Neuroendocrine differentiation in cervical carcinoma

P R Savargaonkar, R J Hale, A Mutton, V Manning, C H Buckley

Abstract

Aims—To examine neuroendocrine differentiation, as shown by chromogranin A (CGA) expression, in cervical carcinomas.

Methods—Sixty seven cervical carcinomas were studied and were classified as adenocarcinomas, adenosquamous carcinomas or squamous cell carcinomas based on the assessment of haematoxylin and eosin staining and stains for mucin. Where features of glandular differentiation were identified, sections were also stained for evidence of intestinal type mucin. CGA immunostaining was done and the results were graded on a three point scale: 0, + (1-5% of cells positive) and ++ (>5% of cells positive). These findings were then analysed with respect to lymph node status, tumour differentiation and clinical outcome.

Results—There were 32 adenocarcinomas, 18 adenosquamous carcinomas and 17 squamous cell carcinomas. Positive staining was seen in 14 (20.9%) cases, of which four were strongly positive. All but one case were either adenocarcinomas or adenosquamous carcinomas. There was a trend for CGA positivity to be related to intestinal differentiation but this failed to reach statistical significance. No correlation could be demonstrated between CGA staining and lymph node status, tumour differentiation and clinical outcome.

Conclusions—Neuroendocrine differentiation is common in cervical carcinomas where there is evidence of glandular differentiation. Whilst the numbers in this study are relatively small, the presence of neuroendocrine cells in otherwise typical carcinomas does not seem to have any association with clinical behaviour.

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neuroendocrine differentiation both overall and within each tumour subtype (squamous, adenosquamous and adenocarcinoma); (2) the relation of neuroendocrine differentiation to tumour differentiation and lymph node status; and (3) the effect of the presence or absence of this differentiation on the clinical outcome.

Methods

Sixty seven cases of cervical carcinoma treated from 1969 to 1990 at St Mary's Hospital, Manchester, were studied. In most cases (n=64) material from Wertheim's hysterectomy specimens was available.

Representative sections from the tumours were stained with haematoxylin and eosin and periodic acid Schiff/alcan blue, with and without diastase predigestion, and were then classified according to the criteria of Buckley and Fox.² Thirty two were adenocarcinomas, 18 adenosquamous carcinomas and 17 squamous cell carcinomas.

Cases showing features of glandular differentiation were also stained with PB/KOH/PAS to identify the presence of intestinal type mucin.³

Immunostaining was performed using a standard avidin biotin complex (Dako, High Wycombe, UK) alkaline phosphatase technique. A rabbit antihuman monoclonal antibody to chromogranin A (Dako) was supplied at a concentration of 3 g/l in a solvent of 0.1 NaCl and 15 mM NaN₃ and was used at a dilution of 1 in 50 in Tris buffered saline.

Incubation was performed for 50 minutes at room temperature. Positive and negative controls were used throughout.

The presence of diffuse cytoplasmic staining was assessed. Positivity was graded on a three point scale: 0, + and ++ for no positivity, positivity in 1-5% cells and positivity in >5% of cells, respectively. The presence or absence of cytoplasmic staining in the adjacent normal endocervical columnar and ectocervical squamous epithelium was also noted where this was present.

The presence or absence of lymph node metastases, tumour differentiation and the survival status of the patients (alive and well; died of disease; lost to follow up) was noted. A minimum follow up period of three years was available in all cases.

The results were analysed using a χ^2 analysis with Yates' correction where appropriate. A significant correlation between two parameters was taken at the 95% confidence limit, where $p < 0.05$.

Results

Fourteen (20.9%) cases expressed chromogranin A (table 1). In four cases the tumour

Department of
Reproductive
Pathology,
St Mary's Hospital,
Manchester
P R Savargaonkar
C H Buckley

Department of
Pathology,
Stepping Hill Hospital,
Stockport, Cheshire
R J Hale

North Manchester
General Hospital,
Manchester
A Mutton
V Manning

Correspondence to:
Dr R J Hale,
Department of
Histopathology, Stepping
Hill Hospital, Stockport
SK2 7JE.

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Neuroendocrine differentiation in carcinomas of the cervix is unusual, but is well recognised. It is seen in neoplasms ranging from well differentiated carcinoid tumours to poorly differentiated small cell carcinomas. These tumours morphologically resemble their counterparts at other sites of the body—for example, in the gastrointestinal tract and lung. Isolated neuroendocrine cells are, however, also present in the common types of cervical carcinoma, but are not evident on routine haematoxylin and eosin preparations.¹

Sixty seven cases of cervical carcinoma were studied to elucidate: (1) the prevalence of

Table 1 Chromogranin A staining pattern in each tumour type and overall

Tumour type	Chromogranin A staining			Total
	None (–)	Weak (+)	Strong (++)	
Adenocarcinoma	24	5	3	32
Adenosquamous carcinoma	13	5	0	18
Squamous cell carcinoma	16	0	1	17
Overall	53	10	4	67

(–) = no positive cells; (+) = 1–5% positive; (++) = >5% positive.

Table 2 Chromogranin A staining and clinical outcome

Clinical outcome	Chromogranin A staining			Total
	None (–)	Weak (+)	Strong (++)	
Alive and well	35	5	3	43
Died of disease	13	5	1	19
Lost to follow up	5	0	0	5
Total	53	10	4	67

(–) = no positive cells; (+) = 1–5% positive; (++) = >5% positive.

contained numerous chromogranin A positive cells and these were regarded as strongly positive (++) (figure). In 10 cases far fewer chromogranin A positive cells were present and these were regarded as weakly positive (+).

Table 1 shows the correlation between staining for chromogranin A and the different tumour subtypes. In all but one case the chromogranin A positive cells were in either adenocarcinomas or adenosquamous carcinomas ($p = 0.08$).

There was no significant correlation between staining for chromogranin A and tumour differentiation or lymph node status either overall or in each of the three individual tumour subtypes ($p > 0.1$). In three cases biopsy material only was available and so the nodal status was not known.

Table 2 compares the clinical status with the chromogranin A staining pattern. Follow up was not available in five cases. While there was a trend for outcome to be worse where the tumours stained positively for chromogranin A, this failed to reach statistical significance ($p = 0.07$), both overall and in each individual tumour subtype.

Where chromogranin A staining was analysed with respect to evidence of intestinal differentiation, there was a trend for the two factors to show some positive correlation, but this did not reach statistical significance ($p = 0.07$).

An adenocarcinoma showing strong positivity for chromogranin A.

Endocervical columnar epithelium was present in 31 cases, with occasional chromogranin A positive cells being present in 28. In one of the three cases where these cells were not seen, very little of the normal endocervical epithelium was present. Staining for chromogranin A within the ectocervix was also noted in eight cases. These demonstrated rather weak and diffuse cytoplasmic staining, predominantly in the basal cells. Individual, clear cut positive cells, as noted within the endocervix or the positive tumour, were not seen.

Discussion

Argyrophil and argentaffin cells are well described in the female genital tract. Fox and colleagues have reported occasional argyrophil cells in the normal endocervix⁴ and these cells have also been observed in normal and abnormal endocervix as well as ectocervix.⁵ The presence of these neuroendocrine cells in the normal endocervix was confirmed in our study, but the significance of the rather diffuse positivity seen in the ectocervix is uncertain and may not represent genuine positivity for chromogranin A.

It has been suggested that these neuroendocrine cells within the normal cervix give rise to the neuroendocrine tumours.⁶ This does not, however, explain the occurrence of combined tumours showing both squamous and neuroendocrine differentiation,⁷ or glandular and neuroendocrine differentiation.⁸ Undifferentiated basal stem cells⁸ and mesodermal totipotential precursor cells⁹ have been proposed as the cell of origin. Endodermal stem cells have also been put forward as a possible cell of origin.⁹ Animal experiments have shown that all cells of the small bowel epithelium of the mouse, including enteroendocrine cells, develop from a common undifferentiated precursor, the crypt base-columnar cell.¹⁰ It is possible that this may be extrapolated to tumours of the cervix. Hence, the commoner subtypes of cervical carcinoma may arise from undifferentiated stem cells and, therefore, can not only show areas of glandular and squamous differentiation, but may also contain neuroendocrine cells.^{11–13}

Demonstration of neuroendocrine differentiation can be achieved by several methods. We chose immunohistochemical staining for chromogranin A because of the simplicity of the technique and the relative ease of interpretation. Grimelius reactivity is similar in terms of specificity to chromogranin A, but the latter is more sensitive.¹⁴ Chromogranin A is also more specific than neurone specific enolase.¹⁵

In the present study 14 (20.9%) of 67 cases were positive for chromogranin A, with the highest proportion of positivity being noted in adenosquamous carcinomas and adenocarcinomas (27.7% and 25%, respectively). Only one case of squamous cell carcinoma contained chromogranin A positive cells. This case was a small cell, non-keratinising, squamous cell carcinoma and was the only poorly differentiated tumour in the whole study group

which showed strong chromogranin A positivity. On review, the tumour was composed predominantly of small undifferentiated cells, many of which stained strongly for chromogranin A. Occasional small foci showing possible squamoid differentiation were present, but no definite keratinisation was seen. This patient had lymph node metastases at the time of diagnosis and subsequently developed disseminated disease and died. The clinical behaviour, the histological appearances, and the immunohistochemical findings suggest that this tumour should perhaps have been regarded as a neuroendocrine carcinoma showing focal squamous differentiation, rather than a squamous cell carcinoma.

The remaining three cases showing strong immunoreactivity for chromogranin A were all adenocarcinomas, were well differentiated, had no lymph node metastases, and were alive and well. Of these three cases, two contained goblet cells which stained positively for intestinal type mucin (PB/KOH/PAS),³ indicating enteric differentiation in these tumours. This is an interesting finding as neuroendocrine differentiation is also frequently identified in tumours of the gastrointestinal tract.¹⁷

Previous studies have investigated the prognostic significance of neuroendocrine differentiation in tumours of the gastrointestinal tract and lung,^{17,18} and these have suggested that such differentiation is an indicator of poor prognosis. In the study on colorectal carcinoma¹⁷ the prognosis was related to the degree of chromogranin A reactivity such that tumours containing numerous neuroendocrine cells behave significantly worse than those lacking neuroendocrine cells. Neuroendocrine carcinomas of the cervix, as confirmed by neuroendocrine markers, are also regarded as highly aggressive tumours,^{1,19} with subclinical haematogenous and lymphatic metastases being frequent even in stage I disease. Early adjuvant chemotherapy has been advocated in such cases.¹⁹ Neuroendocrine features in poorly differentiated carcinomas of the cervix are also regarded as indicators of poor prognosis.¹⁶ In our study, however, there is only a trend that suggests that the demonstration of neuroendocrine differentiation, as measured by chromogranin A positivity, has any prognostic significance. In view of the small numbers in the study we cannot draw any strong conclusions, but our results suggest that a similar analysis on a larger group of cases may be interesting. This apparent contrast with the results of previous studies^{1,12,19} may be partly explained by the fact that these authors have concentrated on either poorly differentiated carcinomas or on neuroendocrine carcinomas which can be recognised easily, even on routinely stained haematoxylin and eosin sections. It would seem, therefore, that neuroendocrine differentiation in poorly differentiated carcinomas and well differentiated neuroendocrine carcinomas (carcinoid tumours) may be indicative of a poor prognosis, but the mere presence of neuroendocrine cells within an otherwise typical cervical carcinoma

may not necessarily be associated with aggressive behaviour. It is also interesting to note that the presence of neuroendocrine cells within cervical adenocarcinomas does not seem to confer a bad prognosis, even when the presence of intestinal differentiation is taken into consideration. This contrasts somewhat with the findings in colorectal carcinomas where neuroendocrine differentiation has been shown to be an indicator of poor prognosis.¹⁷

The one poorly differentiated carcinoma with strong chromogranin A positivity, as discussed earlier, would best have been regarded as a neuroendocrine carcinoma; its aggressive behaviour confirming the poor prognosis for these tumours as described by Barrett *et al.*¹⁶

In summary, neuroendocrine cells are present in the normal endocervix. The presence of neuroendocrine cells in the common types of cervical carcinoma is not unusual (14/67, 20.9%). They are seen in both adenocarcinomas and adenosquamous carcinomas. Strong positivity is seen mainly in adenocarcinomas, especially those which are of enteric type, but this does not seem to be of prognostic significance.

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