Prognostic significance of FOXM1 in oral squamous cell carcinoma patients treated by docetaxel-containing regimens

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Abstract. Forkhead box protein M1 (FOXM1) is an oncoprotein that is involved in cell proliferation, differentiation and aging, and overexpression of FOXM1 is thought to be associated with the development and progression of various types of cancer. The expression of FOXM1 was retrospectively examined in tumor tissues taken from 56 oral squamous cell carcinoma (OSCC) patients by immunohistochemical staining. All of these patients received docetaxel (Doc)-containing regimens as treatments against OSCC. The association between FOXM1 expression and the clinicopathological characteristics and prognosis of these patients was then examined. FOXM1 was expressed in the nucleus and cytoplasm of OSCC tissues samples. There was a significant association between FOXM1 expression in tumor tissues and N classification (P=0.0395), stage (P=0.004), therapeutic efficacy (P=0.0113) and outcome (P=0.0134) of patients. However, FOXM1 expression had no association with patients' sex, age or T classification. Additionally, high expression of FOXM1 in tumor cells was associated with a shorter overall survival (P=0.0257) of patients. Multivariate analysis also revealed that elevated expression of FOXM1 was a predictor of patients' poor survival (P=0.0327). The results suggested that high expression of FOXM1 in OSCC tumors may result in reduced therapeutic effects and poor clinical outcomes of patients receiving Doc-based treatment regimens.

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Introduction

Oral squamous cell carcinoma (OSCC) is the most common malignant tumor of the head and neck, which represents approximately 90% of all oral neoplasms affecting the oral cavity (1). OSCC is the 8th most common cancer worldwide in terms of occurrence, which is more prevalent (approximately 4%) in men than in women (2%) (2,3). An increased incidence and prevalence of OSCC has been reported particularly in developing countries in recent years. The annual occurrence rate of oral cancer is about 300,000 worldwide (4-6), with over 11,000 new cases each year in Japan (7). Despite recent advances in surgery and chemoradiation therapies, only 50% of the OSCC patients survive 5 years after the diagnosis (8,9). OSCC typically shows poor prognosis at the advanced stage of the disease; and probably due to the heterogeneous nature of the disease, it shows differential outcomes to the same treatment. Because early diagnosis is crucial for the successful treatment of OSCC, development of promising biomarkers is necessary for its detection at an early stage (7).

Some cancers can develop resistance to a particular chemotherapy that was effective initially. Although chemoresistance can be caused by multiple mechanisms, the markers involved in the chemoresistance-related mechanisms can help to predict the response of OSCC to a certain chemotherapeutic agent. The efficacy of neoadjuvant chemotherapy (NAC) for OSCC remains to be elucidated, but it could be improved by the detection of the biomarkers related to chemoresistance. Many researchers have identified new molecular predictors and biomarkers that are useful for understanding the response of tumors to certain anticancer agents. The detection of thymidylate synthase (TS), dihydropyrimidine dehydrogenase (DPD), thymidine phosphorylase (TP) and orotate phosphoribosyltransferase (OPRT) as the predictive factors of the response to treatment with 5-fluorouracil (5-FU) is one such example (10,11).

Docetaxel (Doc) or Taxotere (N-debenzoyl-N-tart-butoxycarbonyl-10-deacetyl taxol) is a semi-synthetic taxane developed from a non-cytotoxic precursor of 10-deacetyl baccatin III obtained from the needles of the European yew tree *Taxus baccata L*. Doc is an effective drug to combat cancer and is used as a first-line treatment or as an adjuvant therapy for various cancers including OSCC (12). Doc showed a 22.2% response rate as a single-agent therapy in advanced/recurrent head and neck cancer patients (13). Doc binds with microtubules, thereby interrupting their normal function during mitosis, which

eventually causes cell death. Chemotherapeutic agents with different mechanisms of antitumor activity (e.g., 5-FU, cisplatin, etc.) than Doc are sometimes used with Doc as an effective combined chemotherapy against various types of cancers. We previously carried out a clinical trial of Doc and S-1 combination therapy against OSCC (14). Moreover, we treated patients with locally advanced OSCC with Doc-containing regimens as an NAC in our hospital, which showed promising results. Recently, we carried out a microarray analysis of Doc-resistant OSCC cells established in our laboratory and identified a few genes potentially related to Doc resistance. One of those genes was Forkhead box protein M1 (FOXM1).

FOXM1, a member of the FOX family of transcription factors, is characterized by a 100-amino acid winged-helix DNA binding domain (15). FOXM1 is a human proto-oncogene that plays a key role in cell cycle progression, mitosis, differentiation and aging (16,17). Moreover, it has already been reported that overexpression of FOXM1 is related to the development and progression of various cancers, and it is often associated with a poor prognosis and poor outcome in patients (18-20). Furthermore, FOXM1 amplification is reported to be responsible for gefitinib-resistance in non-small cell lung cancer (NSCLC) and for acquired resistance of herceptin and paclitaxel in breast cancer (21,22). Therefore, FOXM1 may be closely associated with the resistance of cancers cells to various chemotherapeutic agents including Doc. Several studies have reported the association between Doc resistance and high expression of FOXM1 in different cancer types (20,23,24). However, the relationship between high expression of FOXM1 and Doc resistance in OSCC is still unknown. We have to clarify further whether FOXM1 expression can be clinically used as a predictive factor for the response of OSCC patients to Doc-containing chemotherapies.

In the present study, we tried to examine the potential value of FOXM1 as a prognostic factor for OSCC patients receiving a Doc-containing chemotherapy.

Materials and methods

Patients and specimens. In the present study, we retrospectively used tissue samples taken from a total of 56 patients with OSCC who visited Yamaguchi University Hospital between August 2004 and September 2012. Most of these patients were in stage II or III of OSCC and were not diagnosed with distant metastasis at the first visit to our hospital. All patients had a diagnosis of squamous cell carcinoma and had not been treated for OSCC previously. The clinicopathological characteristics of the patients are shown in Table I. All patients received Doc 40-50 mg/m^2 by superselective intra-arterial infusion on day 1 and S-1 65 mg/m^2 or tegafur/uracil (UFT) 300-400 mg/body on day 1-14 (14 days). Surgical operation was carried out 1-2 weeks after the administration of the combination chemotherapy mentioned above. Tissue specimens were collected from all 56 patients by biopsy before they received any treatment. We performed a surgical operation when the tumor was resectable. However, we selected this chemotherapy with Doc for the patients who had a hope of functional preservation (limited operation) or a refusal of extended surgery after discussion with patients. So, the potential for selection bias of patients is unavoidable. This study was conducted according to

Table I. Patient characteristics (n=56).

Characteristics	No. of patients, n (%)
Sex	
Male	34 (60.7)
Female	22 (39.3)
T classification	
1	6 (10.7)
2	29 (51.8)
3	13 (23.2)
4	8 (14.3)
N classification	
0	46 (82.1)
1	5 (8.9)
2	4 (7.1)
3	1 (1.8)
Stage	
I	5 (8.9)
II	29 (51.8)
III	12 (21.4)
IV	10 (17.9)
Tumor differentiation	
Well	38 (67.8)
Moderately	15 (26.8)
Poorly	(3) 5.4
Therapeutic effect	
CR	11 (19.6)
PR	37 (66.1)
SD	8 (14.3)
Outcome	
Alive	47 (83.9)
Death	9 (16.1)
FOXM1 expression in tumor cell cytoplasm	
Low	35 (62.5)
High	21 (37.5)
Age (years)	Median=67; Min-max=30-83

FOXM1, Forkhead box protein M1; CR, complete response; PR, partial response; SD, stable disease.

the ethical standards of the Institutional Review Board (IRB) of Yamaguchi University Hospital.

Immunohistochemical staining and evaluation. Tissue specimens were fixed in phosphate-buffered 10% formalin, embedded in paraffin, and 4 μm-thick tissue sections were prepared. These tissue sections were deparaffinized in xylene and rehydrated in graded ethanol (70-100%). After washing with phosphate buffered saline (PBS), the sections were microwaved in an antigen retrieval solution and allowed to cool down gradually. After immersion of slides for 30 min

Table II. Associations between FOXM1 expression and clinicopathological factors in oral squamous cell carcinoma treated patients with a docetaxel-containing regimen.

| Characteristics | FOXM1 expression in tumor cells | | | P-value |
	Low expression, (n=35, 62.5%)	High expression (n=21, 37.5%)	Total (n=56)	
Sex				0.7614
Male	21	13	34	
Female	14	8	22	
Age (years)				0.5307
≥65	19	14	33	
<65	16	7	23	
T classification				0.0539
T1+T2	25	7	32	
T3+T4	10	14	24	
N classification				0.0395
0	30	11	41	
N1+N2+N3	5	10	15	
Stage				0.0113
I+II	23	4	27	
III+IV	12	17	29	
Tumor differentiation				0.0523
Well	27	11	38	
Moderately+Poorly	8	10	18	
Therapeutic efficacy				0.004
CR+PR	34	14	48	
SD	1	7	8	
Outcome				0.0134
Alive	34	13	47	
Death	1	8	9	

FOXM1, Forkhead box protein M1; CR, complete response; PR, partial response; SD, stable disease.

in methanol containing 0.3% H_2O_2 at room temperature, the sections were washed again in PBS. A Dako REAL™ Peroxidase-Blocking Solution (S2023, Dako; Agilent Technologies GmbH, Waldbronn, Germany) was used for 15 min as a blocking reagent to reduce nonspecific binding. Then the sections were incubated overnight at 4˚C with anti-FOXM1 rabbit polyclonal antibody (1:250; ab137647, Abcam, Cambridge, UK). After washing in PBS, a secondary antibody solution (EnVision+ System HRP; Dako; Agilent Technologies GmbH) was applied for 60 min at room temperature, and the sections were incubated with diaminobenzidine using a REAL™ EnVision™ Detection System kit (K5007, Dako; Agilent Technologies GmbH). After a tap-water wash, the sections were lightly counterstained with hematoxylin (Muto Pure Chemicals, Tokyo, Japan), immersed in graded alcohol (70-100%) and xylene and finally mounted and cover slipped. In the case of negative controls, the slides were incubated without any FOXM1 antibody.

We evaluated FOXM1 expression as the mean percentage of positive tumor cells observed in at least five random fields of each section at x400 magnification. The intensity of the FOXM1-immunoreaction was scored as follows:

1+, weak; 2+, moderate; and 3+, intense. The final score or the FOXM1-immunohistochemical staining score was calculated by multiplying the percentage of positive tumor cells with the staining intensity (25). High expression was defined as a score of ≥111.7 (the highest score for normal tissue including a dysplastic lesion), and low expression was defined as a score of <111.7. All the specimens were evaluated by three authors (KH, TF and YU), who had no knowledge of the patient's clinical status. The tissue samples were also stained with hematoxylin and eosin (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for histological evaluation.

Statistical analysis. Fisher's exact test was used to estimate the associations between FOXM1 and different clinicopathological parameters of patients. The Kaplan-Meier method was used to calculate overall survival (OS), and the log-rank test was used to compare between different groups. Univariate and multivariate analyses were performed using the Cox proportional hazards model. P<0.05 was considered to indicate a statistically significant difference. Statistical analyses were performed using the StatView software (version 5.0J; SAS Institute, Inc., Cary, NC, USA).
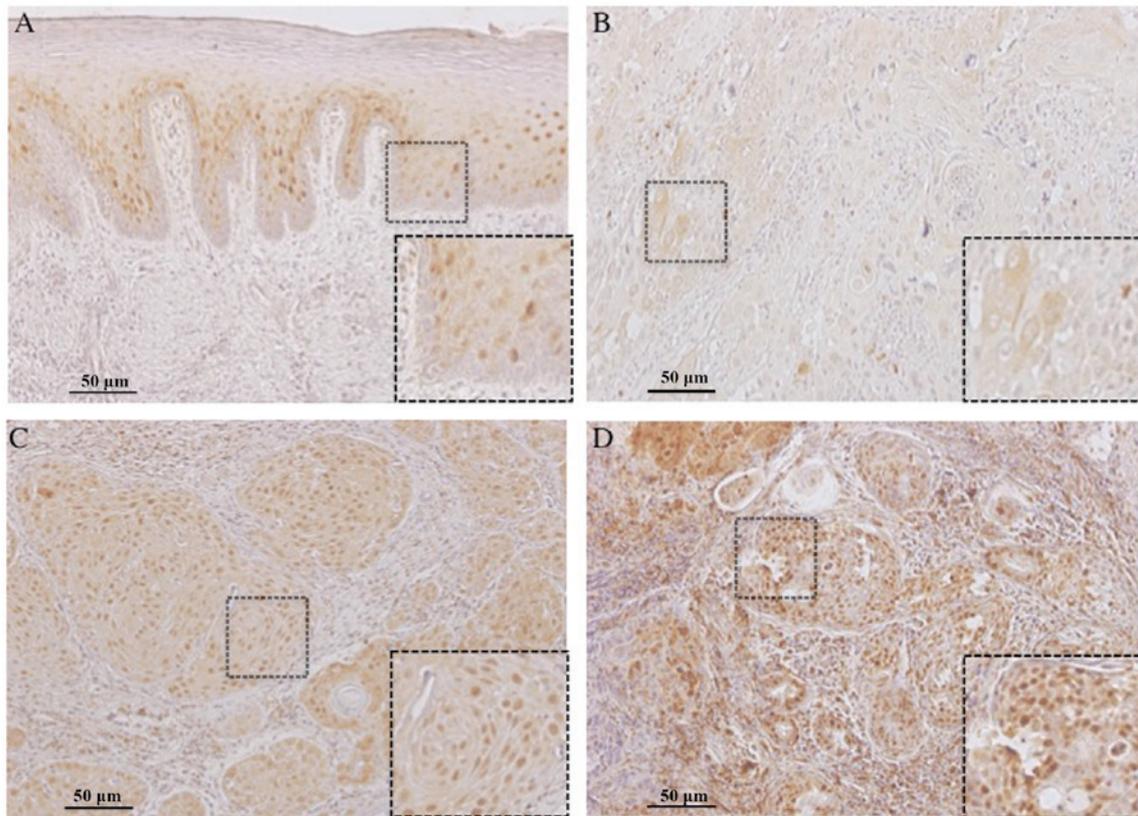
Figure 1. FOXM1 expression in normal tissues and OSCC tumors detected by immunohistochemistry. (A) A dysplastic lesion is visible, and FOXM1 expression is observed in the nucleus of the dysplastic lesion. (B) Low expression of FOXM1 in OSCC tissues. (C) Moderate expression of FOXM1 in OSCC. (D) High expression of FOXM1 in OSCC. A weak to strong positive immunoreaction was detected in the cytoplasm and nucleus of OSCC cells. Scale bars, 50 μm. FOXM1, Forkhead box protein M1; OSCC, oral squamous cell carcinoma.

Results

Patients and tumor characteristics. Table I summarizes the clinicopathological data of 56 OSCC patients who participated in this study. All of the patients were treated with a Doc-containing regimen. The median follow-up time was 8.6 years, and the median age was 67 years (range 30-83 years). Clinical stages I, II, III and IV were diagnosed in 5, 29, 12 and 10 patients, respectively. All tissue specimens were collected before the primary treatment, and there were adequate histologic materials available for immunohistochemical analysis of those patients.

FOXM1 expression in tumor cells and clinicopathological features. Table II summarized the association between the status of FOXM1 expression and the clinicopathological characteristics of the patients. FOXM1 expression was observed in the nucleus of normal oral tissues adjacent to tumors and both in the cytoplasm and nucleus of OSCC tumors (Fig. 1). In the case of normal tissues adjacent to tumors, the immunohistochemistry scores for FOXM1 ranged from 24.2 to 111.7 (mean, 74.2). The FOXM1 expression level in primary OSCCs ranged from 18.7 to 217.6 (mean, 98.7), which was significantly higher than those in normal oral tissues (P<0.001, Fig. 2). Among 56 patients with OSCC, 35 patients (62.5%) showed low expression (<111.7) of FOXM1 and 21 patients (37.5%) showed high expression (≥111.7) (Table II). No correlation was found between FOXM1 expression and
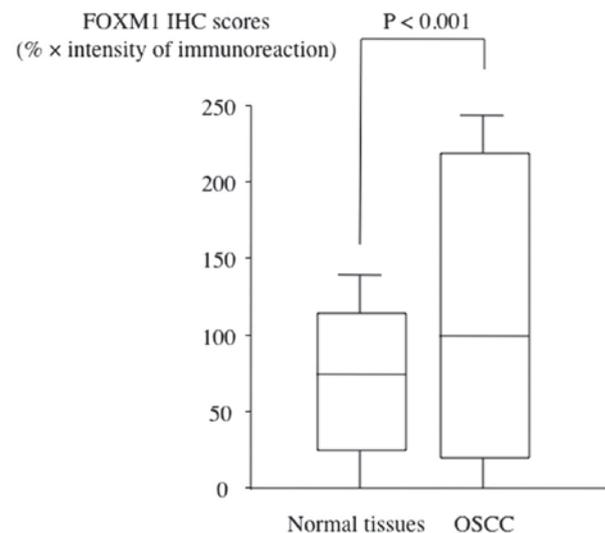
Figure 2. FOXM1 expression pattern in normal and dysplastic tissues (n=56), and OSCC (n=56) tumors. A significantly higher expression of FOXM1 was observed in OSCC tumors when compared with normal oral tissues (P<0.001, as indicated). The results are presented as the mean ± standard deviation. IHC, immunohistochemistry; FOXM1, Forkhead box protein M1; OSCC, oral squamous cell carcinoma.

sex, age, T classification or tumor differentiation of OSCC patients. However, a significant association was observed between FOXM1 expression and N classification (P=0.0395),
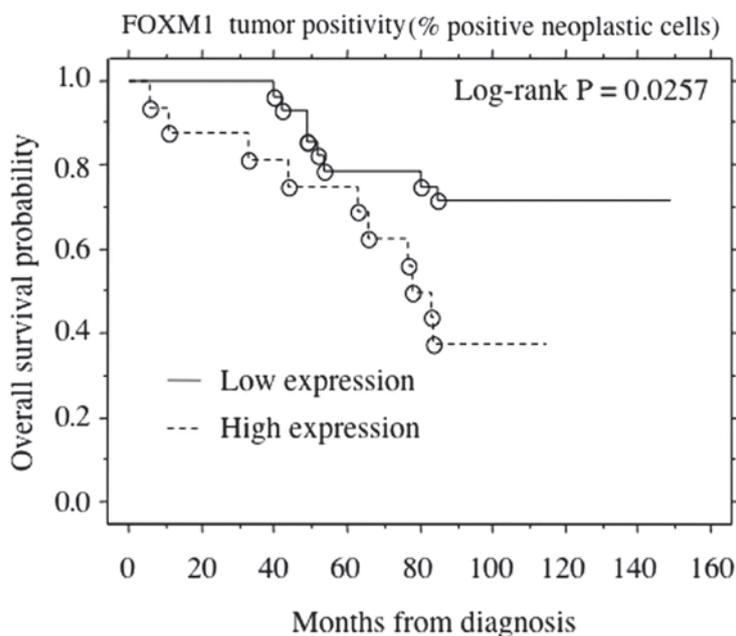
Figure 3. Correlation between OS and FOXM1 expression in OSCC. Kaplan-Meier curves revealed a significant association between high expression of FOXM1 in tumor cells and a shorter OS (log-rank; P=0.0257). OS, overall survival; FOXM1, Forkhead box protein M1; OSCC, oral squamous cell carcinoma.

therapeutic efficacy (P=0.0040), stage (P=0.0113) and patient outcome (P=0.0134; Table II).

FOXM1 expression and survival time. A total of 47 patients survived, and 9 patients died during the study period with a median follow-up time of 8.6 years. The relationship between FOXM1 expression and patients' OS was analyzed by a Kaplan-Meier curve. There was a significant association between high expression of FOXM1 in tumor cells and shorter OS (P=0.0257; Fig. 3). Moreover, a Cox proportional hazards model was applied to estimate the effect of FOXM1 expression on OSCC patient survival. A univariate Cox regression analysis identified T classification, stage, tumor differentiation, therapeutic effect and the expression of FOXM1 as significant prognostic factors. Using a multivariate analysis, T classification, therapeutic effect and the expression of FOXM1 were found to be independent prognostic factors for overall survival (Table III). Collectively, the results indicate that FOXM1 expression may act as an independent predictor for poor patient prognosis.

Discussion

FOXM1 has vital roles in adult tissue homeostasis, cell proliferation, cell differentiation, apoptosis and aging as well as in the pathogenesis of human cancers (16,17,26). Normal cells show a lower expression of FOXM1 than cancer cells. Dysfunction of FOXM1 inhibits cell differentiation, which may finally lead to the malignant transformation of undifferentiated cells (27). Moreover, upregulated expression of FOXM1 has been observed in a number of cancers including hepatocellular carcinoma, breast cancer, non-small cell lung carcinomas and glioblastomas as well as prostate, cervical and gastric cancer (21,28-33). Recent studies have strongly suggested that overexpression of FOXM1 could be correlated

with cancer progression and might be a potential prognostic biomarker for cancer patients (28-33). In addition, the prognostic significance of FOXM1 expression is clearly seen in various cancers including renal, liver, pancreatic and lung cancer, by using The Cancer Genome Atlas or The Human Protein Atlas. These days, Doc, a semisynthetic taxane drug with a notable anticancer effect, has been extensively used to treat various cancers. However, acquired resistance to Doc is one of the major obstacles to the application of Doc containing regimens to treat cancers. It was reported that FOXM1 is related to Doc resistance in several cancer types; nevertheless, few studies have investigated the association between FOXM1 and Doc resistance (20,23,24).

In gastric cancer, FOXM1 is reported to mediate Doc-resistance by upregulating the microtubule-destabilizing protein stathmin (23). Okada *et al* (20) also reported the relationship between FOXM1 overexpression and Doc chemoresistance in gastric cancer cells. Moreover, FOXM1 expression is also associated with paclitaxel resistance in several cancers (26,34-36). However, until now, no link between FOXM1 expression and Doc resistance has been reported in OSCC.

In recent years, we have treated OSCC patients with a Doc-containing regimen as an NAC (Doc plus S-1 or UFT) in our hospital, but the number of patients in each trial was small. In the present study, we aimed to investigate the usefulness of FOXM1 in predicting the response of these 56 OSCC patients to an NAC with a Doc-containing regimen.

In this study, upregulated expression of FOXM1 was detected in OSCC cells compared to normal tissues (Fig. 2). Moreover, overexpression of FOXM1 was significantly associated with therapeutic efficacy, N classification and stage, patient outcome (Table II) and shorter OS (Fig. 3). We also observed that OSCC patients with low expression of FOXM1 responded well (CR or PR) to NAC treatments with a Doc-containing

Table III. Univariate and multivariate analysis of overall survival.

Variables	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
Sex						
Male vs. Female	1.074	0.573-1.984	0.9746			
Age (years)						
>65 vs. <65	1.172	0.648-1.867	0.6894			
T classification						
T3+T4 vs. T1+T2	2.687	1.056-6.834	0.0379	1.874	1.014-2.931	0.0463
N classification						
N0 vs. N1+N2+N3	0.99	0.353-2.788	0.9847			
Stage						
Stage III+IV vs. Stage I+II	2.808	1.051-7.501	0.0394	2.576	0.745-6.945	0.0871
Tumor differentiation						
Well vs. Moderately+Poorly	0.174	0.012-0.928	0.0427	0.132	0.010-0.847	0.1678
Effect						
CR+PR vs. NC	0.111	0.036-0.345	0.0001	0.105	0.021-0.282	0.0354
FOXM1 expression						
High vs. Low	2.765	1.087-7.033	0.0327	1.867	0.946-5.393	0.0472

CI, confidence interval; FOXM1, Forkhead box protein M1; CR, complete response; PR, partial response; NC, normal control.

regimen than those with high FOXM1 expression (Table II). Additionally, multivariate analysis showed that high expression of FOXM1 was a predictive factor of reduced survival (P=0.0327) (Table III). These findings suggest that high expression of FOXM1 might be associated with Doc resistance and poor prognosis in OSCC. Thus, examining the FOXM1 expression pattern in biopsy samples might help to determine the most effective treatment strategies for OSCC patients.

FOXM1 promotes drug resistance in cancers by targeting and mediating several molecules [e.g., XIAP, survivin, nibrin or NBS1; kinesin-like protein (KIF) 20A; stathmin, etc.] involved in DNA repair, metastasis, cell invasion, migration and mitosis (23,36-38). It is assumed that FOXM1 and Doc might have overlapping roles in the progression of mitosis, and FOXM1 might target other molecules involved in regulation of mitosis to ensure Doc resistance (23). Therefore, identification of those molecules is essential to understand the FOXM1-mediated resistance of Doc. It was reported that agents that suppress FOXM1 expression can reverse the acquired docetaxel resistance in cancer cells. For example, proteasome inhibitor thiostrepton and cell-penetrating adenosine diphosphate ribosylation factor (ARF) peptide are reported to inhibit the FOXM1 functions that lead to the reversal of Doc resistance and reduced tumor cell proliferation *in vitro*, respectively (23,39). Therefore, the use of FOXM1 inhibitors might be promising as new anticancer therapeutics in cancer patients with elevated FOXM1 expression or Doc resistance.

In this study we showed that FOXM1 could be a potential prognostic marker for OSCC treated with a Doc-containing regimen. Molecularly targeted therapies against FOXM1 may have promising therapeutic benefits for the successful treat-

ment of cancer. Our results agree with most of the previous findings on the association between FOXM1 overexpression and patients' response to DOC based chemotherapies in different types of cancers. Further studies are needed to clarify the clinical importance of FOXM1 and to understand the detailed mechanism of Doc-resistance and FOXM1 expression in OSCC in both *in vitro* and *in vivo* models.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

KH designed the experimental study, analyzed the data and wrote the manuscript. TF carried out the immunohistochemical experiments, collected and evaluated the data and assisted with writing and revising the manuscript. HM was involved in data collection and analysis. KM collected and analyzed the data, and revised and edited the manuscript.

Ethics approval and consent to participate

The present study was approved by the ethical standards of the Institutional Review Board (IRB) of Yamaguchi University Hospital (Ref. H24-125). Due to the retrospective nature of the present study, the requirement for informed consent was waived by the IRB.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Lawoyin JO, Lawoyin DO and Aderinokun G: Intra-oral squamous cell carcinoma in Ibadan: A review of 90 cases. Afr J Med Med Sci 26: 187-188, 1997.
2. Exarchos KP, Goletsis Y and Fotiadis DI: A multiscale and multiparametric approach for modeling the progression of oral cancer. BMC Med Inform Decis Mak 12: 136, 2012.
3. Parkin DM, Bray F, Ferlay J and Pisani P: Global cancer statistics, 2002. CA Cancer J Clin 55: 74-108, 2005.
4. Rautava J, Luukkaa M, Heikinheimo K, Alin J, Grenman R and Happonen RP: Squamous cell carcinomas arising from different types of oral epithelia differ in their tumor and patient characteristics and survival. Oral Oncol 43: 911-919, 2007.
5. Funk GF, Karnell LH, Robinson RA, Zhen WK, Trask DK and Hoffman HT: Presentation, treatment, and outcome of oral cavity cancer: A National Cancer Data Base report. Head Neck 24: 165-180, 2002.
6. Mehrotra R, Singh MK, Pandya S and Singh M: The use of an oral brush biopsy without computer-assisted analysis in the evaluation of oral lesions: A study of 94 patients. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 106: 246-253, 2008.
7. Tanaka T, Tanaka M and Tanaka T: Oral carcinogenesis and oral cancer chemoprevention: A review. Pathol Res Int 2011: 431246, 2011.
8. Inagi K, Takahashi H, Okamoto M, Nakayama M, Makoshi T and Nagai H: Treatment effects in patients with squamous cell carcinoma of the oral cavity. Acta Otolaryngol Suppl: 25-29, 2002.
9. Shingaki S, Takada M, Sasai K, Bibi R, Kobayashi T, Nomura T and Saito C: Impact of lymph node metastasis on the pattern of failure and survival in oral carcinomas. Am J Surg 185: 278-284, 2003.
10. Kamoshida S, Shiogama K, Shimomura R, Inada K, Sakurai Y, Ochiai M, Matuoka H, Maeda K and Tsutsumi Y: Immunohistochemical demonstration of fluoropyrimidine-metabolizing enzymes in various types of cancer. Oncol Rep 14: 1223-1230, 2005.
11. Miyoshi Y, Uemura H, Ishiguro H, Kitamura H, Nomura N, Danenberg PV and Kubota Y: Expression of thymidylate synthase, dihydropyrimidine dehydrogenase, thymidine phosphorylase, and orotate phosphoribosyl transferase in prostate cancer. Prostate Cancer Prostatic Dis 8: 260-265, 2005.
12. Bissery MC, Guénard D, Guéritte-Voegelein F and Lavelle F: Experimental antitumor activity of taxotere (RP 56976, NSC 628503), a taxol analogue. Cancer Res 51: 4845-4852, 1991.
13. Inuyama Y, Kataura A, Togawa K, Saijo S, Satake B, Takeoda S, Konno A, Ebihara S, Sasaki Y, Kida A, et al: Late phase II clinical study of RP56976 (docetaxel) in patients with advanced/recurrent head and neck cancer. Gan To Kagaku Ryoho 26: 107-116, 1999 (In Japanese).
14. Ueyama Y, Okafuji M, Harada K, Mano T, Mihara M, Uchida K, Horinaga D and Wada N: Clinical phase I trial of S-1 in the combination with DOC using super-selective intra-arterial infusion with oral cancer. Gan To Kagaku Ryoho 36: 395-399, 2009 (In Japanese).
15. Wierstra I and Alves J: FOXM1, a typical proliferation-associated transcription factor. Biol Chem 388: 1257-1274, 2007.
16. Tuteja G and Kaestner KH: SnapShot: Forkhead transcription factors I. Cell 130: 1160, 2007.
17. Tuteja G and Kaestner KH: Forkhead transcription factors II. Cell 131: 192, 2007.
18. Liu M, Dai B, Kang SH, Ban K, Huang FJ, Lang FF, Aldape KD, Xie TX, Pelloski CE, Xie K, et al: FoxM1B is overexpressed in human glioblastomas and critically regulates the tumorigenicity of glioma cells. Cancer Res 66: 3593-3602, 2006.
19. Yau C, Wang Y, Zhang Y, Foekens JA and Benz CC: Young age, increased tumor proliferation and FOXM1 expression predict early metastatic relapse only for endocrine-dependent breast cancers. Breast Cancer Res Treat 126: 803-810, 2011.
20. Okada K, Fujiwara Y, Takahashi T, Nakamura Y, Takiguchi S, Nakajima K, Miyata H, Yamasaki M, Kurokawa Y, Mori M and Doki Y: Overexpression of forkhead box M1 transcription factor (FOXM1) is a potential prognostic marker and enhances chemoresistance for docetaxel in gastric cancer. Ann Surg Oncol 20: 1035-1043, 2013.
21. Xu N, Zhang X, Wang X, Ge HY, Wang XY, Garfield D, Yang P, Song YL and Bai CX: FoxM1 mediated resistance to gefitinib in non-small-cell lung cancer cells. Acta Pharmacol Sin 33: 675-681, 2012.
22. Carr JR, Park HJ, Wang Z, Kiefer MM and Raychaudhuri P: FoxM1 mediates resistance to herceptin and paclitaxel. Cancer Res 70: 5054-5063, 2010.
23. Li X, Yao R, Yue L, Qiu W, Qi W, Liu S, Yao Y and Liang J: FOXM1 mediates resistance to docetaxel in gastric cancer via up-regulating Stathmin. J Cell Mol Med 18: 811-823, 2014.
24. Li X, Qiu W, Liu B, Yao R, Liu S, Yao Y and Liang J: Forkhead box transcription factor 1 expression in gastric cancer: FOXM1 is a poor prognostic factor and mediates resistance to docetaxel. J Transl Med 11: 204, 2013.
25. Lok GT, Chan DW, Liu VW, Hui WW, Leung TH, Yao KM and Ngan HY: Aberrant activation of ERK/FOXM1 signaling cascade triggers the cell migration/invasion in ovarian cancer cells. PLoS One 6: e23790, 2011.
26. Laoukili J, Stahl M and Medema RH: FoxM1: At the crossroads of ageing and cancer. Biochim Biophys Acta 1775: 92-102, 2007.
27. Wang Z, Banerjee S, Kong D, Li Y and Sarkar FH: Down-regulation of Forkhead Box M1 transcription factor leads to the inhibition of invasion and angiogenesis of pancreatic cancer cells. Cancer Res 67: 8293-8300, 2007.
28. Kalinichenko VV, Major ML, Wang X, Petrovic V, Kuechle J, Yoder HM, Dennewitz MB, Shin B, Datta A, Raychaudhuri P and Costa RH: Foxm1b transcription factor is essential for development of hepatocellular carcinomas and is negatively regulated by the p19ARF tumor suppressor. Genes Dev 18: 830-850, 2004.
29. Bektas N, Haaf At, Veeck J, Wild PJ, Lüscher-Firzlaff J, Hartmann A, Knüchel R and Dahl E: Tight correlation between expression of the Forkhead transcription factor FOXM1 and HER2 in human breast cancer. BMC Cancer 8: 42, 2008.
30. Gialmanidis IP, Bravou V, Amanetopoulou SG, Varakis J, Kourea H and Papadaki H: Overexpression of hedgehog pathway molecules and FOXM1 in non-small cell lung carcinomas. Lung Cancer 66: 64-74, 2009.
31. Kalin TV, Wang IC, Ackerson TJ, Major ML, Detrisac CJ, Kalinichenko VV, Lyubimov A and Costa RH: Increased levels of the FoxM1 transcription factor accelerate development and progression of prostate carcinomas in both TRAMP and LADY transgenic mice. Cancer Res 66: 1712-1720, 2006.
32. Chan DW, Yu SY, Chiu PM, Yao KM, Liu VW, Cheung AN and Ngan HY: Over-expression of FOXM1 transcription factor is associated with cervical cancer progression and pathogenesis. J Pathol 215: 245-252, 2008.
33. Li Q, Zhang N, Jia Z, Le X, Dai B, Wei D, Huang S, Tan D and Xie K: Critical role and regulation of transcription factor FoxM1 in human gastric cancer angiogenesis and progression. Cancer Res 69: 3501-3509, 2009.

34. Wang X, Wu E, Wu J, Wang TL, Hsieh HP and Liu X: An anti-mitotic and antivascular agent BPR0L075 overcomes multidrug resistance and induces mitotic catastrophe in paclitaxel-resistant ovarian cancer cells. PLoS One 8: e65686, 2013.
35. Zhao F, Siu MK, Jiang L, Tam KF, Ngan HY, Le XF, Wong OG, Wong ES, Gomes AR, Bella L, *et al*: Overexpression of forkhead box protein M1 (FOXM1) in ovarian cancer correlates with poor patient survival and contributes to paclitaxel resistance. PLoS One 9: e113478, 2014.
36. Khongkow P, Gomes AR, Gong C, Man EP, Tsang JW, Zhao F, Monteiro LJ, Coombes RC, Medema RH, Khoo US and Lam EW: Paclitaxel targets FOXM1 to regulate KIF20A in mitotic catastrophe and breast cancer paclitaxel resistance. Oncogene 35: 990-1002, 2016.
37. Nestal de Moraes G, Delbue D, Silva KL, Robaina MC, Khongkow P, Gomes AR, Zona S, Crocamo S, Mencalha AL, Magalhães LM, *et al*: FOXM1 targets XIAP and Survivin to modulate breast cancer survival and chemoresistance. Cell Signal 27: 2496-2505, 2015.
38. Khongkow P, Karunarathna U, Khongkow M, Gong C, Gomes AR, Yagüe E, Monteiro LJ, Kongsema M, Zona S, Man EP, *et al*: FOXM1 targets NBS1 to regulate DNA damage-induced senescence and epirubicin resistance. Oncogene 33: 4144-4155, 2014.
39. Radhakrishnan SK, Bhat UG, Hughes DE, Wang IC, Costa RH and Gartel AL: Identification of a chemical inhibitor of the oncogenic transcription factor forkhead box M1. Cancer Res 66: 9731-9735, 2006.