Histol Histopath (1989) 4: 457-458

Histology and Histopathology

Nucleolar organiser regions in colonic dysplasia. A preliminary study

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Summary. As a preliminary investigation in the evaluation of the argyrophilic nucleolar organiser region (AgNOR) technique in colonic dysplasia, quantitation of AgNORs was carried out in biopsies of normal rectal mucosa and tubulovillous adenomas. The AgNOR counts in the lower third of the normal crypts were approximately twice those in the surface mucosa but there was no significant difference between counts in normal crypt bases and adenomas. It is concluded that the AgNOR technique is unlikely to be of value in the assessment of colonic dysplasia.

Key words: Colon, Dysplasia, Nucleolar organiser regions.

Introduction

Quantitation of nucleolar organiser regions visualised by silver staining (AgNORs) has attracted recent interest both as a means of distinguishing benign from malignant tumours and in tumour grading. The technique has been shown to provide useful information in a number of tumours including non-Hodgkin's lymphomas and melanocytic skin tumours (Crocker et al., 1987 a,b,c). There have been fewer attempts to evaluate AgNORs in areas of borderline malignancy and the diagnosis of dysplasia in ulcerative colitis is an obvious potential application.

To be of practical value in the diagnosis of dysplasia in inflammatory bowel disease it would be necessary for the AgNOR technique to assist in the interpretation of equivocal findings. If the assessment of mucosa indefinite for dysplasia were to be possible it should also be possible to distinguish normal from unequivocally neoplastic mucosa. With this in view, a preliminary investigation was performed in which the AgNOR technique was applied to biopsies of normal rectal mucosa and to biopsies showing low grade dysplasia, represented by tubulovillous adenomas.

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Materials and methods

Ten biopsies of normal rectal mucosa and ten biopsies of tubulovillous adenomas were retrieved from our files. Paraffin sections were cut at 3 μm and stained for 40 minutes in a solution consisting of two parts of a 50% silver nitrate solution to one part of a 2% gelatin in 1% aqueous formic acid solution (v/v). Staining was performed at room temperature. After staining slides were washed, dehydrated, cleared and mounted in a synthetic medium. AgNORs were visualized as black intra-nuclear dots and the number of dots per nucleus counted manually under a × 100 oil immersion objective using a Celltrac electronic cell counter.

If the normal biopsies 200 nuclei of the surface and upper third of the crypts and 200 nuclei of the basal third of the crypts were counted while in the adenomas 200 nuclei from a representative area were counted. The mean AgNOR count in each group was then calculated.

Results

In the normal surface epithelium most nuclei contained one or two large dots of argyrophilic material (Fig. 1a). The nuclei of cells in the crypt bases and in the tubulovillous adenomas contained a larger number of smaller dots scattered throughout the nuclei (Fig. 1 b,c). The mean numbers of AgNORs are shown in Fig. 2. The group mean for the normal surface epithelium was 1.78 (SD 0.19) whilst the mean for the crypt bases was 3.45 (SD 0.41) and the tubulovillous adenomas 3.61 (SD 0.49). The latter two values are not significantly different ($p > 0.2$).

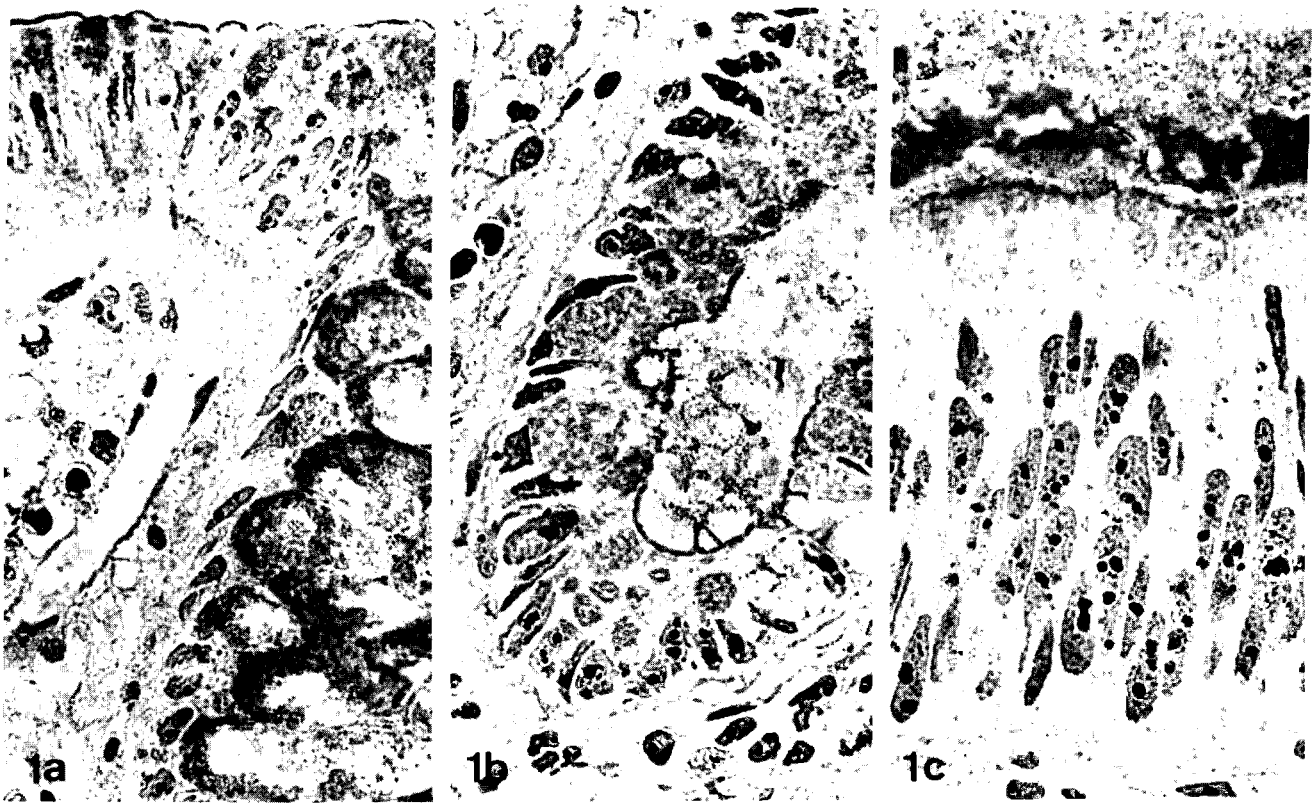

Fig. 1. AgNORs stained as black dots in **a.** Colonic surface epithelium; **b.** Colonic crypt bases; **c.** Neoplastic polyp. × 988

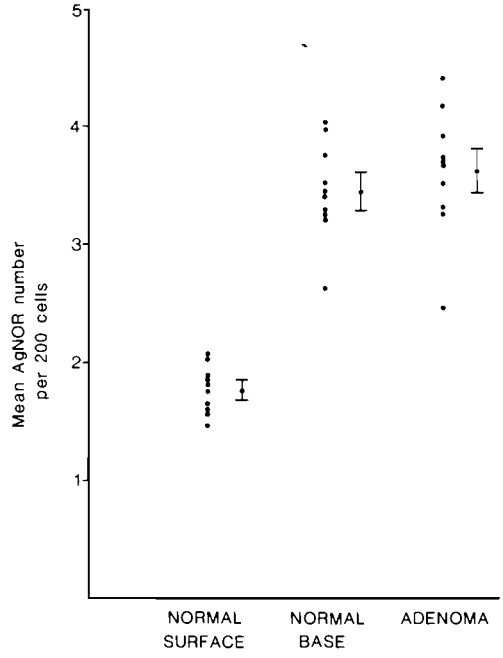

Fig. 2. Mean AgNOR counts in colonic mucosa. Group mean ± S.D. on the right.

Discussion

The nature of NORS and the demonstration of NOR associated proteins have been reviewed elsewhere (Underwood and Giri, 1988; Walker, 1988) but the reason for varying numbers of AgNORs in cells remains uncertain. It has been suggested that the mean number of AgNORs in cells may be correlated with the degree of nuclear and cellular activity (Ploton et al., 1986).

In the standarised classification of dysplasia in inflammatory bowel disease (Riddell et al., 1983) mucosa in which dysplasia is indefinite is accorded categories of indefinite probably negative, indefinite probably positive and indefinite unknown. If the AgNOR technique were to have routine application it should contribute information in this area of diagnostic difficulty.

In this preliminary study we have shown that in the normal colonic mucosa high AgNOR counts correlate with areas of epithelial cell mitotic activity. The tubulovillous adenomas selected showed low grade dysplasia and are representative of mucosa positive for low grade dysplasia according to the classification of Riddell et al (Riddell et al., 1983). We have demonstrated that the mean AgNOR numbers in areas of normal proliferative activity are not significantly different from the numbers in colonic mucosa showing low grade dysplasia.

As it has not been possible to distinguish between normal and unequivocally neoplastic colonic mucosa we consider that the technique is unlikely to be of value in the assessment of indefinite dysplasia in inflammatory bowel disease.

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Accepted April 28, 1989