PCNA immunohistochemical expression (evaluated with the PC10 monoclonal antibody) seems to be related to cellular proliferation in many normal tissues and neoplasms, such as gastrointestinal lymphomas, central nervous system tumours, lung neuroendocrine neoplasms, and prostatic carcinomas. However, in other tumours, like breast and gynaecological neoplasms, PC10 expression seems aberrant and not strictly related to proliferative activity. 

Various factors unrelated to cell proliferation may influence the immunohistochemical expression of PCNA, including post-transcriptional regulation (and deregulation) of the PCNA gene, long half-life of the PCNA protein, involvement of PCNA protein in DNA repair synthesis, and tissue and section processing-type and intensity strength of the fixatives, fixation time, section heating, immunohistochemical techniques.

Further problems in PCNA immunohistochemical staining, as in other kinetic quantitive immunohistochemical studies, concern evaluation and scoring methods. Should we use quantitative or semiquantitative methods? How can the scoring be done? 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 localise PCNA. Different staining patterns may be seen with different antibodies, and this may add to confusing and 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" and especially with clinical data. The possibility that PCNA immunostaining may have diagnostic or prognostic value is intriguing and carefully performed clinicopathological 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.

The interest of pathologists in interphase silver stained nucleolar organiser regions (AgNORs) has increased recently. 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. Thus, this parameter might also have prognostic importance.

Nucleolar organiser regions (NORs) are chromosomal regions that contain ribosomal gene segments. 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 microscopy, AgNORs appear as well defined black dots, which in interphase cells are exclusively distributed throughout 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 content 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 by some authors. Looking in fact at the micrographs reported—for example, by Gier et al (breast carcinoma)

AgNOR quantification in tumour pathology: What is actually evaluated?

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Method for grading breast cancer

Parham and colleagues have proposed a new and "simplified" method for grading breast cancer, and claim that it is superior to the Bloom and Richardson method, 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. We have shown in a study of over 1500 patients that histological grade, using this method, provides powerful prognostic information, and in combination with tumour size and lymph node involvement the Nottingham Prognostic Index which can be used by clinicians to stratify patients for the

6. 6 Denezzini M, Farabegoli F, Treré D. Relationship between interphase AgNOR distribution and nucleolar size in cancer cells. Histochim J (in press).
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