Papers

Expression of E-cadherin in oesophageal carcinomas from the UK and China: disparities in prognostic significance

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Abstract

Aims—To study the expression and prognostic significance of the cell adhesion molecule E-cadherin in oesophageal tumours from the UK (low risk area) and China (high risk area).

Methods—E-cadherin expression was measured immunohistochemically in resected tumours from 17 patients in the UK with adenocarcinoma, 23 patients from the UK with squamous carcinoma, and 30 patients from China with squamous carcinomas who survived for five years postoperatively and compared with similar tumours from patients in the same regions who did not survive (140 tumours in all).

Results—Normal squamous epithelial cells and well differentiated areas of tumours showed membranous staining for E-cadherin expression. Cytoplasmic staining, heterogeneous staining, or an absence of staining was seen in dysplastic epithelium and in less well differentiated areas of tumours. Only one of 140 primary tumours had homogeneous membranous expression. In tumours from UK patients with adenocarcinoma ($p = 1.00$) and from Chinese patients with squamous carcinomas ($p = 0.06$) there was no correlation between E-cadherin absence and non-survival. In tumours from UK patients with squamous carcinomas there was a significant correlation between absence of E-cadherin and non-survival ($p = 0.009$). Tumours from UK patients with squamous carcinoma who survived were significantly less likely to be E-cadherin absent than those from Chinese patients with squamous carcinomas who survived ($p = 0.007$). Multivariate analysis ($n = 37$ UK, paired data) showed that absence of E-cadherin in the primary tumour was a weak independent prognostic factor for non-survival (30% significance level; $p = 0.26$; odds ratio = 3.56). In UK nodal metastases there was no correlation between E-cadherin expression and survival.

Conclusions—Squamous carcinomas from UK patients differed from both adenocarcinomas from UK patients and carcinomas from Chinese patients with respect to E-cadherin expression and prognostic significance. In tumours from UK patients, E-cadherin absence in the primary carcinoma (a weak independent prognostic factor) but not metastases correlated with non-survival.

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In some areas of China, the annual mortality rate from oesophageal cancer is 133 per 100 000 people.¹ Rates in the UK and many Western countries range from 2-15 per 100 000 people.¹ Survival of UK patients with squamous carcinoma (the almost exclusive histological type found in China) is better than that of those with adenocarcinoma. Survival is better for women but declines with age.² Oesophageal cancer has a high metastatic potential and poor prognosis. The majority of patients has disseminated disease at presentation.³

E-cadherin is a transmembrane, cell-cell, homophilic adhesion molecule present at zonulae adherentes of all epithelia and is essential for cell polarity and differentiation. In malignancy, E-cadherin may act as an anti-metastasis molecule.⁴⁻⁹ Downregulation of E-cadherin expression has been shown to be inversely correlated with differentiation and advanced stage in bladder,¹⁰ breast,^{11 12} cervical,^{13 14} colorectal,¹⁵ endometrial,¹⁶ gastric,¹⁷ kidney,¹⁸ lung,¹⁹ pancreatic,²⁰ and prostate²¹ cancers.

However, there have been few previous studies of E-cadherin in vivo in oesophageal cancer. Material from small numbers of tumours^{8 22-26} or cell lines²⁷ only have been used. In addition, E-cadherin expression has been related to stage and grade of tumours only, rather than to survival. The present study investigated E-cadherin expression in an archival series of UK and Chinese patients with a five year post-operative survival, compared with that in

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Table 1 Patient details and histopathological staging and grading of resected tumours

	Mean age (range)	Sex (M/F)	T1	T2	T3	T4	N0	N1	NX	G1	G2	G3
Group 1												
17 UK adenocarcinoma survivors	66.8 (46–78)	16/1	6	1	10	0	12	5	0	0	10	7
17 UK adenocarcinoma non-survivors	60.8 (45–75)	16/1	0	1	15	1	1	14	2	0	2	15
Group 2												
23 UK squamous carcinoma survivors	56.9 (31–76)	6/17	3	2	18	0	14	9	0	0	13	10
23 UK squamous carcinoma non-survivors	63.8 (55–72)	6/17	1	0	21	1	10	12	1	0	7	16
Group 3												
30 Chinese squamous carcinoma survivors	57.8 (44–72)	18/12	1	11	18	0	14	0	16	0	10	20
30 Chinese squamous carcinoma non-survivors	66.4 (54–79)	18/12	0	8	22	0	10	15	5	1	2	27

matched patients who died of their tumour within five years.

Methods

Localised tumours were resected from 80 UK (1977–89) and 60 Chinese (1979–88) patients. Cases were matched into two national groups where a five year survivor was paired with a non-survivor by matching tumour type (squamous carcinoma or adenocarcinoma), sex, and as closely as possible, age, and year of operation. Cause of death was confirmed as oesophageal cancer for the non-survivor group. None of the cases died within 30 days of surgery. Seventeen UK adenocarcinoma survivors, 23 UK squamous carcinoma survivors, and 30 Chinese squamous carcinoma survivors were paired with equal numbers of matched non-survivors, giving groups 1, 2, and 3, respectively. Patient details and staging of resected tumours are given in table 1.

Sections (3 µm) were cut from archival wax blocks of primaries and nodal metastases (where available). E-cadherin was detected immunohistochemically, following microwave treatment (0.01 M citrate buffer, pH 6, at 650 W for three five-minute cycles), using Vectastain Elite ABC and DAB Peroxidase Substrate Kits (Vector Laboratories, Peterborough, UK). The primary antibody was anti-E-cadherin (mouse monoclonal antihuman, Clone HECD-1; R & D Systems, Abingdon, UK), diluted 1/100 in phosphate buffered saline (PBS), pH 7.4; incubation was for 30 minutes at room temperature. Positive and negative controls were parallel sections of normal colon in PBS, with and without primary antibody, respectively.

Distribution of E-cadherin was classified as membranous, membranous and cytoplasmic or cytoplasmic. Staining was classified as positive if > 90% of cells were stained, heterogeneous if 10–90% were stained, and negative if < 10% were stained. Staining intensity was ignored. Staining was assessed independently by two observers with no knowledge of survival status. Consensus was achieved easily.

Histological type, staging and grade (G) were reported according to the tumour, node, metastasis (pTNM) classification of the UICC.²⁸

UNIVARIATE STATISTICAL ANALYSIS

Analysis of matched primary tumours of survivors and non-survivors for E-cadherin in all three groups, and group 1 combined with group 2 used McNemar's test of symmetry.²⁹ Comparison of groups 2 and 3 and investigation of associations between E-cadherin and staging variables in the UK and Chinese groups used χ^2 tests, or Fisher's exact test²⁹ where frequencies were small. Secondary nodal metastases were analysed using Fisher's exact test.

MULTIVARIATE STATISTICAL ANALYSIS

Multivariate statistical analysis was performed on group 1 plus group 2: 37 survivors/37 non-survivors with complete data. Logistic regression for 1–1 matched data³⁰ analysed E-cadherin, T, N, and G by a stepwise selection of the significant explanatory variables for survival (SAS software, The SAS Institute, Cary, North Carolina, USA).

Results

Normal squamous oesophageal epithelium had a banded pattern of E-cadherin expression (fig 1). All layers, except negatively stained flattened squames, showed a membranous staining pattern, with some cytoplasmic staining in basal and parabasal layers. Normal glandular epithelium showed membranous staining with some cytoplasmic staining in the proliferative region. Dysplastic squamous (fig 2) and glandular epithelia showed weakened membranous staining and increased cytoplasmic expression, with negative basal layers in many cases. There was no difference in expression between UK and Chinese epithelia. When reporting tumours, E-cadherin in normal epithelia acted as an internal positive control.

Results of staining of primary tumours are shown in table 2. Only one of 140 tumours had a positive, membranous staining pattern (from a Chinese survivor). Membranous staining was seen most often in the better differentiated areas, with cytoplasmic staining, or negative staining, in the less well differentiated areas. An area from an adenocarcinoma with a positive, membranous staining pattern is shown in fig 3. Nodal metastases were assessed for E-cadherin (table 2) expression. Two of 54 metastases (from one UK adenocarcinoma survivor and

Figure 1 E-cadherin immunoreactivity of normal oesophageal squamous epithelium. Basal (arrowed) and parabasal cells show cytoplasmic/membranous staining. Prickle cell membranes are stained, with expression lessening as the cells approach the lumen.

Figure 2 E-cadherin immunoreactivity of dysplastic oesophageal squamous epithelium is restricted to cytoplasmic staining (basal layer arrowed).

Figure 3 Membranous E-cadherin staining of a well differentiated area of an adenocarcinoma; UK survivor.

Figure 4 Primary squamous carcinoma negative for E-cadherin; UK non-survivor.

one UK adenocarcinoma non-survivor) had positive, membranous staining. Numbers were small but by inspection any change in expression from that of the primary tumour to that of a derived secondary was variable. In UK survivors, 12 maintained the same expression, one had decreased expression, and one increased expression. In UK non-survivors, 19 maintained the same expression, two had decreased expression, and four had increased expression; in Chinese non-survivors, six maintained the same expression, four had decreased expression, and five had increased expression (data not shown). Comparison can be made between a squamous negatively expressing primary (fig

4) and its derived positively expressing secondary (fig 5).

UNIVARIATE STATISTICAL ANALYSIS

Analysis showed a disparity in results from the primary tumours. In group 1 (UK adenocarcinoma) there was no correlation between the absence of E-cadherin and non-survival ($p = 1.00$). However, in group 2 (UK squamous carcinoma) tumours from non-survivors were significantly more likely not to express E-cadherin ($p = 0.009$). In group 3 (Chinese squamous carcinoma), there was no correlation between the absence of E-cadherin and non-survival ($p = 0.06$). Comparison of group 2 with group 3 showed that tumours from UK squamous survivors were significantly less likely to be E-cadherin absent than those from Chinese survivors ($p = 0.007$), and in the non-survivors, the tumours from Chinese patients had a greater proportion of E-cadherin absent ($p = 0.10$). When considering all UK cancers (group 1 plus group 2), absence of E-cadherin significantly correlated with non-survival ($p = 0.02$). When E-cadherin was absent, UK and Chinese cancers were significantly more likely to be G3 ($p = 0.08$ and $p = 0.04$, respectively). In the UK nodal metastases there was no association between absence of E-cadherin and non-survival.

Table 2 Results of E-cadherin expression (present/absent) of primary tumours and nodal metastases

	Tumours		Nodal metastases	
	Present	Absent	Present	Absent
Group 1				
17 UK adenocarcinoma survivors	16	1	3	2
17 UK adenocarcinoma non-survivors	16	1	13	1
Group 2				
23 UK squamous carcinoma survivors	23	0	9	0
23 UK squamous carcinoma non-survivors*	16	7	8	3
Group 3				
30 Chinese squamous carcinoma survivors	22	8	–	–
30 Chinese squamous carcinoma non-survivors	14	16	8	7

*One of 12 nodal metastases not assessable for E-cadherin because of insufficient tissue.

Figure 5 Nodal metastasis positive for E-cadherin, derived from the negative primary squamous carcinoma shown in fig 4.

MULTIVARIATE STATISTICAL ANALYSIS

This analysis used data from group 1 plus group 2 (37 UK matched pairs: three NX cases not analysed). Using a 5% significance level, N1 rather than N0 ($p = 0.01$; odds ratio = 4.97) followed by T3/4 rather than T1/2 status ($p = 0.03$; odds ratio = 11.64) were selected for non-survival.

It was not until a 30% significance level was chosen that E-cadherin was brought into the model as a third significant explanatory variable for non-survival. N1 rather than N0 ($p = 0.04$; odds ratio = 3.83) was followed by T3/4 rather than T1/2 ($p = 0.04$; odds ratio = 10.55), and then E-cadherin ($p = 0.26$; odds ratio = 3.56). A patient with an E-cadherin absent tumour was approximately four times more likely to be a non-survivor compared with a patient with an E-cadherin present tumour.

Discussion

Pairing survivors and non-survivors eliminated tumour type, age, and sex as prognostic factors. Presumably, survivors had less aggressive tumours, although four UK survivors did die of recurrent disease. Demographics and staging of the groups were well matched except for the disparity in tumour types. Adenocarcinomas are rare in China and were not represented. Squamous carcinomas found in China and the West may have different aetiologies, alcohol and tobacco being major determinants in the West but diet and other environmental factors being more important in China. However, once cancers have developed, there do not appear to be significant differences in the inherent behaviour of the tumours.¹

In normal oesophageal epithelium, the least differentiated but more mobile cells have cytoplasmic staining for E-cadherin, with development of membranous staining as cells differentiate. Loss of expression by squames could permit desquamation. Dysplastic epithelia showed cytoplasmic staining, or a negative pattern, probably in association with poorer differentiation (also reported previously in dysplastic Barrett's oesophagus).^{8 25 26} In oesopha-

geal tumours, membranous staining was associated with better differentiation and cytoplasmic or negative staining with poorer differentiation. When present, a cytoplasmic or negative staining pattern is an early event in oesophageal dysplasia/tumorigenesis.

The disordered E-cadherin expression seen in the majority of oesophageal carcinomas could help to explain their high metastatic potential, as probably it reflects a reduction or loss of cell-cell adhesion (as has been demonstrated in squamous cell lines).²⁷ Several authors⁹ have considered that cytoplasmic E-cadherin must be non-functional, cell adhesion and polarity requiring a membranous site. Jankowski *et al* suggest that there may be molecular alterations in E-cadherin in Barrett's oesophagus and adenocarcinoma.⁸

It is of particular interest that a significant relation between E-cadherin absence and non-survival exists for UK squamous carcinomas but not for UK adenocarcinomas or Chinese squamous carcinomas. Adenocarcinomas and squamous carcinomas are known to differ biologically (in terms of both the transversion/transition spectrum of mutations³¹ and the immunohistochemical expression³² of the tumour suppressor gene, p53) and, therefore, they may have different aetiologies: the former are also more aggressive clinically.² In survivors and non-survivors from the UK and China there were also discrepancies in E-cadherin status. Perhaps the squamous carcinomas found in China (high risk) and the West (low risk) are different disorders in terms of expression of E-cadherin and aetiology. Mutation of p53 is also known to be variable in squamous oesophageal tumours; in high risk areas of China, expression is 87.2%,³³ in lower risk areas of China 64%,³³ in Germany 67.4%,³⁴ and in the UK 64%.³² Expression of p53 is a significant prognostic indicator in Chinese³³ but not in European cancers.^{32 34} E-cadherin loss and other accumulated genetic events may have different roles to play in the tumorigenesis and metastasis of these three distinct oesophageal malignancies.

E-cadherin expression in the primary, not the secondaries, is the factor relating to survival. Comparison of E-cadherin of an individual primary with its derived secondary gave variable results; the latter had predominantly the same expression but expression could either be reduced or increased. Previous reports have found E-cadherin expression in metastases equal to, or often greater than, the primary,²⁵ abnormal or absent,⁸ or reduced in tumours with extensive nodal metastases.^{23 24} Differences in expression between primary and secondary tumours could reflect a primary heterogeneity, or instability of E-cadherin expression. Abnormality might allow detachment from the primary followed by re-expression and adhesion in the secondary site, perhaps under local influences.⁹

Multivariate analysis of UK primary tumours showed absence of E-cadherin was a weak prognostic indicator for non-survival, behind node positivity and increased depth of invasion but ahead of grade. E-cadherin is only

one factor in cell–cell, cell–matrix adhesion. A single abnormality in a complex system might be unlikely to have an independent prognostic effect. Further studies of other factors, such as catenins^{9–23} may lead to a better understanding of tumorigenesis and metastasis.

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