

ORIGINAL ARTICLE

Increased E2F-1 expression via tumour cell proliferation and decreased apoptosis are correlated with adverse prognosis in patients with squamous cell carcinoma of the oesophagus

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Background: The retinoblastoma (Rb) pathway, which governs cell cycle progression, is frequently genetically altered in cancer, causing deregulated expression of the E2F-1 transcription factor, which promotes DNA synthesis and cell cycle progression. Recent studies show that E2F-1 also participates in apoptosis induction in a p53 dependent or independent manner. Despite its crucial role and paradoxical effects on cell turnover, the function of E2F-1 in human cancer is unclear.

Aims: To evaluate E2F-1 expression using immunohistochemistry in 43 surgically resected oesophageal squamous cell carcinoma (OSCC) specimens.

Methods: This study analysed the association of E2F-1 with tumour cell proliferation and apoptosis and the upstream regulators modulating these processes, and its impact on patient outcome. Tumour cell proliferation and apoptosis were assessed as percentage of MIB-1 positive or apoptotic cells (MIB-1 labelling index (MI) and apoptotic index (AI)), respectively.

Results: Entire specimens showed abnormal expression of one or more upstream regulators of pRb/E2F-1. Although E2F-1 positivity was not associated with the expression of upstream regulators, it showed a linear and positive correlation with MI but not AI. Patients with high MI, low AI, or high E2F-1 positivity had significantly shorter recurrence free survival. By multivariate analysis, high MI and low AI were independently associated with recurrence free survival, but E2F-1 was not.

Conclusions: Increased cell proliferation and decreased apoptosis are associated with adverse prognosis in patients with OSCC. Although E2F-1 remains a controversial prognostic factor, its expression was closely associated with tumour cell proliferation and might influence clinical outcome, mainly via cell cycle progression.

With the recent explosion of knowledge about the cell cycle, large numbers of genes that govern its regulation have been identified and characterised. Multiple components, such as cyclins, cyclin dependent kinases (CDKs), CDK inhibitors, retinoblastoma protein (pRb), and p53 are implicated in the transition from one cell cycle phase to another. Furthermore, these proteins can physically interact with each other in a network in which the relative proportions of the antiapoptotic and proapoptotic molecules determine the sensitivity of tumour cells to cell death stimuli. For example, D-type cyclins bind and activate CDK4/CDK6, which phosphorylate pRb, and CDK inhibitors such as p16 and p21 can inhibit the function of CDKs in the cell cycle. In addition, p53 responds to different forms of cellular stress by targeting and activating genes, including the p21 gene, involved in growth arrest and cell death.¹

"E2F-1 DNA binding sites have been identified in the promoter regions of many genes involved in DNA replication or cell cycle control, and drive cells from G1 into the S phase"

The transcription factor E2F-1 is the best characterised member of the E2F family and has been identified as a key downstream target in the pRb pathway.¹⁻³ E2F-1 DNA binding sites have been identified in the promoter regions of many genes involved in DNA replication (for example, dihydrofolate reductase, thymidine kinase, and DNA polymerase α) or cell cycle control (for example, cyclin A, cyclin E, and CDK2), and drive cells from G1 into the

S phase.⁴ E2F-1 has a transactivation domain in its C-terminal region, which is responsible for binding to pRb, a member of the pocket protein family of tumour suppressors.⁵⁻⁶ Hypophosphorylated pRb binds to E2F-1 and thereby downregulates its activity, suggesting a model in which pRb restricts cell cycle progression by restraining E2F-1. Evidence has shown that transcriptional activation by E2F-1 is important in promoting cell proliferation, and that loss of pRb mediated control of E2F-1 activity leads to the progression of DNA synthesis. Furthermore, the fact that overexpression of E2F-1 results in malignant transformation of cultured cells and produces tumours in nude mice supports its putative role as an oncogene.⁷⁻⁹

Despite its clear importance in allowing cell cycle progression, several studies have also suggested a role for E2F-1 in apoptosis.¹⁰⁻¹⁵ Moreover, the role of E2F-1 as a direct tumour suppressor is supported by the observation that E2F-1 deficient mice have impaired apoptotic function and an increased incidence of cancer.¹⁶⁻¹⁷ Although several in vitro studies have demonstrated these paradoxical biological roles of E2F-1, only a few reports have discussed their impact on the prognosis of human tumours. Previous studies showed that E2F-1 overexpression is associated with worse prognosis in urothelial carcinoma of urinary bladder and squamous cell carcinoma of the tongue,¹⁸⁻¹⁹ therefore suggesting that E2F-1 acted as a tumour suppressor in these tumours.

Abbreviations: AI, apoptotic index; CDK, cyclin dependent kinase; HPV, human papillomavirus; LI, labelling index; MI, MIB-1 labelling index; OSCC, oesophageal squamous cell carcinoma; pRb, retinoblastoma protein

In contrast, some recent reports have shown a positive correlation between E2F-1 and proliferation indices, and that increased expression of E2F-1 is associated with an adverse prognosis in patients with cancers of the lung, breast, and pancreas.^{20–22} Furthermore, a recent study has shown that E2F-1 acts as a growth promoter in breast carcinoma and superficial transitional cell carcinomas of the urinary bladder, whereas it seems to act as a growth suppressor in colon and prostate cancer.¹¹ These findings imply a tissue specific effect, so that the role of E2F-1 needs to be investigated in other tumours.

In view of the crucial and paradoxical role of E2F-1 in cell turnover, we wanted to determine whether E2F-1 expression is associated with cell proliferation or apoptosis—which have been identified as prognostic indicators—in oesophageal squamous cell carcinoma (OSCC).^{23–25} Therefore, we evaluated the E2F-1 status in 43 surgically resected OSCC specimens from patients who had not been treated with chemotherapy or radiotherapy and analysed its association with tumour cell proliferation, apoptosis, and the molecules that regulate these two processes. In addition, we also analysed the clinical impact of E2F-1 status on patient outcome.

METHODS

Patients

We studied 43 patients with OSCC who had been treated between 1986 and 1999. Our study was approved by the human subjects committee of Teikyo University Ichihara Hospital, Japan, and all the patients' information was dealt with anonymously. Table 1 shows the data on age, sex, stage, and grading. This cohort comprises 43 curative resection specimens that were obtained from our previous study.²⁶ All the patients underwent transthoracic oesophagectomy, and none had received irradiation or chemotherapy before or after surgery. No distant metastases were present at the time of surgery. The tumours were classified histologically using the World Health Organisation criteria. We also classified the tumours using the TNM staging of oesophageal carcinoma. All of the surgical specimens were confirmed to have tumour free resection margins by pathological evaluation. The recurrence free survival time was from October 1986 to August 2002. At the end of the follow up period, 13 of the 43 patients were still alive. Median follow up after oesophageal resection for the nine patients censored was 1680 days (range, 414–4470). Median overall survival was 1027 days for the whole group.

Immunohistochemical staining

Immunohistochemistry was performed as described previously²⁶; antigen retrieval was achieved by microwaving in

citrate buffer (pH 7.0 for cyclin D1 and E2F-1, pH 6.0 for others). Primary monoclonal antibodies were directed against p16^{INK4a} (clone G175–405; 1/150 dilution; Pharmingen, Heidelberg, Germany), cyclin D1 (clone G124–326; 1/100 dilution; Santa-Cruz Biotechnology, Santa Cruz, California, USA), both phosphorylated and underphosphorylated pRb (clone G3–245; 1/500 dilution; Pharmingen), E2F-1 (clone KH95; 1/100 dilution; Santa-Cruz), and MIB-1 (1/100 dilution; Immunotech, Marseilles, France). We used lymphoid cells, fibroblasts, or non-neoplastic epithelial cells that showed positive nuclear staining as positive controls. For the negative controls, the primary antibodies were omitted. The p21 immunostaining data were taken from our previous study.²⁶ Two observers without knowledge of the clinical data calculated the percentage of cells showing positive nuclear staining. Five representative fields were examined for each specimen, and more than 1000 tumour cells (200 for each field) were counted by microscopy with a high power ($\times 400$) objective. The percentage of tumour cells positive for MIB-1 was recorded as the MIB-1 labelling index (MI, number of MIB-1 stained cells/100 tumour cells). We also counted the p16, pRb, p21, cyclin D1, and E2F-1 labelling indices (LIs) as the percentage of cells with positive nuclear staining for each antibody. All slides in the same batch were independently scored two or three times and all the batches were coded and scored blind at least twice. All immunostaining was interpreted in a similar manner by the two observers.

Detection and interpretation of apoptosis

The data on tumour cell apoptosis, detected by the terminal deoxynucleotidyl transferase mediated dUTP nick end labelling method, were taken from our previous study.²⁶

HPV DNA detection and mutation analysis of the p53 gene

The data concerning the presence of human papillomavirus (HPV) DNA and the HPV subtypes seen, evaluated by the polymerase chain reaction, were taken from our previous study,²⁶ as were the data on the mutational analysis of exons 5–8 of the p53 gene.²⁶

Statistical analysis

The data were analysed with the StatView 5.0 computer software package (SAS Institute Inc, Tokyo, Japan). For statistical analysis, clinicopathological variables were categorised as follows: T1/T2 versus T3/T4, N0 versus N1, stages I/II versus III/IV, grades G1 versus G2/G3, median ages ≥ 61 versus < 61 years, and female versus male. Immunohistochemical variables were categorised using a cutoff value for subsequent statistical analysis as follows: normal pRb (pRb LI, ≥ 20) versus decreased pRb (Rb LI, < 20), normal cyclin D1 (cyclin D1 LI, < 10) versus increased cyclin D1 (cyclin D1 LI, ≥ 10), normal p16 (p16 LI, ≥ 70) versus decreased p16 (p16 LI, < 70), normal p21 (p21 LI, ≥ 10) versus decreased p21 (p21 LI, < 10) expression. Similar cutoff points for these proteins have been established independently by other groups.^{25–27–28} We also categorised tumours by p53 status—normal versus abnormal (immunohistochemical overexpression or missense mutation or deletion). For intervariable assessment, the unpaired Mann-Whitney U test for continuous variables and Fisher's exact F test, where appropriate, were applied. Associations within continuous variables were examined with the non-parametric Spearman test. Survival analysis was carried out according to Kaplan-Meier methodology and Cox regression analysis. Differences at $p < 0.05$ were considered to be significant.

Table 1 Patients' clinicopathological details (n = 43)

Clinicopathological parameter	
Age (years)	Range, 37.0–83.0; median, 60
Sex (female/male)	6 (14.0%)/37 (86.0%)
Tumour depth (Tis/T1/T2/T3)	2 (4.7%)/10 (23.3%)/5 (11.6%)/26 (60.5%)
Lymph node metastasis (N0/N1)	20 (46.5%)/23 (53.5%)
Histological grade (G1/G2/G3)	12 (27.9%)/22 (51.2%)/14 (32.6%)
Stage (0/I/IIa/IIb/III)	2 (4.7%)/7 (16.3%)/11 (25.6%)/6 (14.0%)/17 (39.5%)
Additional treatment (radiotherapy or radio/chemotherapy; yes/no)	0/43 (100%)

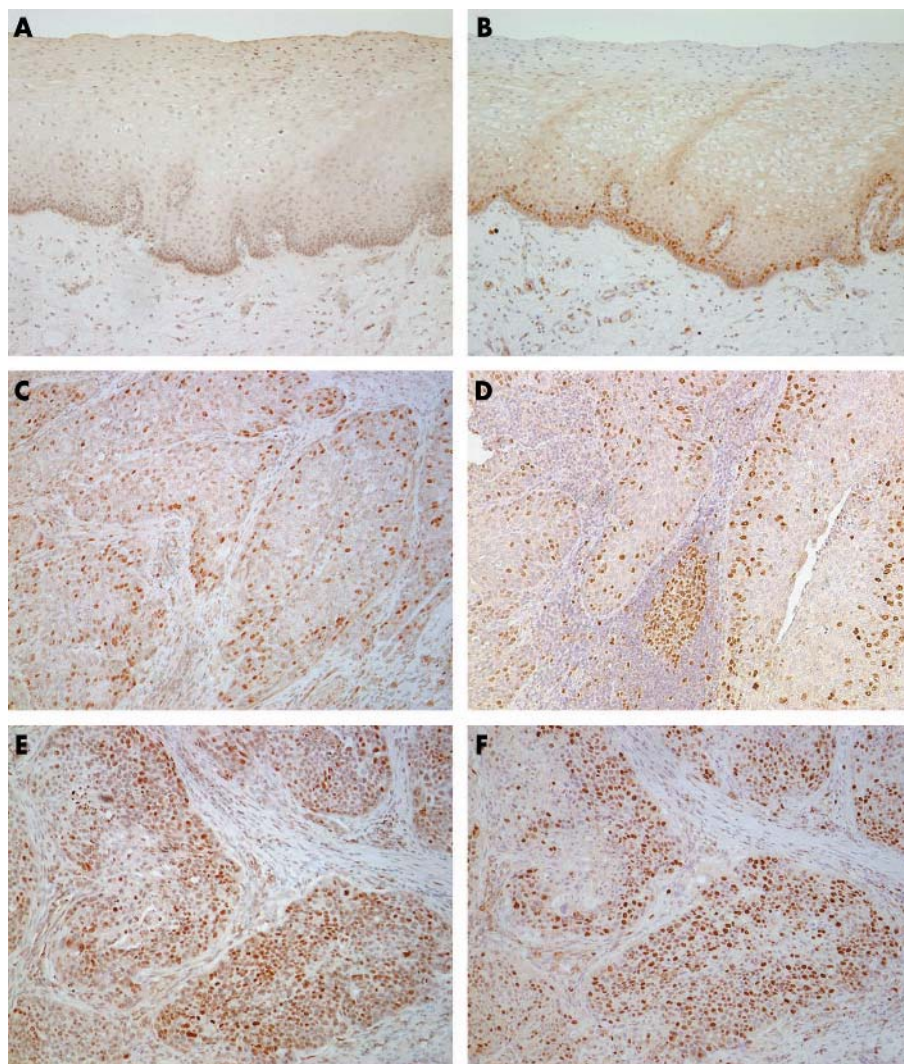


Figure 1 Representative results of E2F-1 and Ki-67 immunohistochemistry. In non-tumorous squamous epithelium, basal and parabasal cells showed (A) faint and sparse nuclear E2F-1 staining and (B) a low degree of Ki-67 positivity. Well differentiated squamous cell carcinoma showed a low degree of positivity for (C) E2F-1 and (D) Ki-67. Poorly differentiated squamous cell carcinoma showed a high degree of positivity for (E) E2F-1 and (F) Ki-67. Active stromal fibroblasts and infiltrating active lymphocytes in tumour and non-tumour specimens occasionally showed positive staining.

RESULTS

E2F-1 status and its association with the clinicopathological parameters

E2F-1 was expressed both in normal squamous epithelium and OSCC (fig 1). In normal squamous epithelium, E2F-1 staining was detected mainly in the basal and parabasal areas

and its staining pattern was faint and sparse. In OSCC, E2F-1 staining was confined mainly to the tumour cell nuclei throughout the entire specimen. Active stromal fibroblasts and infiltrating active lymphocytes in tumour and non-tumour specimens occasionally showed positive staining.

Table 2 Molecular parameters of the tumours (n = 43)

Molecular parameters	Positivity (%)	Altered	Normal
Mean (SD; median) MI	46.8 (15.8; 47)		
Mean (SD; median) AI	3.28 (2.62; 3.2)		
Mean (SD; median) E2F-1 LI	27.2 (17.1; 25)		
pRB status	47.8 (31.4)	Decreased, 6 (14.0%)	37 (86.0%)
Cyclin D1 status	23.3 (27.6)	Increased, 23 (53.5%)	20 (46.5%)
p16 status	29.6 (31.9)	Decreased, 33 (76.7%)	10 (23.3%)
p21 status	22.3 (21.8)	Decreased, 16 (37.2%)	27 (62.8%)
p53 status (overall)		Abnormal, 24 (55.8%)	19 (44.2%)
Immunohistochemistry		Increased, 14 (32.6%)	29 (67.4%)
Missense mutation/deletion		20 (46.5%)	23 (53.5%)
HPV infection status		Positive, 18 (41.9%)	Negative, 25 (58.1%)

AI, apoptotic index; HPV, human papillomavirus; LI, labelling index; MI, MIB-1 labelling index; pRb, retinoblastoma protein.

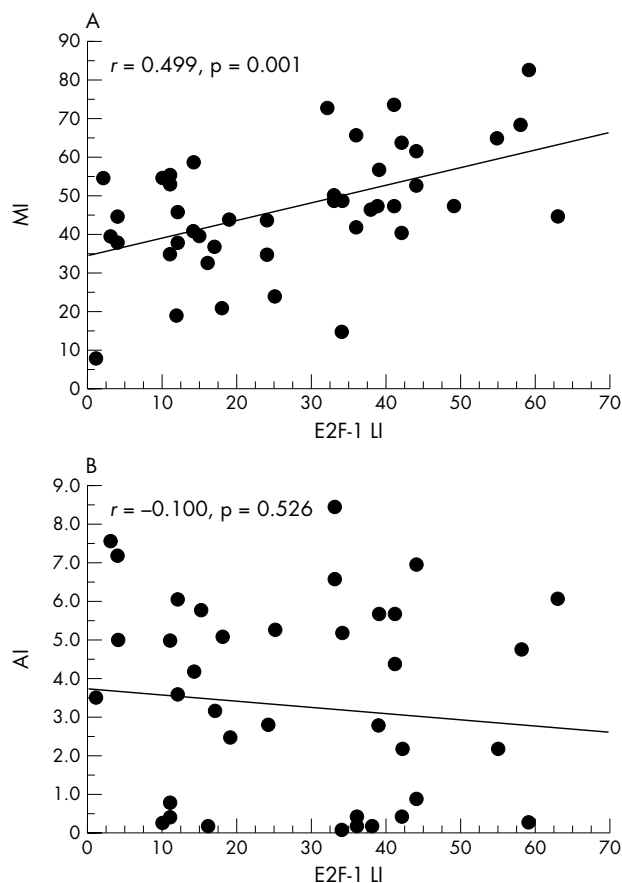


Figure 2 Scatter plots and linear regression fit lines showing the correlation between E2F-1 labelling index (LI) and (A) MIB-1 LI (MI) and (B) apoptotic index (AI). There was a significant positive correlation between E2F-1 LI and MI ($r = 0.499$, $p = 0.001$).

In the tumours, the mean E2F-1 LI was 27.2 (SD, 17.1; range, 1–63; table 2). There were no significant differences for E2F-1 status between the clinicopathological subgroups classified by histological grade, tumour depth, and lymph node involvement using the Mann-Whitney U test.

Upstream regulators of tumour cell proliferation/apoptosis and their association with clinicopathological parameters

Table 2 summarises the immunohistochemical status of upstream regulators of the cell cycle and apoptosis. Using immunohistochemistry, six cases showed decreased or negative pRB expression, 23 showed increased cyclin D1 expression, 33 showed decreased p16 expression, and 16 showed decreased p21 expression. In a previous analysis,²⁶ we detected immunohistochemical p53 overexpression in 14 cases and missense mutations or deletions in exons 5–8 of the p53 gene in 20. Overall, 24 cases showed abnormal p53 status (immunohistochemical overexpression or deletion/mutation). HPV infection was seen in 18 cases, which included 14 cases harbouring HPV-16 or HPV-18 infection. We found no significant correlation between these factors and the clinicopathological parameters using Fisher's exact F test.

The relation between E2F-1 and upstream regulators of tumour cell proliferation/apoptosis

We searched for an association between pRb, cyclin D1, p16, p21, p53, and HPV infection and E2F-1 LI by means of the Mann-Whitney U test, but no association was found.

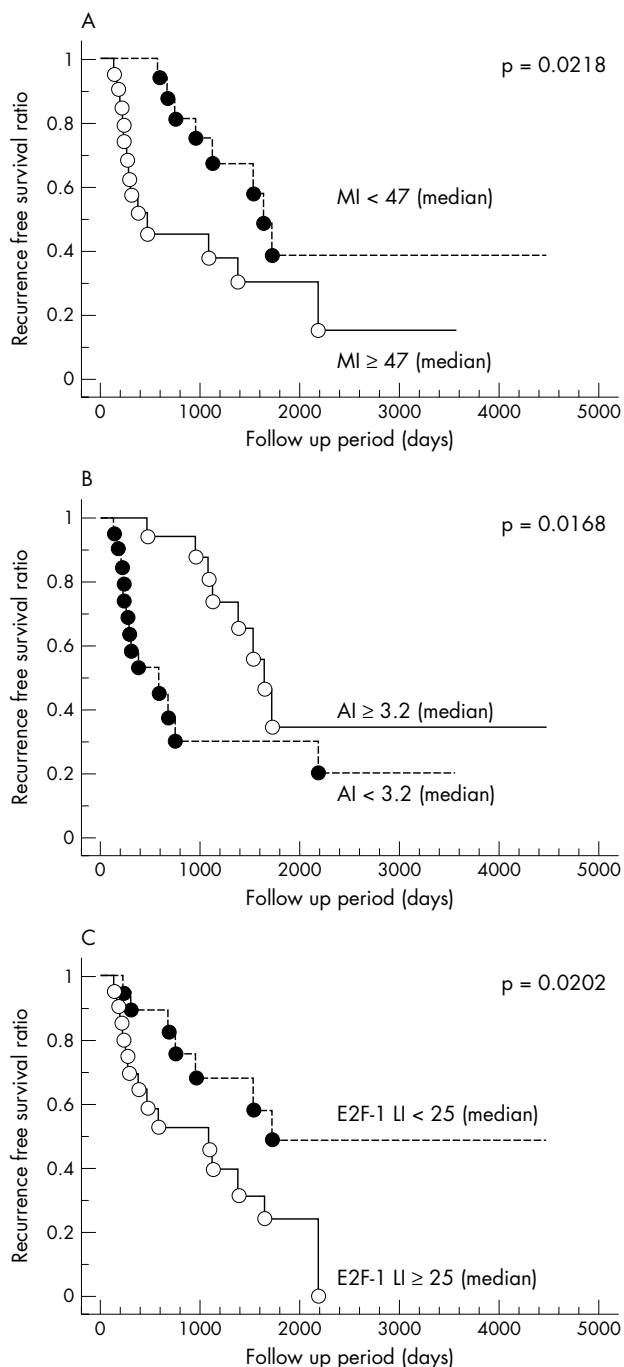


Figure 3 Kaplan-Meier postoperative survival curves of patients with oesophageal squamous cell carcinoma ($n = 43$). (A) The recurrence free survival rate of patients with high MIB-1 labelling index (MI) tumours was significantly lower than that of patients with low MI tumours ($p = 0.0218$). (B) The recurrence free survival rate of patients with low apoptotic index (AI) tumours was significantly lower than that of patients with high AI tumours ($p = 0.0168$). (C) There was also a significant association between high E2F-1 labelling index (LI) and worse clinical outcome ($p = 0.0202$).

Tumour kinetic parameters (MI and AI) and their relation to clinicopathological parameters

The median MI and AI values were 47% (range, 8–83%) and 3.2% (range, 1–8.5%), respectively (table 2). MI and AI showed no significant differences between the clinicopathological subgroups classified by histological grade, tumour depth, or lymph node involvement by Fischer's exact F test.

The relation between tumour kinetic parameters and regulators of tumour cell proliferation/apoptosis

Tumours with normal p21 expression showed a significantly higher AI ($p = 0.0083$) than those with decreased p21 expression (Mann-Whitney U test), but this was not the case for MI ($p = 0.679$). We found no significant associations between pRb, cyclin D1, p16, p53, or HPV infection and MI or AI.

The relation between tumour kinetic parameters and E2F-1 expression

Spearman's correlation efficiency test showed a strong positive correlation between E2F-1 LI and MI ($r = 0.499$, $p = 0.001$; fig 2A). No association was found between E2F-1 LI and AI ($r = -0.100$, $p = 0.526$; fig 2B).

Survival analysis

To determine the prognostic impact of each parameter, patients were stratified according to the dichotomised variables (table 3; fig 3). Univariate survival analysis showed that the median recurrence free survival of the 17 patients with pTis/T1/T2 tumours was 1385 days, whereas that of the 26 patients with pT3 tumours was only 433 days ($p = 0.0026$). The median recurrence free survival of the 20 patients who presented with stage N0 disease was 977 days, whereas that of the 23 patients with stage N1 was only 581 days ($p = 0.0007$).

For survival analysis, we categorised tumours by MI (high MI (median, ≥ 47) v low MI (< 47)), AI (high AI (median, ≥ 3.2) v low AI (< 3.2)), and E2F-1 positivity (high E2F-1 LI (median, ≥ 25) v low E2F-1 LI (< 25)). Interestingly, patients with high MI or low AI tumours showed poor overall recurrence free survival. The 21 patients with low MI tumours had a median recurrence free survival of 1102 days, whereas the 23 patients with high MI tumours had a median recurrence free survival of 291 days ($p = 0.0275$ by Cox regression and $p = 0.0218$ by Kaplan-Meier methodology; fig 3A). The median recurrence free survival of the 21 patients with high AI tumours was 1111 days, whereas that of the 22 patients with low AI tumours was only 365 days ($p = 0.0216$ by Cox regression and $p = 0.0168$ by Kaplan-Meier methodology; fig 3B). Finally, the median recurrence free survival of the 21 patients with low E2F-1 LI tumours was 956 days, whereas that of the 22 patients with high E2F-1 LI tumours was only 326 days. There was a significant association between high E2F-1 LI and poor patient outcome ($p = 0.0420$ by Cox regression and $p = 0.0202$ by

Kaplan-Meier methodology; fig 3C). However, Cox multivariate analysis showed that both MI and AI were independent prognostic factors ($p = 0.0039$ and $p = 0.0047$, respectively), but E2F-1 was not ($p = 0.2078$) (table 4). In contrast to these parameters, pRb, p16, p21, cyclin D1, and p53 status and HPV infection were not associated with patient outcome.

DISCUSSION

We found that E2F-1 expression was higher in OSCC than in the corresponding adjacent non-tumorous squamous epithelium, and tumours with high E2F-1 positivity had significantly higher growth indices as assessed by immunohistochemistry. These results suggest that the underlying mechanisms involved in E2F-1 expression are upregulated in OSCC compared with non-tumorous squamous epithelium. Recent studies have shown that E2F-1 expression in some human tumours is increased at the transcriptional level without localised gene amplification.^{4 7 8 29} Therefore, one possible explanation for our observation is that E2F-1 expression is increased in OSCC by a similar mechanism. In addition, previous in vitro experiments have shown that E2F-1 binding activity and the mRNA value fluctuate throughout the cell cycle, reaching a peak at the G1-S phase boundary.⁷ Accordingly, it can be speculated that E2F-1 positive cells defined in OSCCs are mainly in the G1-S phase as a result of upregulated progression of the cell cycle.

Although E2F-1 positivity was not associated with the expression of its upstream regulators, it showed a positive linear correlation with MIB-1 immunostaining and patients with increased E2F-1 expression showed a worse clinical outcome. Recent studies of surgical specimens from various tumours have shown that tumour E2F-1 positivity positively correlates with MIB-1 immunopositivity.^{11 20-22} These findings agree with our data, and support the hypothesis that E2F-1 expression is associated with tumour cell proliferation in OSCC.

As a result of pRb phosphorylation, activated E2F-1 initiates the transcription of genes that determine the progression of the cell cycle and others that activate apoptosis. The Rb gene is frequently mutated or deleted in many human cancers, and in tumours with an intact Rb gene there is usually some other abnormality of the upstream regulators in this pathway. For example, overexpression of cyclin D1 and mutation/deletion or methylation of the p16 CDKI gene, resulting in its functional deficiency, are common events in OSCC.²⁸ To examine whether such deregulated upstream regulators result in increased E2F-1 expression, we attempted to examine the combined results by classifying the patients into those with a normal and those with an altered p16/cyclin D1/pRb status. However, 42 of the 43 cases harboured one or more disruption in this pathway, including decreased p16 expression (33 of 43), increased cyclin D1 expression (23 of 43), and decreased/negative pRb expression (six of 43). In addition, the only patient with a normal p16/

Table 3 Univariate regression analysis, Cox proportional hazards model

	RR	95% CI	p Value
Age (≥ 60 years)	0.731	0.296 to 1.744	0.4646
Sex (male)	0.737	0.134 to 2.487	0.4611
pT (pT3 or 4)	3.191	0.038 to 0.672	0.0123*
Lymph node metastasis (positive)	3.419	0.030 to 0.602	0.0087*
Grade (G2 or G3)	0.539	0.248 to 2.210	0.5900
MI (≥ 47)	2.205	0.152 to 0.895	0.0275*
AI (< 3.2)	2.297	0.142 to 0.857	0.0216*
E2F-1 LI (≥ 25)	2.857	0.139 to 0.882	0.0259*
p21 LI (< 10)	0.712	0.199 to 1.501	0.2413
p16 LI (< 70)	0.352	0.608 to 3.440	0.7250
pRb LI (< 20)	0.712	0.492 to 4.570	0.4765
Cyclin D1 (≥ 10)	0.079	0.409 to 2.282	0.9367
p53 mutation (positive)	1.222	0.718 to 4.156	0.2218
HPV infection (positive)	1.053	0.654 to 4.094	0.2924

*Significant.
AI, apoptotic index; CI, confidence interval; HPV, human papillomavirus; LI, labelling index; MI, MIB-1 labelling index; RR, relative risk.

Table 4 Multivariate regression analysis, Cox proportional hazards model

	RR	95% CI	p Value
pT (pT3 or 4)	6.215	0.000 to 0.053	0.0002*
Lymph node metastasis (positive)	2.833	0.009 to 0.398	0.0037*
MI (≥ 47)	3.255	0.005 to 0.307	0.0039*
AI (< 3.2)	2.504	0.014 to 0.464	0.0047*
E2F-1 LI (≥ 25)	0.747	0.148 to 1.515	0.2078

*Significant.
AI, apoptotic index; CI, confidence interval; LI, labelling index; MI, mitotic index; RR, relative risk.

cyclin D1/pRb status was infected with HPV-16. Although we could not demonstrate the association between the E2F-1 expression and these factors, the entire tumour specimens harboured deregulated upstream signals of the pRb/E2F-1 pathway, which probably resulted in the release of E2F-1 from pRb mediated repression, thus promoting tumour cell cycle progression.

“Increased E2F-1 expression appeared to be associated with cell cycle progression, but had no apparent influence on tumour cell apoptosis in our study”

Some previous in vitro studies have shown that E2F-1 overexpression directly activates the expression of p14^{ARF}, which inhibits MDM2 mediated p53 degradation. A recent study showed that the sequential transfer of the wild-type p53 and E2F-1 genes into oesophageal cancer cells induces tumour cell apoptosis via this E2F-1/ARF/MDM/p53 pathway.²⁷ However, increased E2F-1 expression appeared to be associated with cell cycle progression, but had no apparent influence on tumour cell apoptosis in our study. Interestingly, a recent study of E2F-1 in non-small cell lung carcinoma yielded similar results.²¹ The authors suggested a model whereby tumours with a deregulated E2F-1/pRb network cannot promote p53 dependent apoptosis under conditions of p53 mutation or MDM2 overexpression. Although we did not investigate MDM2 expression in detail, 24 cases showed abnormal p53 status and six had decreased/negative pRb expression. Overall, 28 cases were considered to have abnormal p53 or pRb status, so that E2F-1-mediated apoptosis was probably defective in these cases. In addition, we found that 18 patients had high risk HPV infection, which is similar to the infection rate seen in Asian countries.³⁰ The mechanisms by which the HPV viral oncoproteins E6 and E7 overcome normal cell cycle control involve inactivation of two major tumour suppressor proteins: p53 and pRb.³¹ E7 inhibits the pRb pathway, which may contribute to the release of E2F-1 from pRb mediated repression, thus promoting tumour cell proliferation. In addition, because E6 inhibits the function of p53 by directing its ubiquitination and degradation, p53 induced apoptosis may have been defective in a considerable proportion of our cases. Furthermore, a recent study showed that the CDKN2A (p16^{INK4A} and p14^{ARF}) gene was affected through mutations, deletions, and promoter hypermethylation in more than 80% of OSCC cases.³² It is possible that such genetic alterations affected E2F-1 mediated apoptosis in a considerable proportion of our patients. Recently, an in vitro study by Yang *et al* reported that E2F-1 mediated apoptosis in oesophageal cancer cell lines is not dependent on p53 expression, but is related to the differential expression of Bcl-2 family member proteins.³³ Thus, further studies of E2F-1 mediated apoptosis in OSCC should investigate not only its association with p53, but also with these apoptosis related factors.

The E2F-1 LI was not significantly related to lymph node metastasis or a worse clinical stage. Clinical staging categorises the degree of cancer advancement, based on features of tumour spread, invasion, and metastasis. Although it has been reported that E2F-1 can regulate cell proliferation, there is no evidence of direct regulation of gene transcription of proteins that promote tumour invasiveness or metastasis. Our data are thus consistent with the known functions of E2F-1—integration of upstream signals from a variety of cell cycle regulators and promotion of cell cycle progression.

In conclusion, cell proliferation and apoptosis appear to be significantly associated with clinical outcome in patients with OSCC who have not undergone chemotherapy or

Take home messages

- Cell proliferation and apoptosis are associated with clinical outcome in patients with oesophageal squamous cell carcinoma (OSCC)
- E2F-1 expression in OSCC is positively associated with tumour cell proliferation but not with apoptosis
- Patients with increased E2F-1 expression had significantly shorter recurrence free survival, so that E2F-1 might have influenced clinical outcome mainly by its effects on cell cycle progression

radiotherapy. E2F-1 expression is positively associated with tumour cell proliferation and not with apoptosis. Our results were consistent with previous studies demonstrating that E2F-1 upregulates the cell cycle and promotes cell cycle progression directly or indirectly. In addition, patients with increased E2F-1 expression had a significantly shorter recurrence free survival time. Thus, in our series, E2F-1 might have influenced the clinical outcome mainly via cell cycle progression.

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