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Cathepsin-D expression in cervical carcinoma and its prognostic significance

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Abstract. An immunohistochemical study was made of cathepsin-D protein expression in each of the three main types of uterine cervical carcinoma (squamous carcinoma, adenosquamous carcinoma and adenocarcinoma) with particular reference to lymph node status and prognosis. Of the 61 cases, 54.1% showed cytoplasmic staining in more than 2.5% of tumour cells counted. Cathepsin-D expression was significantly higher in adenocarcinoma (mean −3.128) than in squamous carcinoma and adenosquamous carcinoma (mean −3.709, $p=0.047$ using logit transformation). Cathepsin-D had no prognostic value in any of the three tumour types. No relationship was found between cathepsin-D staining and lymph node status and there was no advantage in adding cathepsin-D values to lymph node status. These results suggest that immunostaining for cathepsin-D protein expression is unlikely to be of use as a prognostic marker.

Key words: Cathepsin-D – Cervical carcinoma – Prognosis

Introduction

Cathepsin-D is an oestrogen-induced lysosomal aspartyl protease (endopeptidase) involved in degradation of endocytosed proteins. Its production in human breast cancer cell lines is specifically increased by oestrogens and inhibited by anti-oestrogens (Tandon et al. 1990).

Proteases such as cathepsin-D have been proposed as possible tumour agents involved in invasion and metastasis and their quantification in carcinomas has been suggested to be of prognostic significance (Garcia et al. 1986; Liotta 1988; Rochefort et al. 1988).

In node-negative (but not node-positive) breast cancer, a high level of cytosolic cathepsin-D is a significant predictor of reduced disease-free survival (Tandon et al. 1990). Increased cathepsin-D concentration in tumour cytosol has also been shown to be strongly related to shorter metastasis-free survival and reduced disease-free survival particularly in this category of patients (Spyratos et al. 1989).

In contrast it has been shown that in lymph node-positive (but not node-negative) breast carcinoma, positive cathepsin-D staining is associated with a significant prognostic advantage. In addition, positive cathepsin-D staining in oestrogen receptor-positive (but not receptor-negative) breast carcinoma is associated with significantly prolonged survival (Henry et al. 1990).

High levels of cathepsin-D expression are also found in poorly differentiated gastric adenocarcinoma (Saku et al. 1990), metastatic ovarian carcinoma (Reich et al. 1984) and poorly differentiated endometrial carcinoma (Maudelonde et al. 1990; Nazeer et al. 1992).

We performed an immunohistochemical analysis of cathepsin-D protein expression in primary cervical carcinoma with particular respect to its prognostic significance.

Materials and methods

Sixty-one cases of stage IB/IIA cervical carcinoma treated by Wertheim's hysterectomy at St Mary's Hospital, Manchester, were used. None of the patients had received prior radiotherapy.

Different cervical tumour types have been shown to have different behavioral patterns and, therefore, the cases used were selected to represent each of the three main tumour types (squamous carcinoma, adenosquamous carcinoma and adenocarcinoma).

The presence or absence of lymph node metastases was noted. A minimum of 5 year follow-up data was available for all patients.

Tissue was formalin-fixed, paraffin-processed and wax embedded. Using both haematoxylin and eosin and periodic acid-Schiff/Alcian blue with and without diastase predigestion, the tumours were typed according to the criteria of Buckley and Fox (1989).

Immunostaining was performed using a standard avidin biotin complex (Dako) alkaline phosphatase technique. A mouse monoclonal antibody (anti-human spleen cathepsin-D, clone NCL-CDm, Novocastra, UK) was used at a concentration of 1:150 and incubated for 50 min at room temperature.

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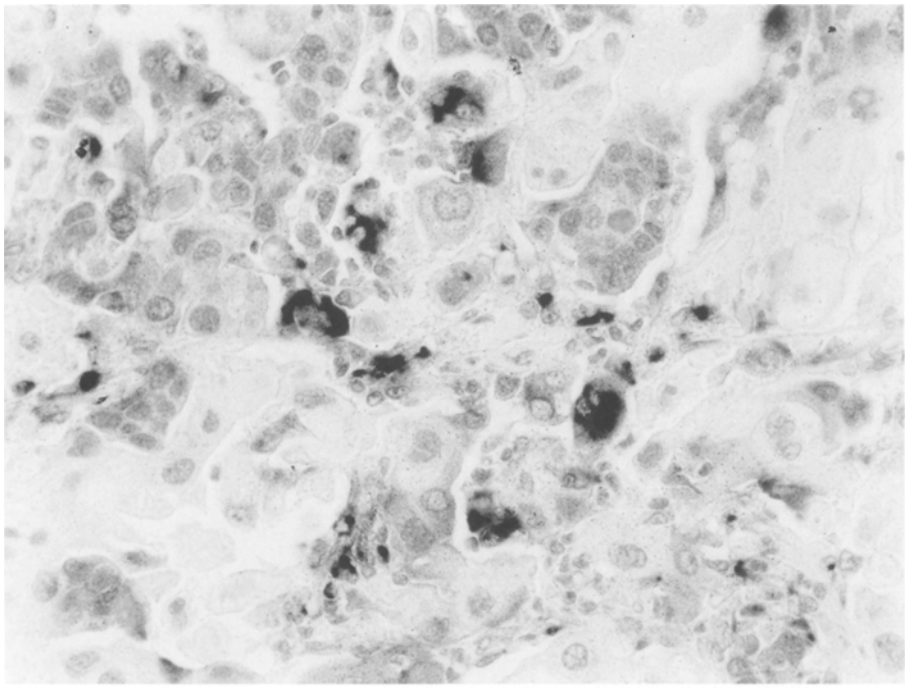

Fig. 1. Cathepsin-D expression in a primary cervical adenosquamous carcinoma (×375)

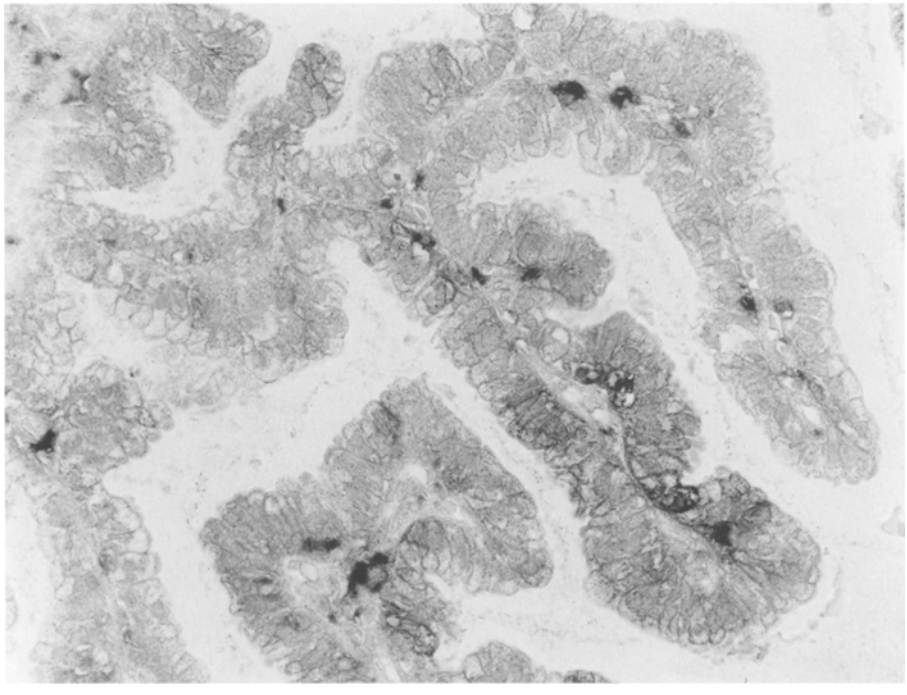

Fig. 2. Cathepsin-D expression in a primary cervical adenocarcinoma (×375)

Tissue macrophages present in all cases were used as positive controls and negative controls involved the omission of the primary antibody.

Cytoplasmic staining (Figs. 1, 2) was graded quantitatively as the percentage of positively staining tumour cells, 1000 carcinoma cells having been counted in each case.

The results were tabulated and analysed using logit transformation with chi-square, analysis of variance and linear discriminant analysis. A significance level of 0.05 ($p<0.05$) was used for all tests.

Results

The distribution of cases by tumour type are presented in Table 1. Cytoplasmic staining was detected in all 61 cases but the numbers of positively stained tumour cells in each case varied from 0.2% to 21.4% (mean 4.5%). For the purpose of some statistical procedures, cases with more than 2.5% of tumour cells staining positively were regarded as positive. Using this threshold, 54.1% of cases demonstrated positive staining. The numbers

Table 1. Distribution of cases by tumour type and numbers staining positively for cathepsin-D

Tumour type	Staining for cathepsin-D				Total
	+ve		−ve		
	No.	(%)	No.	(%)	No.
Squamous carcinoma	11	(55)	9	(45)	20
Adenosquamous carcinoma	10	(48)	11	(52)	21
Adenocarcinoma	12	(60)	8	(40)	20
Overall	33	(54)	28	(46)	61

staining for cathepsin-D in each tumour type are shown in Table 1.

Cathepsin-D expression was higher in adenocarcinoma than in squamous carcinoma and adenosquamous carcinoma ($p = 0.047$ using logit transformation).

The mean and median percentages of tumour cells staining positively for cathepsin-D in each tumour type with respect to lymph node status and outcome are given in Table 2.

The percentage death rate with respect to cathepsin-D staining in patients with and without lymph node metastases in each tumour type and overall are shown in Table 3.

Cathepsin-D had no clear prognostic value either overall or in each of the three individual tumour types, whether or not lymph node status was also taken into consideration.

Table 3. Percentage death rate with respect to cathepsin-D staining in patients with and without lymph node metastases in each tumour type and overall

Tumour type	Staining for cathepsin-D	
	Positive	Negative
	% Death rate	% Death rate
Lymph node metastases present		
Squamous	50% (1/2)	43% (3/7)
Adenosquamous	33% (2/6)	0% (0/5)
Adenocarcinoma	67% (2/3)	75% (3/4)
Overall	45% (5/11)	38% (6/16)
Lymph node metastases absent		
Squamous	44% (4/9)	50% (1/2)
Adenosquamous	25% (1/4)	67% (4/6)
Adenocarcinoma	0% (0/9)	0% (0/4)
Overall	23% (5/22)	42% (5/12)
Total		
Squamous	45% (5/11)	44% (4/9)
Adenosquamous	30% (3/10)	36% (4/11)
Adenocarcinoma	17% (2/12)	38% (3/8)
Overall	30% (10/33)	39% (11/28)

Discussion

To our knowledge, the present study is the first to investigate cathepsin-D expression and its prognostic implications in cervical carcinoma.

All 61 cases contained at least some tumour cells staining positively for cathepsin-D and when an arbitrary threshold was used, 54.1% of all cervical carcinomas showed definite cytoplasmic staining.

In breast cancer, increased cathepsin-D expression has been correlated with a poor prognosis in some studies (Rochefort et al. 1988; Spyratos et al. 1989; Tandon et al. 1990) but the reverse has been shown by others (Henry et al. 1990). Well-differentiated gastric adenocarcinoma shows loss of cytoplasmic staining for cathepsin-D whereas poorly differentiated gastric adenocarcinoma and signet-ring cell carcinoma show strong, diffuse cytoplasmic staining (Saku et al. 1990). It has been shown that metastatic ovarian carcinoma results in extremely high levels of cathepsin-D (Reich et al. 1984). Endometrial carcinomas have shown a correlation between levels of cathepsin-D and both depth of myometrial invasion and degree of differentiation (Maudelonde et al. 1990; Nazeer et al. 1992).

We have shown that cathepsin-D expression was higher in adenocarcinoma than in squamous carcinoma and adenosquamous carcinoma ($p = 0.047$ using logit transformation). Cathepsin-D had no clear value, however, in predicting outcome in any of the three tumour types. No significant relationship was found between cathepsin-D staining and lymph node status and there was no advantage in adding cathepsin-D values to lymph node status.

The identification of patients at high risk of recurrence and death from disease is of great importance in early stage cervical carcinoma because, whilst lymph node status is a major factor in predicting outcome (Hale

Table 2. Mean and median percentages of tumour cells staining positively or cathepsin-D in each tumour type with respect to lymph node status and outcome

Tumour type	Alive		Dead	
	Node +ve	Node −ve	Node +ve	Node −ve
	[Mean % (Median %)]		[Mean % (Median %)]	
Squamous	1.8 (1.3)	5.3 (4.2)	2.3 (1.9)	3.7 (3.6)
Adenosquamous	3.2 (2.2)	5.7 (3.4)	7.6 (7.6)	2.1 (1.5)
Adenocarcinoma	7.5 (7.5)	5.7 (4.0)	6.9 (2.0)	–

et al. 1991a, b), in absolute terms equal numbers of deaths occur in node negative and node positive disease (Thomas and Dembo 1991). It is, therefore, important to try to identify those patients who lack lymph node metastases but who still have a poor prognosis and may benefit from early adjuvant therapy (Thomas and Dembo 1991; Hale et al. 1992). Our study suggests that cathepsin-D is unlikely to be of use in this respect.

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