Nucleolar organiser regions in adenocarcinoma in situ of the endocervix

J E CULLIMORE, T P ROLLASON,* T MARSHALL†

*From the Birmingham and Midland Hospital for Women, and the Departments of *Pathology, and †Social Medicine, University of Birmingham, Birmingham*

SUMMARY The AgNOR technique was used to analyse 11 cases of adenocarcinoma in situ of the endocervix and five examples of healthy cervixes to assess whether areas of "increased nuclear activity" could be located adjacent to the malignant tissue. Areas of adenocarcinoma in situ had significantly more AgNOR staining dots than apparently normal bordering areas ("transitional areas") and areas of endocervical epithelium remote from adenocarcinoma in situ. There were no significant differences between AgNOR counts in transitional areas and areas remote from adenocarcinoma in situ, and between these areas and histologically normal cervixes.

These observations provide no support for the hypothesis that areas of glandular atypia of lesser severity or zones of "increased nuclear activity" exist adjacent to adenocarcinoma in situ.

Adenocarcinoma in situ is believed to be a precursor of invasive cervical adenocarcinoma. It is an uncommon lesion, although its true incidence has probably been underestimated.¹ Various authors have found that glandular atypia of the cervix was strongly associated with cervical squamous intraepithelial neoplasia (CIN),² and one group observed glandular atypia of lesser severity than adenocarcinoma in situ in crypts adjacent to areas of CIN3 in 15% of cases.³ Although we have not observed such a high incidence of cervical glandular atypia in association with CIN3, we might have overlooked most glandular atypias. Given the observation by the same workers that adenocarcinoma in situ was found separated from normal crypts by intervening areas of lesser grades of atypia, we investigated whether there are areas adjacent to adenocarcinoma in situ which show differences in the numbers of nucleolar organiser regions (NORs), as measured by the silver impregnation (AgNOR) method. For if it could be shown that there was a measurable change in nuclear "activity", this might support the concept of the existence of a transitional zone of pre-neoplastic mucosa which is not easily recognisable on routine histological staining. We also felt that it would be useful to determine whether the preferred sites for neoplastic transformation—that is, the crypts adjacent to the squamocolumnar junction—differed from the rest of the endocervix by using the same technique in healthy cervixes.

Material and methods

Cases of classic adenocarcinoma in situ were identified by one of us (TPR) from the pathology files of the Birmingham and Midland Hospital for Women. Sections 3 µm thick were cut from routinely processed paraffin wax blocks. These were dewaxed in xylene and hydrated through ethanols to double distilled deionised water. A staining solution was prepared which consisted of gelatin dissolved in 10 g/l aqueous formic acid at a concentration of 20 g/l, mixed with 500 g/l aqueous silver nitrate (1 volume gelatin/formic acid to 2 volumes silver nitrate). This mixture was poured over tissue sections which were left in a humidity chamber in the dark for one hour at room temperature. The silver colloid was then washed off with deionised water and sections were counterstained with haematoxylin. Sections were dehydrated to xylene and mounted in synthetic medium. The sections were viewed under a × 100 oil immersion lens using a green filter and counts were made of discrete AgNOR dots within nuclei. In counting each cell nucleus, the focus control was carefully adjusted to allow the dots to be counted. One hundred cells were counted in each area chosen. Large mulberry shaped aggregates were always counted as one dot.

Sections of histologically normal cervixes were stained and AgNOR counts were made in endocervical epithelium (i) immediately adjacent to and beneath the squamocolumnar junction and (ii) at least 5 mm distant from the squamocolumnar junction along the axis of the endocervical canal. Cells were

Table 1 Mean numbers of AgNOR dots within nuclei with median numbers in different areas of diseased cervixes

Case No	Adenocarcinoma in situ	Transitional	Distant	Partially affected glands	
				Adenocarcinoma in situ	Normal
1	4.9 (5)	1.4 (1)	1.5 (1)	3.9 (3)	1.0 (1)
2	3.6 (4)	1.9 (2)	1.0 (1)	2.7 (3)	1.2 (1)
3	4.0 (4)	1.3 (1)	1.3 (1)	3.5 (3)	1.5 (1)
4	7.5 (8)	1.2 (1)	1.1 (1)	9.0 (8)	1.3 (1)
5	1.3 (1)	1.3 (1)	1.2 (1)		
6	4.9 (5)	1.1 (1)	1.1 (1)		
7	5.3 (5)	1.4 (1)	1.2 (1)		
8	4.5 (4)	0.6 (1)	0.9 (1)		
9	6.1 (6)	1.1 (1)	1.2 (1)	5.4 (5)	1.0 (1)
10	5.2 (5)	0.9 (1)	1.1 (1)	4.9 (5)	1.1 (1)
11	3.9 (4)	1.2 (1)	1.3 (1)		

chosen "randomly" within the defined areas but the selection method excluded a standard random sampling technique.

ADENOCARCINOMA IN SITU

Counts were made in the following areas:

- In endocervical epithelium showing obvious adenocarcinoma in situ. In every case these areas were closely related to the endocervical border of the squamocolumnar junction.
- In endocervical epithelium not more than 1 mm away from the neoplastic cells, subsequently referred to as "transitional" areas.
- In histologically normal endocervical crypts at a distance of at least 5 mm further along the axis of the endocervical canal, subsequently referred to as "distant" areas.
- In all cases where crypts were partially affected by adenocarcinoma in situ these were included in the counting to include transitional areas as close as possible to the neoplastic cells.

The differences between AgNOR counts in different areas were assessed by analysis of variance, using a square root transformation of the counts.⁴

Results

Eleven cases of cervical adenocarcinoma in situ and five examples of normal cervixes from hysterectomy specimens removed for non-cervical pathology were assessed. After counting 100 cells in each category the

Table 2 Mean and median numbers of AgNOR dots in normal cervixes

Case No	At squamocolumnar junction	> 5 mm from squamocolumnar junction
1	1.3 (1)	1.2 (1)
2	0.9 (1)	1.0 (1)
3	1.4 (1)	1.6 (1)
4	0.9 (1)	0.7 (1)
5	1.0 (1)	0.9 (1)

mean number of AgNOR dots in each cell nucleus was calculated. The results are recorded in tables 1 and 2 and illustrated in figs 1-3. In every case of adenocarcinoma in situ except one the numbers of AgNOR dots within cell nuclei of the neoplastic epithelium were increased compared with those of normal controls (table 1). The differences in AgNOR counts between adenocarcinoma in situ and all other areas were significant ($p < 0.01$). The AgNOR staining dots in the histologically normal areas usually appeared as well defined rounded areas; in the areas with adenocarcinoma in situ the AgNORs were smaller, sometimes irregular, and dispersed throughout the whole nucleus.

There were no significant differences between transitional and distant areas, including those transitional areas immediately adjacent to adenocarcinoma in situ tissue which occupied only part of a crypt. There were no significant differences between distant areas in diseased cervixes and normal cervixes. There were also no significant differences in AgNOR counts at different sites in the group of normal cervixes examined (table 2).

Discussion

Nucleolar organiser regions (NORs) are chromosomal segments in which ribosomal RNA is encoded and are the interphase equivalent of the pars amorpha within the nucleolus. NORs can be located by silver staining, hence the term AgNORs. The stain identifies acidic proteins associated with the NOR. In some malignant diseases AgNOR counts are significantly greater than in normal or benign conditions at the same site, and this may be in part because in malignancy NORs become dispersed throughout the nucleus. The AgNOR count is therefore more likely to be a measure of nucleolar dispersal than of an absolute increase in nucleolar material. It has been suggested that AgNOR counting can be used to distinguish between high and low grade lymphomas⁵ and benign melanocytic lesions and malignant melanoma⁶ among

Fig 1 *Adenocarcinoma in situ of cervical gland crypts stained by AgNOR technique. Multiple small NORs are seen in nuclei.*

other conditions.^{7,8} All these studies have concentrated on conditions distinguished for the purpose of investigation by standard histological techniques. It might therefore be argued that the AgNOR technique has simply been used to confirm an already established diagnostic method. In this study we used the technique simply to attempt to detect epithelial change adjacent to adenocarcinoma in situ with no predetermined histological differences evident. To our knowledge, this is the first time the technique has been used in this way.

It has been suggested that in areas adjacent to adenocarcinoma in situ of the cervix there is a transitional zone of epithelial atypia of lesser grade than adenocarcinoma in situ, which separates adenocarcinoma in situ from histologically normal epithelium.⁹ The extent of these areas was not stipulated by the authors. While we certainly do not deny the existence of glandular atypias of lesser grade than adenocarcinoma in situ, we have not been able to confirm such atypia commonly occurring adjacent to

areas of adenocarcinoma in situ, which usually distinctly abut on adjacent histologically normal glands. The presence of a broad zone of neoplastic potential, however, might have implications for the treatment of these lesions, especially if such a zone extends more than a few millimetres from the microscopically diseased areas. In our experience clearly identifiable glandular atypia of lesser severity or grade than adenocarcinoma in situ is no more common than adenocarcinoma in situ itself, and the differentiation of the conditions is difficult. As the results of AgNOR counting show no appreciable difference in counts between areas immediately adjacent to adenocarcinoma in situ and distant from it this offers no support for the possibility that we may be failing to appreciate subtle histological changes in glands adjacent to adenocarcinoma in situ. This series examined typical cases of adenocarcinoma in situ, unlike that of Brown and Wells, who looked for glandular atypias in patients with CIN3. To date, we have not applied the AgNOR technique to examine

Fig 2 Histologically normal gland crypt stained by AgNOR technique. A solitary large NOR is seen in nuclei.

Fig 3 Mean numbers of Agnor dots in each cell in different areas and significance of differences.

the cervical crypts in cases of CIN3, but if there was a broad glandular change indicative of pre-malignancy we might expect to see lesser degrees of glandular atypia in adenocarcinoma in situ more readily than in cases of CIN3.

We failed to appreciate any difference in AgNOR staining at differing sites within the cervixes of normal subjects and, therefore, there seems to be no intrinsic difference in the degree of nuclear activity as measured by the AgNOR method at different sites in the endocervix in the normal subject.

We would not extend our findings on this small group of cases using this additional technique to deny the presence of a transitional zone adjacent to adenocarcinoma in situ or CIN, but the present study in our opinion adds no weight to the debate in favour of its existence.

JE Cullimore is supported by a grant from the Heath Endowment Fund, Birmingham University.

References

- 1 Boon ME, Baak PA, Kurver PJH, Overdiep SH, Verdonk GW. Adenocarcinoma in situ of the cervix: an underdiagnosed lesion. *Cancer* 1981;48:768-73.
- 2 Ostor AG, Pagano R, Davoren RAM, Fortune DW, Chanen W, Rome R. Adenocarcinoma in situ of the cervix. *Int J Gynecol Pathol* 1984;3:179-90.
- 3 Brown LJR, Wells M. Cervical glandular atypia associated with squamous intraepithelial neoplasia; a pre-malignant lesion? *J Clin Pathol* 1986;39:22-8.
- 4 Armitage P. *Statistical methods in medical research*. Oxford: Blackwell Scientific Publications, 1971:354.
- 5 Crocker J, Nar P. Nucleolar organiser regions in lymphomas. *J Pathol* 1987;151:111-18.
- 6 Crocker J, Skilbeck N. Nucleolar organiser region associated proteins in melanocytic lesions of skin; a quantitative study. *J Clin Pathol* 1987;40:885-9.
- 7 Walker RA. The histopathological evaluation of nucleolar organiser region proteins. *Histopathology* 1988;12:221-3.
- 8 Underwood JCE, Giri DD. Nucleolar organiser regions as diagnostic discriminants for malignancy. *J Pathol* 1988;155: 95-6.
- 9 Wells M, Brown LJR. Glandular lesions of the uterine cervix; the present state of our knowledge. *Histopathology* 1986;10:777-92.

Requests for reprints to: Dr J E Cullimore, Department of Obstetrics and Gynaecology, Clinical Sciences Building, Leicester Royal Infirmary, Infirmary Square, Leicester LE1 5WW, England.