Correspondence

expression (evaluated with the PC10 monoclonal antibody) seems to be related to cellular proliferation in many normal tissues and to neoplasms, such as gastro-intestinal lymphomas, central nervous system tumours, lung neuroendocrine neoplasms, and prostatic carcinomas. However, in other tumours, like breast and gastric carcinomas, PC10 expression seems absent and not strictly related to proliferative activity. 1,7

Various factors unrelated to cell proliferation may influence the immunohistochemical expression of Ki67, including de-transcriptional regulation (and deregulation) of the PCNA gene, 8 long half-life of the PCNA protein, 9 involvement of PCNA protein in DNA repair, 10 and tissue section processing—type and toughness of the fixatives, fixation time, section heating, immunohistochemical techniques. 11

Further problems in PCNA immunohistochemical staining, as in other kinetic quantitative immunohistochemical studies, concern evaluation and scoring methods. 1415 Should we use quantitative or semiquantitative parameters? Which cells should be counted? Which tumour areas should be evaluated (the most positive or random selected areas?) Which immunoreactive cells should be evaluated (all positive cells or only the most intensely stained?)

Particular attention should be also drawn to the kind of antibody used to localize PCNA. Different staining patterns may be seen with different antibodies, and this may add to the confusing results.

In our opinion PCNA immunostaining should be evaluated with great caution and in some fields even with scepticism. More work is needed to assess the extent and range of PCNA staining in different tissues and lesions (neoplastic and non-neoplastic). PCNA counts should be evaluated concurrently with the different anti-PCNA available antibodies and the results should be compared with the "proliferation fraction" (especially with clinical data. The possibility that PCNA immunostaining may have diagnostic or prognostic value is intriguing and carefully performed clinical pathological studies are needed to assess this possibility further. This will be the only way to know if we are faced with an interesting but clinically worthless tool or with an important test to be added to the routine evaluation of neoplasms.


AgNOR quantification in tumour pathology: What is actually evaluated?

The interest of pathologists in interphase silver stained nucleolar organiser regions (AgNORs) has increased since it was shown that malignant cells frequently have higher AgNOR numbers compared with corresponding benign or normal cells. Moreover, interphase AgNOR numbers are closely related to cell proliferative activity, suggesting that this parameter might also have prognostic importance.

Nucleolar organiser regions (NORs) are chromosomal domains containing single NORs. NORs are associated with a group of argyrophilic proteins, and can be visualised by silver staining in routinely processed cytological and histological samples. At light microscopic level AgNORs appear as well defined black dots, which in interphase cells are exclusively distributed through the lighter stained nucleoli. Each black dot corresponds, at the ultrastructural level, to a fibrillar centre with the surrounding dense fibrillar component. The number of AgNORs in quiescent cells is generally low (most lymphocytes and stromal cells have only one), while in proliferating cells, such as cancer cells, a high AgNOR count is present.

Over the past six years the silver staining technique has become widespread among pathologists, but the lack of a standardised staining protocol has led to misinterpretation of structures and their staining intensity. 2,3 Looking in fact at the micrographs reported—for example, by Giri et al. (breast carcinoma) 4 other, (colonic carcinoma) 5 Cheville et al. (renal carcinoma) 6 and Kaneko et al. (lung carcinoma) 7—it is evident that not just the AgNORs, but the whole nucleoli have been stained by silver and counted as NORs.

The selective visualisation of AgNORs is subject, apart from the fixative used, to the temperature and temporal length of the staining reaction. These two variables are inversely related to each other: the higher the temperature, the shorter the time required for NOR silver staining. As the staining reaction is prolonged beyond the time for selective visualisation of NORs, all the other nucleolar structures are progressively stained, until the whole nucleolus appears homogeneously stained. Therefore, the visualisation of NORs appears to be more cumbersome than expected before evident that different nucleolar structures have been stained and counted in various laboratories, and this has caused disagreement about AgNOR numbers reported in individual studies on the same neoplastic lesions.

In a recent investigation it was shown that the total interphase AgNOR area was closely related to the whole nucleolar area stained by silver when staining was prolonged beyond the optimal time for selective interphase NOR visualisation. 8 To obtain comparable data between different laboratories the whole nucleolus ought to be silver stained and the area occupied by the silver stained nucleoli per cell measured using image analysis instead of AgNOR counting. Because AgNOR area and nucleolar area are so closely linked, the morphometric analysis of silver stained nucleoli will certainly have the same clinical and biological relevance demonstrated for interphase AgNORs.

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Method for grading breast cancer

Parham and colleagues 1 have proposed a new and "simplified" method for grading breast cancer that is superior to the Bloom and Richardson method, 2 which they rightly criticise for its lack of precision. We agree entirely with this criticism, but are rather surprised that they do not refer to our recent publication in which, for precisely this reason, we have devised modifications which provide objective criteria for the evaluation of the three morphological components of histological grade. 3 We have shown in a study of over 1500 patients that histological grade, using this method, provides powerful prognostic information, and in combination with other factors, such as hormone receptors and the Nottingham Prognostic Index which can be used by clinicians to stratify patients for