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ORIGINAL ARTICLE

Clinical relevance of Fas expression in oesophageal squamous cell carcinoma

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Aims: To determine the extent of Fas expression in oesophageal squamous cell carcinomas (ESCCs) from Chinese patients and to correlate Fas expression with clinicopathological prognostic parameters.
Methods: Clinicopathological data were collected from 58 patients with ESCC who underwent oesophagectomy and had no prior radiotherapy or chemotherapy. Immunostaining was performed on the primary tumours. Expression of Fas was correlated with patients' demographics, tumour characteristics and stage, R category of surgery, and patients' survival.
Results: The actuarial survival rates of all patients at two and five years after surgery were 48% and 14%, respectively. Fas expression was detected in 89.7% of ESCCs. Higher Fas expression recorded on a four point scale correlated with better tumour differentiation ($p < 0.01$), but not with other patient or tumour variables. Importantly, higher Fas expression was associated with better survival ($p = 0.0317$).
Conclusions: These findings suggest that Fas activated apoptosis is important in the pathogenesis of ESCC. This molecular pathway may be a potential therapeutic target for ESCC.

Fas is a cell surface receptor and a major regulator of apoptosis.[1] It has been defined as a tumour suppressor gene. Together with its ligand (FasL), Fas is a key element in the homeostasis of peripheral lymphocytes and many other types of cells.[2 3] Alterations in expression of Fas and FasL have been reported in malignant lymphomas[4 5] and other solid tumours, including oesophageal squamous cell carcinoma (ESCC).[6–10]

> ''Fas mediated apoptosis may play a part in the natural mechanism of cellular turnover in squamous epithelium''

In the normal oesophagus, Fas is expressed in the oesophageal epithelium, and the intensity of Fas staining increases from the basal or lower prickle cell layer to the upper squamous epithelium, where apoptosis occurs.[10] Because squamous epithelial cells also coexpress FasL, Fas mediated apoptosis may play a part in the natural mechanism of cellular turnover in squamous epithelium.[11] Immunohistochemical studies on the expression of the Fas protein in ESCC have shown that Fas is downregulated in these tumours.[7–10] However, there are no available data on the expression of Fas in ESCC in the Chinese population. The prognostic relevance of Fas expression in ESCC is also unknown. The aims of our present study were to (1) document Fas expression in ESCC, (2) correlate Fas expression with clinicopathological variables, and (3) assess its role as a prognostic factor.

PATIENTS AND METHODS

Fifty eight surgically resected ESCC specimens obtained at oesophagectomy from January 1996 to December 1998 were selected from the department of pathology, The University of Hong Kong, at Queen Mary Hospital, Hong Kong. Clinical information on patients' demographics, tumour characteristics and stage, and patient outcome were derived from a prospectively collected database. All patients who had undergone surgical resection during the period but without prior neoadjuvant chemotherapy or radiotherapy were included. They were all ethnic Chinese from the local community. The specimens were processed for routine histopathology. The tissue blocks were fixed in 10% buffered formalin and embedded in paraffin wax. ESCCs were divided into three grades: well differentiated, moderately differentiated, and poorly differentiated according to the World Health Organisation classification.[12] The ESCCs were staged according to the TNM classification.[13] The R category of the residual tumour was classified according to the UICC system, where R_0 indicates macroscopic and microscopic tumour clearance, R_1 indicates microscopic residual tumour, and R_2 indicates macroscopic residual tumour.[14]

The expression of Fas was examined by immunohistochemistry in the 58 ESCC tumour specimens and corresponding morphologically normal non-tumour oesophageal mucosal tissues from the proximal resection margin.

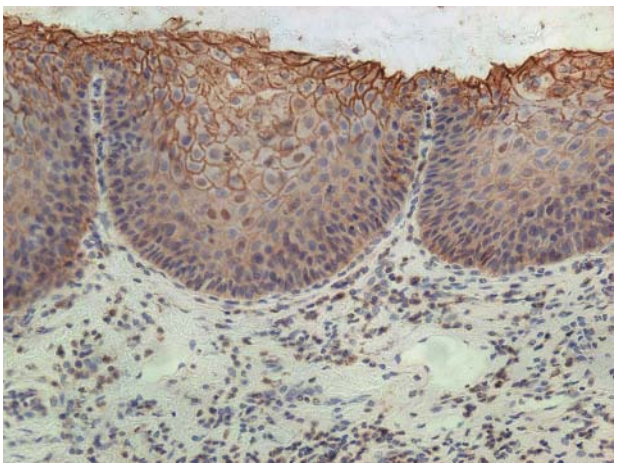

Figure 1 The morphologically normal oesophageal epithelium shows a pronounced decrease in Fas expression from the surface to the prickle cell layer. The basal and suprabasal prickle cells show almost no Fas expression.

Abbreviations: ESCC, oesophageal squamous cell carcinoma; FasL, Fas ligand

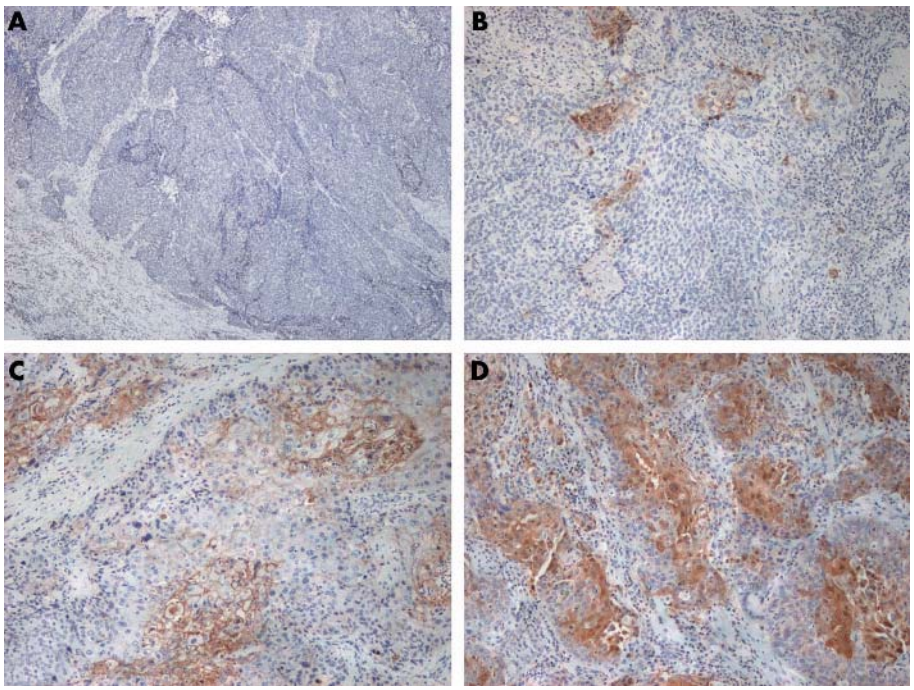

Figure 2 Fas expression in oesophageal squamous cell carcinoma showing (A) negative (−), (B) +, (C) ++, and (D) Fas +++ immunostaining.

Immunohistochemistry

Paraffin wax embedded sections were cut from one of the representative tumour blocks and the morphologically normal mucosal tissue from the proximal resection margin from each case of ESCC. They were mounted on to triethoxysilylpropylamine (TESPA; Sigma Chemicals, St Louis, Missouri, USA) coated glass slides. Sections (4 μm thick) were dried overnight in a 42℃ incubator and then used immediately or kept at room temperature for later use. They were dewaxed in xylene and then hydrated in graded alcohol.

Immunostaining was performed using the three step Dako LSAB+ horseradish peroxidase system (K0690; Dako, Glostrup, Denmark) and the Fas C-20 (SC-715) primary antibody, which is specific for Fas of human origin (Santa Cruz Biotechnology Inc, Santa Cruz, California, USA). Fas C-20 is a rabbit polyclonal IgG antibody that recognises an epitope mapping to the C-terminus of human Fas. A dilution of 1/600 (0.33 μg/ml) gave optimal results.

Antigen retrieval or unmasking by microwave pretreatment was performed according to the manufacturer's (Dako) recommendations. After dewaxing and hydration, all paraffin wax embedded sections were placed in plastic microwave penetrable jars containing 10mM citrate buffer at pH 6.0, and heated in a microwave oven (H2800; Energy Beam Sciences, Agawam, Massachusetts, USA) at 95℃ for 10 minutes. All tissue sections were blocked with 0.1% avidin followed by 0.1% biotin for 10 minutes each using the Biotin Blocking kit (X0590; Dako), then treated with 3% hydrogen peroxide for 30 minutes. Before the application of the primary antibody, sections were further blocked at room temperature for 30 minutes with 10–20% non-immune serum derived from the same species of animal as the secondary antibody. The sections were incubated with Fas C-20 primary antibody at the predetermined optimal dilution of 1/600 for one hour. The slides were then incubated at 37℃ for 20 minutes with biotinylated secondary antibody solution from the kit. This was followed by three washes with phosphate buffered saline. Afterwards, the signal was amplified by incubation with the streptavidin–enzyme conjugate at 37℃ for 20 minutes, followed by three washes with phosphate buffered saline. The signals were visualised under the microscope after incubating with the substrate chromogen solution (3,3′-diaminobenzidine) at room temperature for not more than two minutes. The reaction was stopped when signals on the morphological normal epithelium or lymphocytes could be seen under the microscope. After visualisation, the tissue sections were counterstained to highlight the nuclei with Lillian and Mayer's haematoxylin.

Cells showing granular brown staining were considered positive and expression was assessed on a four point system based on the proportion of positive cells as follows: −, < 5% of cells positive; +, 5–10% cells positive; ++ 10–20% cells positive; and +++, > 20% cells positive.

Statistical analyses

Comparisons between groups were performed using the ANOVA test or Kruskal-Wallis H test for continuous variables, and Fisher's exact test or Kendall's τ – b test for categorical variables where appropriate. Survival curves were analysed using the Kaplan–Meier method and comparison between groups was performed using the log rank test. Multivariate analysis for survival was performed using the Cox proportional hazard model. A p value of less than 0.05 was regarded as significant. Statistical analysis was performed using SPSS package version 11.0 for windows (SPSS Inc, Chicago, Illinois, USA).

RESULTS

Fas expression in the non-tumorous, morphologically normal oesophageal epithelium was higher in differentiated cell layers such as the superficial layer and the upper part of the prickle cell layer. It gradually decreased and was negative in the basal cell layer (fig 1).

Fas was expressed by the ESCC tumour cells in 52 of the 58 cases (fig 2). It was notable that the ESCC tumour cells at the periphery of the tumour islands were frequently Fas negative, whereas the centrally located tumour cells expressed Fas strongly. In six cases, the tumour cells were Fas negative, whereas the normal epithelium and infiltrating lymphocytes were positive.

Table 1 Correlation between clinicopathological features and Fas expression

Feature	Total	Fas −	Fas +	Fas ++	Fas +++	p Value
Mean (SD) age (years)	63.5 (9.4)	57.7 (11.8)	70.7 (6.0)	63.3 (9.9)	63.0 (8.0)	0.08
Sex (M/F)	48/10	5:1	5:2	20:4	18:3	0.90
Differentiation						
Well	18	0	0	5	13	<0.01
Moderately	24	1	3	15	5	
Poorly	16	5	4	4	3	
T stage						
T1	2	0	1	0	1	0.46
T2	3	0	0	1	2	
T3	37	5	6	15	11	
T4	16	1	0	8	7	
N stage						
N0	23	4	3	7	9	0.37
N1	35	2	4	17	12	
M stage						
M0	55	6	7	22	20	1.00
M1	3	0	0	2	1	
Stage						
I	1	0	0	0	1	0.45
II	18	4	4	3	7	
III	36	2	3	19	12	
IV	3	0	0	2	1	
Location						
Cervical	5	1	1	3	0	0.83
Upper	5	1	0	2	2	
Middle	32	3	5	11	13	
Lower	14	1	1	7	5	
Double primary	2	0	0	1	1	
R category						
R0	41	5	6	16	14	0.77
R1/R2	17	1	1	8	7	
Tumour size (cm)						
Median	5.5	6.0	4.5	5.45	6.0	0.49
Minimum	2.0	3.5	3.0	2.0	3.0	
Maximum	11	9.5	8.0	10.5	11.0	

Table 1 shows Fas expression in relation to various clinicopathological parameters. Using the four point scale, tumour differentiation was highly correlated with Fas expression ($p < 0.01$). However, there was no association between Fas expression and patients' demographics, size and location of the tumour, TNM stage, and R category of resection.

Figure 3 shows the patients' survival in relation to Fas expression. Patients with no expression or weakly (− or +) expressed Fas had significantly worse median survival (11.8 months) than those with moderately or strongly (++ or +++) expressed Fas (28.2 months) ($p = 0.0317$). Multivariate analysis for prognostic factors was carried out by means of Cox proportional hazard analysis using the following variables: age, sex, tumour differentiation, T stage, N stage, overall TNM stage, R category of resection, and Fas expression (M stage was excluded because of colinearity with other TNM parameters). Using a forward selection algorithm with likelihood ratio criteria, Fas expression and overall TNM stage were independent prognostic factors. High Fas expression correlated with better survival (hazard ratio, 0.639; 95% confidence interval, 0.442 to 0.925), whereas advanced disease stage was associated with poor survival (hazard ratio, 2.635; 95% confidence interval, 1.378 to 5.038).

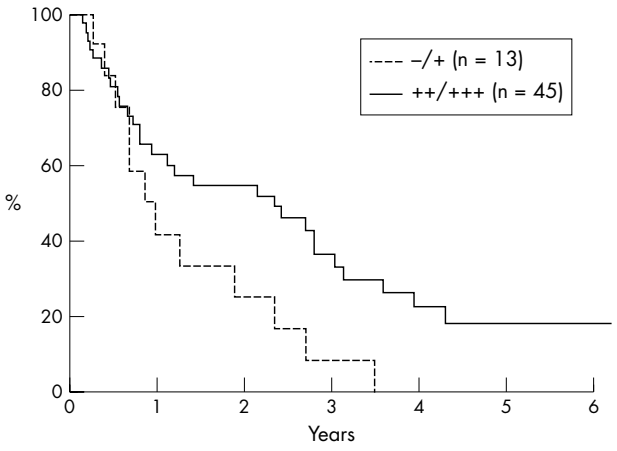

Figure 3 Correlation of Fas expression in oesophageal squamous cell carcinoma with patients' survival. Patients with no or weak Fas expression (− or +) had a median survival of 11.8 months and those with moderate or strong Fas expression (++ or +++) had a median survival of 28.2 months ($p = 0.0317$).

DISCUSSION

Despite recent advances in the treatment of ESCC,[15 16] this disease remains a highly lethal malignancy, mainly because presentation is at a late disease stage when the tumour is often not amenable to surgical resection. The two and five year actuarial survival rates of patients in our current study were 48% and 14%, respectively. New approaches to the treatment of ESCC, such as neoadjuvant chemotherapy or chemoradiotherapy, have no definite benefits over surgical resection alone.[15 16]

Immunostaining for Fas in normal oesophageal epithelium was consistent with the results of a previous study.[11] Because squamous epithelial cells also coexpress FasL, Fas mediated apoptosis probably plays a part in the natural turnover of the squamous epithelium. Recently, polymorphisms of the Fas and FasL genes have been implicated in the pathogenesis of ESCC in Chinese.[17]

Take home messages

- In oesophageal squamous cell carcinoma (ESCC) Fas expression significantly correlated with better tumour differentiation, but not with other patient or tumour variables
- Higher Fas expression was significantly associated with better survival
- These findings suggest that Fas activated apoptosis is important in the pathogenesis of ESCC, and that the Fas mediated apoptotic pathway may be a potential therapeutic target in ESCC

However, the results of immunostaining for Fas in ESCC using a four point scale did not correlate with the clinicopathological parameters examined, except for tumour differentiation. Keratinisation is the hallmark of well differentiated ESCC, and keratinising cells of ESCC have been found to be more apoptotic.[18] As shown here, tumour cells in well differentiated ESCC had higher Fas expression, similar to that of normal epithelium. It appears that Fas mediated apoptosis is often retained despite the malignant transformation process.

> ''Our findings suggest that Fas activated apoptosis limits the growth of oesophageal squamous cell carcinoma''

Fas activated cytotoxic T cells are present in ESCC.[19] These activated T cells express FasL,[20 21] which could trigger the Fas mediated apoptotic pathway of the tumour cells if the Fas system were intact. We found that the ESCC tumour cells at the periphery of the tumour islands were frequently negative for Fas. Presumably, any Fas expressing tumour cells at the periphery of tumour islands would be eliminated by Fas activated cytotoxic T cells migrating from the stroma. In this way, the access of cytotoxic cells to tumour cells most susceptible to attack could be physically prevented by the less susceptible ones, thus maintaining a higher proportion of Fas expressing cells than would otherwise be present. This could also explain why higher Fas expression in ESCC correlated with better survival. Further studies should be carried out to correlate prognosis with Fas expression in ESCC, the degree of cytotoxic T cell infiltration, and FasL expression in these T cells.

In summary, our study showed that better tumour differentiation correlated with higher Fas expression. More importantly, higher Fas expression was associated with longer survival of patients. These findings suggest that Fas activated apoptosis limits the growth of ESCC, although further work is necessary to establish whether this molecular pathway is indeed functional and could be exploited for therapeutic advantages.

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