Comparative Study of PCNA and Ki-67 Immunohistochemical Staining in Psoriasis, Basal Cell Carcinomas and Squamous cell Carcinomas

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Background: **Immunostaining to identify nuclear antigen provides a convenient way of assessing proliferative kinetics in hyperplastic/tumor tissue.**

Objective: The object of this study is to find out whether there are any differences in the expression of proliferation related protein among psoriasis, basal cell carcinoma and squamous cell carcinoma by immunohistochemical evaluation on the PCNA (proliferative cell nuclear antigen) and Ki-67.

Methods: **The detection of PCNA and Ki-67 were done by immunohistochemical methods (avidin-biotin immunoperoxidase methods) using respective monoclonal antibodies in the paraffin embeded tissues from psoriasis (17 cases), basal cell carcinomas (15 cases) and squamous cell carcinomas (10 cases).**

Results: **The labelling indices of PCNA were 14.2±4.0% in psoriasis, 10.9±5.5% in basal cell cardinoma and 28.0±7.8% in squamous cell carcinoma, while the labelling indices of Ki-67 were 15.7±3.8% in psoriasis, 11.2±6.1% in basal cell carcinoma and 30.3±9.4% in squamous cell carcinoma.**

Conclusion: **1. Interpretation of Ki-67 staining was easier than that for PCNA, mainly because cell morphology was better preserved and the distinction between hyperplastic/tumor and nontumor cell was clear.**

2. PCNA and Ki-67 counts had strong correlation to each other (r=0.979).

3. Our immunohistochemical results of PCNA and Ki-67 suggested that proliferative activity was more marked in psoriasis than basal cell carcinoma.

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Key Words: PCNA, Ki-67, Psoriasis, Basal cell carcinoma, Squamous cell carcinoma

Cell kinetic information may be a useful adjunct to histologically based tumor classifications and contribute to the understanding of a range of non neoplasic conditions[1]. Counting mitoses in routine hematoxyline and eosin stained sections provides the simplest assessment of cell proliferation. Newer, often more informative, techniques are flow cytometry, computerized image analysis, autoradiography with tritiated thymidine, or immunohistochemistry using bromodeoxyuridine, proliferative cell nuclear antigen (PCNA) or Ki-67 antibodies. Immunohistochemical methods of assessing cell proliferation have particular advantages over other techniques because of the maintenance of cellular and tissue architecture, the relative simplicity of the methodology and the rapidity of results; niether **in vivo** nor **in vitro** labelling is required and the use of radioactivity in avoided[1]. Immunohistology would be even more useful if available antibodies to cell cycle related

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antigens were applicable to conventionally fixed and processed tissue.

Proliferative cell nucleus antigen (PCNA), also known as cyclin, is expressed in proliferating cells and is present in greatest concentration during the S-phase. A number of antibodies to PCNA identify proliferating cells.

The monoclonal antibody Ki-67 identifies a nuclear antigen in proliferating cells in the G_1, S, G2 and M phase of the cell cycle[2-4]. Thus,Ki-67 antibodg counts provide an estimate of the total tumor growth fraction, whereas the bromodeoxyuridine and 3H thymidine techniques and analysis of DNA histograms are used to estimate the tumor S-phase fraction. Most Ki-67 antibodies are not reactive with routine formalin-fixed paraffin-embeded tissue.

In this study, we assessed in normal and in a variety of tumors the reactivity of a new commerically available antibody to PCNA and Ki-67 which are reactive with formalin-fixed, paraffin-embeded tissue[5]. This paper gives a comparative analysis of the proliferation activity determined by PCNA and Ki-67 immunostaining in hyperplastic and tumor tissues.

MATERIALS AND METHODS

Seventeen cases of psoriasis, fifteen cases of basal cell carcinomas which were classified on the basis of their predominant patterns, and ten cases of squamous cell carcinomas were collected from the files of Soonchunhyang University Hospital, department of pathology. For the control, five specimens of normal skin from various sites were included. The details of specimens included are listed in table1.

Tissue was fixed in neutral-buffered formalin followed by mercuric chloride post-fixation and embeded in paraffin. Sections (4μm) were cut and mounted on lysin-coated slides. For immunohistochemical staining, the tissue sections were treated with xylene to remove paraffin, rehydrated through graded alcohols, and then immunostained by a avidin-biotin-peroxidase technique. After blocking the endogenous peroxidase activity, sections were incubated in mouse Ig G monoclonal antibody against Ki-67 (MIB-1; DAKO Denmark) diluted 1:50 with phosphate-buffered saline for 60 min at 37°C, in biotinylated rabbit anti-mouse Ig G (ready to use; Nichirei, Japan) for 10 min at room temperature, and then in peroxidase-conjugated avidin (ready to use; Nichirei, Japan) for 5 min at room temperature. Between incubations, tissue sections were rinsed for 5 min at room temperature in phosphate-buffered saline. After immunostaining sections counterstained with Mayer's haematoxylin, and mounted in Glycergel (DAKO). To confirm the identity of the Ki-67 positive cells in the tissues, serial sections were immunostained using antibody against PCNA (PC 10, DAKO, Denmark) diluted 1:20. Immunostaining steps for PCNA was also performed using a avidin-biotin-peroxidase technique.

Sections were counted at high power (× 400) using an eyepiece graticule, and eight fields/specimens were chosen at random. In each case, nuclei from 800 and 1000 cells were counted respectively for PCNA and Ki-67. The labelling index was calculated on the percentage of positive stainning cell nuclei. To take account of intra-lesional heterogeneity, a semi-quantitative grading system was used in which the whole of a section was assessed at lower power (× 100)

RESULTS

The labelling indexes of PCNA were 14.2± 4.0% in psoriasis, 10.9±5.5% in basal cell carcinoma and 28.0±7.8% in squamous cell carcinoma, while the labelling indexes of Ki-67 were 15.7 ±3.8% in psoriasis, 11.2±6.1% in basal cell carcinoma and 30.3±9.4% in squamous cell carcinoma.

Interpretation of Ki-67 staining was easier than that for PCNA, mainly because cell morphology was better preserved and the distinction between tumor and nontumor cell was clear (Fig. 1,2,3)

Mean Ki-67 counts were slightly higher than PCNA counts (Table 1) but were not statistically significant (p value = 0.19, two sample Student *t*-test). Both antibodies gave similar results with a correlation coefficient of 0.979.

DISCUSSION

PCNA in an evolutionarily highly conserved 36 kDa acidic nuclear protein, essential for DNA synthesis[6]. It in the nuclear antigen detected in proliferating cells by auto-antibodies in patients with systemic lupus erythematosus. Several mono-

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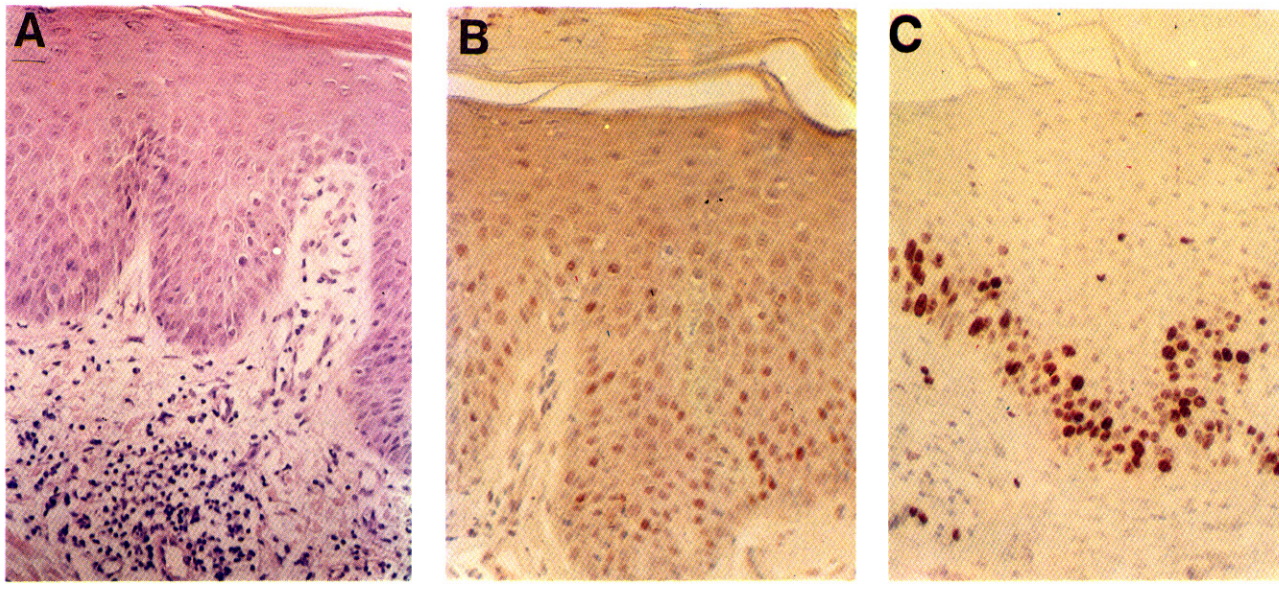

Fig. 1A-C. H&E and Immunohistochemical staining in psoriasis. (A) H&E, (B) PCNA, (C) Ki-67, paraffin section. Cellular morphologic features are better preserved in Ki-67 preparation (× 200).

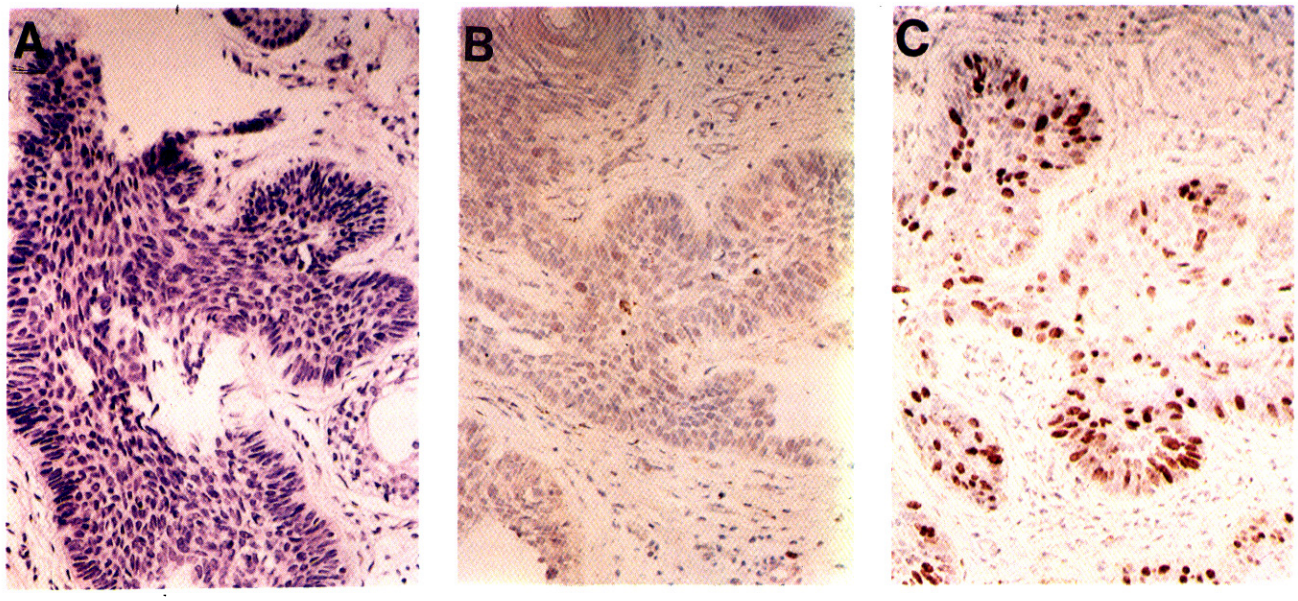

Fig. 2A-C. H&E and immunohistochemical staining in basal cell carcinoma. (A)H&E, (B) PCNA, (C) Ki-67, paraffin section. Nodular basal cell carcinoma showing maximal staining at the periphery of the tumor islands for PCNA and Ki-67 (× 200).

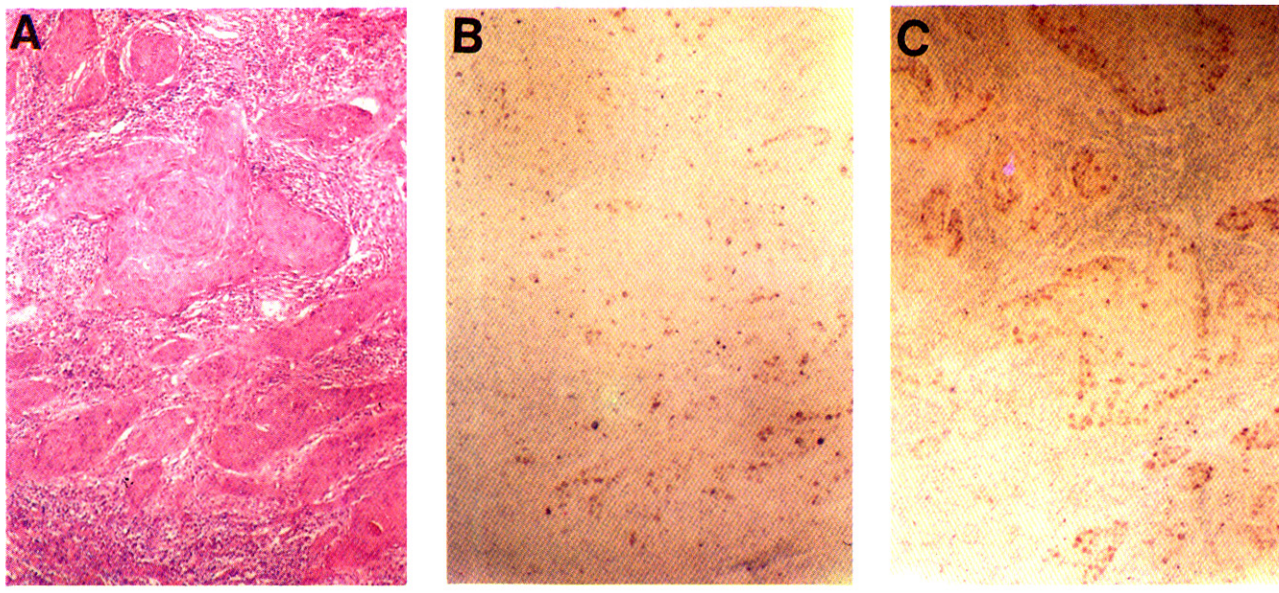

Fig. 3A-C. H&E and immunohistochemical staining in squamous cell carcinoma. (A) H&E, (B) PCNA, (C) Ki-67, paraffin section. Varying numerical densities of PCNA and Ki-67 positive nuclei; peripheral and central localization of proliferating cells (× 200).

clonal antibodies have recently been generated to genetically engineered PCNA[14], one of these, designated PC10, recognized PCNA in conventionally fixed and processed histological material. PC10 immunostaining has recently fixed and processed histological material. PC10 immunostaining has recently been employed to demonstrate the proliferative compartment of paraffin-embeded tissues[1]. Immunohistochemical methods of assessing cell proliferation have advantages over other techniques because the architecture of the tissue is maintained, and thus the individual proliferating cells can be visualized in their context. So far, the best known antibody directed against a component of the cell cycle, Ki-67, is only of use in fresh frozen material, limiting the assessment of architectural features and precluding retrospective studies[7,8].

Recently, L. Form and colleagues[9] used a different Ki-67 antibody, MIB-1, which successfully stained proliferating cells in formalin-fixed paraffin sections. The monoclonal antibody MIB-1 reacts with the Ki-67 nuclear antigen which is associated with cell proliferation. This antibody recognizes native Ki-67 antigen and recombinant peptide corresponding to a 1002 bp Ki-67 cDNA fragment. The antibody can be used on formalin-fixed paraffin-embeded tissue in contrast to the antibody Ki-67 which only works on frozen material.

In this study we have produced several significant results. PCNA and Ki-67 immunostaining can demonstrate the proliferative compartment of conventionally fixed and processed tissues. Our mean Ki-67 counts were slightly higher than those obtained with PCNA, although statistically not significant. Ki-67 marks all phases of the cycle except Go, whereas PCNA is considered primarily an S-phase-associated protein. Theoretically, Ki-67 counts should be greater than PCNA counts. In contrast, in Dervan's study[10] mean PCNA counts were slightly greater than those for Ki-67. Therefore, a number of theoretical, technical and practical questions need further clarification. This questions can be answered by appropriate survival studies only after uniformly acceptable methods for technical preparation and for evaluation of results are defined. In our study, the interpretation of Ki-67 staining was easier than that for PCNA, mainly because cell morphology was better preserved and the distinction between tumor and nontumor cell was clear. PCNA positivity usually had a slightly coarsly granular or clumped appearance, and nucleoli usually were not stained intensely. Nuclei showed varying intensities of PC-

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Table 1. Labelling indices of PCNA and Ki-67 immunostaining in psoriasis, basal cell carcinomas and wquamous cell carcinomas.

Lesion	No of cases	PCNA(PC10)	Ki-67(MIB-1)V
		Labelling index(%)(mean±SD[a])	
Normal skin	5	1.5±0.1	1.7±0.1
Psriasis	17	14.2±4.0	15.7±3.8
Basal cell ca.	15	10.9±5.5	11.2±6.1
nocular	8	12.9	13.2
sclerosing	3	10.8	11.4
sup.spreading	2	9.2	9.3
adenoid	2	10.7	10.9
Squamous cell ca.	10	28.4±7.4	30.3±9.4

* a:$p<0.05$

NA staining, from weak to intense. In contrast Ki-67 nucler positivity was more uniformly granular or homogenously dense and nucleoli were often positive(Fig. 1.2.3). The PCNA and Ki-67 cornts strongly correlated with each other(r= 0.979). There remain some doubts as to the relationship betwcen PCNA and Ki-67 expression and cell proliferation in the context of at least some forms of neoplasia. Our immunohistochemical results of PCNA and Ki-67 counts strongly correlated with each other(r=0.979), There remain some doubts as to the relationship between PCNA and Ki-67 suggested that mean proliferative activities were more marked in psoriasis than in basal cell carcinomas. It may be that this can be explained in part by the PCNA and Ki-67 growth fraction displaying a wide variation in basal cell carcinomas[11].

The rather high Ki-67 labelling index in basal cell carcinomas seems to be at odds with the clinical observation of a slowly growing tumor. However, it must be kept in mind that the actual tumor volume is not only determined by cell division but also by loss of cells. Single necrotic cells or even mass necrosis of parts of a basal cell carcinoma are a common finding. Furthermore, nuclear Ki-67 expression only indicates that a cell is outside Go or early G1 phase, but the velocity of tumor growth is also dependent on the duration of the individual phases of the cell cycle. The duration of S phase of a basal cell carcinoma tumor cell is about twice that of basal keratinocytes of the epidermis[12]. owing to a decreased rate of DNA synthesis in basal cell carcinomas. A fraction of tumor cells might be blocked in G0 or early G1 phase (Ki-67 negative) or in late G1 and late G2 phase (Ki-67 positive).

In addition it is possible that the control of PCNA and Ki-67 expression may be deregulated, within and adjacent to same types of tumors, perhaps by the autocrine secretion of growth factors.

Further studies will be needed to reinforce the finding that the number of positive cells of PCNA and Ki-67 were more marked in psoriasis than in basal cell carcinoma, and to delineate more precisely their biololgic role.

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