labelling. PCNA immunohistochemical expression (evaluated with the PC10 monoclonal antibody) seems to be related to cellular proliferation in many normal tissues and other neoplasms, such as gastrointestinal lymphomas, central nervous system tumours, lung neuroendocrine neoplasms, and prostatic carcinomas. However, in other tumours, like breast and gynaecological neoplasms, PCNA (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 strength of the fixatives, fixation time, section heating, immunohistochemical techniques.

Further problems in PCNA immunohistochemical staining, as in other kinetic quantitative immunohistochemical studies, concern evaluation and scoring methods. Should we use quantitative or semiquantitative or should we only count the positive areas? 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 to the proliferating cell nuclear antigen (PCNA) especially with clinical data. The possibility that PCNA immunostaining may have diagnostic or prognostic value is intriguing and carefully performed clinicopathological studies alone need 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 grown rapidly. 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 components 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 microscopic level, 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 number 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 interested authors. Looking in fact at the micrographs reported—for example, by Giri et al (breast carcinoma)16 Ofer et al (colonic carcinoma)17 Cheville et al (prostatic carcinoma)1 and Kaneko et al (lung carcinoma),2 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. Therefore, the staining reaction is prolonged beyond the time for selective visualisation of NORs, all the other nuclear structures are progressively stained, until the whole nucleolus appears homogeneously stained. Therefore, it is evident that different nuclear 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. 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 strongly correlated, the morphometric analysis of silver stained nucleoli will certainly have the same clinical and biological relevance demonstrated for interphase AgNORs.
AgNOR quantification in tumour pathology: what is actually evaluated?

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doi: 10.1136/jcp.46.2.189-a

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