original article

Class III β-tubulin, but not ERCC1, is a strong predictive and prognostic marker in locally advanced head and neck squamous cell carcinoma


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Background: Recent researches revealed that class III β-tubulin (TUBB3) is a prognostic marker in various tumors and role of TUBB3 in head and neck squamous cell carcinoma (HNSCC) is not defined yet. We analyzed the significance of TUBB3 expression along with p53 and ERCC1 in locally advanced HNSCC patients receiving cisplatin-based induction chemotherapy.

Materials and methods: Retrospective review of medical records at Seoul National University Hospital between 1998 and 2007 was carried out. Immunohistochemical stain of TUBB3, p53, and ERCC1 was done in paraffin-embedded tumor tissue. We assessed response to treatment, progression-free survival (PFS), overall survival (OS), and cancer-specific survival (CSS).

Results: Eighty-five patients with oropharyngeal, hypopharyngeal, and laryngeal cancers received induction chemotherapy with 5-fluorouracil (5-FU) and cisplatin (n = 55), or 5-FU, cisplatin, and docetaxel (Taxotere) (n = 30). Eighty-three received definitive treatment after induction chemotherapy, where 62 received radiotherapy and 21 received surgery. TUBB3-positive patients showed lower response rate than TUBB3-negative patients (69% versus 88%, P = 0.039). Shorter median PFS was observed in TUBB3-positive group (12 versus 47 months, P = 0.001). Shorter median OS was observed in TUBB3-positive group (30 versus 59 months, P = 0.072). TUBB3 status significantly influenced CSS (35 months versus not reached, P = 0.017). Positive p53 status was related to poorer OS and CSS. ERCC1 showed no influence on chemotherapy response, PFS, OS, and CSS.

Conclusion: TUBB3 is a predictive and prognostic marker along with well-known p53 in HNSCC patients receiving cisplatin-based induction chemotherapy. Clinical impact of ERCC1 is not evident in this setting.

Key words: β-Tubulin, ERCC1, head and neck cancer, locally advanced, prognosis

introduction

Molecular and genetic alterations are important in pathogenesis of head and neck squamous cell carcinoma (HNSCC). These molecules include epidermal growth factor receptor, cyclin D1, p16, p53, c-myc, and human papillomavirus (HPV) [1–6]. p53 and HPV are also important as prognostic markers [7–9]. In HNSCC, locally advanced disease represents an especially difficult management problem requiring multimodality therapy. Favorable physiologic outcome and prognosis with induction chemotherapy established the treatment scheme of platinum-based induction chemotherapy followed by definitive treatment with either radiotherapy (RT) or operation in locally advanced HNSCC [10]. Docetaxel in induction regimen seems to confer additional benefit in terms of response rate (RR), and improved survival with docetaxel, cisplatin, and 5-FU (DCF) chemotherapy especially in unresectable patients was shown in previous trials [11–13]. For induction chemotherapy, p53 is a single well-known pathologic predictive marker and role of HPV status is under research [14, 15].

Besides, class III β-tubulin (TUBB3) has been shown to have a role in chemotherapy resistance and it is a predictive marker in chemotherapy for various tumors. Role of TUBB3 was actively investigated in non-small-cell lung cancer (NSCLC), and it is shown that they are linked to resistance to antitubulin agents including taxanes [16, 17]. TUBB3 is also a prognostic factor in NSCLC and ovarian cancer [18, 19]. But the role of TUBB3 in HNSCC is not determined yet. Considering relatively similar treatment strategies and chemotherapeutic agents used between NSCLC and HNSCC, TUBB3 in HNSCC also can give important information on treatment planning. But we can
assume role of TUBB3 to be different in HNSCC from NSCLC considering HNSCC’s distinct pathophysiology. Recently, excision repair cross-complementation group 1 (ERCC1) is also investigated in NSCLC. It is a prognostic factor for resected NSCLC and a predictor for lack of benefit from platinum-based adjuvant chemotherapy [20, 21]. Role of ERCC1 in HNSCC is under evaluation and still controversial and there is a report suggesting that patients with low ERCC1 expression can earn benefit from induction chemotherapy [22].

We analyzed the significance of TUBB3, p53, and ERCC1 in locally advanced HNSCC patients receiving induction chemotherapy. Chemotherapy regimen was confined to 5-FU and cisplatin (FP) and modified DCF. Our aim is to identify the role of TUBB3 and ERCC1 as prognostic and predictive markers to chemotherapy along with p53, a well-known prognostic and predictive marker.

**materials and methods**

**patients and treatment plan**

All cases of adult (≥18 years) patients referred to our center from January 1998 to December 2007 diagnosed with locally advanced HNSCC and treated with induction DCF or FP chemotherapy, whose paraffin-embedded tumor tissue at diagnosis was available, were included in this retrospective study. Tumor stage ranged from II to IV according to the American Joint Committee on Cancer staging [23]. Disease location was limited to oropharynx, hypopharynx, and larynx. Nasopharyngeal carcinoma, paranasal sinus cancer, and oral cavity cancer were excluded. Data regarding patient demographics, dose intensity of chemotherapy, response to treatment, progression-free survival (PFS), overall survival (OS), and cancer-specific survival (CSS) were obtained by medical record review.

Induction regimens consisted of cycles of modified DCF and FP chemotherapy. Thirty patients received DCF comprised of docetaxel (Taxotere, Sanofi Aventis, Paris, France) (70 mg/m²) on day 1, cisplatin (40 mg/m²) on days 1 and 2 and infusional 5-FU (1200 mg/m²) on days 1, 2, and 3. Fifty-five patients received FP chemotherapy composed of infusional 5-FU (1200 mg/m²) for four consecutive days and cisplatin (40 mg/m²) for 2 days. Each regimen was repeated every 3 weeks. Chemotherapy was discontinued in patients who did not respond after two cycles. Four patients received only one cycle, whereas 13 patients received two cycles; 64 patients received three cycles and four patients received four cycles. Hematological and non-hematological adverse events were evaluated. Management of adverse events and subsequent dose reduction of chemotherapeutic agents was carried out in a conventional manner. Forty-two patients received full dose-intense chemotherapy, whereas 43 patients needed modification of dose or chemotherapy interval.

Followed by induction chemotherapy, patients received definitive treatment if possible. Operation was carried out in 21 patients and RT was applied for 62 patients. Two patients did not receive definitive treatment due to disease progression. Decision of modality of definitive treatment was derived from tumor board whose members include radio-oncologist, otorhinolaryngologist, and medical oncologist. Nineteen patients received adjuvant RT after operation on physician’s decision. Additional operation was carried out for residual disease after RT if there is a chance for cure. Patients whose disease progressed during treatment so that curative treatment cannot be applied received palliative chemotherapy of various regimens using bleomycin, methotrexate, docetaxel, 5-FU, and cisplatin or supportive care only.

Responses were classified as complete response (completely disappeared demonstrable lesion and PR as more than 30% decrease in the sum of the longest diameter for all target lesions on imaging study), partial response, stable disease SD was defined as less than a 30% reduction and less than a 20% increase in the sum of the longest diameter for all target lesions, or progressive disease PD more than 20% increase in the sum of the longest diameter for all target lesions or newly developed lesions according to RECIST [24]. PFS was defined as a period from first induction chemotherapy to documentation of disease progression or death from any cause. OS was calculated from diagnosis to death from any cause. Cancer-specific survival (CSS) was calculated from diagnosis to death related to HNSCC. The study protocol was reviewed and approved by the institutional review board of Seoul National University Hospital. The recommendations of the Declaration of Helsinki for biomedical research involving human subjects were also followed.

**immunohistochemical staining for TUBB3, p53, and ERCC1**

Immunohistochemistry (IHC) was carried out as previously described [22, 25, 26]. Eighty-five sections (4 µm) from formalin-fixed paraffin-embedded tissue were analyzed for TUBB3, p53, and ERCC1. Each paraffin section was dewaxed, followed by antigen retrieval. Antigen retrieval was carried out using 0.1 M (pH 6.0) buffer and microwaved for 15 min. Endogenous peroxidase activity was blocked using 3% peroxidase for p53. Then slides were incubated with mouse mAb for TUBB3 (Goveaux, Princeton, NJ, USA, clone TUJ1, 1 : 1200), mouse mAb for p53 (Dako, Glostrup, Denmark, clone DO7, 1 : 100), and mouse mAb for ERCC1 (GeneTex Inc., San Antonio, TX, USA clone 8F1, 1 : 300). The antibodies use and subsequent steps were carried out according to the manufacturer’s instructions. Antibody binding was detected by means of detection kit of Ultravision (Thermo scientific, Erembodegem, Belgium) for TUBB3 and ERCC1 and Vectastain Elite ABC kit (Vector laboratories, Burlingame, CA, USA) for p53. Mayer’s hematoxylin was used as the counterstain. Various normal and cancer tissue microarray blocks were included as external controls. H-score of >3 for TUBB3 and 9 for ERCC1 was considered positive [27]. p53 was considered positive if >10% of tumor cells are stained for p53.

**microscopic analysis**

Tumor staining was assessed by a trained pathologist (YKJ) who had no knowledge of patient’s clinical data under the light microscope at ×200 and ×400 magnifications. TUBB3 and ERCC1 tumor staining (cytoplasm for TUBB3 and nuclei for ERCC1) intensity was graded on a scale of 0–2 and 0–3, respectively, using adjacent nonmalignant cells as a reference. The percentage of positive tumor cells was evaluated and a proportion score was attributed for TUBB3 and ERCC1, respectively (0 if 0%, 0.5 if 1%–9%, 1 if 10%–24%, 2 if 25%–49%, 3 if 50%–74%, and 4 if ≥75%). This proportion score was then multiplied by the staining intensity to obtain a final semiquantitative H-score for TUBB3 and ERCC1 [28]. Percentage of positive tumor nuclei for p53 was assessed.

**statistical analysis**

The variables of inclusion in the model were age, gender, tumor location, stage, chemotherapy regimen, dose intensity of chemotherapy, response to induction chemotherapy, modalities of definitive treatment, PFS, CSS, and OS. Statistical analysis of 2 × 2 contingency tables of categorical variables was carried out using Pearson’s χ² test or Fisher’s exact test, as appropriate. Odds ratio with confidence interval for categorical outcome was calculated using binary logistic regression model. Median durations of PFS, OS, and CSS were calculated using the Kaplan–Meier method and comparisons between groups were made using log-rank tests. Multivariate analysis was carried out using a logistic regression model for response and Cox regression models for PFS, CSS, and OS. Factors with P values <0.15 in univariate analysis were examined with multivariate regression.
models. All statistical tests were two sided, with significance defined as \( P < 0.05 \). All analysis was carried out using SPSS for Windows Version 12.0 (SPSS Inc., Chicago, IL, USA).

**results**

**patient characteristics**

This study included two females and 83 males with a median age of 61.6 years. Twenty-seven patients had their tumor in oropharynx, 29 in hypopharynx, and 29 in larynx. Stages II, III, and IV were diagnosed in 12.9%, 28.4%, and 57.7%, respectively. Thirty patients received DCF induction regimen and 55 patients received FP regimen as induction chemotherapy (Table 1). During follow-up of median 61.2 months, 46 patients died and 43 patients had PD. Thirty three of 46 deaths were attributable to HNSCC.

**TUBB3, p53, and ERCC1 by IHC**

The expressions of TUBB3 and p53 were evaluable in 80 and 83 patients, respectively (Figure 1). Thirty-two patients had H-score ≥10 and were assumed positive for TUBB3, and 34 patients showed in >10% of tumor tissues and were assumed positive for p53. TUBB3 and p53 expressions were not different according to tumor stage, and p53 was less frequently expressed in oropharyngeal cancers compared with cancers of other locations (18.5% in oropharynx and 48.3% and 51.7% in hypopharynx and larynx, respectively, \( P = 0.025 \)). There were no other single baseline clinical factors associated with TUBB3 expression. TUBB3 and p53 expressions were not different between two induction chemotherapy regimens. TUBB3 and p53 expressions had correlation and TUBB3 expression was more frequently observed in patients with p53 expression (\( P = 0.003 \)) (Table 1).

Fifty-one patients had H-score ≥10 and were assumed positive for ERCC1, and there were no baseline clinical factors associated with ERCC1 expression. ERCC1 expression did not have correlation with p53 or TUBB3.

**correlations between RR of induction chemotherapy, PFS, OS, and CSS and expressions of TUBB3, p53, and ERCC1**

Overall RR to induction chemotherapy was 80% and the RR was higher with DCF regimen than with FP regimen (97% versus 71%, \( P = 0.004 \)). ERCC1 showed no impact on RR (\( P = 0.419 \)). Patients with TUBB3 expression had lower chance to respond to induction chemotherapy (69% versus 88%, \( P = 0.040 \)). This was true for patients who received FP chemotherapy (\( P = 0.040 \)). Impact of TUBB3 on DFP induction regimen cannot be showed because all patients assessable for TUBB3 showed response to DCF regimen. p53 expression did not show impact on RR. In multivariate analysis considering chemotherapy regimen, only TUBB3 was a negative predictive marker for RR (\( P = 0.043 \)).

Median PFS was 24.9 months, and no clinical factors predicted PFS. ERCC1 did not influence PFS (\( P = 0.275 \)). Median PFS was significantly shorter in patients with TUBB3 expression (12 versus 47 months, \( P = 0.001 \)) (Figure 2). This was true for both FP regimen group (\( P = 0.007 \)) and DCF regimen group (\( P = 0.030 \)) (Figure 3). p53 was not a predictive factor for PFS. In multivariate analysis, only TUBB3 was related to shorter PFS (\( P = 0.002 \)).

Median OS was 52.9 months, and there was no statistically significant clinical factor associated with OS. ERCC1 did not predict OS (\( P = 0.470 \)). TUBB3-positive patients showed tendency for shorter OS but this was not statistically significant (\( P = 0.072 \)). p53 expression was related to shorter OS (\( P = 0.012 \)). In multivariate analysis, only p53 was a prognostic factor for OS (\( P = 0.035 \)).

Thirteen patients died without evidence of HNSCC and regardless of HNSCC treatment. When CSS was calculated, no single clinical parameter had prognostic value. ERCC1 was not a prognostic factor (\( P = 0.889 \)). TUBB3 was a poor prognostic marker (median CSS not reached versus 35 months, \( P = 0.017 \)). TUBB3 was a prognostic marker in DCF group, but it was not a prognostic marker in FP group (\( P = 0.025 \) and \( P = 0.184 \), respectively). p53 was also a poor prognostic marker (median CSS not reached versus 35 months, \( P = 0.015 \)) (Figure 2).

Among patients with p53 expression, patients showing coexpressions of TUBB3 and p53 had shorter median CSS than patients without TUBB3 expression (27 versus 57 months, ...
In multivariate analysis, only TUBB3 was related to CSS ($P = 0.032$). No interaction was observed between p53 and TUBB3 expressions on any clinical outcomes. Dose intensity of chemotherapy had no impact on RR, PFS, OS, and CSS.

**discussion**

Role of TUBB3 and ERCC1 is under active investigation in NSCLC. They are assumed as a predictive marker for chemotherapy and a prognostic marker at the same time. Because both the molecules confer significant information on selection of chemotherapy in NSCLC, they are of particular interest. As in NSCLC, platinum and taxane are major chemotherapeutic agents in HNSCC. In this sense, we are interested in TUBB3 and ERCC1 in HNSCC. Especially, TUBB3 is of interest in HNSCC because recent clinical trend has seen increased use of taxane-based therapy [29].

The results of this study suggest that high TUBB3 expression in locally advanced HNSCC is associated with lower response to

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**Figure 1.** Representative examples of TUBB3 and ERCC1 immunostains (×400). (A) TUBB3 H-score 8. (B) TUBB H-score <4. (C) ERCC1 H-score 12. (D) ERCC1 H-score <10.

**Figure 2.** Progression-free survival (PFS) (upper) and cancer-specific survival (CSS) (lower) of patients according to class III $\beta$-tubulin (TUBB3)/p53 positive (black line) and TUBB3/p53 negative (orange line) ($P = 0.001$ and $P = 0.137$ for PFS, respectively; $P = 0.017$ and $P = 0.015$ for CSS, respectively).
induction chemotherapy, faster disease progression, and poorer survival. These findings consistently imply that TUBB3 is a poor prognostic marker and reflects aggressive tumor biology. Especially for CSS, TUBB3 is a strong predictive marker and has similar effect as p53, which is the single most important predictive histopathologic marker in HNSCC known thus far. While TUBB3 predicted RR and PFS, role of p53 was not evident for PFS and RR on the other hand.

Interesting finding in this study is the role of TUBB3 according to chemotherapy regimen, with or without docetaxel. In subgroup analysis, TUBB3 was a prognostic factor only in DCF group, and it was the same for p53. Otherwise, TUBB3 was a predictive marker for response in terms of RR and PFS in FP regimen too. To summarize, the role of TUBB3 was not limited to patients with docetaxel-containing regimen, although it is well known to be inherent in taxane resistance.

High coexpression rate of p53 and TUBB3 in this study implies a linkage between p53 and TUBB3. But additional poor prognostic impact of TUBB3 on p53-positive patients suggests TUBB3 is not mere a molecule contained in p53-related pathway but has a distinct role in poor prognosis. This is to be evaluated in further researches. Considering the different impact of p53 expression by IHC and p53 mutational status by PCR on clinical outcomes, parallel comparison of p53 mutational status and TUBB3 IHC expression is also necessary.

Although ERCC1 expression was frequently observed in HNSCC, it did not have influence on chemotherapy response, PFS, OS, and CSS. The role of ERCC1 was also tested with diverse cut-off H-score values, with no meaningful results. The true meaning of this finding requires further analysis in large number of patients.

In our study, the analysis for response mainly focused on induction chemotherapy. Because of heterogeneity in definitive treatment, impact of TUBB3 on RT could not be evaluated. The fact whether TUBB3 is associated with resistance to RT needs verification.

In conclusion, TUBB3 status should be considered in treatment of locally advanced HNSCC. TUBB3 in HNSCC is a prognostic and predictive marker for chemotherapy. Tumor biology and response to chemotherapy of TUBB3-positive HNSCC requires exact evaluation in prospective trials. And poor prognosis of TUBB3- and p53 double-positive HNSCC patients mandates trials with newer chemotherapeutic agents including targeted agents in this tumor.

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references


