International Journal of Radiation Oncology biology • physics

www.redjournal.org

Clinical Investigation: Head and Neck Cancer

FADD Expression as a Prognosticator in Early-Stage Glottic Squamous Cell Carcinoma of the Larynx Treated Primarily With Radiotherapy

Michiel L. Schrijvers, M.D.,[*,†] Wouter J. Pattje, M.Sc.,[†,‡] Lorian Slagter-Menkema, B.Sc.,[*,†] Mirjam F. Mastik, B.Sc.,[†] Johan H. Gibcus, Ph.D.,[†] Johannes A. Langendijk, M.D., Ph.D.,[‡] Jacqueline E. van der Wal, M.D., D.M.D., Ph.D.,[†] Bernard F.A.M. van der Laan, M.D., Ph.D.,[*] and E. Schuuring, Ph.D.[†]

*Departments of *Otorhinolaryngology/Head and Neck Surgery, †Pathology, and ‡Radiation Oncology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands*

Received Jul 31, 2011, and in revised form Jul 31, 2011. Accepted for publication Sep 27, 2011

Summary

This study demonstrates that increased expression of (p) FADD is associated with a better local control in T1-T2 glottic squamous cell carcinoma of the larynx primarily treated with radiotherapy. These findings suggest that (p) FADD might be new prognostic biomarker to predict local recurrence after radiotherapy in early stage glottic carcinoma.

Purpose: We recently reported on the identification of the Fas-associated death domain (FADD) as a possible driver of the chromosome 11q13 amplicon and the association between increased FADD expression and disease-specific survival in advanced-stage laryngeal carcinoma. The aim of this study was to examine whether expression of FADD and its Ser194-phosphorylated isoform (pFADD) predicts local control in patients with early-stage glottic carcinoma primarily treated with radiotherapy only.

Methods and Materials: Immunohistochemical staining for FADD and pFADD was performed on pretreatment biopsy specimens of 92 patients with T1−T2 glottic squamous cell carcinoma primarily treated with radiotherapy between 1996 and 2005. Cox regression analysis was used to correlate expression levels with local control.

Results: High levels of pFADD were associated with significantly better local control (hazard ratio, 2.40; 95% confidence interval, 1.04−5.55; $p = 0.040$). FADD overexpression showed a trend toward better local control (hazard ratio, 3.656; 95% confidence interval, 0.853−15.663; $p = 0.081$). Multivariate Cox regression analysis showed that high pFADD expression was the best predictor of local control after radiotherapy.

Conclusions: This study showed that expression of phosphorylated FADD is a new prognostic biomarker for better local control after radiotherapy in patients with early-stage glottic carcinomas.

Keywords: FADD, Larynx, Prognostic marker, Radiotherapy, Squamous cell carcinoma

Reprint requests to: E. Schuuring, Ph.D., Department of Pathology (HPC EA10), University Medical Center Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands. Tel: +31 50 3619623; Fax: +31 50 361 9107; E-mail: e.schuuring@umcg.nl

Conflict of interest: none.

Supplementary material for this article can be found at www.redjournal.org.

Int J Radiation Oncol Biol Phys, Vol. 83, No. 4, pp. 1220−1226, 2012
0360-3016
doi:10.1016/j.ijrobp.2011.09.060

Introduction

In squamous cell carcinoma of the head-and-neck region (HNSCC), DNA amplification of the chromosome 11q13 region is observed in approximately 30% of all HNSCCs and is therefore one of the most frequently observed genomic abnormalities (1). In HNSCC 11q13 amplification is associated with the presence of lymph node metastases, decreased disease-specific survival, and decreased overall survival (OS) (2). Recently, we reported that the commonly amplified 11q13 region in oropharyngeal/laryngeal carcinomas contains at least 6 genes, FADD (Fas-associated death domain) (MORT1), PPFIA1 (LIPRIN), ORAOV1 (TAOS1), FGF19, cortactin (CTTN), and cyclin D1 (CCND1), that are amplified and overexpressed in almost all carcinomas with 11q13 amplification (3). Of all genes in this 11q13 amplicon, FADD was not only amplified the most, but overexpression and amplification of FADD also correlated with increased FADD protein expression, suggesting that FADD is a key gene in the 11q13 amplicon (3).

Originally, FADD (Fas [TNFRSF6]–associated via death domain) was reported as a proapoptotic adaptor molecule that recruits caspases 8 and 10 to promote formation of the death-inducing signal complex (4). The recruitment of these caspases to the death-inducing signal complex leads to intracellular processing and activation of caspases, eventually resulting in cleavage of downstream targets and apoptosis. More recently, an alternative function for FADD was described because many studies showed that FADD also plays an important role in growth and regulation of the cell cycle (5, 6). Nuclear localization of FADD has been ascribed to FADD phosphorylation at Ser194 (pFADD), and the highest levels of pFADD are observed at the G2/M phase of the cell cycle (7). In addition, treatment of cells *in vitro* with agents blocking the G2/M transition resulted in a significant accumulation of pFADD (8, 9). Finally, expression of a Ser194–phosphomimicking FADD mutant caused G2/M cell cycle arrest (9). Altogether, these data suggest a key role for FADD/pFADD in cell cycle control. Because previous studies showed that cells arrested in the G2/M phase are most radiosensitive (10), FADD is amplified in more than 30% of HNSCCs, and pFADD is mainly expressed at the G2/M phase of the cell cycle, we hypothesize that glottic carcinomas with overexpression of pFADD will have better local control (LC) after radiotherapy alone.

In a series of 167 advanced-stage oropharyngeal/laryngeal carcinomas, we found that increased levels of both FADD and pFADD were significantly associated with a worse disease-specific survival and positive lymph node status (3, 11). In agreement with this finding, in HNSCC, FADD overexpression and pFADD overexpression in adenocarcinomas of the lung were both associated with decreased OS (12). In prostate cancer, pFADD expression was associated with progression of disease (9). In our series of 167 advanced-stage oropharyngeal/laryngeal carcinomas, we could not evaluate the prognostic value for LC after radiotherapy because of the presence of many confounding parameters that might influence the clinical outcome, such as the variety of different (combined) treatment modalities, different subsites in the head-and-neck region, and differences in extended disease (*e.g.*, N+) and tumor size (3, 11).

In this study we examined the prognostic value of FADD and pFADD expression in patients with laryngeal carcinoma primarily treated with radiotherapy alone. To evaluate the effect of FADD/pFADD expression on clinical outcome after radiotherapy, we limited the number of possible interfering variables by selecting a homogeneous study population. Of all patients with laryngeal carcinoma treated at our institution between 1997 and 2004, we selected 92 patients with early-stage (pT1/pT2) glottic carcinoma of the larynx who were treated primarily with radiotherapy with curative intent. Because early-stage glottic carcinomas rarely have regional lymph node metastasis on initial diagnosis and because FADD expression has been associated with the presence of lymph node metastasis in advanced-stage HNSCC as well (11), the analysis of early-stage glottic cancer will restrict our validation to LC after radiotherapy. Immunohistochemical staining of FADD and pFADD on pretreatment biopsy specimens will be correlated with LC and OS.

Methods and Materials

Patients and tissues

The selection of patients and samples was described in detail previously (13). In brief, between 1997 and 2004, 638 patients were diagnosed with laryngeal squamous cell carcinoma in the northern part of the Netherlands (comprising >10 medical centers) and treated at our institute. Demographic and clinicopathologic data, such as sex, age, pretreatment hemoglobin level, T status, N status, and current and past tobacco use and alcohol use, were retrospectively collected by reviewing the patient charts. The inclusion criteria for this study were (*1*) histologically proven squamous cell carcinoma, (*2*) localization in the glottis, (*3*) cT1 and cT2, (*4*) no evidence of distant metastasis (cM0), (*5*) curative treatment with radiotherapy alone, and (*6*) no other previous treatment. From the 360 patients with T1/T2 glottic carcinomas, 157 formalin-fixed, paraffin-embedded pretreatment biopsy specimens were collected and revised by an experienced pathologist. Tissue specimens with sufficient tumor cells for immunohistochemical staining were available from 92 patients. The pretreatment characteristics are summarized in Table 1. Informed consent was given by all patients included in the study.

Radiotherapy and follow-up

In all patients radiotherapy was delivered with megavoltage equipment by use of 6-MV photons as reported previously (13). T1 tumors were treated with a total dose of 66 Gy at 2 Gy per fraction, 5 times per week. T2 tumors were generally treated with 6 fractions per week, to a total dose of 70 Gy in 6 weeks. In case of elective irradiation of the neck nodes, a total dose of 46 Gy was given on the primary planning target volume with an additional boost of 70 Gy on the primary tumor and pathologic lymph nodes (as detailed in the Appendix available online at redjournal.org).

After completing radiation, patients were followed up every 3 months in the first and second year and every 6 months in the third, fourth, and fifth year. After 5 years without evidence of disease, patients were discharged from follow-up. Twenty-one patients in whom a local recurrence developed after radiotherapy underwent salvage by total laryngectomy; one patient received palliative therapy because of inoperable recurrent tumor. A second primary developed in 9 patients (10%), in the lung ($n = 4$), head-and-neck region ($n = 4$), or colon ($n = 1$). During follow-up, 26 patients (28%), of whom 7 died of disease. All follow-up data are summarized in Table 2.

Table 1 Patient characteristics: Glottic cancer treated with primarily radiotherapy

	Data (n = 92) [n (%)]
Sex	
Male	82 (89%)
Female	10 (11%)
Age (y)	
Median (range)	65 (40–86)
Primary symptom	
Hoarse voice	88 (96%)
Other	4 (4%)
T status	
1	50 (54%)
2	42 (46%)
N status	
0	90 (98%)
1	1 (1%)
X	1 (1%)
Hemoglobin level (mmol/L)	
Median	9.1
Past tobacco use (cigarettes per day)	
0	4 (4%)
1–20	45 (49%)
>20	20 (22%)
Unknown	23 (25%)
Present tobacco use (cigarettes per day)	
0	40 (3%)
1–20	29 (32%)
>20	11 (12%)
Unknown	12 (13%)
Past alcohol use (units per day)	
0	25 (27%)
1–6	47 (51%)
>6	3 (3%)
Unknown	17 (18%)

Immunohistochemistry

Immunohistochemical staining and scoring for FADD, pFADD, cortactin, and cyclin D1 was performed as reported previously (3, 11) by use of the murine monoclonal antibody A66-2 (BD Biosciences, Franklin Lakes, NJ), rabbit polyclonal antibody against Ser194 pFADD (BD Biosciences), murine monoclonal antibody 30/cortactin (BD Biosciences), and rabbit anti-human monoclonal antibody SP4 (Lab Vision/Neomarkers, Fremont, CA), respectively (examples are shown in Fig. E1 available online at redjournal.org). For the scoring of the immunostaining of pFADD and cyclin D1, the percentage of tumor cells with nuclear staining was determined. Cutoff values of percentages for dichotomization for pFADD were determined by use of receiver operating curve analyses, and the best sensitivity/specificity ratio associated with LC was found at a cutoff level of 71%. Percentages of positive staining above the cutoff level were considered as high expression and those below as negative/low expression. Scoring was performed by two independent observers without knowledge of clinical data. The discordant cases were reviewed by all observers, and scores were reassigned on consensus of opinion. Detailed information is presented in the Appendix available online at redjournal.org.

Table 2 Patient characteristics: Follow-up

Characteristics	Data (n = 92) [n (%)]
Events during follow-up	
Any	43 (47%)
Local recurrence	22 (24%)
Regional recurrence	3 (3%)
Second primary	9 (10%)
Death	26 (28%)
Death of disease	7 (27%)
Death not of disease	19 (73%)
Time to first event (mo)	
Mean	19
Median (range)	13 (0–98)
Time to follow-up (mo)	
Mean	49
Median (range)	40 (1–119)
Time to local recurrence (mo)	
Mean	14
Median (range)	11.5 (2–46)
Time to regional recurrence (mo)	
Mean	11
Median (range)	12 (6–16)
Time to second primary (mo)	
Mean	26
Median (range)	10 (0–68)
Time to death (mo)	
Mean	33
Median (range)	28 (3–98)

Statistical analysis

Associations between expression of FADD, pFADD, cyclin D1, and cortactin were analyzed with a chi-square test. Follow-up time was calculated from the date of diagnosis until the date of the last follow-up visit. Local recurrence was defined as tumor recurrence at the primary tumor site and was calculated from the date of diagnosis until the date of local recurrence diagnosis or to the last follow-up. OS was defined from the date of diagnosis to the date of death or to last follow-up.

Kaplan-Meier survival analysis and Cox regression analysis adjusted for expression of FADD and pFADD, as well for sex, age, hemoglobin level, T status, N status, tobacco use, and alcohol use, were performed with LC and OS as endpoints. Only variables showing an association with LC or OS on univariate analysis ($p < 0.10$) were included in the multivariate Cox regression analysis. Alcohol consumption was excluded from the multivariate analysis because of the large number of missing values (17 missing [18%]). All statistical analyses were performed with SPSS software, version 16.0 (SPSS, Chicago, IL).

Results

FADD and pFADD are not associated with clinicopathologic features

Immunohistochemical staining of FADD was mainly cytoplasmic and very homogeneous across the tumor (Fig. E1A). High levels of FADD were detected in 21 of 92 cases (23%). The

Table 3 Patient and disease characteristics in total and related to local recurrence after radiotherapy

Characteristics	Total	Local recurrence [*n* (%)]	Univariate HR (95% CI)	*p* value
FADD expression				
Low	71	20 (28%)	3.656 (0.853−15.663)	$p = 0.081$
High	21	2 (10%)	1	
pFADD expression				
Low	30	11 (37%)	2.403 (1.041−5.548)	$p = 0.040$
High	62	11 (18%)	1	
Cyclin D1 expression				
Low	43	12 (28%)	1.292 (0.558−2.991)	$p = 0.550$
High	44	10 (23%)	1	
Cortactin expression				
Low	42	14 (33%)	1.890 (0.762−4.687)	$p = 0.169$
High	39	7 (18%)	1	
Sex				
Female	10	1 (10%)	1	$p = 0.323$
Male	82	21 (26%)	2.751 (0.370−20.470)	
Age				
<65 y	46	11 (24%)	1	$p = 0.695$
≥65 y	46	11 (24%)	1.183 (0.511−2.738)	
Hemoglobin level				
High	70	15 (21%)	1	$p = 0.232$
Low	22	7 (32%)	1.731 (0.704−4.257)	
T status				
T1	50	9 (18%)	1	$p = 0.137$
T2	42	13 (31%)	1.906 (0.814−4.463)	
N status				
N1	1	0 (0%)	1	$p = 0.704$
N0	91	22 (24%)	20.570 (0.00−125000000) 10	
Current tobacco use				
Yes	40	8 (20%)	1	$p = 0.237$
No	40	12 (30%)	1.718 (0.701−4.215)	
Past tobacco use				
No	4	0 (0%)	1	$p = 0.524$
Yes	65	16 (25%)	21.941 (0.002−295955)	
Alcohol use				
No	25	9 (18%)	1	$p = 0.037$
Yes	50	13 (31%)	4.816 (1.10−21.087)	

Abbreviations: CI = confidence interval; FADD = Fas-associated death domain; pFADD = Ser194-phosphorylated isoform of Fas-associated death domain; HR = hazard ratio.

immunostaining of pFADD was distributed more heterogeneously within the tumor tissues and predominantly found within the nucleus (Fig. E1B, available online at redjournal.org). High nuclear pFADD levels were observed in 62 of 92 cases (67%). No significant association was found between FADD and pFADD expression (results not shown), which is in agreement with the different morphologic distributions. No significant associations were found between FADD or pFADD expression and sex, age, hemoglobin level, T stage, N stage, current and past tobacco use, and use of alcohol (data not shown).

Expression of FADD and pFADD predicts increased LC in early-stage glottic carcinoma

Univariate Cox regression analysis on dichotomized groups showed that high pFADD expression was associated with better LC (hazard ratio [HR], 2.40; 95% confidence interval [CI], 1.04−5.55; $p = 0.040$), whereas borderline significance for the association with this endpoint was found for high FADD expression (HR 3,66; 95% CI, 0.85−15.66; $p = 0.081$) (Table 3). Kaplan-Meier survival analysis showed similar results for both pFADD ($p = 0.033$) and FADD ($p = 0.060$) (Figs. 1A and 1B).

Interestingly, no local recurrence developed in any of the 12 patients with high expression of both FADD and pFADD, whereas a local recurrence developed in 22 of the 80 patients with low expression of either FADD, pFADD, or both (27.5%) ($p = 0.025$) (Fig. 1C). Multivariate Cox regression analysis showed that high pFADD expression was the strongest independent prognostic factor for LC after radiotherapy (HR, 2.72; 95% CI, 1.17−6.29; $p = 0.020$) (Table 4).

Expression of FADD or pFADD does not predict better OS

Kaplan-Meier survival analysis showed that high expression of FADD ($p = 0.213$) and high expression of pFADD ($p = 0.788$)

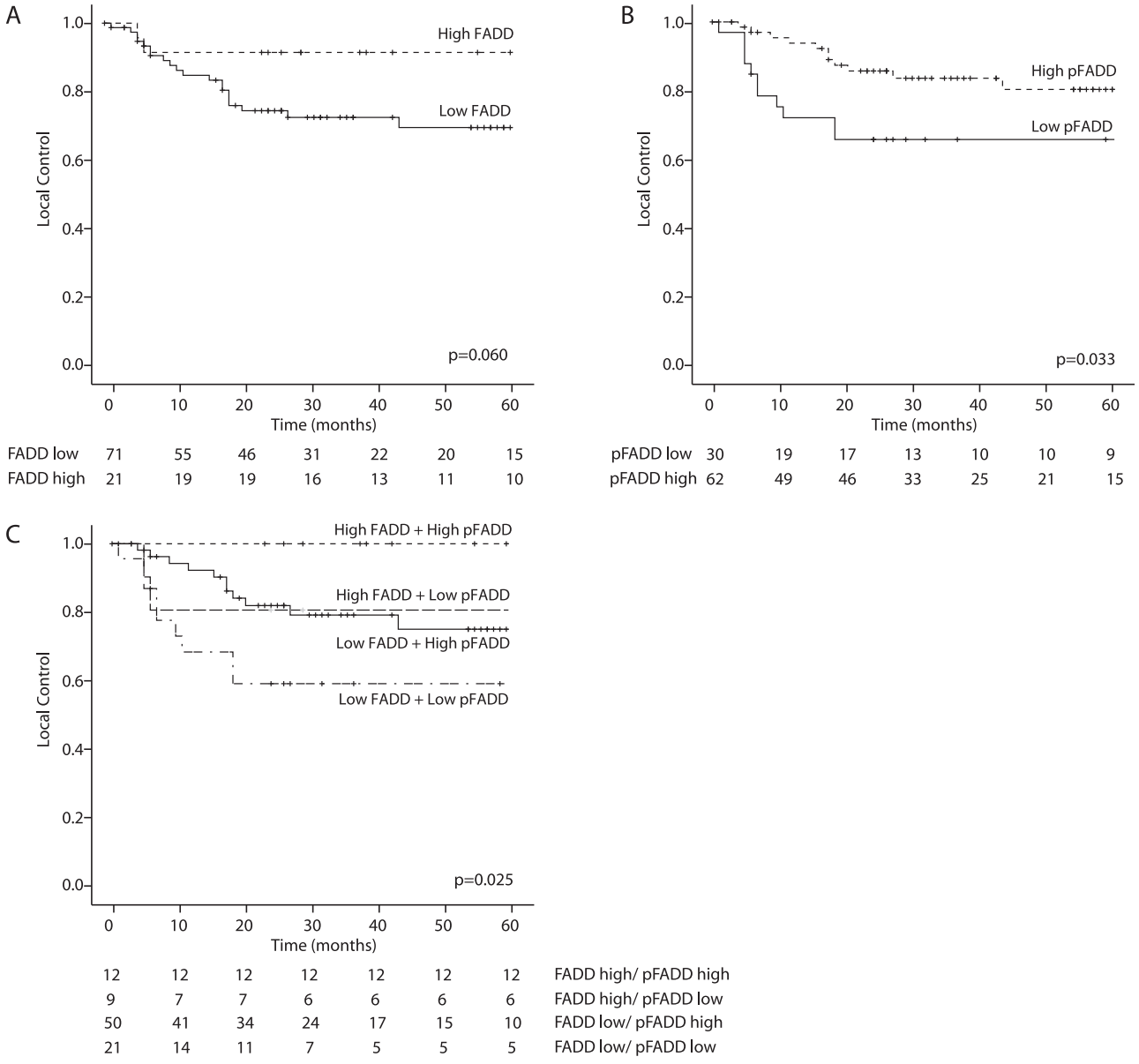

Fig. 1. Local control (LC) rate as a function of (A) Fas-associated death domain (FADD), (B) Ser194-phosphorylated isoform of Fas-associated death domain (pFADD), and (C) combination of FADD and pFADD. Patients overexpressing FADD show a trend toward better LC. Patients overexpressing pFADD show significantly better LC. Patients overexpressing both FADD and pFADD have an LC rate of 100%.

were not associated with OS (Fig. 2). Cox regression analysis also showed that high expression of FADD (HR, 1.94; 95% CI, 0.67−5.65; $p = 0.223$) and high expression of pFADD (HR, 1.12; 95% CI, 0.49−2.55; $p = 0.788$) were not associated with OS.

Cyclin D1 and cortactin are not associated with LC

Because cortactin and cyclin D1 are frequently co-amplified and consequently co-overexpressed with FADD in HNSCC (3, 11) and have been associated with poor prognosis and/or response to therapy (3, 14, 15), we immunostained the same series for cortactin and cyclin D1 (examples are shown in Figs. E1C and E1D, available online at redjournal.org). Increased expression of cyclin D1 and cortactin was observed in 43 of 92 cases and 39 of 92 cases, respectively. Univariate Cox regression analysis (Table 3) and Kaplan-Meier survival analysis (Fig. E2, available online at redjournal.org) showed that high expression of cyclin D1 and high expression of cortactin were not associated with LC in early-stage glottic cancer.

Discussion

Laryngeal squamous cell carcinoma is the most common type of HNSCC and accounts for approximately 20% of all newly diagnosed HNSCCs. Nowadays, most patients with Stage T1/T2 laryngeal carcinoma are treated with radiotherapy because of better laryngeal function after treatment compared with surgery. The 5-year LC and OS rates for T1/T2 laryngeal carcinoma vary

Table 4 Patient characteristics related to local recurrence after radiotherapy

Characteristics	Multivariate HR (95% CI)	p value
Low FADD expression	4.227 (0.981−18.320)	$p = 0.053$
Low pFADD expression	2.715 (1.172−6.287)	$p = 0.020$

Abbreviations: CI = confidence interval; FADD = Fas-associated death domain; pFADD = Ser194-phosphorylated isoform of Fas-associated death domain; HR = hazard ratio.
Only variables showing a correlation with local control on univariate analysis ($p < 0.10$) were included (pFADD and FADD). Alcohol use was excluded because of the large number of missing values.

between 69% and 94% and between 63% and 82%, respectively (16). Unfortunately, except for TNM status, no good clinicopathologic factors are available to predict clinical outcome in early-stage laryngeal carcinoma treated with radiotherapy. The clinical relevance is obvious, because in case of recurrent disease after radiotherapy, salvage laryngectomy is mandatory, resulting in loss of vocal cord function and therefore quality of life. Thus molecular tumor markers could be useful to predict local recurrence and survival in patients with early-stage glottic carcinoma treated with radiotherapy.

Associations between expression of either FADD or pFADD with clinical outcome have been described in different tumor types. Chen *et al.* (12) showed that FADD overexpression and pFADD overexpression were both associated with decreased OS in patients with adenocarcinoma of the lung. Shimada *et al.* (9) reported that expression of pFADD was associated with progression of prostate carcinoma. In a previous study we found an association between FADD and pFADD expression and clinical outcome in a group of mainly advanced-stage laryngeal carcinomas (3, 11). No associations could be performed between FADD/pFADD expression and response to therapy because of the heterogeneity of the patient series. In this study we selected a homogeneous series of patients with T1/T2 glottic carcinoma treated with radiotherapy only. Our data showed that high expression of FADD and, even more significantly, pFADD overexpression were associated with better LC. No association with OS was found. These data strongly suggest that overexpression of pFADD is associated with better radiosensitivity.

FADD is part of the chromosome 11q13 amplicon (3). We have shown that not only FADD but also several other genes in the amplicon (PPFIA1, TPCN2, FLJ442258, ORAOV1, FGF19, CTTN/cortactin, CCND1/cyclin D1) are overexpressed in HNSCC carcinomas with 11q13 amplification (3). Two of these genes (cyclin D1 and cortactin) have been studied extensively (1). Cyclin D1 has been associated with response to radiotherapy previously in breast cancer both in a clinical study (15, 17, 18) and in an *in vitro* model (14), and cortactin has been associated with worse clinical outcome in HNSCC (11) and cell migration *in vitro* (19). Immunostaining of the same series of 92 glottic cancers showed no association between either cortactin or cyclin D1 expression and LC (Table 3 and Fig. E2). The lack of this association for cyclin D1 is not consistent with the observed response to therapy in breast cancer but is concordant with studies in HNSCC that failed to associate cyclin D1 expression and clinical outcome (20). In this study we only selected early-stage (T1/T2) glottic carcinomas, in which the rate of regional or distant metastasis is relatively low (only 1 of 92), which results from the less developed lymphatic drainage system of the glottic larynx. The lack of the observed association between cortactin and LC in this series of early-stage glottic carcinomas is therefore in good agreement with cortactin's function in cell migration and invasion (19). This analysis showed that not cyclin D1 or cortactin but high pFADD expression is associated with LC in our series of early-stage glottic carcinomas.

Our data also suggest that the expression of pFADD might mediate the sensitivity of tumor cells to radiotherapy. However, at present, we can only speculate how. Besides the role of FADD in apoptosis, more recently, phosphorylation of FADD (pFADD) at serine 194 was reported to be involved in cell cycle progression (4, 5, 7, 8). Hua *et al.* (5) showed that phosphorylation of FADD is essential for growth/proliferation in T cells. Shimada *et al.* (9)

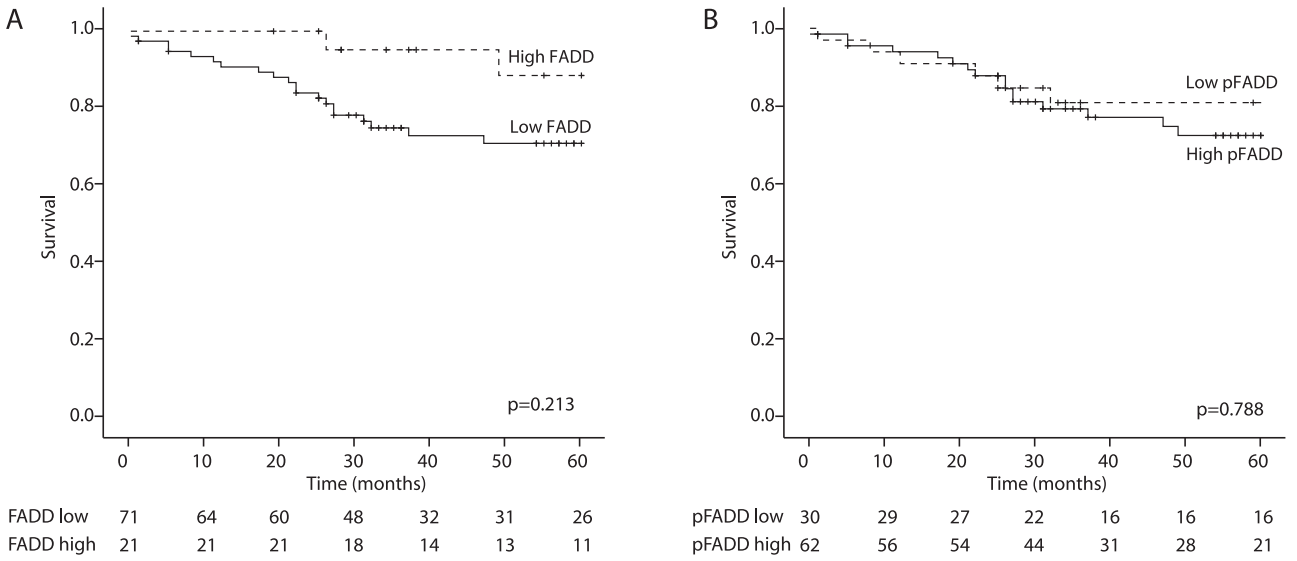

Fig. 2. Overall survival (OS) as a function of (A) Fas-associated death domain (FADD) and (B) Ser194-phosphorylated isoform of Fas-associated death domain (pFADD). No significant correlation was found between either FADD or pFADD overexpression and OS.

and Alappat *et al.* (8) have shown that phosphorylation of FADD caused more cells to be arrested in the G2/M phase of the cell cycle. A possible explanation for this is that cells in the G2/M phase are more radiosensitive than cells in other phases of the cell cycle (10). Furthermore, the fact that phosphorylation of FADD is associated with nuclear localization in our tumors and is involved in G2/M arrest in *in vitro* models (9) suggests that FADD plays an important role in sensitizing these tumors for radiation. However, whether pFADD itself triggers cells into G2/M arrest or cells at G2/M arrest show expression of pFADD has to be tested in future experiments by knocking down or expressing FADD and pFADD mutants in HNSCC cell lines and determining RT response directly by the classical clonogenic survival assay.

Conclusions

We showed that increased expression of pFADD/FADD is associated with better LC in early-stage glottic carcinoma treated with radiotherapy alone. A possible explanation is that pFADD expression is associated with more arrested cells in the radiosensitive G2/M phase of the cell cycle. Our findings suggest that pFADD might be a new prognostic biomarker to predict local recurrence in T1/T2 glottic carcinoma treated with radiotherapy.

References

1. Schuuring E. The involvement of the chromosome 11q13 region in human malignancies: Cyclin D1 and EMS1 are two new candidate oncogenes—A review. *Gene* 1995;159:83-96.
2. Akervall JA, Jin Y, Wennerberg JP, et al. Chromosomal abnormalities involving 11q13 are associated with poor prognosis in patients with squamous cell carcinoma of the head and neck. *Cancer* 1995;76:853-859.
3. Gibcus JH, Menkema L, Mastik MF, et al. Amplicon mapping and expression profiling identify the Fas-associated death domain gene as a new driver in the 11q13.3 amplicon in laryngeal/pharyngeal cancer. *Clin Cancer Res* 2007;13:6257-6266.
4. Chinnaiyan AM, O'Rourke K, Tewari M, Dixit VM. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. *Cell* 1995;81:505-512.
5. Hua ZC, Sohn SJ, Kang C, Cado D, Winoto A. A function of Fas-associated death domain protein in cell cycle progression localized to a single amino acid at its C-terminal region. *Immunity* 2003;18:513-521.
6. Osborn SL, Sohn SJ, Winoto A. Constitutive phosphorylation mutation in Fas-associated death domain (FADD) results in early cell cycle defects. *J Biol Chem* 2007;282:22786-22792.
7. Alappat EC, Feig C, Boyerinas B, et al. Phosphorylation of FADD at serine 194 by CKIalpha regulates its nonapoptotic activities. *Mol Cell* 2005;19:321-332.
8. Alappat EC, Volkland J, Peter ME. Cell cycle effects by C-FADD depend on its C-terminal phosphorylation site. *J Biol Chem* 2003;278:41585-41588.
9. Shimada K, Matsuyoshi S, Nakamura M, Ishida E, Konishi N. Phosphorylation status of Fas-associated death domain-containing protein (FADD) is associated with prostate cancer progression. *J Pathol* 2005;206:423-432.
10. Pawlik TM, Keyomarsi K. Role of cell cycle in mediating sensitivity to radiotherapy. *Int J Radiat Oncol Biol Phys* 2004;59:928-942.
11. Gibcus JH, Mastik MF, Menkema L, et al. Cortactin expression predicts poor survival in laryngeal carcinoma. *Br J Cancer* 2008;98:950-955.
12. Chen G, Bhojani MS, Heaford AC, et al. Phosphorylated FADD induces NF-kappaB, perturbs cell cycle, and is associated with poor outcome in lung adenocarcinomas. *Proc Natl Acad Sci U S A* 2005;102:12507-12512.
13. Schrijvers ML, van der Laan BF, de Bock GH, et al. Overexpression of intrinsic hypoxia markers HIF1alpha and CA-IX predict for local recurrence in stage T1-T2 glottic laryngeal carcinoma treated with radiotherapy. *Int J Radiat Oncol Biol Phys* 2008;72:161-169.
14. Coco Martin JM, Balkenende A, Verschoor T, Lallemand F, Michalides R. Cyclin D1 overexpression enhances radiation-induced apoptosis and radiosensitivity in a breast tumor cell line. *Cancer Res* 1999;59:1134-1140.
15. Yoo SS, Carter D, Turner BC, et al. Prognostic significance of cyclin D1 protein levels in early-stage larynx cancer treated with primary radiation. *Int J Cancer* 2000;90:22-28.
16. Mendenhall WM, Werning JW, Hinerman RW, Amdur RJ, Villaret DB. Management of T1-T2 glottic carcinomas. *Cancer* 2004;100:1786-1792.
17. Xia F, Powell SN. The molecular basis of radiosensitivity and chemosensitivity in the treatment of breast cancer. *Semin Radiat Oncol* 2002;12:296-304.
18. Akervall JA, Michalides RJ, Mineta H, et al. Amplification of cyclin D1 in squamous cell carcinoma of the head and neck and the prognostic value of chromosomal abnormalities and cyclin D1 overexpression. *Cancer* 1997;79:380-389.
19. van Rossum AG, Moolenaar WH, Schuuring E. Cortactin affects cell migration by regulating intercellular adhesion and cell spreading. *Exp Cell Res* 2006;312:1658-1670.
20. Morshed K, Skomra D, Korobowicz E, Szymanski M, Polz-Dacewicz M, Golabek W. An immunohistochemical study of cyclin D1 protein expression in laryngeal squamous cell carcinoma. *Acta Otolaryngol* 2007;127:760-769.