

Review

# Expression of proliferating cell nuclear antigen in polyps from large intestine

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Colon cancer has a high incidence in the world, especially in Western countries, and the incidence is also increasing in China in recent years. To reduce the incidence, it is essential to identify individuals at high risk and to eradicate risk factors. Although colorectal neoplasia is a multi-stage process, hyperproliferation is the main factor leading to the initiation of carcinogenesis<sup>[1]</sup>. In animal models of colonic cancer, an increased colonic proliferative rate resulted from inherited differences, pharmacological or surgical intervention, which increase the susceptibility of the colonic mucosa to carcinogens<sup>[2]</sup>. On the other hand, interventions that decrease colonic proliferation are associated with decreased susceptibility to carcinogens<sup>[3]</sup>. PCNA (proliferating cell nuclear antigen) is an accessory protein of DNA polymerase and is thought to play an important role in the elongation or replication of the DNA chain. Its accumulation in the nucleus during the G-1 and S stages of the cell cycle has been reported<sup>[4]</sup>, and the labeling index, which is the percentage of PCNA-positive cells, has been reported to be correlated with the proliferative activity and the prognosis of various malignant tumors<sup>[5]</sup>. Thus it is valuable to assess what role PCNA plays during the transformation of the colonic polyps to colonic cancer.

## PCNA AREA RATE AND PCNA LABELING INDEX

Fujishima<sup>[6]</sup> has measured the PCNA labeling index

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(PCNA-LI) by visual inspection and the PCNA area rate (PCNA-AR), determined with the newly developed image processor for analytical pathology (IPAP), in tissue samples obtained by biopsy and polypectomy under endoscopic observation. Samples from 20 patients with serrated adenoma, 10 subjects with normal mucosa of the large intestine, 9 patients with hyperplastic polyp, 11 with tubular adenoma low-grade atypia, 15 with tubular adenoma in high-grade atypia, and 15 with well differentiated adenocarcinoma were studied. In serrated adenoma, the crypts were divided into upper, middle, and lower zones with each zone examined microscopically. In the lower zone of crypt of the serrated adenoma, the PCNA-LI and PCNA-AR were found to be approximate to the values of tubular adenoma, indicating the presence of high proliferative activity in the bottoms of crypts. Determination of the pattern of distribution of PCNA-positive cells indicated the presence of a proliferative zone in the lower region or bottom of the serrated adenoma. However, 5 of the 20 serrated adenomas exhibited an irregular or widely extended proliferative zone, and 2 were complicated by cancer. These findings indicated that serrated adenoma is also a highly proliferative tumor and that it may be complicated by cancer if atypia is increased and disturbance of the proliferative zone is present. The PCNA-AR and PCNA-LI increased in the following order: normal mucosa of the large intestine, hyperplastic polyp, tubular adenoma with low grade atypia, and tubular adenoma with high-grade atypia and adenocarcinoma, in proportion to the degree of atypia (Table 1).

**Table 1 Colonic mucosa pathology and PCNA ( $\bar{x}\pm s$ , %)**

Pathological diagnosis	PCNA-area rate	PCNA-labeling index
Normal colonic mucosa	12.8±2.5	32.8±5.8
Hyperplastic polyp	18.2±5.7	27.6±10.3
Tubular adenoma with low-grade atypia	31.0±8.1	40.9±11.8
Tubular adenoma with high-grade atypia	44.6±10.1	59.2±9.8
Adenocarcinoma	69.2±11.8	74.3±13.6
Serrated adenoma (upper zone)	16.4±7.5	25.6±6.1
Serrated adenoma (middle zone)	31.9±9.9	40.4±8.9
Serrated adenoma (lower zone)	49.6±11.6	60.2±10.1

The difference of both PCNA-AR and PCNA-LI between tubular adenoma with low-grade atypia

and hyperplastic polyp was significant ( $P < 0.01$ ), as were the differences between tubular adenoma with high-grade atypia and tubular adenoma with low-grade atypia ( $P < 0.01$ ), and between adenocarcinoma and tubular adenoma with high-grade atypia ( $P < 0.01$ ). Proliferative activity of epithelial tumor cells was evaluated by Bielicki *et al.*<sup>[7]</sup> with immunohistochemistry and anti-PCNA monoclonal antibodies in alcohol fixed, paraffin embedded sections of 44 colonic adenomas, including 33 tubular, 5 villous and 6 tubulovillous adenomas. The mean PCNA-LI was  $24.7\% \pm 10.9\%$ ,  $24.8\% \pm 6.2\%$  and  $24.8\% \pm 14.0\%$  in tubular, villous and tubulovillous adenomas respectively. In 12 tubular adenomas with dysplasia the mean PCNA index in areas with dysplasia was significantly higher ( $38.2\% \pm 11.5\%$ ) as compared to areas without dysplasia ( $17.0\% \pm 8.9\%$ ;  $P < 0.05$ ). The results indicate that PCNA-LI of epithelial tumor cells is significantly increased in adenomas with high grade of dysplasia irrespective of histological type or size of the tumour.

#### DISTRIBUTION PATTERN OF PCNA-POSITIVE CELLS

Carr *et al.*<sup>[8]</sup> compared PCNA immunoreactivity in hyperplastic polyps, adenomas, and inflammatory cloacogenic polyps of the human colon and rectum using paraffin embedded tissue. The monoclonal antibody PC10 was used to demonstrate PCNA immunoreactivity in 88 polypoid lesions from 68 patients. Cases in which immunoreactivity was completely absent were excluded, leaving 32 hyperplastic polyps, 31 adenomas, and seven inflammatory cloacogenic polyps for analysis. Labelling indices for the upper and lower third of each lesion and for adjacent normal mucosa were calculated. It was found that the upper third labelling indices for adenomas were substantially higher than those for hyperplastic polyps or normal mucosa, whereas those for the upper thirds of hyperplastic polyps and normal mucosa did not differ greatly. The differences between the lower third samples were not significant. In 16 (50%) hyperplastic polyps positive cells persisted onto the luminal surface. Some adenomas showed the most intense staining and the highest labelling indices in the upper third, with strong staining of surface cells; this pattern was not seen in the other lesions. The inflammatory cloacogenic polyps did not show a consistent pattern of immunoreactivity. They concluded that differences in cell kinetics between adenomas, hyperplastic polyps, and normal mucosa may be shown in formalin fixed, paraffin embedded tissue using PC10 as a marker of proliferative activity. PCNA expression also persists into the

upper portion of hyperplastic polyps. Assuming that hyperplastic polyps are hypermature lesions with a slower rate of cell migration, this finding suggests that there may be an alteration in PCNA protein metabolism. Fujishima *et al.*<sup>[6]</sup> has compared the upper, middle and lower zones of serrated adenomas, both the PCNA area rate and PCNA labeling index increased from the upper zone to the lower zone. The difference in both the PCNA-AR and PCNA-LI was very significant ( $P < 0.01$ ). Meanwhile, the values in the upper zone of serrated adenomas were higher than those in the normal mucosa of large intestine, and those in the middle zone and lower zone were similar to those in tubular adenomas with low-grade atypia and tubular adenomas with high-grade atypia respectively. In the distribution pattern of PCNA-positive cells, the lower crypt type was predominant in the normal mucosa of the large intestine and in the hyperplastic polyps. Whereas 75% cases of tubular adenoma with low-grade atypia were upper crypt type, 71% of the cases of tubular adenoma with high-grade atypia had labelled cells distributed in all the layers, and 100% of adenocarcinomas had labeled cells distributed in all layers. Of the serrated adenomas, 75% were the lower crypt type, with PCNA-positive cells being distributed in the lower to the bottom regions of the crypt. The remaining 25% were the total crypt type. Thus, labeled cells were predominantly located in the lower regions of serrated adenomas, although some were distributed throughout the crypts. Two cases of total crypt type of serrated adenoma were complicated by cancer.

#### PCNA, A MARKER OF CELL KINETICS

Tranchina *et al.*<sup>[9]</sup> has evaluated proliferating cell index in colonic adenomas, using anti PCNA-PC10 antibody. The results have been compared by grading. A high proliferating grade has been shown in patients with multiple adenomas. This finding suggests a prognostic value of proliferating cell index. In Adam's study<sup>[10]</sup>, colon cancer was induced in 40 Sprague Dawley rats using a 10-week course of 1, 2 dimethylhydrazine (DMH). Twenty animals received cimetidine in their drinking water, commencing from the 5th week after concluding the course of DMH. After five weeks treatment the animals were sacrificed with the colon and rectum excised. Tumors were assessed histologically for depth of invasion, inflammatory cell response and stained for proliferating cell nuclear antigen (PCNA), as a measure of tumor proliferative index. PCNA staining was measured by a computerized image analysis system. There were 25 tumors in the cimetidine treated group and 20 in

controls. In the control group, 10% of the tumors were benign, 35% malignant polyps, 40% invading through submucosa and 15% invading through the bowel wall, as opposed to 40%, 44%, 8% and 8%, respectively in the cimetidine group (Chi-square test,  $P = 0.002$ ). The mean proliferative index was 27.9% for control tumors and 23.1% for the cimetidine treated tumors (t test,  $P = 0.002$ ). It is concluded that cimetidine inhibits colon cancer cellular proliferation and slows down early tumor invasion in this animal model. Winde *et al*<sup>[11]</sup> studied PCNA and KI-67 proliferation indices (PI) by point counting. Prostaglandin PG E2 and PGF2 alpha were quantified by time-resolved competitive fluorescence immunoassay. All patients responded to sulindac therapy within 6 to 24 weeks. Complete adenoma reversion was achieved in 60 and 87 percent of patients after 48 weeks at 53 mg and 67 mg of sulindac per day per patient on average, respectively. Reversion was evident compared with the control group. Dose reduction by one-sixth to one-eighth of the usual oral dose was significant (Mann's trend test,  $P < 0.05$ ). PCNA and KI-67 PIs of adenomatous and flat mucosa were significantly reduced (Wilcoxon's test,  $P < 0.05$ ). Correlation of PCNA and KI-67 PIs indicates similar reaction of different tissue structures (Spearman's rank correlation test,  $P < 0.01$ ). Nonsteroidal anti-inflammatory drug-induced redifferentiation from high-grade to low-grade dysplasia occurred in all but two patients. Tissue-PGE2 levels were greatly reduced. Unwanted, curable side effects were rare (gastritis,  $n = 2$ ), and laboratory controls were within detection limits. These indicated that low-dose rectal sulindac maintenance therapy was highly effective in achieving complete adenoma reversion without relapse in 87 percent of patients after 33 months. Rectal FAP phenotype should be crucial for the surgical decision. Colectomy with ileorectal anastomosis and regular chemoprevention might be a promising alternative to pouch procedures. Chemoprevention of FAP-related tumors with lower incidence via dysplasia reversion may be possible in the future. Shpitz *et al*<sup>[12]</sup> also believed that proliferating cell nuclear antigen is a marker of cell kinetics in aberrant crypt foci, hyperplastic polyps, adenomas, and adenocarcinomas of the human colon. They made serial sections with paraffin-embedded ACF (aberrant crypt foci) stained with a monoclonal antiproliferating cell nuclear antigen (PCNA) antibody and macroscopic lesion. The PCNA-labelling index (PCNA-LI), expressed as a ratio of positively stained nuclei to total nuclei counted, was calculated separately for basal, middle, and upper colonic crypt compartments. A

comparison of the PCNA-LI was made for each compartment in normal mucosa, and hyperplastic lesions. A stepwise increase in the PCNA-LI was observed during neoplastic progression of colonic lesions. The two most important variables of increased cell proliferation, expressed as PCNA-LI per crypt compartment, were the presence of dysplasia and the size of dysplastic lesions. Their conclusions are as follows. In colorectal carcinogenesis, all stages of malignant progression are characterized by hyperproliferation with upward expansion of proliferative compartment. In vitro uptake of bromodeoxyuridine and expression of proliferating cell nuclear antigen (PCNA) were evaluated histochemically by Risio *et al*<sup>[13]</sup> in rectal mucosa of control subjects with colorectal neoplasia in large intestine adenomas and adenocarcinomas. Both labeling indices increased progressively along the path of tumor progression, as did the difference between them (PCNA labeling indices were always greater than those of bromodeoxyuridine). The correlation between them was fairly close in the controls and in adenomas with low-grade dysplasia, whereas no significant linear correlation was noted in adenomas with high-grade dysplasia or in adenocarcinomas. The progressive increase in PCNA would thus seem to be related to both hyperproliferation and neoplastic deregulation of PCNA synthesis. In the mucosa of subjects with colorectal neoplasia, PCNA labeling revealed hyperproliferation but not the surface-wards shift of the proliferative compartment detected by bromodeoxyuridine. PCNA expression, therefore, is not a sufficiently sensitive marker of the risk of tumor transformation in the intestinal mucosa.

#### PCNA AND ONCOGENE EXPRESSION AND DNA PLOIDY

p53 is a nuclear phosphoprotein which controls normal cell growth. Normal p53 protein can not be detected by standard immunohistochemical staining and the over-expression found in neoplastic cells correlates with the presence of point mutations of evolutionary conserved regions of the p53 gene. Pignatelli *et al*<sup>[14]</sup> examined the expression of p53 protein in a series of 36 colorectal adenomas (13 tubular, 17 tubulovillous, 6 villous) showing different degrees of dysplasia (11 mild, 19 moderate, 6 severe), using the polyclonal antibody CM1 which recognises p53 protein in conventionally fixed and processed histological material. They found that 15 out of 36 colorectal adenomas showed p53 immunoreactivity although the staining was very focal in 4 positive cases (26%) (less than 0.1% positive cells). More than 80% of severely

dysplastic adenomas showed strong p53 immunoreactivity, and this over-expression was correlated with increased cell proliferative rate as detected by the proliferating cell nuclear antigen (PCNA) staining, p53 nuclear staining was also seen in 8 out of 11 (65%) colorectal adenocarcinomas as previously shown. Their data suggest that the p53 gene mutation with the subsequent over-expression of the protein, occurs in colorectal adenomas and may therefore be a fundamental genetic event underlying the dysplasia and loss of proliferative control that are characteristic of adenomas with malignant potential. Tomita<sup>[15]</sup> investigated the colonic adenoma-adenocarcinoma progression sequence and analyzed DNA ploidy on hyperplastic polyps to adenocarcinomas. DNA ploidy data were then compared with immunocytochemical staining for proliferating cell nuclear antigen (PCNA). In hyperplastic polyps to villous adenomas, all cases were diploid except one aneuploid villous adenoma. In three adenomas, diploid in situ adenocarcinomas were present. As diploid percentage decreased from hyperplastic polyps to villous adenomas, aneuploid percentage increased. In adenocarcinomas, the Dukes classification corresponded well to DNA ploidy status. All four stage A carcinomas were diploid, whereas three cases each of stage C1 and C2 carcinomas were aneuploid or multiploid. A surprising finding was that S-phase percentage in adenocarcinomas was not parallel with PCNA-positive tumor cell numbers. It is concluded that multistep adenoma-adenocarcinoma progression is partially reflected in DNA ploidy pattern from hyperplastic polyps to villous adenomas. In adenocarcinomas, the Dukes classification parallels well with the DNA ploidy status from stage A diploid to stage D aneuploid, but is not accompanied by increasing PCNA-positive cell numbers.

#### PCNA AND TYROSINE KINASE

Tyrosine kinase and a number of growth factors, especially EGF- $\alpha$  are known to stimulate proliferation of cells in the gastrointestinal tract, including colon. In humans increased colonic mucosal proliferative activity has been observed in numerous premalignant lesions including adenomatous polyps and ulcerative colitis. In the present study Malecka-Panas *et al*<sup>[16]</sup> determined the differences of proliferative patterns in patients with adenomatous polyps, ulcerative colitis and colonic adenocarcinoma as reflected by rectal mucosa tyrosine kinase, EGF receptor tyrosine kinase and PCNA to evaluate the role of tyr-k in colonic mucosal cell proliferation during carcinogenic

process. The study population comprised 40 patients, aged 17-74 years (mean 57). Of them, 10 patients had adenomatous polyps, 10 ulcerative colitis in remission phase, 10 colon adenocarcinoma and 10 healthy controls. After informed consent 6-8 rectal mucosal biopsy specimens were obtained at 10 cm from the anal verge at the beginning of colonoscopy examination and at least 10 cm away from any macroscopic mucosal changes. They found that mean PCNA labeling indices in patients with colon adenocarcinoma, adenomatous polyps, ulcerative colitis and healthy controls were  $27.6\% \pm 5.75\%$ ;  $12.8\% \pm 6.76\%$ ;  $10.9\% \pm 5.34\%$  and  $1.5\% \pm 0.97\%$  respectively. PCNA labeling index in rectal mucosa of patients with adenomatous polyps, ulcerative colitis and colon cancer was significantly higher ( $P < 0.01$ ) than that in the control group. An upward expansion of the proliferative compartment was also observed in patients with premalignant and malignant colon conditions. Total tyrosine kinase activity was elevated by 219%, 224% and 600% in the rectal mucosa of patients with polyps, ulcerative colitis and colorectal carcinoma respectively as compared with the control group. EGF receptor tyrosine kinase was increased in colonic mucosa by 35.2% in patients with adenomatous polyps, by 40.6% in patients with ulcerative colitis and 123% in patients with colon cancer. They concluded that increased values of this enzyme in the above mentioned groups of patients may suggest that tyrosine phosphorylation represents an early sign of colonic mucosa susceptibility for cancer development. Overall, EGF receptor-associated tyrosine kinase plays an important role in the development of hyperproliferative state of the colon mucosa and colon carcinogenesis.

The first step in multistage colonic carcinogenesis are increased cell proliferation and an upward shift of the proliferation zone of colonic crypts. In the present study, progression in cell kinetics was followed up at sequential stages of colonic carcinogenesis, starting with aberrant crypt foci (ACF), the earliest putative preneoplastic lesions, hyperplastic and dysplastic polyps, and invasive carcinomas<sup>[12]</sup>. In humans, increased colonic mucosal proliferative activity and expansion of proliferative compartment have been observed in numerous premalignant lesions including adenomatous polyps, familial polyposis coli and ulcerative colitis<sup>[17]</sup>.

Furthermore, the colonic mucosa of individuals with colon carcinoma has been demonstrated to be diffusely hyperproliferative as compared to that from normal individuals<sup>[18]</sup>. It also suggested that

colonic hyperproliferation, including an increased proliferative rate and expansion of proliferative zone could be used as an intermediate marker of colon cancer risk, therefore PCNA detection helps with early diagnosis of large intestinal cancer and assessment of the prognosis of patients.

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