Virchows Archiv A Pathol Anat (1991) 419:493–497

Virchows Archiv A
Pathological Anatomy and Histopathology

Silver-stained nucleolar organizer regions in the normal, hyperplastic and neoplastic endometrium

Kenji Niwa[1], Yasuhiro Yokoyama[1], Takuji Tanaka[2], Hidehiro Mori[1], Hideki Mori[2], and Teruhiko Tamaya[1]

Departments of [1] Obstetrics and Gynaecology and [2] Pathology, Gifu University School of Medicine, 40 Tsukasa-Machi, Gifu 500, Japan

Summary. The numbers of silver-stained nucleolar proteins (AgNORs) were counted in hyperplastic and neoplastic lesions of the endometrium and compared with those of normal proliferative and secretory phase endometrium. In glandular cells in the normal menstrual cycle, the mean number of AgNORs in proliferative phase endometrium (3.8) was significantly higher than that in secretory phase endometrium (2.7, $P<0.05$). The mean number of AgNORs in well-differentiated endometrioid type adenocarcinoma (5.5) was significantly higher than that in both complex hyperplasia without cytological atypia (3.6, $P<0.01$), and simple hyperplasia (3.3, $P<0.01$). Mean AgNOR counts in complex hyperplasia with cytological atypia were greater than those in both complex hyperplasia without cytological atypia ($P<0.05$) and simple hyperplasia ($P<0.05$). Thus, complex hyperplasia with cytological atypia appears to be a direct precursor of well-differentiated endometrioid type adenocarcinoma. These findings suggest that the mean numbers of AgNORs are increased in neoplastic changes in the endometrium, and the one-step colloid method for AgNORs may therefore be a simple and useful technique to examine proliferative activity in neoplastic and pre-neoplastic endometrial cells.

Key words: Menstrual cycle – Hyperplasia – Carcinoma – Nucleolar organizer regions – Endometrium

Introduction

Nucleolar organizer regions (NORs) are sites where ribosomal DNA is localized. During the interphase, they correspond to loops of DNA (rDNA) encoded for ribosomal RNA (rRNA) production (Alberts et al. 1983). A two-step silver staining method for NORs detection has been utilized for the study of genetic disorders. In 1980, Howell and Black described a one-step colloid method, which is simple and reproducible, for demonstration of NORs, by means of the argyrophilia of their associated proteins (AgNORs).

Recently, the diagnostic value of counting AgNORs in malignancy has been emphasized (Crocker and Paramjit 1987). This technique has been used in the investigation of various malignant tissues, including the lymphoid system (Crocker et al. 1988), pleural methothelioma (Ayres et al. 1988), breast neoplasms (Smith and Crocker 1988), small round cell tumours (Egan et al. 1987), fibroblastic tumours of childhood (Egan et al. 1988a), melanocytic tumours (Fallowfield et al. 1988), other skin neoplasms (Denham and Salibury 1988; Egan and Crocker 1988), and nasal transitional tumours (Egan et al. 1988b), uterine cervical dysplasia (Rowlands 1988), pre-neoplastic endometrium (Coumbe et al. 1990; Wilkinson et al. 1990) and rat liver pre-neoplastic lesions (Tanaka et al. 1989).

In the human endometrium, a clear distinction between hyperplasia and well-differentiated endometrioid type adenocarcinomas may sometimes be difficult. Therefore, we have evaluated the argyrophil staining technique for NORs as a method for distinguishing pre-neoplastic and neoplastic lesions in the endometrium. As the endometrium changes morphologically during the menstrual cycle, change of AgNOR counts in glandular cells was examined in the normal menstrual cycle.

Materials and methods

Twenty-three specimens with known endometrial pathology and 21 normal endometrial tissues from women with uterine leiomyomas were studied. These specimens were obtained by biopsy or hysterectomy in the Department of Obstetrics and Gynaecology, Gifu University School of Medicine, from April 1989 to July 1990. The pathological diagnosis was made using Scully's criteria (1982) by more than two pathologists; there were 5 early proliferative phase, 3 mid-proliferative phase, 3 late-proliferative phase, 3 early-secretory phase, 4 mid-secretory phase, 3 late secretory phase, 10 simple hyperplasia, 5 complex hyperplasia without cytological atypia, 3 complex hyperplasia with cytological atypia, 5 well-differen-

Offprint requests to: K. Niwa

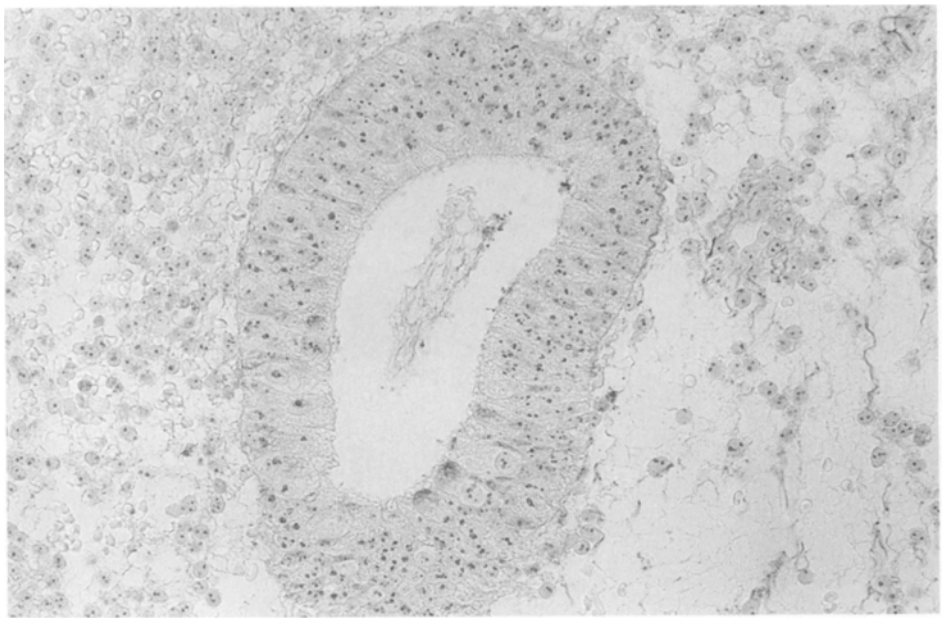

Fig. 1. Early proliferative phase of the endometrium. Four or five silver dots are present in each nucleus. Silver colloid stain, ×800

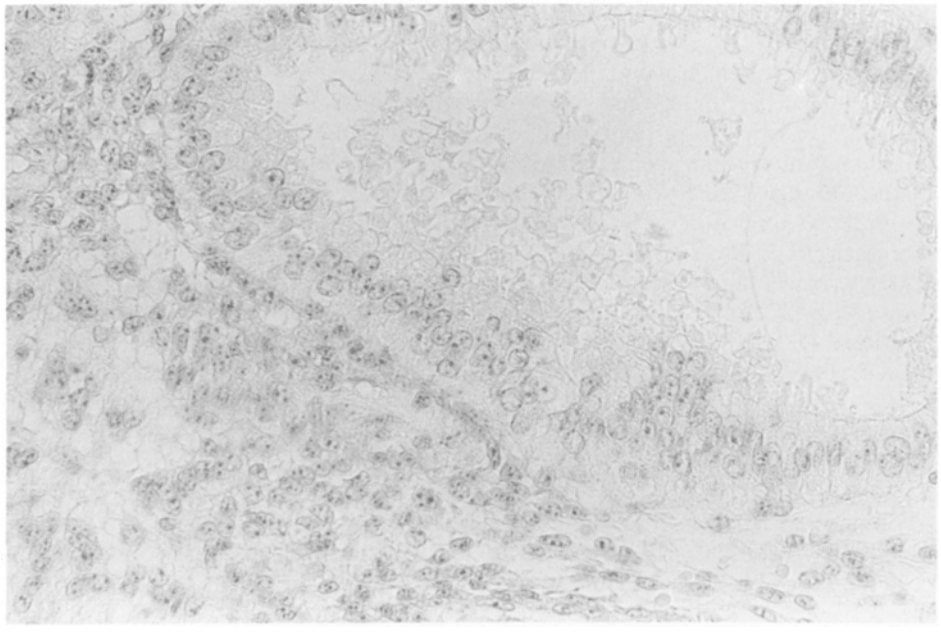

Fig. 2. Mid-secretory phase of the endometrium. Three or four silver dots are seen in each nucleus. Silver colloid stain, ×800

tiated endometrioid type adenocarcinomas. Atypical endometrial lesions without myometrial invasion were diagnosed as "complex hyperplasia with cytological atypia".

AgNOR staining was performed as reported by Howell and Black (1980): briefly, 3-μm-thick sections of routinely processed formalin-fixed, paraffin-embedded blocks were cut, dewaxed in xylene and then hydrated through ethanol to distilled water. A solution of gelatine at a concentration of 2 g/dl in 1% aqueous formic acid was mixed, 1:2 volumes, with 50% aqueous silver nitrate. After filtration with Millipore (0.22 μm), the filtrate was dropped onto the sections, which were left for 60 min in the dark at room temperature. The sections were then washed with distilled water, counterstained with Mayer's haematoxylin and mounted in a synthetic medium.

Sections were examined under a ×100 oil immersion lens. In all the specimens, the numbers of AgNOR dots in more than 100 cells of each lesion were randomly counted. Nucleolar aggregates of AgNORs were counted as a single AgNOR dot. The resulting data were analysed by Student's *t*-test.

Results

AgNORs were clearly recognized as black dots in cell nuclei (Figs. 1–6). The mean numbers of AgNORs of proliferative and secretory phase endometrium are summarized in Table 1. The mean number of AgNORs in proliferative phase endometrium tended to be higher than in the secretory phase. The numbers of AgNORs tended to decrease with the progression of the menstrual cycle.

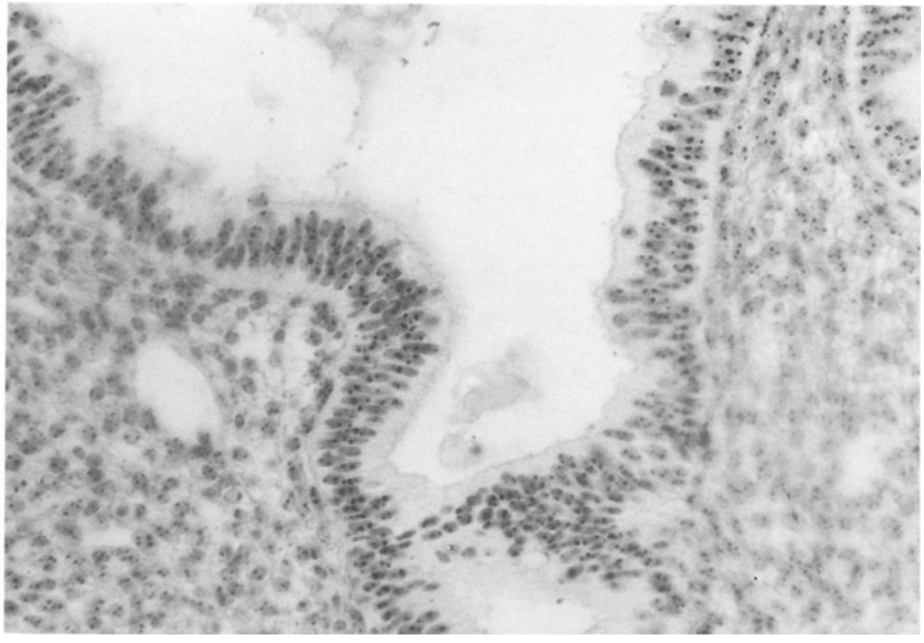

Fig. 3. Simple hyperplasia of the endometrium. Two or three silver dots are observed in each nucleus. Silver colloid stain, ×960

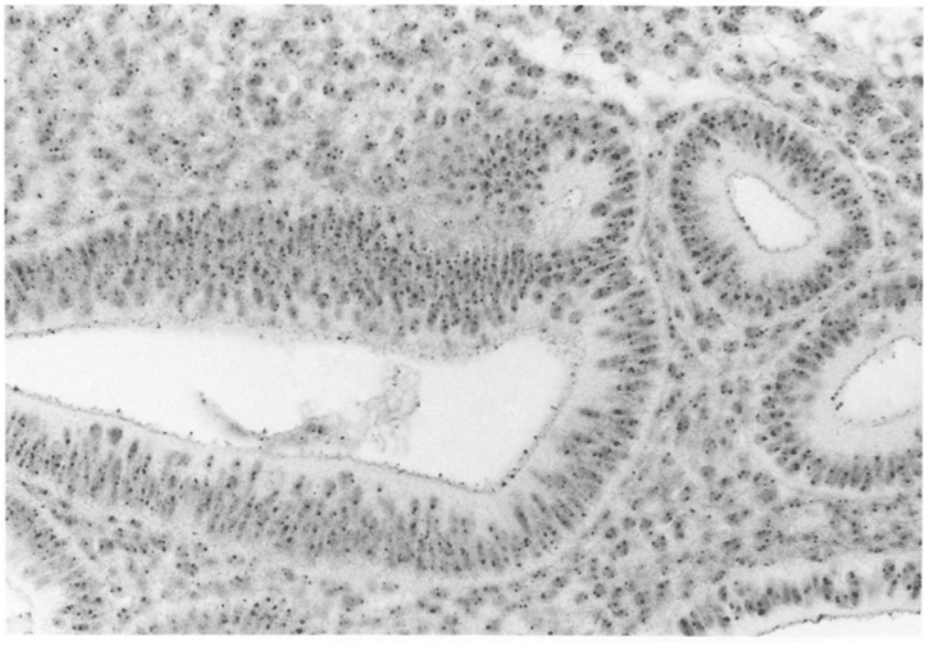

Fig. 4. Complex hyperplasia without cytological atypia of the endometrium. Three or four sliver dots are recognized in each nucleus. Silver colloid stain, ×800

The mean numbers of AgNORs in the hyperplastic and neoplastic endometrial lesions are summarized in Table 2. The mean number of AgNORs in well-differentiated endometrioid type adenocarcinomas was significantly higher than that in complex hyperplasia without cytological atypia and simple hyperplasia. The number in complex hyperplasia with cytological atypia was significantly higher than that in complex hyperplasia without cytological atypia and simple hyperplasia. The numbers of AgNORs tended to increase with advance of neoplastic changes.

Discussion

The results of this study show that the mean numbers of AgNORs in pre-neoplastic and neoplastic lesions tended to increase with increasing neoplastic changes: those in well-differentiated endometrioid type adenocarcinoma and complex hyperplasia with cytological atypia were significantly higher than those in complex hyperplasia without cytological atypia and in simple hyperplasia. Complex hyperplasia with cytological atypia is considered to be a direct precursor of well-differentiated

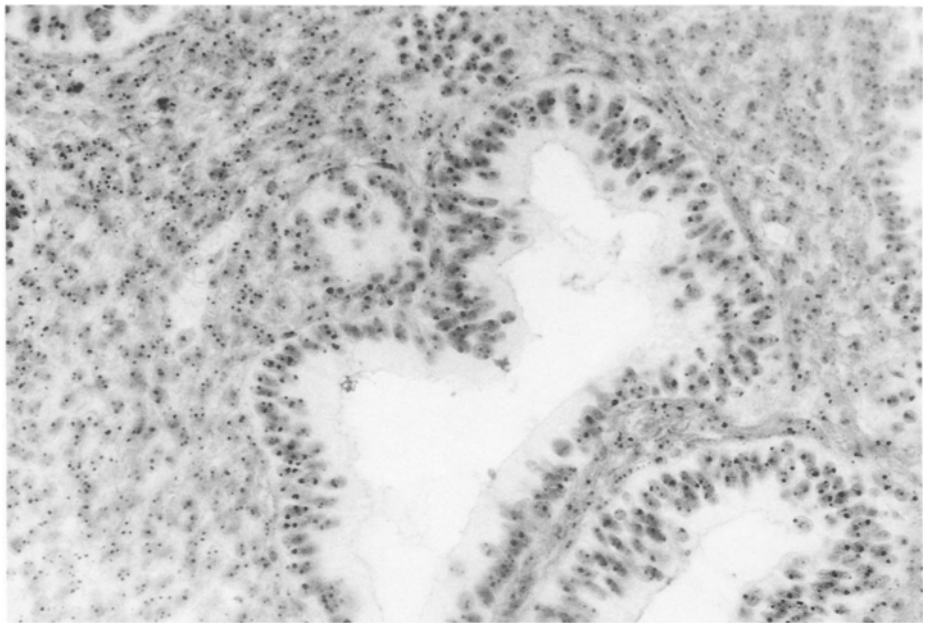

Fig. 5. Complex hyperplasia with cytological atypia of the endometrium. Four or five silver dots are present in each nucleus. Silver colloid stain, ×800

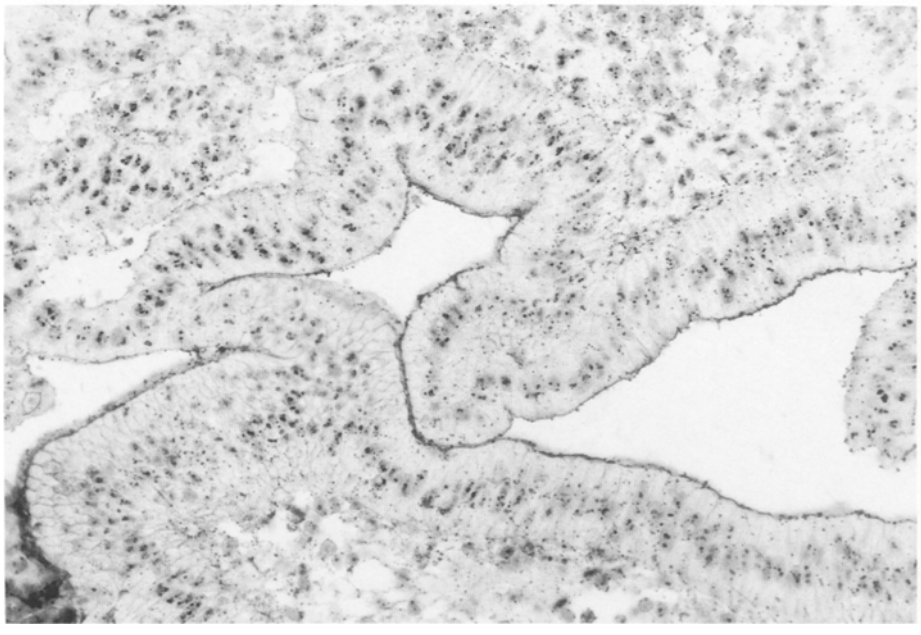

Fig. 6. Well-differentiated endometrioid type adenocarcinoma of the endometrium. Four or five silver dots are present in each nucleus. Silver colloid stain, ×960

endometrioid type adenocarcinoma (Scully 1982). The results of AgNOR counts in hyperplastic and neoplastic endometrial lesions tend to confirm this hypothesis. The number of cases was too small for statistical analysis, but the increased numbers of AgNORs in neoplastic changes are clear in the present study. Further studies of AgNORs in pre-neoplastic endometrial lesions and other types of endometrioid adenocarcinoma will be necessary. The number of AgNORs has also been shown to be of diagnostic significance in malignancy and in pre-neoplastic or neoplastic lesions of the rat liver (Tanaka et al. 1989), human cirrhotic and carcinomatous livers (Crocker and McGovern 1988), uterine cervical dysplasia (Rowlands 1988) and uterine endometrial pre-neoplastic lesions (Coumbe et al. 1990; Wilkinson et al. 1990). In these reports, the mean numbers of AgNORs tended to increase with neoplastic change.

The number of AgNORs is thought to reflect the degree of cellular proliferation (Hall and Levison 1990; Quinn and Wright 1990). The number of AgNORs correlates with that of cells labelled with the monoclonal antibody Ki-67, which is considered to be a marker of cell proliferation (Hall et al. 1988). In experimental carcinogenesis of the rat liver, the mean number of AgNORs was also reported to correlate with the bromodeoxyuridine-labelling index, which reflects the cell proliferation (Tanaka et al. 1989). Moreover, DNA flow cytometry in non-Hodgkin's lymphomas shows a good

Table 1. Silver-stained nucleolar organizer regions (AgNORs) per nucleus in proliferative and secretory endometrial lesions

Endometrium	Number of specimens examined	Number of AgNORs
Glandular cells in the proliferative phase	11	3.8±0.7[a, *]
Early proliferative phase	5	4.0±0.7
Mid-proliferative phase	3	3.7±0.5
Late proliferative phase	3	3.6±0.3
Glandular cells in the secretory phase	10	2.7±0.4
Early secretory phase	3	2.9±0.5
Mid-secretory phase	4	2.6±0.8
Late secretory phase	3	2.4±0.5

[a] Mean±SD
* Significant differences are present between glandular cells in the proliferative and secretory phase ($P<0.05$)

Table 2. AgNORs per nucleus in hyperplastic and neoplastic endometrial lesions

Endometrium	Number of specimens examined	Number of AgNORs
Simple hyperplasia	10	3.3±1.4[a]
Complex hyperplasia without cytological atypia	5	3.6±0.5
Complex hyperplasia with cytological atypia	3	4.5±0.6*
Well differentiated endometrioid type adenocarcinoma	5	5.5±0.7**

[a] Mean±SD
* Significantly higher than those in complex hyperplasia without cytological atypia and in simple hyperplasia ($P<0.05$)
** Significantly higher than those in complex hyperplasia without cytological atypia and in simple hyperplasia ($P<0.01$)

correlation between the mean numbers of AgNOR dots per nucleus and the percentage of S-phase cells (Crocker and Egan 1988). Therefore, AgNOR counts may contribute to the assessment of cell proliferation in some tissues.

The mean number of AgNORs of glandular cells in the proliferative phase endometrium is higher than that in the secretory phase. In the normal menstural cycle, progesterone is secreted during the secretory phase and can be suggested to the number of AgNORs in the endometrium.

As shown in the present study, AgNOR staining is simple and useful for the evaluation of neoplastic changes and proliferative activities in the human endometrium.

References

Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD (1983) Molecular biology of the cell. Garland, New York, pp 424–426

Ayres J, Crocker JG, Skilbeck NQ (1988) Differentiation of malignant from normal and reactive mesothelial cells by the argyrophil technique for nucleolar organiser region associated proteins. Thorax 43:366–370

Boldy DAR, Crocker J, Ayres JG (1989) Application of the Ag-NOR method to cell imprints of lymphoid tissue. J Pathol 157:75–79

Coumbe A, Mills BP, Brown CL (1990) Nucleolar organiser regions in endometrial hyperplasia. Pathol Res Pract 186:254–259

Crocker J, Egan MJ (1988) Correlation between NOR sizes and numbers in non-Hodgkin's lymphomas. J Pathol 156:233–239

Crocker J, McGovern J (1988) Nucleolar organiser regions in normal, cirrhotic, and carcinomatous livers. J Clin Pathol 41:1044–1048

Crocker J, Paramjit N (1987) Nucleolar organizer regions in lymphomas. J Pathol 151:111–118

Crocker J, MaCartney JC, Smith PJ (1988) Correlation between DNA flow cytometric and nucleolar organizer region data in non-Hodgkin's lymphomas. J Pathol 154:151–156

Denham PL, Salisbury JR (1988) Nucleolar organizer region counts in keratoacanthomas and squamous cell carcinomas (abstract). J Pathol 154:70–71

Egan MJ, Crocker J (1988) Nucleolar organizer regions in cutaneous tumours. J Pathol 154:247–253

Egan MJ, Raafat F, Crocker J, Smith K (1987) Nucleolar organizer regions in small cell tumours of childhood. J Pathol 153:275–280

Egan MJ, Raafat F, Crocker J, Smith K (1988a) Nucleolar organiser regions in fibrous proliferations of childhood and infantile fibrosarcoma. J Clin Pathol 41:31–33

Egan M, Ransden K, Crocker J (1988b) Diagnostic significance of mean numbers of nucleolar organizer regions in benign and malignant transitional tumours of nose. Histopathology 12:579–581

Fallowfield ME, Dodson AR, Cook MG (1988) Nucleolar organizer regions in melanocytic dysplasia and melanoma. Histopathology 13:95–99

Hall PA, Levison DA (1990) Review: assessment of cell proliferation in histological material. J Clin Pathol 43:184–192

Hall PA, Crocker J, Watts A, Stansfeld AG (1988) A comparison of nucleolar organizer region staining and Ki-67 immunostaining in non-Hodgkin's lymphoma. Histopathology 12:373–381

Howell WM, Black DA (1980) Controlled silver staining of nucleolar organiser regions with protective colloidal developer: a one-step method. Experientia 36:1014

Quinn CM, Wright NA (1990) The clinical assessment of proliferation and growth in human tumours: evaluation of methods and applications as prognostic variables. J Pathol 160:93–102

Rowlands DC (1988) Nucleolar organising regions in cervical intraepithelial neoplasia. J Clin Pathol 41:1200–1202

Scully RE (1982) Definition of endometrial carcinoma precursors: Clin Obstet Gynecol 25:39–48

Smith R, Crocker J (1988) Evaluation of nucleolar organizer region-associated proteins in breast malignancy. Histopathology 12:113–125

Tanaka T, Takeuchi T, Nishikawa A, Takami T, Mori H (1989) Nucleolar organizer regions in hepatocarcinogenesis induced by N-2-fluorenylacetamide in rats: comparison with bromodeoxyuridine immunohistochemistry. Jpn J Cancer Res 80:1047–1051

Wilkinson N, Buckley CH, Chawner L, Fox H (1990) Nucleolar organiser regions in normal, hyperplastic, and neoplastic endometrium. Int J Gynecol Pathol 9:55–59