Clinicopathological and Prognostic Significance of FOXM1 Expression in Esophageal Squamous Cell Carcinoma

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Abstract. Background: Esophageal squamous cell carcinoma (ESCC) has a poor prognosis because invasion and metastasis are prevalent. To improve diagnosis, it is important to identify and characterize tumor-specific molecular markers in ESCC. FOXM1 is overexpressed and correlates with pathogenesis in a variety of human malignancies. We aimed to investigate the clinical significance of FOXM1 overexpression in ESCC.

Patients and Methods: FOXM1 expression was assessed in ESCC specimens from 174 curatively-resected cases. The relationships between FOXM1 expression, clinicopathological parameters, and prognoses were examined. Results: Immunohistochemical analysis showed that 94 (54.0%) tumors were positive for FOXM1 expression. FOXM1 positivity did not correlate with any clinicopathological parameter. However, FOXM1-positive cases had poorer prognoses than FOXM1-negative ones (p=0.0037, log-rank test). In multivariate analysis, the following were independent prognostic factors: pT, pN, neoadjuvant chemotherapy, and FOXM1 expression (hazard ratio=1.69, 95% confidence interval=1.06-2.75, p=0.027). Conclusion: FOXM1 may be a novel prognostic factor in patients with ESCC who undergo curative resection.

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Patients and Methods. The present study included 174 patients with pathologically-confirmed primary ESCC (Table I) who underwent curative surgical resection at Osaka University Hospital between 2001 and 2007. The study population included 19 women and 155 men; the median age was 64 years (range=46 to 81 years). All patients underwent subtotal esophagectomy via right thoracotomy with two-or three-field lymphadenectomy. Non-curative resection was excluded, and curative (R0) resection was achieved for all patients. No patients died of postoperative complications. The 63 patients with lymph node metastasis at initial diagnosis received neoadjuvant chemotherapy (NAC), which consisted of two courses of 5-fluorouracil, cisplatin, and adriamycin. After surgery, patients were surveyed every three months by physical examination and serum tumor markers (squamous cell carcinoma antigen, carcinoembryonic antigen), every six months by computed tomographic scanning and abdominal ultrasonography, and every year by endoscopy until tumor recurrence. Patients with tumor recurrence received chemotherapy or chemoradiotherapy as long as they were able to tolerate it. The mean overall survival (OS) was 46.3 months, and the mean recurrence-free survival (RFS) was 42.8 months.
Immunohistochemical analysis. FOXM1 expression was evaluated by immunohistochemistry of 4-μm-thick sections of 10% formalin-fixed and paraffin-embedded tissue blocks, as described previously (12). For staining, tissue slides were de-paraffinized in xylene and then rehydrated using graded ethanol. For antigen retrieval, slides were autoclaved in 10 mM citrate buffer (pH 6.0) at 110˚C for 20 min. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 20 min. Non-specific binding was blocked with 0.1% hematoxylin. Sites of antibody binding were visualized with the ABC peroxidase detection system (Vector Laboratories, Burlingame, CA, USA). Finally, sections were incubated in 3,3'-diaminobenzidine tetrahydrochloride with 0.05% H2O2 for 1 min and counterstained with 0.1% hematoxylin. One representative slide with the deepest tumor invasion was selected from each patient and subjected to immunohistochemistry. Out of these, 94 (54.0%) were positive for FOXM1 expression (Figure 1A). Similarly, patients with FOXM1-positive tumors exhibited poorer OS than those with negative tumors (5-year OS rate was 42.8% versus 64.8%; p=0.0037; Figure 1B). In contrast, none of the samples of normal squamous epithelium exhibited substantial FOXM1 staining, although some basal cells exhibited faint nuclear immunostaining (Figure 1C). FOXM1-positive cells were detected in various parts of the tumors, including the surface, central, and deep areas of the esophagus.

Correlation between FOXM1 expression and clinicopathological parameters. Table II lists the correlations between FOXM1 expression and various clinicopathological parameters. No significant correlations were observed between FOXM1 expression and other parameters, including age, sex, histology, use of NAC, or depth of tumor invasion (Table II).

Results

FOXM1 expression in ESCC. A total of 174 samples (Table I) that contained both cancerous and non-cancerous lesions were evaluated for FOXM1 expression by immunohistochemistry. Out of these, 94 (54.0%) were positive for FOXM1 expression; staining was mainly cytoplasmic, with faint nuclear staining in tumor cells (Figure 1A). The remaining 80 (46.0%) samples were negative for FOXM1 expression (Figure 1B). In contrast, none of the samples of normal squamous epithelium exhibited substantial FOXM1 staining, although some basal cells exhibited faint nuclear immunostaining (Figure 1C). FOXM1-positive cells were detected in various parts of the tumors, including the surface, central, and deep areas of the esophagus.
Figure 1. FOXM1 expression determined by immunohistochemical staining. A: Representative FOXM1-positive esophageal squamous cell carcinoma exhibiting staining mainly in the cytoplasm of tumor cells (magnification ×200). B: Representative FOXM1-negative esophageal squamous cell carcinoma exhibiting almost no staining of tumor cells (magnification ×200). C: Representative normal squamous epithelium that was negative for FOXM1 expression except in a few basal cells (magnification ×100). Scale bars, 100 μm.
negative tumors. In univariate analysis, the following were significantly associated with OS: pT [hazard ratio (HR)=2.48, 95% confidence interval (CI)=1.56-4.05, \( p<0.0001 \)], pN (HR=3.56, 95% CI=2.01-6.93, \( p<0.0001 \)), NAC (HR=2.36, 95% CI=1.52-3.66, \( p=0.0001 \)), and FOXM1 expression (HR=1.95, 95% CI=1.24-3.15, \( p=0.0034 \)) (Table III). The four parameters that showed statistical significance (\( p<0.05 \)) in univariate analysis were entered into multivariate analysis. Multivariate analysis revealed that pN was the poorest prognostic factor (HR=2.77, 95% CI=1.54-5.42, \( p=0.0004 \)), followed by NAC (HR=1.97, 95% CI=1.26-3.10, \( p=0.0031 \)), pT (HR=1.69, 95% CI=1.06-2.75, \( p=0.027 \)) (Table III).

**Discussion**

In the present study, we investigated the expression of FOXM1 in ESCC tissues. To our knowledge, this is the largest series of samples analyzed for FOXM1 expression in ESCC to date. Our analysis revealed that FOXM1 expression in ESCC is an independent prognostic indicator for OS. This finding is consistent with previous reports (7, 11, 12, 15, 16). In our series, patients with advanced ESCC received NAC. Thus, NAC became a strong prognostic factor for OS. As far as we are aware, there is just one report on the association between FOXM1 and ESCC in clinical samples (17). In that study, Hui et al. reported that FOXM1 overexpression was associated with pathological stage, but not with prognosis of patients with
FOXM1 is a proliferation-associated transcription factor with important roles in cell proliferation, differentiation, and apoptosis (5, 6, 18). However, the mechanism by which FOXM1 signaling induces tumor growth is not well-understood. Multiple pathways cross-talk with the FOXM1 pathway, including the phosphatidylinositol 3-kinase/protein kinase B (Akt) (19, 20), nuclear factor-κB (21), sonic hedgehog (22), extracellular signal-regulated kinase (23), cyclooxygenase-2 (24), epidermal growth factor receptor (25, 26), vascular endothelial growth factor (27, 28), avian myelocytomatosis virus oncogene cellular homolog (c-MYC) (29, 30), p53 (31, 32), and hypoxia-inducible factor-1 pathways (33). Thus, these reports strongly suggest that FOXM1 is centrally-involved in tumor aggressiveness. In our analysis FOXM1 expression was associated not only with OS but also RFS, this phenomenon was consistent with these mechanisms.

Overexpression of FOXM1 in tumor cell lines is correlated with resistance to apoptosis and to premature senescence induced by oxidative stress, which is strongly implicated in resistance to chemotherapy (34). Recent studies show that FOXM1 is overexpressed in a variety of human cancer types and is crucially implicated in tumorigenesis (3, 8-10, 35, 36). Furthermore, down-regulation of FOXM1 leads to inhibition of cell growth, migration, and invasion in several cancer types (36-38). These results suggest that FOXM1 may play a crucial role in the development and progression of human cancer. Therefore, although more studies are required, inactivation of FOXM1 may represent a promising strategy for developing novel and selective anticancer therapies.

In conclusion, here we examined the expression of FOXM1 protein in ESCC specimens and investigated correlations between FOXM1 overexpression and clinicopathological characteristics. Patients that were positive for FOXM1 expression had worse prognoses. Thus, evaluation of FOXM1 expression might help identify a subset of patients with ESCC who need more intensive treatment.

References


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