Correspondence

Clinical value of interphase argyrophilic nucleolar organiser regions in transitional cell bladder tumours

In the interesting article on interphase argyrophilic nucleolar organiser regions (AgNORs) in transitional cell bladder tumours,[1] the authors conclude that the AgNOR score gives additional cell kinetic information, but that the clinical value of this technique is limited. Neither mean AgNOR count nor nucleoli numbers could distinguish between patients with superficial or invasive tumours because of considerable overlap. This was also the case when the AgNOR numbers of grade II and III tumours were compared.

Recently, we performed a study on the AgNOR pattern in urinary cytology, where the final diagnosis was done in each case by subsequent histological examination of bladder biopsies.[2] We found that AgNOR precipitations increased in parallel with the grade of malignancy, thus confirming the results of Korneyev *et al*.[1] There was also considerable overlap of the AgNOR counts between the different diagnostic groups. When special attention was given to the arrangement of the AgNORs, we found that low grade urothelial carcinomas contained small precipitations, sometimes arranged in clusters, when compared with normal cells. However, high grade carcinomas often had heterogeneous AgNOR features—for example, precipitations with great variations of size and shape, and peculiar forms with angulations, often caused by the confluation or arrangement in clusters.

The recognition of large and irregularly clustered silver precipitations, which we called the "heterogeneous AgNOR pattern", is an important element for differential diagnosis. This pattern was often found in high grade urothelial carcinomas, but was rarely seen in low grade tumours, and was absent in non-neoplastic cells. Joining together the parameters "mean AgNOR count", "range of the AgNOR count", and presence of a "heterogeneous AgNOR pattern" in a linear discriminant analysis, the algorithm could correctly differentiate between non-neoplastic lesions and low grade or high grade carcinoma in 84.3% of cases. In accordance with other studies,[3 4] these results show that it may be very useful both for differential diagnosis and prognosis to take into account peculiar structural features of the silver precipitations and thus to differentiate between various AgNOR configurations. In general, it is easy to separate different AgNOR types and this procedure is highly reproducible. But AgNOR configurations can also be described by computer algorithms. By applying principles of topology to digitalised images, it is possible to differentiate between various AgNOR configurations by quantification of the parameters of the component tree.[5]

In summary, we believe that AgNOR morphology should be taken into consideration for diagnostic and prognostic studies and we think that this additional information will improve the clinical value of the AgNOR technique.

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1 Korneyev IA, Mamev NN, Kozlov VV, *et al*. Interphase argyrophilic nucleolar organiser regions and nucleolus counts in transitional cell bladder tumours. *J Clin Pathol: Mol Pathol* 2000;**53**:129–32.
2 Cia EMM, Trevisan M, Metze K. Ag-NOR technique: a helpful tool for the differential diagnosis in urinary cytology. *Cytopathology* 1999;**10**:30–9.
3 Metze K, Lorand-Metze I . Methods for analysing AgNORs. *J Clin Pathol* 1999;**52**:550.
4 Metze K, Lobo AM, Lorand-Metze I. Nucleolus organizer regions (AgNORs) and total tumor mass are independent prognostic parameters for treatment-free period in chronic lymphocytic leukemia. *Int J Cancer* 2000;**89**: 440–3.
5 Metze K, Souza Filho W, Leite NJ, *et al*. Quantification of the component tree—a new method for image analysis in pathology. *Pathol Int* 2000;**50**:A205.

The authors' reply

In their letter, K Metze and colleagues have suggested that the differentiation ability and prognostic value of interphase argyrophilic nucleolar organiser region (AgNOR) staining in patients with transitional cell carcinoma can be improved by the use of image analysis of AgNOR morphology. It appears that the highly reproducible computer based description of AgNOR configurations is a promising tool to assist in clinical decision making.

Although the improvement of diagnostic and predictive powers remains the ultimate goal in AgNOR staining, it is important to remember that the AgNOR configuration pattern in tumour cells is a secondary phenomenon of unclear origin, and further studies are needed to investigate its characteristics, especially with regard to tumour prognosis. In addition, the AgNOR count in the nucleoli of interphase cells is mainly dependent on the content of nucleolin, nucleophosmin, and RNA polymerase. Thus, the analysis of primary silver stained aggregates based on the calculation of the single separately lying silver grains as standard units reflects the degree of pre-rRNA transcription and pre-rRNA processing in the tumour cells. These features presumably become less evident with the suggested image AgNOR configuration analysis.

Joining together parameters is a valuable approach. In our retrospective study, we have found that the combination of AgNOR distribution (variation coefficient of AgNOR score) with pathological (tumour grade) and clinical (tumour size) criteria yields additional power to discriminate between superficial and invasive bladder neoplasms.

In conclusion, K Metze *et al* have confirmed the importance of peculiar AgNOR morphology and future research is needed to determine the biological role of AgNOR configurations as well as to elucidate the powerful indicators of prognosis in transitional cell carcinomas.

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Correction

Rapid real time PCR to distinguish between high risk human papillomavirus types 16 and 18. Cubie HA, Seager AL, McGoogan E, *et al. J Clin Pathol: Mol Pathol* 2001;**54**:24–29.

The authors would like to apologise for an error that occurred in the writing of this paper. In table 1 (page 25) two of the primer sequences were incorrect.

- The sequence of GP6+ should have been as follows: GAAAAATAAACTGTAAATCATATTC.
- The sequence of TS165 should have been as follows: TTTGTTACTGTTGTTGATACTACA.

All experiments reported in this study were performed with the correct primers and experimental data do not change as a result of this error.

Notice

Therapeutic Filtration

Wolfson Conference Centre, Hammersmith Hospital, London, UK

6 July 2001

Topics include: inflammatory response to cardiopulmonary bypass—the role of filtration; leucocyte depletion of blood cardioplegia; haemofiltration during cardiopulmonary bypass; the bioartificial liver; leucocyte filtration in the treatment of inflammatory bowel disease; therapeutic filtration and adsorption applications of PEPA membranes; leucodepletion for lung transplantation; total filtration approach to cardiac surgery.

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