Histol Histopath (1991) 6: 531-534

Histology and Histopathology

Evaluation of nucleolar organizer region-associated proteins in endometrial pathology

M.F. Mauri, M.L. Fibbi, V. Lo Re, M. Albertoni and P. Canevini
Department of Morbid Anatomy and Histopathology, Ospedale Civile Bollate, Bollate, Italy

Summary. In the current study the argyrophil staining technique for NOR proteins (Ag-NORs) has been performed on cases of different endometrial lesions, trying to find an aid in differentiating atypical hyperplasia from well differentiated carcinoma in biopsy specimens.

We conclude that the Ag-NOR count, even though in endometrial carcinoma is significantly exceeding that of atypical hyperplastic endometrium, could be a misleading discriminator, because of a wide overlap of values in individual cases.

Key words: Nucleolar organizer regions, Hyperplasia of endometrium, Carcinoma of endometrium

Introduction

The silver-staining technique for interphase NOR at light microscopic level has recently been widely applied in histopathology, since the number of NORs per nucleus appears to correlate with cell proliferation and cellular ploidy (Underwood and Giri, 1988) and may be an indicator of the degree of malignancy in tumours (Lewin, 1980).

In the present study the pattern of Ag-NOR distribution in normal, hyperplastic and neoplastic endometrium has been assessed in order to evaluate the usefulness of this parameter in differentiating atypical hyperplasia from well differentiated endometrioid adenocarcinoma in tissues obtained from curettage.

In therapeutic terms this distinction is extremely important, particularly because in many cases the recognition of an invasive well differentiated endometrioid adenocarcinoma may be very difficult, indeed often impossible, in curettings.

Offprint requests to: Dr. Vincenzo Lo Re, Servizio Anatomia ed Istologia Patologica, Ospedale Civile, Via Piave 20, 20021 Bollate, Milano, Italy

Materials and methods

Fifty-six biopsy specimens from the same number of patients who underwent endometrial curettage were selected from routine histological files. The tissues studied had been previously diagnosed on the basis of routine H&E stain, according to the terminology recommended by the International Society of Gynecological Pathology for the various types of hyperplasia and by the WHO classification for the tumours. We included 10 cases of normal proliferative endometrium, 33 cases of endometrial hyperplasia (10 of simple type, 10 of complex type and 13 of complex type with cytological atypia) and 13 cases of well differentiated adenocarcinoma of endometrioid type. All specimens were formalin fixed and paraffin embedded. All patients in whom a diagnosis of adenocarcinoma was made had also been subjected to hysterectomy, which revealed 5 cases with myometrial invasion, not recognized in the biopsy.

One section from each specimen was examined independently by two pathologists at $\times 100$ magnification under oil immersion and for each section 50 nuclei were studied, randomly selected. Ag-NORs were subdivided into small Ag-NORs(SNs) with diameter <1 μm and large Ag-NORs(LNs) with diameter >1 μm as described in literature (Ploton et al., 1986). The count of the number of clustered dots within each LN was not attempted. LNs and SNs were counted separately. Total numbers of Ag-NORs (TNs) were also recorded. For each case the mean number of SN, LN and TN, + SD was calculated. The inter-observer error was insignificant. The data were analyzed by means of analysis of variance.

Results

Well defined black silver-stained nuclear dots were observed in all specimens included in this study (Figs. 1, 2). The results obtained are shown in Table 1.

Table 1. Mean number of LNs, SNs and TNs ± SD in proliferative, hyperplastic and neoplastic endometrium and statistical analysis.

	LNs	SNs	TNs
PROLIFERATIVE	1.13 ± 0.14	1.24 ± 0.38	2.37 ± 0.46
SIMPLE HYPERPLASIA	1.17 ± 0.09	0.95 ± 0.14	2.12 ± 0.18
COMPLEX HYPERPLASIA	1.15 ± 0.08	1.02 ± 0.37	2.17 ± 0.43
COMPLEX HYPERPLASIA WITH CYTOLOGICAL ATYPIA	1.05 ± 0.21	1.16 ± 0.28	2.22 ± 0.34
CARCINOMA	1.47 ± 0.51	3.07 ± 0.91	4.54 ± 0.92
Statistical analysis	**LNs**	**SNs**	**TNs**
PROLIFERATIVE vs SIMPLE HYPERPLASIA vs COMPLEX HYPERPLASIA WITH AND WITHOUT ATYPIA	$p > 0.05$	$p > 0.05$	$p > 0.05$
COMPLEX HYPERPLASIA WITH CYTOLOGICAL ATYPIA vs CARCINOMA	$p < 0.01$	$p < 0.01$	$p < 0.01$

Fig. 1. Endometrial carcinoma, well differentiated. All-nuclei contain multiple LN and SN. × 1,000

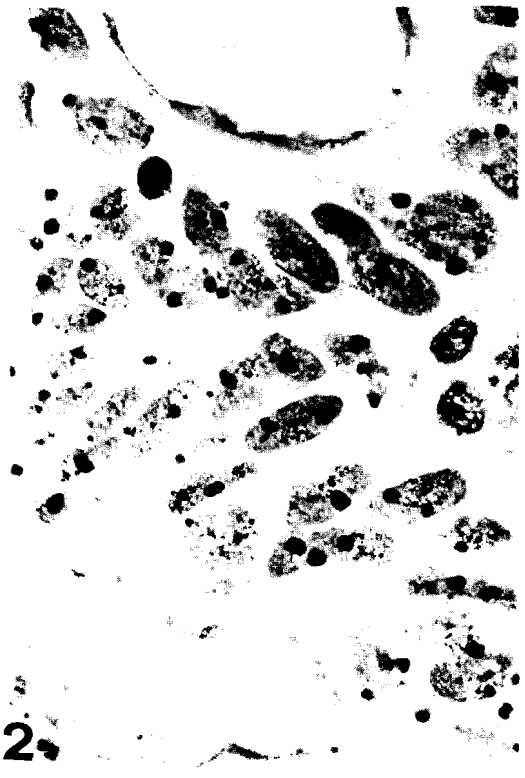

Fig. 2. Complex hyperplasia with cellular atypia. Many cells of back-to-back arranged glands show more than two Ag-NORs per nucleus. × 1,000

Comparison of the mean between proliferative and hyperplastic endometrium (including the simple and complex type with and without cytological atypia) showed a wide overlap of values, whereas there was a clear gap between atypical hyperplasia and carcinoma, with statistically highly significant differences ($p < 0.01$) for SNs, LNs and TNs. Nevertheless, the mean Ag-NOR count in cases of adenocarcinoma invading

Table 2. Mean number of Ag-NORs (large, small structures and total number) per cell nucleus in normal proliferative, hyperplastic and neoplastic endometrium.

DIAGNOSIS	CASE no.	LNs	SNs	TNs
PROLIFERATIVE	1	1.54	1.76	3.3
	2	1.06	1.28	2.34
	3	1.08	1.60	2.68
	4	1.04	1.24	2.28
	5	1.16	1.56	2.72
	6	1.16	1.18	2.34
	7	0.98	1.02	2.00
	8	1.04	0.52	1.56
	9	1.12	0.98	2.10
	10	1.12	1.24	2.36
SIMPLE HYPERPLASIA	1	1.14	0.84	1.98
	2	1.26	1.18	2.44
	3	1.26	1.10	2.36
	4	1.26	0.94	2.20
	5	1.16	0.78	1.94
	6	1.10	1.10	2.20
	7	1.20	0.80	2.00
	8	1.08	1.02	2.10
	9	1.02	0.82	1.84
	10	1.26	0.90	2.16
COMPLEX HYPERPLASIA	1	1.34	1.78	3.12
	2	1.08	1.20	2.28
	3	1.14	1.44	2.58
	4	1.18	0.90	2.08
	5	1.18	1.12	2.30
	6	1.14	0.86	2.10
	7	1.18	0.88	2.06
	8	1.14	0.56	1.70
	9	1.12	0.58	1.70
	10	1.00	0.82	1.82
COMPLEX HYPERPLASIA WITH CYTOLOGICAL ATYPIA	1	0.90	1.38	2.28
	2	1.14	1.32	2.46
	3	0.92	0.70	1.62
	4	1.04	1.48	2.52
	5	1.26	1.38	2.64
	6	0.72	1.50	2.22
	7	1.34	1.38	2.72
	8	1.20	1.04	2.24
	9	1.12	0.74	1.86
	10	1.00	1.18	2.18
	11	1.06	1.18	2.24
	12	0.74	0.94	1.68
	13	1.28	0.94	2.22
CARCINOMA	1	1.28	3.48	4.76
	2	1.32	2.12	3.44
	3	1.24	2.34	3.58
	4	1.92	4.82	6.74
	5	1.72	2.30	4.02
	6	2.12	3.14	5.26
	7	1.26	3.98	5.24
	8	1.38	3.86	5.24
with myometrial invasion	9	1.44	1.94	3.38
	10	1.26	3.84	5.10
	11	1.48	3.50	4.98
	12	1.44	2.40	3.84
	13	1.24	2.16	3.40

the myometrium did not significantly differ from that in cases of adenocarcinoma confined within the endometrium. Moreover, we emphasize the frequent overlap of values in individual cases among the lesions considered (Table 2).

Discussion

The number of Ag-NORs seems to be an index of cell proliferation or of cellular ploidy and in all investigations it has been stressed that such a number is far greater in cells from malignant lesions than in those from benign or non neoplastic conditions (Likowsky et al., 1987; Howat et al., 1989; Egan et al., 1988a,b; Mauri et al., 1990). Numerous reports have endeavoured to evaluate the usefulness of Ag-NOR quantifications in the study of various neoplastic and borderline lesions (Croker and Egan, 1988; Egan and Croker, 1988; Egan et al., 1988a; Morgan et al., 1988; Smith and Croker, 1988; Derenzini et al., 1989; Eusebi et al., 1989). There are, however, a few reports questioning the usefulness of Ag-NOR counts; they point to some problems regarding both the technique and the evaluation of the results (Howat et al., 1988; Underwood and Giri, 1988; Walker, 1988; Fallowfield and Cook, 1989). Moreover, a wide scatter, with overlapping values, of Ag-NOR counts was noted by authors for some malignant neoplasms and in individual cases this may hinder the distinction between benign and malignant lesions (Suarez et al., 1989).

Both enthusiastic and critical reports of Ag-NOR counts prompted us to evaluate the usefulness of the technique in the field of endometrial pathology, investigated before by some people who reported stimulating results (Wilkinson et al., 1990).

There should not be any difficulty in distinguishing simple hyperplasia, complex hyperplasia with architectural atypia or complex hyperplasia with mild cellular atypia from well differentiated adenocarcinoma of the endometrium. One of the heaviest crosses a histopathologist has to bear is, however, the task of differentiating histologically between a complex hyperplasia with severe cellular atypia and a well differentiated adenocarcinoma. The difficulty arises in the most acute form in curettage material, even if many morphological criteria are described in literature for indicating that the glands in such specimens are neoplastic rather than hyperplastic (Robertson, 1981) and that the best differentiated adenocarcinomas behave biologically as carcinomas, i.e. invading the myometrium (Hendrickson and Kempson, 1980; Fox and Buckley, 1982). Moreover, sometimes cases are encountered in which an invasive adenocarcinoma of the endometrium shows less cellular atypia than does a complex hyperplasia with severe cellular atypia and in which all the commonly accepted criteria of true malignancy are absent. So it is possible in most, but no all, curettage specimens to draw a distinction between complex hyperplasia with

severe cellular atypia and adenocarcinoma.

Therefore our attention was drawn to the problem of demonstrating the usefulness of the Ag-NOR technique in the diagnostic assessment of complex hyperplasia with severe cellular atypia and invasive well differentiated adenocarcinoma of the endometrium. The results of the present report show that Ag-NOR counts may assist in distinguishing carcinoma from endometrial hyperplasia.

Unfortunately, there is a wide overlap of values among the series examined. This indicates that an Ag-NOR count is of limited diagnostic value in the routine histological examination of curettings from hyperplastic and neoplastic endometrium and it should be used with great caution.

Acknowledgements. The authors are indebted to Dr. Luigi Di Bernardino for statistical analysis.

References

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

Derenzini M., Nardi F., Farabegoli F., Ottinetti A., Roncaroli F. and Bussolati G. (1989). Distribution of silver-stained interphase nucleolar organizer regions as a parameter to distinguish neoplastic from non neoplastic reactive cells in human effusions. Acta Ctyol. 33, 491-498.

Egan M. and Croker J. (1988). Nucleolar organizer regions in cutaneous tumours. J. Pathol. 154, 247-253.

Egan M., Raafat F., Croker J. and Smith K. (1988a). Nucleolar organizer regions in fibrous proliferations of childhood and infantile fibrosarcoma. J. Clin. Pathol. 41, 31-33.

Egan M., Ramsden K. and Croker J. (1988b). Diagnostic significance of mean number of nucleolar organizer regions in benign and malignant transitional tumours of nose. Histopathology 13, 579-581.

Eusebi V., Ceccarelli C., Cancellieri A. and Derenzini M. (1989). Nucleolar organizer regions in normal skeletal muscle and benign and malignant rhabdomyoblastic tumours. Tumori 75, 4-7.

Fallowfield M.E. and Cook M.G. (1989). The value of nucleolar organizer region staining in the differential diagnosis of borderline melanocytic lesions. Histopathology 14, 299-304.

Fox H. and Buckley C.H. (1982). The endometrial hyperplasias and their relationship to endometrial neoplasia. Histopathology 6, 493-510.

Hendrickson M.R. and Kempson R.L. (1980). Surgical pathology of the uterine corpus. Bennington J. (ed). W.B. Saunders. Philadelphia. pp 338-357.

Howat A.J., Giri D.D., Cotton D.W.K. and Slater D.N. (1989). Nucleolar organizer regions in Spitz nevi and malignant melanoma. Cancer 63, 474-478.

Howat A.J., Giri D.D., Wright A.L. and Underwood J.C.E. (1988). Silver-stained nucleoli and nucleolar organizer region counts are of no prognostic value in thick cutaneous malignant melanoma. J. Pathol. 156, 227-232.

Lewin B. (1980). Gene expression. 2. Eucaryotic chromosome. 2nd ed. J. Wiley & Sons. New York. pp 875-878.

Likowsky Z., Fabian E. and Smetana K. (1987). Interphasic nucleolus organizer regions visualized by silver reaction in human hepatocytes. Hepatogastroenterology 34, 106-107.

Mauri F.A., Muscara M., Ferrero S., Barbareschi M., Scampini S. and Perrone G. (1990). Ag-NOR counts in endometrial and endocervical carcinomas. Pathologica 82, 399-403.

Morgan D.W., Crocker J., Watts A. and Shenoi P.M. (1988). Salivary gland tumours studied by means of the Ag-NOR technique. Histopathology 13, 553-559.

Ploton D., Menager M., Jeanesson P., Himber G., Pigeon F. and Adnett J.J. (1986). Improvement in the staining and in the visualization of the argyrophilic proteins of the nucleolar organizer regions at the optical level. Histochem. J. 18, 5-14.

Robertson W.B. (1981). The endometrium. Butterworths. London. pp 213-220.

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

Suarez V., Newman J., Hiley C., Croker J. and Collins M. (1989). The value of NOR numbers in neoplastic and non neoplastic epithelium of the stomach. Histopathology 14, 61-65.

Underwood J.C.E. and Giri D.D. (1988). Nucleolar organizer regions as a diagnostic discriminants for malignancy. J. Pathol. 155, 95-96.

Walker R.A. (1988). The histopathologic evaluation of nucleolar organizer region proteins. Histopathology 12, 221-223.

Wilkinson N., Buckley C.H., Chawner L. and Fox H. (1990). Nucleolar organizer regions in normal, hyperplastic and neoplastic endometria. Int. J. Gynecol. Pathol. 9, 55-59.

Accepted May 14, 1991