Pergamon

*Oral Oncol, Eur J Cancer*, Vol. 31B, No. 6, pp. 403–404, 1995
Elsevier Science Ltd
Printed in Great Britain
0964–1955/95 $9.50 + 0.00

0964–1955(95)00037–2

# PCNA and p53

**A. Mighell**

TISSUE MORPHOLOGICAL changes observed under the light microscope are recognised as a comparatively late consequence of key molecular events that have initiated pathological change. With increasing knowledge of molecular control mechanisms, it is understandable that immunohistochemical studies are used to link specific regulatory proteins, in either their normal or mutated forms, with tissue morphological changes. The recent paper by Girod *et al.* [1] considered the patterns of p53 and proliferating cell nuclear antigen (PCNA) immunoreactivity in a range of paraffin-embedded normal, non-malignant, dysplastic and neoplastic oral mucosal tissues. However, the results presented in Figures 1 and 2 are difficult to interpret, and may partially reflect the complex biologies of p53 and PCNA proteins, and the influence of immunohistochemical techniques on patterns of immunoreactivity.

PCNA cannot be considered a good marker of proliferation status. Nuclear PCNA levels are elevated during cell cycling, but increased PCNA nuclear levels can also be induced in the absence of cell cycling, by either growth factors or as a result of DNA damage [2–5]. Interpretation is further confused by the long tissue half-life of PCNA (at least 20 h) [2], which indicates that nuclei can remain PCNA$^+$ long after the PCNA-inducing stimulus has passed. Accordingly, great variation is observed in intensity of PCNA nuclear immunoreactivity, and under certain experimental conditions, up to 100% of some cell populations are PCNA$^+$ [6]. The influence of the type, duration and temperature of tissue fixation on subsequent PCNA immunoreactivity has been reported in detail, and even the temperature and length of tissue section drying on glass slides have been demonstrated to be critical [7–10]. Some of the variables introduced by tissue fixation can be overcome with heat-mediated techniques, such as microwave or autoclave/pressure-cooker antigen retrieval [10–14]. Of particular importance are the temperature achieved, the time period at which that temperature is maintained, and buffer composition. Optimum conditions may vary for different monoclonal antibodies specific to the same protein. Ideally, comparison should be made with patterns of PCNA immunoreactivity in fresh/frozen tissue, but anti-PCNA antibodies are ineffective on unfixed tissue [7, 8]. The complex biology of PCNA, and the influence of immunohistochemical technique on patterns of PCNA immunoreactivity, makes quantification of PCNA$^-$ nuclei within tissue sections difficult. Consideration must be given to the threshold of nuclear immunoreactivity, case selection and number, choice and number of areas to be quantified, the number of cells counted, reproducibility of results, and statistical analysis [15]. The difficulties in interpretation of quantitative studies of PCNA immunoreactivity are reflected in many contradictory, published reports [15], and PCNA cannot be considered a good marker to assess proliferative activity of premalignant and malignant lesions.

Loss of normal p53 function occurs in many neoplasms, and although the precise role of p53 in the carcinogenic pathway has yet to be fully elucidated, p53 mutations occur early in the development of neoplastic disease [16–19]. Correlation between p53 mutation and immunoreactivity labelling indices has been demonstrated. For example, in an investigation of colorectal neoplasms, Baas *et al.* [20] reported that 6 of 7 cases with a p53 labelling index (LI) $>30\%$, had a p53 mutation identified, whereas 6 of 7 cases with a p53 LI $<1\%$ had no mutation. The final case with a LI $<1\%$ had a p53 mutation that resulted in a truncated p53 protein that lacked the target epitope required for immunolocalisation. Accordingly, absence of p53 expression does not always indicate lack of p53 expression. However, p53 immunoreactivity does not necessarily equate with a carcinogenic pathway, particularly where the antibody investigated may detect either wild type or mutant p53. Increased p53 immunoreactivity is observed in normal cells in response to DNA-damaging stimuli, such as ultra-violet light in recreational sun-bathing, and p53 immunoreactivity is observed in non-neoplastic lesions and in association with inflammation [21–23]. As with PCNA immunohistochemistry, technical considerations, such as choice of anti-p53 antibody, tissue fixation and antigen retrieval protocols, can have a marked effect on patterns of p53 immunoreactivity, and p53$^-$ nuclei may be observed in paraffin-embedded normal tissue following heat-mediated antigen retrieval [11, 13, 14, 20, 24]. Many anti-p53 antibodies are effective on fresh/frozen tissue, and investigation of a few fresh/frozen specimens helps validate patterns of p53 immunoreactivity observed in a large series of paraffin-embedded, archival tissue.

The interest in PCNA and p53 proteins as potential immunohistological markers of malignant disease is reflected in the large number of published reports. However, useful interpretation of PCNA and p53 immunohistochemical studies can only be made if full consideration is given to the complex biologies of PCNA and p53, and to the influence of experimental technique.

Correspondence to A. Mighell at Leeds Dental School, Clarendon Way, Leeds LS2 9LU.
Accepted 14 June 1995.

1. Girod SC, Pape H-D, Krueger GRF. p53 and PCNA expression in carcinogenesis of the oropharyngeal mucosa. *Oral Oncol, Eur J Cancer* 1994, **30B**, 419–423.
2. Bravo R, Macdonald-Bravo H. Existence of two populations of cyclin/proliferating cell nuclear antigen during the cell cycle. Association with DNA replication sites. *J Cell Biol* 1987, **105**, 1549–1554.

3. Shivji MKK, Kenny MK, Wood RD. Proliferating cell nuclear antigen (PCNA) is required for DNA excision repair. *Cell* 1992, **69**, 1–20.
4. McCormick D, Hall PA. The complexities of proliferating cell nuclear antigen. *Histopathology* 1992, **21**, 591–594.
5. Hall PA, Hart I, Goodlad R, Coates PJ, Lane DP. Expression of proliferating cell nuclear antigen in non-cycling cells may be induced by growth factors *in-vivo*. *Br J Cancer* 1994, **70**, 244–247.
6. McCormick D, Yu C, Hobbs C, Hall PA, The relevance of antibody concentration to the immunohistological quantification of cell-associated antigens. *Histopathology* 1993, **22**, 543–547.
7. Hall PA, Levision DA, Woods AL, *et al.* Proliferating cell nuclear antigen (PCNA) immuno-localisation in paraffin sections: an index of cell proliferation with evidence of deregulated expression in some neoplasms. *J Pathol* 1990, **162**, 285–294.
8. Tsuji T, Shrestha P, Yamada K, *et al.* Proliferating cell nuclear antigen in malignant and pre-malignant lesions of epithelial origin in the oral cavity and the skin: an immunohistochemical study. *Virchows Arch A Pathol Anat Histopathol* 1992, **420**, 377–383.
9. Wada T, Shimabukuro T, Matsuyama H, Naito K, Skog S, Tribukait B. Optimal conditions of fixation for immunohistochemical staining of proliferating cell nuclear antigen in tumour cells and its cell cycle related immunohistochemical expression. *Cell Prolif* 1994, **27**, 541–551.
10. Wasielewski R von, Werener M, Nolte M, Wilkens L, Georgii A. Effects of antigen retrieval by microwave heating in formalin-fixed tissue sections on a broad panel of antibodies. *Histochemistry* 1994, **102**, 165–172.
11. Cattoretti G, Pileri S, Parravicini C, *et al.* Antigen unmasking on formalin-fixed, paraffin-embedded tissue sections. *J Pathol* 1993, **171**, 83–98.
12. Kubbutat MHG, Cattoretti G, Gerdes J, Key G. Comparison of monoclonal antibodies PC10 and MIB 1 on microwave-processed paraffin sections. *Cell Prolif* 1994, **27**, 553–559.
13. Norton AJ, Jordan S, Yeomans P. Brief, high-temperature heat denaturation (pressure cooking): a simple and effective method of antigen retrieval for routinely processed tissues. *J Pathol* 1994, **173**, 371–379.
14. Taylor CR, Shi SR, Chaiwun B, Young L, Imam SA, Cote RJ. Strategies for improving the immunohistochemical staining of various intranuclear prognostic markers in formalin–paraffin sections: androgen receptor, eostrogen receptor, progesterone receptor, p53 protein, proliferating cell nuclear antigen, and Ki-67 antigen revealed by antigen retrieval techniques. *Human Pathol* 1994, **25**, 263–270.
15. Yu C, Filipe M. Update on proliferation-associated antibodies applicable to formalin-fixed paraffin-embedded tissue and their clinical applications. *Histochem J* 1993, **25**, 843–853.
16. Chung KY, Mukhopadhyay T, Kim J, *et al.* Discordant p53 gene mutations in primary head and neck cancers and in corresponding cancers of the upper aerodigestive tract. *Cancer Res* 1993, **53**, 1676–1683.
17. Nees M, Homann N, Discher H, *et al.* Expression of mutated p53 occurs in tumor-distant epithelia of head and neck cancer patients: a possible molecular basis for the development of multiple tumors. *Cancer Res* 1993, **53**, 4189–4196.
18. Lane DP. The regulation of p53 function. *Int J Cancer* 1994, **57**, 623–627.
19. Wynford-Thomas D, Bond JA, Wyllie FS, Jones CJ. Does telomere shortening drive selection for p53 mutation in human cancer? *Mol Carcinogenesis* 1995, **12**, 119–123.
20. Baas IO, Mulder J-WR, Offerhaus JA, Vogelstein B, Hamilton SR. An evaluation of six antibodies for immunohistochemistry of mutant p53 gene product in archival colorectal neoplasms. *J Pathol* 1994, **172**, 5–12.
21. Hall PA, McKee PH, Menage HD, Dover R, Lane DP. High levels of p53 protein in UV irradiated human skin. *Oncogene* 1993, **8**, 203–207.
22. Pollanen R, Soini Y, Vahakangas K, Paakko P, Lehto VP. Aberrant p53 expression in cervical intraepithelial neoplasia. *Histopathology* 1993, **23**, 471–474.
23. Soini Y, Kamel D, Paakko P, Lehto VP, Oikarinen A, Vahakangas K. Aberrant accumulation of p53 associates with Ki67 and mitotic counts in benign skin lesions. *Br J Dermatol* 1994, **131**, 514–520.
24. Lambkin HA, Mothersill CM, Kelehan P. Variation in immunohistochemical detection of p53 protein overexpression in cervical carcinomas with different antibodies and methods of detection. *J Pathol* 1994, **172**, 13–18.