table 1. Characteristics of Patients with Non-small Cell Lung Cancer

Characteristics	Patients(n=165)
Age (years)	
≤55	83(50.3%)
>55	82(49.7%)
Gender	
Male	87(52.7%)
Female	78(47.3%)
Smoking status	
Yes	88(53.3%)
No	77(46.7%)
Histology	
Adenocarcinoma	109(66.1%)
Squamous cell carcinoma	56(33.9%)
Differentiation	
Well and moderately	87(52.7%)
Poorly	78(47.3%)
TNM Stage	
I	76(46.1%)
IA	30(18.2%)
IB	46(27.9%)
II	23(13.9%)
III	66(40.0%)
Tumor stage	
T1	40(24.2%)
T2	70(42.4%)
T3	24(14.6%)
T4	31(18.8%)
Lymph node metastasis	
No	91(55.2%)
Yes	74(44.8%)

Abbreviation: n, number; TNM, tumor node metastasis; *significant

Table 2. Univariate Survival Analysis of Prognostic Factors Associated with Disease Free Survival (n=165)

PARAMETER	Hazard ratio	95% CI	P value	Median survival time (month)
Gender	0.96	0.60-1.53	0.6	male: 42 female: 53
Age	1.3	0.82-2.05	0.71	≤55: 43 >55: 24
Smoking status	0.66	0.42-1.03	0.07	No:59 Yes:30
FoxM1expression	0.41	0.25-0.68	<0.01*	weak: 45 strong: 24
Histology	1.11	0.70-1.71	0.66	AC: 64 SCC: 66
Tumor stage	0.65	0.42-1.02	0.06	T1 and T2: 45 T3 and T4: 29
Lymph node metastasis	0.37	0.24-0.58	<0.01*	No: 60 Yes: 23
TNM Stage	2.89	1.79-4.67	<0.01*	Stage I/II: 67 Stage III: 24
Differentiation	1.15	0.74-1.78	0.53	Well/moderately differentiation: 60 Poorly differentiation: 36

Abbreviation: n, number; TNM, tumor node metastasis; AC, adenocarcinoma; SCC, squamous cell carcinoma; *significant

Table 3. Multivariable Analysis of Prognostic Factors Associated with Disease Free Survival (n=165)

Variable	Chi-Square	P value	Hazard ratio	95% CI
Age	0.1	0.75	1.08	0.69-1.70
Gender	0.27	0.61	1.15	0.68-1.93
Histology	1.74	0.19	1.43	0.84-2.43
Differentiation	0	0.97	0.99	0.59-1.65
TNM Stage	7.48	0.01*	3.4	1.41-8.17
Tumor	2.88	0.09	1.65	0.93-2.92
Nodal	0.05	0.83	0.92	0.41-2.06
FoxM1expression	4.29	0.04*	1.96	1.04-3.17
Smoking status	0.01	0.94	0.98	0.6-1.51

Abbreviation: n, number; TNM, tumor node metastasis; *significant

the follow-up, 81 patients had local or distant recurrence. There were no substantial differences between cases with weak/negative or strong expression of FoxM1 in demographic and clinical parameters, including age at diagnosis, gender, year of surgery, histology and smoking status. Patients with strong FoxM1 expression had advanced TNM stage (P<0.01), larger tumors (P<0.01), and poorer differentiation (P=0.03), compared with those with weak or negative FoxM1 expression. Meanwhile, those patients with lymph node metastasis had a significantly higher expression of FoxM1 (P<0.01) compared with those patients without lymph node metastasis.

Disease free survival of non-small cell lung cancer patients with FoxM1 expression

Univariate analysis showed that lymph node stage (P<0.01), TNM stage (P<0.01) and FoxM1 expression (P<0.01), each predicted a significantly worse DFS in NSCLC patients (Table 2). Other clinicopathological factors, such as age, gender, smoking status, histology, tumor stage and differentiation were not correlated with DFS. As shown in Figure 1, a DFS analysis using the Kaplan-Meier method revealed that for all the patients, the presence of strong FoxM1 expression, lymph node

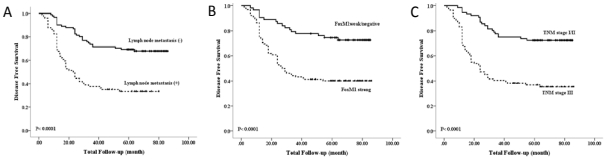

Figure 1. Kaplan-Meier Survival Curves for Disease Free Survival According to Clinicopathological Factors. (A) lymph node metastasis (No.: no metastasis= 91; metastasis= 74); (B) FoxM1 expression (No.: weak/negative expression= 63; strong expression= 102); (C) TNM stage (No.: Stage I/II= 99; Stage III= 66). Abbreviation: No., number

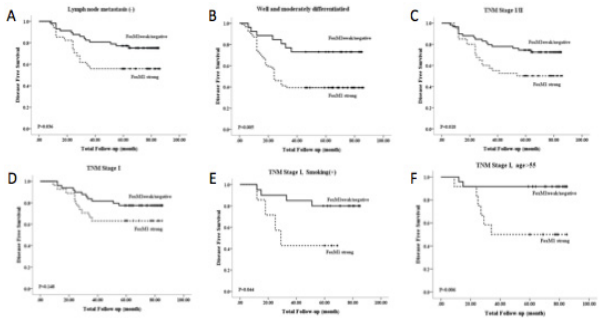

Figure 2. Kaplan-Meier Survival Curves for Disease Free Survival Subgroups According to FoxM1 Expression in Patients with (A) no lymph node metastasis (No.: w/neg= 57; strong= 34), (B) well/moderately differentiation (No.: w/neg= 26; strong= 61), (C) TNM stage I/II (No.: w/neg= 59 ; strong= 40), (D) TNM stage I (No.: w/neg= 49 ; strong= 27), (E) stage I, smoking(+) (No.: w/neg= 20; strong= 7), (F) stage I, age>55 (No.: w/neg= 24; strong= 12). Abbreviation: No., number; w/neg, weak/negative expression

metastasis, advanced TNM stage all correlated with shorter DFS.

Effect of FoxM1 expression on disease free survival with multivariable analysis

With multivariable analysis, TNM stage and FoxM1 expression were significantly associated with recurrence, after adjusting for possible confounders (age, gender, smoking status, histology, differentiation, tumor stage and lymph node metastasis) (Table 3). The crude hazard ratio (HR) of FoxM1 strong expression was nearly two times of that of FoxM1 negative or weak expression (hazard ratio= 1.96, 95% CI, 1.04-3.17, P <0.05), indicating that FoxM1 expression is an independent and significant predictor of lung cancer recurrence.

Subgroup analysis for FoxM1 expression in NSCLC recurrence

In subgroup analysis, no matter at which stage, in those cases with no lymph node metastasis, patients with strong FoxM1 expression were more likely to have recurrence after tumor resection(P=0.04), compared with those with negative/weak FoxM1 expression (Figure 2A). For those cases with well/moderately differentiation, high FoxM1 expression was also associated with poorer DFS (P=0.01) (Figure 2B). For patients with stage I/II, those with strong FoxM1 expression were inclined to have cancer recurrence (P=0.02) (Figure 2C). Although the relationship between FoxM1 expression and recurrence did not reach the significance for patients of stage I (P=0.15), it still showed

the same trend (Figure 2D). Furthermore, among the patients with stage I, the association between FoxM1 expression and recurrence was statistically significant for patients who were smoking (P=0.04) or aged over 55 (P=0 .01) (Figure 2E, 2F).

Discussion

In this study, we demonstrate that FoxM1 expression in NSCLC tumor tissues was associated with lung cancer recurrence, even after adjustment for possible mediating parameters, including clinical, tumor and other variables.

Recently, the prognosis for lung cancer patients has been improved with the development of new agents such as chemotherapeutic drugs and EGFR-targeted agents for adjuvant therapy. Despite these drugs advances, only a small proportion of lung cancer patients could benefit from these drugs and 2 year survival is still very poor (Uchida et al., 2007). Many patients receiving curative tumor resection and adjuvant therapy eventually relapse and die due to their disease. Therefore, new biomarkers for predicting recurrence and interventions aimed at decreasing recurrence in NSCLC are needed to supplement the present adjuvant therapy.

Our findings suggested that FoxM1 was associated with various clinicopathological factors in NSCLC. Some studies also showed the same results. Xia et al. found that a high level of expression of FoxM1 was significantly correlated with clinical staging (P = 0.00), lymph node metastasis (P = 0.01), and histological differentiation (P = 0.02) in pancreatic ductal adenocarcinoma (Xia et al., 2012). Chu et al revealed that high FoxM1 expression was closely correlated with the presence of lymph node metastasis, incidence of liver metastasis, and advanced TNM stage in colorectal cancer (Chu et al., 2012). But Liu et al reported that FoxM1 was only correlated with TNM stage (P=0.01), which is a little different from our findings. This may be due to their sample size is too small (Liu et al., 2011). Moreover, FoxM1 was reported to be correlated with tumor progression and metastasis (Park et al., 2011), which led to the closely relationship with prognosis. It was found to be an independent factor for predicting prognosis in variable tumors. However, no report ever determined the role of FoxM1 in cancer recurrence.

This is the first report concerning FoxM1 in the recurrence of NSCLC. We showed that FoxM1 is an independent prognostic factor for NSCLC recurrence. Therefore, FoxM1 could be used as a biomarker for recurrence in NSCLC patients and help with the therapeutic strategies for NSCLC treatment. Recent randomized controlled trials have shown the usefulness of postoperative adjuvant chemotherapy in stage IB to IIIA NSCLC patients who have undergone curative resections (Douillard et al., 2006). Though surgery alone remains the standard treatment for patients with stage IA NSCLC, our subgroup analysis showed that for those patients with stage I, who was a smoker or aged over 55, if had a strong expression of FoxM1, recurrence rate could be much higher than those with weak/negative FoxM1 expression. Thus, adjuvant therapy including chemotherapy might be of benefit. Moreover, in patients with no lymph node metastasis, no matter at which stage, higher FoxM1 expression was also related with recurrence. This association was still significant when only patients with well/moderately differentiation were evaluated. These results suggested that patients at early stage with no lymph node metastasis, or well/moderately differentiated carcinoma, if strong expression of FoxM1is confirmed, adjuvant therapy and frequent follow-up might be suggested.

Hedgehog-signaling pathway is important in tumor progression and maintenance (Javelaud et al., 2012). It was activated in the NSCLCs, and several molecules involved in this pathway, including PTCH1, SMO, GLI1, were observed to correlate with the increased expression of FoxM1 (Gibbons et al., 2009). Overexpression of FoxM1 upregulated MMP-2 expression and then contributed to the elevated migratory and invasive abilities in oral cavity squamous cell carcinoma (Chen et al., 2009), while down-regulation of FoxM1 inhibited the MMP-2, MMP-9 expression and vascular endothelial growth factor in pancreatic cancer cells (Wang et al., 2007), indicating that FoxM1 is associated with tumor metastasis as well. These lines of in vitro evidence showed that FoxM1 is associated with an aggressive behavior of tumor cells. Our current clinical evidence further supports the hypothesis that the FoxM1 level is inversely correlated with DFS of NSCLC patients. However, further studies should focus on estimating this result in a large sample size and providing the mechanistic insight into the role of FoxM1 in the pathogenesis and progress of NSCLC.

In conclusion, this study showed that FoxM1 expression in tumor tissue had clinical significance for predicting recurrence in patients with NSCLC after tumor surgery. Further studies of FoxM1 are needed to determine its potential treatment strategies in patients with NSCLC and create a composite prognostic index of NSCLC.

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References

Chen CH, Chien CY, Huang CC, et al (2009). Expression of FLJ10540 is correlated with aggressiveness of oral cavity squamous cell carcinoma by stimulating cell migration and invasion through increased FOXM1 and MMP-2 activity. *Oncogene*, **28**, 2723-37.

Chu XY, Zhu ZM, Chen LB, et al (2012). FOXM1 expression correlates with tumor invasion and a poor prognosis of colorectal cancer. Acta Histochem, 2012 Feb 10. *Acta Histochem*, **114**, 755-62.

Douillard JY, Rosell R, De Lena M, et al (2006). Adjuvant vinorelbine plus cisplatin versus observation in patients with completely resected stage IB-IIIA non-small-cell lung cancer (Adjuvant Navelbine International Trialist Association [ANITA]): a randomised controlled trial. *Lancet Oncol*, **7**, 719-27.

Gibbons DL, Lin W, Creighton CJ, et al (2009). Expression signatures of metastatic capacity in a genetic mouse model of lung adenocarcinoma. *PLoS One*, **4**, e5401.

Javelaud D, Pierrat MJ, Mauviel A (2012). Crosstalk between TGF-beta and hedgehog signaling in cancer. *FEBS Lett*, **586**, 2016-25.

Kalin TV, Wang IC, Ackerson TJ, et al (2006). Increased levels of the FoxM1 transcription factor accelerate development and progression of prostate carcinomas in both TRAMP and LADY transgenic mice. *Cancer Res*, **66**, 1712-20.

Kim IM, Ackerson T, Ramakrishna S, et al (2006). The Forkhead Box m1 transcription factor stimulates the proliferation of tumor cells during development of lung cancer. *Cancer Res,* **66**, 2153-61.

Kwok JM, Peck B, Monteiro LJ, et al (2010). FOXM1 confers acquired cisplatin resistance in breast cancer cells. *Mol Cancer Res*, **8**, 24-34.

Lagerwaard FJ, Senan S, van Meerbeeck JP, et al (2002). Has 3-D conformal radiotherapy (3D CRT) improved the local tumour control for stage I non-small cell lung cancer? *Radiother Oncol,* **63**, 151-7.

Liu YQ, Guo RH, Liu LK, et al (2011). Correlation between expression of forkhead box M1 (FOXM1) and clinicopathological features and prognosis in patients with non-small cell lung cancer (NSCLC). *Zhonghua Zhong Liu Za Zhi*, **33**, 426-30.

Martini N, Bains MS, Burt ME, et al (1995). Incidence of local recurrence and second primary tumors in resected stage I lung cancer. *J Thorac Cardiovasc Surg*, **109**, 120-9.

Park HJ, Carr JR, Wang Z, et al (2009). FoxM1, a critical regulator of oxidative stress during oncogenesis. *EMBO J*, **28**, 2908-18.

Park HJ, Gusarova G, Wang Z, et al (2011). Deregulation of FoxM1b leads to tumour metastasis. *EMBO Mol Med,* **3**, 21-34.

Rami-Porta R, Ball D, Crowley J, et al (2007). The IASLC Lung Cancer Staging Project: proposals for the revision of the T descriptors in the forthcoming (seventh) edition of the TNM classification for lung cancer. *J Thorac Oncol*, **2**, 593-602.

Rowell NP, Williams CJ (2001). Radical radiotherapy for stage I/II non-small cell lung cancer in patients not sufficiently fit for or declining surgery (medically inoperable): a systematic review. *Thorax*, **56**, 628-38.

Sobin L, Gospodarowicz M, Wittekind C (2009). TNM Classification of Malignant Tumors,TNM Classification of Malignant Tumors,. 7th Ed Geneva, UICC International Union Against Cancer, pp136-46.

Travis K (2004). Lung cancer screening for all? Not yet, panel says. *J Natl Cancer Inst,* **96**, 900-1.

Uchida A, Hirano S, Kitao H, et al (2007). Activation of downstream epidermal growth factor receptor (EGFR) signaling provides gefitinib-resistance in cells carrying EGFR mutation. *Cancer Sci*, **98**, 357-63.

Wang IC, Meliton L, Tretiakova M, et al (2008). Transgenic expression of the forkhead box M1 transcription factor induces formation of lung tumors. *Oncogene*, **27**, 4137-49.

Wang Z, Banerjee S, Kong D, et al (2007). Down-regulation of Forkhead Box M1 transcription factor leads to the inhibition of invasion and angiogenesis of pancreatic cancer cells. *Cancer Res*, **67**, 8293-300.

Xia JT, Wang H, Liang LJ, et al (2012). Overexpression of FOXM1 is associated with poor prognosis and clinicopathologic stage of pancreatic ductal adenocarcinoma. *Pancreas*, **41**, 629-35.

Xu N, Zhang X, Wang X, et al (2012). FoxM1 mediated resistance to gefitinib in non-small-cell lung cancer cells. *Acta Pharmacol Sin*, **33**, 675-81.

Yoshida Y, Wang IC, Yoder HM, et al (2007). The forkhead box M1 transcription factor contributes to the development and growth of mouse colorectal cancer. *Gastroenterology*, **132**, 1420-31.