Overexpression of E2F-1 Is Associated with Increased Disease-free Survival in Squamous Cell Carcinoma of the Anterior Tongue

Rhonda A. Kwong, Tuan V. Nguyen, Ronaldo J. Bova, James G. Kench, Ian E. Cole, Elizabeth A. Musgrove, Susan M. Henshall, and Robert L. Sutherland

Cancer Research Program [R. A. K., J. G. K., E. A. M., S. M. H., R. L. S.] and Bone and Mineral Research Program [T. V. N.], Garvan Institute of Medical Research, Darlinghurst, NSW 2010; Department of Otorhinolaryngology Surgery, St. Vincent’s Hospital, Darlinghurst, NSW 2010 [R. J. B., I. E. C.]; and Institute of Clinical Pathology and Medical Research, Westmead, NSW 2145 [J. G. K.], Australia

ABSTRACT

Purpose: Overexpression of E2F-1 is associated with increased invasiveness in head and neck squamous cell carcinoma cell lines in vitro, but its significance in vivo is unknown. This study sought to determine the relationship between E2F-1 and retinoblastoma protein (pRb) expression and disease outcome in squamous cell carcinoma (SCC) of the anterior tongue.

Experimental Design: pRb and E2F-1 protein expression was assessed by immunohistochemistry in a cohort of 145 patients with SCC of the anterior tongue. The outcomes examined were time to disease recurrence or death. The relationships between E2F-1 or pRb expression and outcome were assessed by univariate and multivariate Cox’s proportional hazards model, with or without clinicopathological covariates, including nodal status, disease stage, treatment status, and molecular markers (cyclin D1, p16INK4A, and Ki-67) previously measured in this cohort.

Results: On univariate analysis, increased expression of E2F-1 (>35% of positive-stained nuclei) was associated with increased disease-free survival (DFS; hazard ratio [HR]: 0.35; P = 0.04) and increased overall survival (OS; HR: 0.33; P = 0.06). Decreased expression of pRb (<50% positive nuclei) was associated with increased DFS (HR: 1.81; P = 0.06) but not with OS (P = 0.11). However, when considered simultaneously with other significant factors, i.e.

lymph node status, p16INK4A protein expression, and histopathological grade, in the multivariate Cox’s proportional hazards model, the additional contributions of E2F-1 and/or pRb expression to DFS and OS were not statistically significant.

Conclusions: These data demonstrate that in patients with SCC of the tongue, overexpression of E2F-1 is associated with increased DFS and OS. However, this association is not independent of lymph node status, tumor grade, and p16INK4A expression. Among the cell cycle-regulatory molecules studied, p16INK4A expression is the most predictive molecular marker of disease outcome.

INTRODUCTION

Head and neck cancers account for 3% of all newly diagnosed cancers, of which 90% are SCC. More than 50% of head and neck cancers arise in the oral cavity, with the majority representing SCC of the anterior tongue (1). Improvements in surgical technique, radiotherapy protocols, and chemotherapy have reduced morbidity, but locoregional recurrence rates and overall mortality have remained largely unchanged over the past 20 years (2). Regional lymph node status is currently the most important clinical predictor for DFS and OS in patients with HNSCCs. Accuracy of lymph node status as a predictor of clinical outcome is limited because variations in size and location of regional nodal enlargement, both prognostic markers of disease outcome, are unaccounted for in overall regional lymph node staging (3). This potentially limits the accuracy of lymph node status as a sole predictor of clinical outcome and highlights the need for more accurate prognostic markers.

Accrued knowledge of the biology of HNSCC has highlighted specific aberrations in several cell cycle-regulatory genes in human oral cancers. Additional characterization of these molecular defects may identify those patients at risk of an unfavorable outcome, predict the success of newer biological-based therapy, and generate improved follow-up strategies. Identifying molecular and genetic targets of prognostic significance in HNSCC may also help direct novel therapies to those patients whom it is most likely to benefit.

Dysregulation of the normal cell cycle-regulatory machinery is integral to the neoplastic process, and there is now compelling evidence implicating loss of cell cycle control in the development and progression of most human cancers (4). The cyclin D1/p16INK4A/pRb pathway is frequently deregulated in HNSCC. Alteration to this pathway through cyclin D1 overex-

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2 To whom requests for reprints should be addressed, at Cancer Research Program Garvan Institute of Medical Research 384 Victoria Street, Darlinghurst, NSW 2010, Australia. Phone: 612-9295-8322; Fax: 612-9295-8321; E-mail: r.sutherland@garvan.org.au.

3 The abbreviations used are: SCC, squamous cell carcinoma; CI, confidence interval; DFS, disease-free survival; IHC, immunohistochemistry; HNSCC, head and neck squamous cell carcinoma; HR, hazard ratio; OS, overall survival; pRb, retinoblastoma protein; r, correlation coefficient; TNM, tumor-node-metastasis.
pression and p16INK4A mutations and loss of expression have been demonstrated in tongue cancer cell lines and carcinomas (5, 6). Cyclin D1 and cyclin-dependent kinases 4 and 6 regulate the function of the pRb/E2F-1 complex leading to phosphorylation of pRb and release of E2F-1 in late G1 phase. E2F-1 then initiates transcription of genes essential for the progression of cells into S phase. Derangement of the cyclin D1/p16INK4A/pRb pathway releases the inhibitory control exerted by pRb/E2F-1 complex at the G1-S-phase checkpoint, resulting in deregulated S-phase entry and uncontrolled cell proliferation (7).

E2F-1 is a member of the E2F family of transcription factors that regulate progression through checkpoints in the cell cycle (8). E2F-1 has a dual role in cancer development having the capacity to act as both a tumor suppressor gene and an oncogene (8), e.g., E2F-1 enhances proliferation in gastrointestinal carcinomas (9) while conversely stimulating apoptosis in bladder cancers (10) independent of pRb expression (11). Over-expression of E2F-1 in HNSCC cell lines has the capacity to stimulate cell cycle reentry but is also associated with increased invasiveness (12), suggesting a role in metastasis.

Although the association between pRb expression and disease outcomes in HNSCC is controversial (13, 14), the role of E2F-1 in HNSCC remains to be elucidated. Loss of pRb in cancers of the oral cavity was associated with reduced OS in T1 stage cancers in one study (14) but with increased OS in another study involving a more diverse group of HNSCC patients (15). The aim of this study was to determine the relationship between E2F-1 or pRb expression and disease recurrence or death in a previously well-characterized cohort of patients with SCC of the anterior tongue (6).

MATERIALS AND METHODS

Patients and Tissue Samples. We identified 145 patients with primary operable SCC of the anterior tongue treated with curative intent from case records of the Departments of Head and Neck Surgery at St. Vincent’s Hospital and Westmead Hospital (Sydney, New South Wales, Australia) following Ethics Committee approval. Suitable patients included those who had primary tongue cancer and surgery as initial treatment. This cohort includes 145 of the original 148 patients previously examined for the relationship between cyclin D1 and p16INK4A expression and patient outcome (6). Clinical follow-up was recorded to a minimum of 2 years or when the patient was diagnosed with recurrence of disease. This information was obtained from medical records by consultation with the patient’s treating surgeon, and survival data were supplemented from the New South Wales State Register of Births, Deaths, or Marriages. Three patients from the original cohort (6) were omitted because of the lack of an adequate amount of invasive cancer remaining in the archival, paraffin-embedded tissue blocks.

IHC. Four-μm tissue sections were cut from formalin-fixed, paraffin-embedded specimens, which were dewaxed in xylene and rehydrated through graded alcohol concentrations. For E2F-1, unmasking was achieved using EDTA buffer (pH 8.0) solution at high pressure. Endogenous peroxidase activity was then quenched by 0.3% H2O2 treatment. The samples were processed using a Dako Autostainer with E2F-1 antibody (K5H95; Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:150 and biotinylated with 1:200 horse antimouse antibody (Vector Laboratories, Burlingame, CA). The primary antibody was visualized using Vectastain Elite ABC kit (Vector Laboratories), a secondary detection system, for 10 min. Color development was achieved using 3,3-diaminobenzidine (DAB kit; Vector Laboratories), and hematoxylin was used as a counterstain. The MCF-7 breast carcinoma cell line was used as a positive control (16), and MCF-7 cells growth arrested by treatment with the pure antiestrogen ICI 182780 were used as a negative control (Ref. 17; Fig. 1, A and B). Western blots performed on lysates from MCF-7 cells treated with ICI 182 780 for 48 h demonstrated complete loss of E2F-1 protein (data not shown).

For pRb, the slides were treated similarly, although unmasking was achieved using a low pH (pH 6.0) target-retrieval solution (Dako, Carpinteria, CA) in a waterbath at 100°C and processed with pRb antibody (14001A; PharMingen, San Diego CA) at a 1:200 dilution. The positive control for pRb staining was the MCF-7 breast carcinoma cell line, and the negative control was the MDA-MB-468 breast carcinoma cell line (Ref. 18; Fig. 1, C and D). All cell lines were obtained from American Type Culture Collection (Manassas, VA).

Scoring. Each IHC slide was scored by at least two independent blinded observers [R. A. K. (for E2F-1 and pRb), R. J. B. (for pRb), and J. G. K. (for E2F-1)]. Both pRb and E2F-1 were scored according to the percentage of positively stained tumor cell nuclei. A minimum of 12 high power fields of invasive carcinoma were assessed, scored, and averaged for each slide. For E2F-1, scores were ranked as high or low expression for >35 or ≤35% positively stained nuclei, respectively, and pRb was scored as high or low for ≥50 and <50% positively stained nuclei, respectively.

Statistical Analysis. Both univariate and multivariate analyses were performed to assess the association between E2F-1 or pRb expression and DFS and OS in relation to covariates. The covariates considered were: age; tumor stage; nodal stage; grade; pathological stage; and p16INK4A and cyclin D1 expression. Preliminary analyses indicated that the distributions of a number of covariates were highly skewed, and logarithmic transformation failed to normalize the distribution; therefore, in some covariates, the data were reduced into mutually exclusive categories. As a result, the following data were treated as categorical variables: tumor stage, nodal stage, pathological stage, and grade were each reduced into three categories according to the clinical severity of cancer; and p16INK4A protein expression and cyclin D1 were reduced to two categories, 0 or ≥1% positive nuclear staining and 0 or >10% positive nuclear staining, respectively. E2F-1 and pRb expression were analyzed as continuous and dichotomous data. The dichotomization of E2F-1 and pRb expression was based on the 75th percentile distribution and consideration of staining patterns between normal and cancer samples. Additionally, obvious differences in clinical outcome were exhibited between the groups when dichotomized at these levels. The outcome variables were assessed as time-to-event, which was defined as the difference between the time of diagnosis and the time of disease recurrence or death.

In the univariate analysis, event-free survival curves were constructed using the Kaplan-Meier method for each E2F-1 and
pRb category. The differences in survival times between the categories were compared using the two-tailed log-rank statistic. Subsequent analysis involved the use of the Cox’s proportional hazards model, with the SAS procedure of PHREG (19), to estimate the hazards ratio (and its 95% CI) associated with each risk factor and covariate.

In the multivariate analysis, all risk factors and covariates were considered simultaneously in the Cox’s proportional hazards model. In this analysis, statistically significant variables identified by the univariate analysis and, for which data were complete (n = 142), were analyzed in a multivariate model. Separate regression analyses were then performed after each covariate was added to the model until it reached maximal discriminatory power. A backward stepwise selection procedure was used to confirm the final result, with variables being removed from the model according to a partial likelihood ratio test using an entry criterion of \( P < 0.15 \).

RESULTS

Seventy percent of study subjects were males. Their mean age at diagnosis was 59 years (range: 20–87 years), with 63% of subjects being ages \( \geq 65 \) years. The majority (90%) of subjects had early stage tumors (T1 and T2), and 77% had no evidence of regional lymph node metastasis. All patients had undergone primary surgical resection, and 36% of patients had adjuvant radiotherapy (Table 1).

The average duration of follow-up was 60 months (range: 1–190 months), and the cumulative number of patient-years of follow-up was 678.9. During the follow-up period, 34 patients (or 23%) died from their disease, corresponding to an annual mortality incidence of 5.0% (95% CI: 3.3–6.7). The median OS time was 48 months, with the 25th and 75th percentiles being 23 and 80 months, respectively.

Forty-two patients (or 29%) were diagnosed with recurrence of disease, corresponding to an annual incidence of 6.7% (95% CI: 4.6–8.7). The median time to disease recurrence was 44 months, with the 25th and 75th percentiles being 17 and 77 months, respectively.

IHC staining revealed that within tumor cell nests and islands, intensity of E2F-1 staining was generally more marked in the peripheral cell layers than in the middle cellular layers where the cells that were more differentiated and keratinized.
Table 1  Clinicopathological, treatment, and outcome features of 145 patients with SCC of the anterior tongue

<table>
<thead>
<tr>
<th>Clinicopathological parameter</th>
<th>No. of patients</th>
<th>Percentage of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>43</td>
<td>29.7</td>
</tr>
<tr>
<td>Male</td>
<td>102</td>
<td>70.3</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;65</td>
<td>54</td>
<td>37.2</td>
</tr>
<tr>
<td>≥65</td>
<td>91</td>
<td>62.8</td>
</tr>
<tr>
<td>Tumor stage&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>78</td>
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<tr>
<td>II</td>
<td>53</td>
<td>36.6</td>
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<td>III</td>
<td>11</td>
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<td>3</td>
<td>2.0</td>
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<tr>
<td>Lymph node stage&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>113</td>
<td>76.6</td>
</tr>
<tr>
<td>≥N1</td>
<td>32</td>
<td>23.4</td>
</tr>
<tr>
<td>Tumor grade&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well differentiated</td>
<td>38</td>
<td>26.2</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>77</td>
<td>53.1</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>30</td>
<td>20.7</td>
</tr>
<tr>
<td>Adjuvant radiotherapy&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>52</td>
<td>36.0</td>
</tr>
<tr>
<td>No</td>
<td>93</td>
<td>64.0</td>
</tr>
<tr>
<td>Recurrence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>42</td>
<td>29.0</td>
</tr>
<tr>
<td>No</td>
<td>103</td>
<td>71.0</td>
</tr>
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<td>Died of disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>34</td>
<td>23.4</td>
</tr>
<tr>
<td>No</td>
<td>111</td>
<td>76.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> These parameters were determined by pathological analysis according to the TNM system (20).

<sup>b</sup> These parameters were determined by pathological analysis.

<sup>c</sup> All 145 patients had surgery as primary treatment.

was somewhat weaker than that observed between E2F-1 and DFS.

Apart from E2F-1, the following factors were also significantly associated with both DFS and OS: lymph node status; stage of disease; and p16<sub>INK4A</sub> protein expression. In this analysis, lymph node status and p16<sub>INK4A</sub> protein expression exerted the most pronounced effects on both OS and DFS.

When all of the risk factors were considered in the multivariate Cox’s proportional hazards model in Table 3, lymph node status, p16<sub>INK4A</sub> protein expression, and histopathological grade of disease retained their significance for both DFS and OS. Increased DFS was significantly associated with a negative nodal stage (HR: 4.6; 95% CI: 2.2–9.5), positive p16<sub>INK4A</sub> expression (HR: 0.3; 95% CI: 0.2–0.6), and each unit increase in the histopathological grade (HR: 1.9; 95% CI: 1.2–3.1). In the same analysis, for OS, the HR (and 95% CI) associated with a negative lymph node status, a positive p16<sub>INK4A</sub> expression, and each unit increase in histopathological grade was: HR = 7.9 (95% CI: 3.6–17.2); HR = 0.2 (95% CI: 0.1–0.4), and HR = 1.8 (95% CI: 1.0–3.1), respectively. In the presence of the three significant determinants, neither E2F-1 nor pRb was an independent determinant of either OS or DFS, probably because increased E2F-1 expression was correlated weakly with increased p16<sub>INK4A</sub> protein expression (r = 0.19; P = 0.06). The correlation between other variables was also investigated. The only significant correlations between the parameters listed in Table 1 was between pRb and E2F-1 (r = 0.16; P = 0.05).

DISCUSSION

E2F-1 protein expression is a predictor of DFS and OS for patients with HNSCC in the absence of concurrent clinicopathological assessment. At present, the most accurate clinical predictors for DFS and OS are the presence of nodal metastasis and clinical stage that contribute to the TNM staging system for HNSCC (20). Limitations to the predictive ability of the TNM staging system are demonstrated by varied recurrence rates within each TNM substage and additionally confounded by the fact that the majority of patients present with no nodal metastasis at the time of diagnosis (3, 21). Current predictors of DFS and OS are therefore often based on clinical perception and, thus, are subjective and qualitative. Thus, new predictive and quantitative markers would aid management of this disease. The role of E2F-1 as a predictor of disease outcome in HNSCC has not previously been reported. Our study showed that reduced DFS associated with low E2F-1 immunoreactivity. However, aberrant expression of pRb, which is functionally linked with E2F-1, did not significantly influence clinical outcome in this cohort.

The mechanism by which E2F-1 is involved in the progression and pathogenesis of HNSCC warrants additional investigation. Currently, there are no reports of aberrant E2F-1 overexpression in HNSCC. A previous study demonstrated that overexpression of E2F-1 in HNSCC cell lines, through gene transfer, was associated with an enhanced invasive phenotype when the cells were transplanted into the trachea of Scid mice (12), suggesting a potentially oncogenic role. That study also demonstrated that there was no change in the Ki-67 proliferation index or the proportion of cells in S phase in the E2F-1-
transfected cells. Similarly, in our study, no association was demonstrated between E2F-1 and Ki-67 expression (data not shown). However, we found high expression of E2F-1 was associated with improved DFS and OS, suggesting an inhibitory role for E2F-1. These data suggest that E2F-1 may have both oncogenic and tumor inhibitory effects in HNSCC carcinogenesis.

Previous experimental findings suggest that E2F-1 can function either as a tumor suppressor gene or an oncogene depending upon tissue specificity or experimental conditions (22). Overexpression of E2F-1 mRNA is found in invasive ductal carcinomas of the breast (23) and primary gastrointestinal carcinomas (9). E2F-1 protein expression is also up-regulated in small cell lung cancer, the most proliferative and aggressive

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**Table 2** Association between various patients’ characteristics and OS and DFS: results of univariate (unadjusted) Cox’s proportional hazards analysis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Unit</th>
<th>DFS HR 95% CI</th>
<th>OS HR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>+10 yr</td>
<td>1.10 0.88–1.37</td>
<td>1.06 0.83–1.34</td>
</tr>
<tr>
<td>Tumor stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+1</td>
<td>1.16 0.72–1.86</td>
<td>1.55 0.94–2.57</td>
</tr>
<tr>
<td>Nodal stage</td>
<td>0 versus (1,2,3)</td>
<td>3.84 1.90–7.79</td>
<td>6.03 2.91–12.5</td>
</tr>
<tr>
<td></td>
<td>+1</td>
<td>1.40 0.95–2.05</td>
<td>1.83 1.20–2.80</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+1</td>
<td>1.74 1.10–2.77</td>
<td>1.60 0.95–2.69</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+1</td>
<td>1.74 1.10–2.77</td>
<td>1.60 0.95–2.69</td>
</tr>
<tr>
<td>p16</td>
<td>0 versus ≥1</td>
<td>0.38 0.20–0.75</td>
<td>0.23 0.10–0.53</td>
</tr>
<tr>
<td>Cyclin D1</td>
<td>0 versus ≥1</td>
<td>1.22 0.58–2.58</td>
<td>1.18 0.51–2.75</td>
</tr>
<tr>
<td>E2F-1</td>
<td>≤35 versus &gt;35</td>
<td>0.35 0.12–0.98</td>
<td>0.33 0.10–1.10</td>
</tr>
<tr>
<td>pRb</td>
<td>≤50 versus &gt;50</td>
<td>1.81 0.96–3.41</td>
<td>1.76 0.86–3.57</td>
</tr>
</tbody>
</table>

*Boldfaced values signifies statistical significance at P <0.05 level.

These parameters were determined by pathological analysis according to the TNM system (20).

*These parameters were determined by pathological analysis.

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![Fig. 2](image-url) Kaplan Meier curves for DFS and OS of 145 patients with tongue cancer according to E2F-1 and pRb nuclear staining. A, DFS for E2F-1 nuclear staining >35% compared with ≤35%. B, OS for E2F-1 nuclear staining >35% compared with ≤35%. C, DFS for pRb nuclear staining ≥50% compared with <50%. D, OS for pRb nuclear staining ≥50% compared with <50%.
lungs, but is down-regulated in SCCs of the lung (24). However, the role of E2F-1 as a tumor suppressor protein is supported by the development of tumors in E2F-1 homozygous knockout mice. These mice have an increased incidence of tumors, including lung adenocarcinomas and reproductive tract tumors, which have high metastatic potential, and an increased growth rate and invasiveness when compared with tumors in wild-type mice (25). Moreover, overexpression of E2F-1 could arrest the growth of human gliomas in vivo (26), and low E2F-1 protein expression was associated with reduced survival in urinary bladder cancer (10), similar to our data where in our cohort, increased E2F-1 protein expression was associated with prolonged DFS and OS.

Loss of Rb appears to be a rare event in HNSCC. Inactivation of the Rb gene has been implicated in the progression of several neoplasms, including those of the esophagus, lung, urinary bladder, and liver (27). The association between pRb and disease outcome in HNSCC is controversial. Reduced pRb expression has been associated with more aggressive biological behavior and reduced survival in SCC of the oral cavity (14) and larynx (13). In contrast, other studies have demonstrated an association between overexpression of pRb compared with the adjacent normal tissue and adverse outcome in oral cancers (15). In our study, increased levels of pRb expression were observed in 40% of tongue carcinomas, consistent with findings of Pande et al. (15), but this was not significantly associated with disease outcome (P = 0.06) despite the significant cohort size and the site-specific lesion.

E2F-1 has been shown to induce apoptosis, both physiologically (28) and in response to loss of pRb function (29). Reduced expression of pRb was shown in 60% of the patient cohort and was associated with increased DFS (P = 0.06). E2F-1 gene transcription may be inhibited by high levels of pRb because of reduced unbound biologically active E2F-1 through its sequestration in pRb/E2F-1 complexes. E2F-mediated transcription is stimulated by an E2F-1 autoregulatory loop that amplifies the expression of the E2F-1 genes themselves (30). It is also known that E2F-1 binding is necessary to produce a G1-S block. Taken together, these findings suggest that reduced pRb expression through its capacity to bind E2F-1 may result in increased E2F-1 expression and induction of apoptosis, which may explain the association with improved DFS.

E2F-1 has been demonstrated to induce apoptosis independent of endogenous p53, pRb, or p16INK4A status, and its functional activation contributes to proliferation or apoptosis in a tissue-specific manner (22). Additionally, the consequence of pRb activation on E2F-1 expression and activity may also differ during tumorigenesis according to tissue type. Our results did not demonstrate a relationship between E2F-1 and pRb expression (r = 0.16; P = 0.05), suggesting that in HNSCC, the effects of E2F-1 are independent of pRb function.

This study demonstrated a weak association between increased p16INK4A protein expression and increased E2F-1 expression (P = 0.06). p16INK4A and p14ARF tumor suppressors are encoded by the ARF/INK4A locus, one of the most frequently disrupted loci in human cancers (27). The relationship between E2F-1 and the ARF/INK4A locus is complex. The INK4A-encoded protein p16INK4A modulates E2F-1 function through the cyclin D1/p16INK4A/pRb pathway, whereas the ARF-encoded protein, p14ARF, is modulated by E2F-1 at a transcriptional level (31, 32). Roles of p14ARF include stabilizing p53 and inducing p53-responsive genes, including mdm2. p14ARF is not activated directly by DNA damage but from hyperproliferative signals, which include increases in free E2F-1 (33). In addition to cell proliferation, E2F-1 can trigger cells to undergo apoptosis via p14ARF regulation (34) or from ectopic expression (35). In our study, the observed E2F-1 overexpression may be antiproliferative via interaction with the p14ARF/p53/mdm2 pathway.

The importance of E2F-1 is paramount because it links the cyclin D1/p16INK4A/pRb and p14ARF/p53/mdm2 pathways, which determine whether a cell will proliferate or undergo apoptosis, respectively. The ultimate effect of any changes to the cyclin D1/p16INK4A/pRb pathway is the deregulation of E2F-1 transcriptional activity (26). The significance of E2F-1 protein upon carcinogenesis may be masked by the dual involvement in pathways of opposing function in HNSCC. This study suggests that E2F-1 may have a tumor suppressive activity in SCC of the anterior tongue independent of pRb levels. E2F-1 activity may be preferentially directed to the induction of apoptosis via the p14ARF/p53/mdm2 pathway. Additional experiments to clarify this relationship between p14ARF and E2F-1 in HNSCC are required.

**ACKNOWLEDGMENTS**

We thank Drs Andrew Blankin, Darren Saunders, and Professor Ken Ho for critical review of the manuscript.

**REFERENCES**


**Table 3**

<table>
<thead>
<tr>
<th>Determinants of OS and DFS: results from the most parsimonious model</th>
<th>DFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit</td>
<td>HR 95% CI</td>
<td>HR 95% CI</td>
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<tr>
<td>Nodal stage&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 versus (1,2,3)</td>
<td>4.6 2.2–9.5</td>
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<tr>
<td>p16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0 versus ≥1</td>
<td>0.3 0.2–0.6</td>
</tr>
<tr>
<td>Grade&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+1</td>
<td>1.9 1.2–3.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> These parameters were determined by pathological analysis according to the TNM system (20).

<sup>b</sup> These parameters were determined by pathological analysis.
Overexpression of E2F-1 Is Associated with Increased Disease-free Survival in Squamous Cell Carcinoma of the Anterior Tongue

Rhonda A. Kwong, Tuan V. Nguyen, Ronaldo J. Bova, et al.


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