Are AgNORs useful in distinguishing follicular hyperplasia from follicular lymphoma?

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SUMMARY Argyrophilic nucleolar organiser regions (AgNORs) in 28 follicular lymphomas and 30 lymph nodes showing reactive follicular hyperplasia were studied to see if they were helpful in distinguishing follicular lymphoma from follicular hyperplasia in paraffin wax tissue sections. Mean nuclear counts were greater in follicular hyperplasia (3.71 ± 0.83). This difference was significant but counts overlapped so much that they were of no practical value in distinguishing between both conditions. Higher counts may reflect greater proliferative activity in follicular hyperplasia.

Argyrophilic nucleolar organiser regions (AgNORs) have been of theoretical interest to cytogeneticists.¹ Recent reports on the quantification of AgNORs in a variety of tumours have kindled general interest in nucleoli and AgNOR abnormalities in surgical pathology, and in their practical diagnostic usefulness.² In almost all of these studies malignant tumours were found to contain more AgNORs than their benign counterparts.³⁻⁹

The demonstration of follicular monotypic light chain staining distinguishes follicular lymphoma from follicular hyperplasia. Surgical pathologists, however, are occasionally faced with a difficult problem of distinguishing these histologically similar proliferations in routinely processed formalin fixed paraffin wax sections (with no fresh tissue available for κ and λ immunostaining or gene rearrangement studies). To see if quantification of AgNORs might be of value in distinguishing follicular lymphomas from reactive follicular hyperplasia in paraffin wax sections we compared AgNOR counts in both conditions. To see if AgNOR counts might be related to nuclear size we also compared nuclear diameters in both conditions and correlated AgNOR counts with nuclear diameter.

Material and methods

Random follicles from 28 cases of follicular lymphoma (mixed, small cleaved, and large cell/centroblastic-centrocytic) and from 30 lymph nodes showing reactive follicular hyperplasia were examined. These were diagnosed by conventional light microscopy on haematoxylin and eosin stained sections. All specimens were fixed in formalin. Paraffin wax sections (4 μ m thick) were stained for AgNORs as

described by Crocker.⁴ One hundred follicular cells from each case were examined using an oil immersion objective ($\times 100$) and a $\times 10$ eyepiece (total magnification $\times 1000$). Nuclear diameters were also recorded for each cell examined. Using a calibrated eyepiece, the long and short axes of each nucleus were measured. As the shape of the nuclei approximated to a circle the diameter was recorded as the average of the long and short axis. Correlation between nuclear diameter and counts was carried out using Pearson's

Fig 1 AgNOR counts in follicular cells in follicular hyperplasia and follicular lymphoma. + denotes mean count ± 1 standard deviation.

Fig 2 Receiver operating characteristic (ROC) curve. Mean AgNOR scores between 1 and 6 are plotted against the percentage of true positive values and the percentage of false positive values.

correlation coefficient, and significance of intergroup mean counts was estimated with Student's *t* test.

Results

The difference between AgNOR counts in follicular hyperplasia and follicular lymphoma was significant ($p < 0.01$). Follicular cells from reactive hyperplasia had a mean of 3.71 nuclear AgNORs per case (standard deviation, 0.83; median, 3.59; and range, 2.02–5.52). Follicular lymphomas had a mean of 3.11 (standard deviation, 0.9; median, 2.91; and range, 1.62–5.4). Counts in both conditions showed considerable overlap (fig 1). Follicular hyperplasias and follicular lymphomas showed no significant difference in mean nuclear diameters (7.13, SD 0.68 v 7.17, SD 0.90). There was no correlation between AgNOR counts and nuclear diameter ($r = 0.08$; $P = 0.5$).

Discussion

In many studies malignant cells contain more AgNORs than benign proliferations. Nevertheless, the practical diagnostic value of AgNOR counts in borderline or difficult lesions has not been shown.²

In this study the difference between mean counts in both groups was significant (*t* test $p < 0.01$); there was no correlation between counts and nuclear diameter. These results are not useful, however, from a practical point of view, as there was too much overlap between counts. This lack of diagnostic value is clearly shown in the receiver operating characteristic (ROC) curve in fig 2. In the ROC curve, a good diagnostic test with high sensitivity and high specificity will have the curvature located in the top left-hand corner of the plot.¹⁰

Interestingly, counts were higher in the group with reactive hyperplasia. This raises the possibility that increased numbers of AgNORs may reflect cell

proliferation rather than biological malignancy. Hall has shown that AgNOR counts correlate with proliferative activity in malignant lymphomas, as assessed by Ki67 scores.¹¹ Recently, we¹² and Giri¹³ have shown a similar correlation in breast cancer. Weiss has shown that follicular hyperplasia shows greater proliferative activity than follicular lymphoma.¹⁴ Most follicular lymphomas are low grade lymphomas, possibly with low cell proliferation, whereas hyperplastic follicles are highly active units, as can be seen in any reactive lymph node immunostained with monoclonal antibody Ki67.

Estimation of AgNOR counts may prove of diagnostic value in some specific situations and possibly they may be helpful in obtaining cell kinetic information in paraffin wax sections.¹² They are of no value, however, in distinguishing follicular hyperplasia from follicular lymphoma.

This study was supported by the Health Research Board, Eire.

References

- Schwarzacher HG, Wachter F. Nucleolar organiser regions and nucleoli. *Hum Genet* 1983;63:89–99.
- Walker RA. The histopathological evaluation of nucleolar organiser regions. *Histopathol* 1988;12:221–3.
- Crocker J, Ayres J, McGovern J. Nucleolar organizer regions in small cell carcinoma of the bronchus. *Thorax* 1987;42:972–5.
- Crocker J, Skilbeck N. Nucleolar organiser region associated proteins in cutaneous melanotic lesions: a quantitative study. *J Clin Pathol* 1987;40:885–9.
- Crocker J, McGovern J. Nucleolar organiser regions in normal, cirrhotic, and carcinomatous livers. *J Clin Pathol* 1988;41:1044–8.
- Egan MJ, Raafat F, Crocker J, Smith K. Nucleolar organiser regions in fibrous proliferations of childhood and infantile fibrosarcoma. *J Clin Pathol* 1988;41:31–3.
- Egan M, Ramsden K, Crocker J. Diagnostic significance of mean numbers of nucleolar organizer regions in benign and malignant transitional tumours of nose. *Histopathol* 1988;13:579–81.
- Smith R, Crocker J. Evaluation of nucleolar organiser region-associated proteins in breast malignancy. *Histopathol* 1988;12:113–25.
- Morgan DW, Crocker J, Watts A, Sheno PM. Salivary gland tumours studied by means of the AgNOR technique. *Histopathol* 1988;13:553–9.
- Robertson EA, Zweig MH, van Steirteghem AC. Evaluating the clinical efficacy of laboratory tests. *Am J Clin Pathol* 1983;79:78–86.
- Hall PA, Crocker J, Watts A, Stansfeld AG. A comparison of nucleolar organizer region staining and Ki-67 immunostaining in non-Hodgkin's lymphoma. *Histopathol* 1988;12:373–81.
- Dervan PA, Gilmartin LG, Loftus BM, Carney DN. Breast carcinoma kinetics: argyrophilic nucleolar organizer region counts correlate with Ki67 scores. *Am J Clin Pathol* 1989 (in press).
- Giri DD, Nottingham JF, Lawry J, Dundas SAC, Underwood JCE. Silver-binding nucleolar organizer regions (AgNORs) in benign and malignant breast lesions: correlations with ploidy and growth phase by DNA flow cytometry. *J Pathol* 1989;157:307–13.
- Weiss LM, Strickler JG, Medeiros LJ, Gerdes J, Stein H, Warnke RA. Proliferative rates of non-Hodgkin's lymphomas as assessed by Ki-67 antibody. *Hum Pathol* 1987;18:1155–9.

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