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Original contribution

Forkhead box M1 expression in pulmonary squamous cell carcinoma: correlation with clinicopathologic features and its prognostic significance☆

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Summary Forkhead box M1 (FoxM1) transcription factor has been shown to play important roles in regulating the expression of genes that are involved in cell proliferation, differentiation, and transformation by promoting both G_1/S and G_2/M transition. Although it has been reported that the FoxM1 signaling network is frequently deregulated with an up-regulated FoxM1 expression in human malignancies, the role of FoxM1 in lung cancer remains to be determined. We performed immunohistochemical detection of FoxM1 protein in 69 tissue samples from patients with primary pulmonary squamous cell carcinoma using a tissue microarray, and Western blotting was done to confirm the immunohistochemical observations. FoxM1 immunoreactivity was observed in 26 (37.7%) of the 69 squamous cell carcinoma cases. Analysis of the FoxM1 expression in 12 squamous cell carcinoma tissues and 2 normal lung tissues by Western blotting confirmed the immunohistochemical results. A FoxM1 expression was more frequently detected in the moderately or poorly differentiated squamous cell carcinomas than in the well-differentiated squamous cell carcinomas ($P = .008$). The tumors with a positive FoxM1 expression more frequently showed lymph node metastasis ($P = .027$) and an advanced American Joint Committee on Cancer stage ($P = .049$). The Kaplan-Meier survival curves demonstrated that patients with a positive FoxM1 expression had a significantly shorter survival time than those patients with a negative FoxM1 expression ($P = .003$). The multivariate analysis revealed that the FoxM1 expression was an independent poor prognostic factor ($P = .018$). A subset of pulmonary squamous cell carcinoma with a FoxM1 expression was associated with progressive pathologic features and an aggressive clinical course.

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1. Introduction

Non–small cell lung cancer (NSCLC) constitutes 80% to 85% of all the primary lung cancer cases, and adenocarcinoma (AC) and squamous cell carcinoma (SCC) are the 2 major histopathologic subtypes of this group [1]. Although many reports have described biologic markers for NSCLC, SCC and AC have different pathogenetic pathways and distinct biologic characteristics. Consistently, gefitinib was less effective against SCC than AC, despite the fact that SCC showed higher epidermal growth factor receptor expression [2]. Therefore, to improve the poor prognosis of patients with SCC, biologic markers that can predict prognosis and response toward a specific therapy should be established in the treatment of SCC.

The development of pulmonary SCC is a multistep process that includes the gain of function mutations that activate the cell cycle-promoting Ras/mitogen-activated protein kinase signaling pathway [3]. Activated mitogen-activated protein kinase kinase has recently been shown to directly phosphorylate Forkhead box M1 (FoxM1) protein, and this contributes to its transcriptional activation [4]. The FoxM1 transcription factor has been shown to play important roles in regulating the expression of genes involved in cell proliferation, differentiation, and transformation [5].

It has recently been reported that alterations in FoxM1 signaling are associated with tumorigenesis in some cancers [6-14]. FoxM1 is ubiquitously expressed in all proliferating mammalian cells, whereas its expression is extinguished in cells that are undergoing terminal differentiation [15]. The up-regulated expression of FoxM1 prevents differentiation, and this ultimately guides undifferentiated cells toward malignant transformation [7]. Furthermore, Kim et al [9] showed that FoxM1 is essential for the proliferation and development of lung cancer in urethane-induced mouse lung tumors. Depletion of the FoxM1 levels in the A549 lung cancer cell line by short interfering RNA (siRNA) transfection caused diminished DNA replication and mitosis; it decreased the expression of the cell cycle-promoting *cyclin A2* and *cyclin B1* genes, and it reduced the anchorage-independent growth of cell colonies on soft agar. These data show that FoxM1 stimulates the proliferation of tumor cells during progression of lung cancer. Based on these published reports, we wanted to examine the role of FoxM1 in the development and proliferation of human pulmonary SCC, which is one of the most important members of NSCLC.

We performed immunohistochemical detection of FoxM1 protein in pulmonary SCC tissue samples by a using tissue microarray (TMA) to determine whether the immunohistochemical expression of FoxM1 could provide useful information as a novel therapeutic or prognostic option for treating primary pulmonary SCC. This study is the first study to critically determine the clinicopathologic roles of the expression of FoxM1 in patients with pulmonary SCC.

2. Materials and methods

2.1. Patients and tissues

Tissue samples were obtained from 69 Korean patients who underwent surgical resection for primary pulmonary SCC at Dong-A University Medical Center, Busan, South Korea, from 2000 to 2004. No preoperative chemotherapy or radiotherapy had been performed in any of these cases. Standard lobectomy and lymph node dissections were performed in every case. The cases having any other malignancies that occurred before or after the primary lung cancer were excluded from our study. At the time of performing thoracotomy, the mediastinal lymph nodes were dissected as completely as possible, and this included the ipsilateral, paratracheal, lower mediastinal, subcarinal, and N1 areas to arrive at an accurate pathologic staging. The clinical records, pathologic reports, and follow-up information were also obtained when available. The institutional review board at Dong-A University Medical Center approved our study, and written informed consent was obtained from all the patients for surgery and to use their resected samples for research. The hematoxylin and eosin–stained slides were reviewed in each case to confirm the original diagnosis, which was based on the World Health Organization criteria [16]. According to the location of the primary tumor site, the cases were classified into a central type and a peripheral type [17]. The postoperative pathologic staging was determined according to the guidelines of the American Joint Committee on Cancer (AJCC) [18].

2.2. Construction of TMA

One-millimeter cores were removed from the SCCs that had previously been formalin fixed and paraffin embedded. For all the arrays, 3 cores of different areas of the tumor were removed from each case, and these were put in a new blank recipient paraffin block in a previously described manner [19], and 4-μm-thick sections were taken for all the immunohistochemical staining. Full cross-sections from the paraffin blocks were used for 5 of the SCCs along with the adjacent normal lung tissue to confirm the staining patterns seen on the TMA.

2.3. Immunohistochemistry

Immunohistochemical staining for FoxM1 was performed on the TMA slides by using the avidin-biotin-peroxidase complex method. Deparaffinization of all sections was performed through a series of xylene baths, and rehydration was performed with a series of graded alcohol solutions. To enhance the immunoreactivity, we performed microwave antigen retrieval at 750 W for 30 minutes in citrate buffer (pH 6.0). After blocking the endogenous peroxidase activity with 5% hydrogen peroxidase for 10 minutes, incubation with the