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Spermidine/Spermine N^1-Acetyltransferase, a New Biochemical Marker for Epithelial Proliferation in Rat Bladder

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We examined the activity of spermidine/spermine N^1-acetyltransferase (SAT), a rate-limiting enzyme of the biodegradation of polyamines, in *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine-induced transitional cell carcinoma (TCC) and melamine-induced papillomatosis of rat bladder, and compared the activity to that of ornithine decarboxylase (ODC). Both activities were higher in both lesions than in control rats. The difference between SAT and ODC activities in cancerous tissue and papillomatosis was not significant. Cells stained for proliferating cell nuclear antigen (PCNA) were abundant in papillomatosis. TCC had areas with much PCNA. The results indicated that an elevation of SAT activity occurs in both reversible and irreversible proliferation of bladder epithelium and could be important in bladder carcinogenesis.

Key words: Spermidine/spermine N^1-acetyltransferase — Ornithine decarboxylase — Polyamine metabolism — Rat bladder carcinoma — Rat bladder papillomatosis

Polyamines, which are involved in epithelial cell proliferation, participate in the promotion of mouse skin tumorigenesis.[1] Ornithine decarboxylase (ODC), a rate-limiting enzyme of polyamine biosynthesis, is a marker of tumor promotion, including carcinogenesis in the bladder.[2,3] ODC activity is high in carcinomas induced experimentally by chemical carcinogens[4,5] and carcinomas obtained from patients.[6,7] Spermidine/spermine N^1-acetyltransferase (SAT) activity increases when cells are induced to proliferate by various mitogens.[8,9] SAT is a rate-limiting enzyme of the biodegradation of polyamines.[10] It transfers the acetyl moiety from acetyl coenzyme A to spermidine and spermine, producing N^1-acetyl derivatives of these polyamines. Polyamine oxidase, which is abundant in many kinds of cells, oxidizes the resulting N^1-acetylspermidine and N^1-acetylspermine to putrescine and spermidine, respectively.

In this study, we examined SAT activity in neoplastic epithelial cells in the bladder of rats treated with *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) and in hyperplastic epithelial cells in the bladder of rats treated with melamine. We found that SAT activity was very high in both kinds of lesions.

Thirty-seven male F344 rats, 6 weeks old at the start of the experiment, were purchased from Charles River Japan, Inc., Atsugi. The animals were kept in plastic cages with wood chip bedding in an animal room with a 12-h light, 12-h dark cycle under controlled temperature and humidity. The 20 control rats in group 1 were fed a basal chow (Oriental MF; Oriental Yeast Co., Tokyo) without test chemicals for 8 weeks. The 12 rats in group 2 were given the basal chow mixed with 3% melamine (Wako Pure Chemical Industries, Osaka) for 8 weeks. The 5 rats in group 3 were given the basal chow and drinking water containing 0.05% BBN (Tokyo Kasei Co., Tokyo) for 10 weeks and then were maintained without BBN for 28 weeks. All animals had free access to the chow and water during the experiment.

At the end of the experiment, the rats were killed under ether anesthesia and the bladder was rapidly removed. The epithelia of 14 bladders from group 1 and 6 bladders from group 2 were stripped off, suspended in 0.5 ml of 50 m*M* Tris (pH 7.5) containing 0.25 *M* sucrose, and disrupted by sonication for 30 s (Sonifier). In group 1, the epithelia from 2 or 3 rats were pooled as one sample. Bladder tumors of the 5 rats in group 3 were suspended in 5 volumes of the same buffer and disrupted by sonication in the same way. The sonicated suspensions were centrifuged at 100,000*g* for 30 min and the supernatant was assayed for ODC and SAT activity by measurement of the amount of radioactive putrescine produced from [5-^{14}C]ornithine[11] and the amount of acetyl moiety transferred from [1-^{14}C]acetyl coenzyme A to spermidine,[12] respectively. To assess the levels of proliferating cell nuclear antigen (PCNA) in the nuclei of the epithelial cells, the remaining bladders from groups 1 and

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2 were inflated by intraluminal injection of 10% phosphate-buffered formalin (pH 7.4) for fixation before their removal. Some of the bladder tumors in group 3 were fixed in the same way. PCNA in nuclei was stained by the avidin-biotin-peroxidase method with a monoclonal antibody against PCNA.[13] The bladders and tumor tissues were stained with hematoxylin and eosin and examined histologically. Diffuse papillary hyperplasia of the bladder epithelium, a reversible change referred to as papillomatosis, was found together with bladder calculi in all rats examined from group 2. Bladder tumors obtained from group 3 were found histologically to be transitional cell carcinoma (TCC). The histological appearance of bladders from the controls (group 1) was normal. Differences in values for ODC and SAT activities in bladder lesions were examined for statistical significance by means of Student's t test.

Table I shows the activities of SAT and ODC in normal mucosa, papillomatosis, and TCC. Both activities were high in both kinds of lesions, with ODC activity increased more. The differences in values of SAT and ODC between papillomatosis and TCC were not significant.

PCNA is present in nuclei of cells in the G1 and S phases. Cells stained for PCNA were abundant throughout the basal, intermediate, and surface layers of cells in papillomatosis, but the most highly labeled cells were located in the basal layer (Fig. 1). There were few in normal bladder epithelium. TCCs had areas with a high rate of PCNA staining.

Table I. Activities of ODC and SAT in Bladder Lesions of Rats

Group	Type of samples	No. of samples	SAT (pmol/mg protein/10 min)	ODC (pmol/mg protein/h)
1	Control	6	93.8 ± 9.4[a]	51.6 ± 33.1
2	Papillomatosis	6	247.8 ± 90.7[b]	351.4 ± 69.9[b]
3	TCC	5	268.1 ± 98.9[b]	495.5 ± 217.6[b]

a) Values are mean±SD.
b) Significantly different from the control (group 1), $P<0.001$.

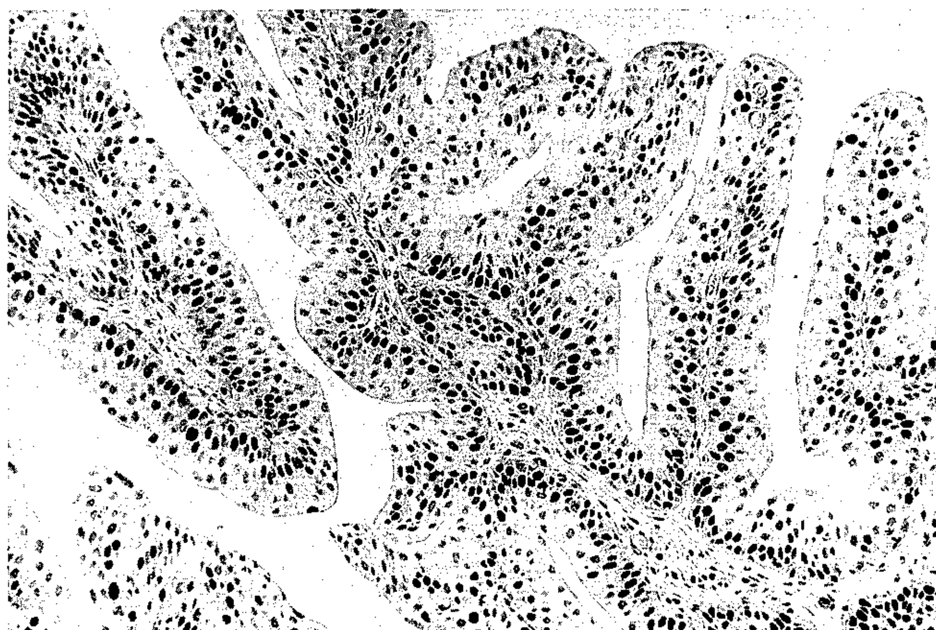

Fig. 1. Specimen of rat papillomatosis. In all layers, particularly in the basal layer of the epithelium, many cells are stained for PCNA. Avidin-biotin-peroxidase method, ×200.

Many investigations of chemical carcinogenesis, and in particular, of the characteristics of tumor promotion in two-stage carcinogenesis, have focused on ODC activity. In rat bladder carcinogenesis, urine, a tumor promoter in rat heterotopic bladder carcinogenesis, induces ODC activity.[14] Here we found that both SAT and ODC activities were high in reversible and irreversible proliferating cells of the bladder epithelium. Short-term oral administration of uracil to rats induces bladder papillomatosis, which is a reversible change.[15,16] Long-term oral administration of uracil induces bladder carcinomas with urinary calculi.[17,18] Melamine also induces bladder carcinomas associated with urinary calculi during long-term oral administration to rats.[19,20] Therefore, our results indicated that SAT is implicated in bladder carcinogenesis. Hepatocarcinogens such as thioacetamide and dialkylnitrosamines increase acetylase activity.[12,21] In humans, the level of N^1-acetylspermidine is higher in cancerous portions of colon mucosa than in portions that look normal[22]; the level is higher in the 24 h urine of cancer patients than in that of healthy subjects.[23] A high spermidine/spermine ratio has been observed in many kinds of cancers,[24] suggesting that SAT activity increases in cancer cells, accelerating the conversion of spermine to spermidine. These results and ours suggest that elevation of SAT activity and the resulting accelerated interconversion of polyamines are important steps in carcinogenesis. The SAT activity is therefore a marker of cell proliferation caused by either genotoxic or nongenotoxic chemicals.

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