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The effects of organ resection on rat urinary bladder carcinogenesis

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Abstract The effect of unilateral nephrectomy, orchiectomy or partial hepatectomy on the growth of chemically induced rat bladder tumors was investigated. Male F344 rats were treated with 0.05% *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BBN) for 5 weeks, and surgical resection of one of these organs was performed 2 weeks after the completion of BBN administration. Histological evaluation of the bladder 24 weeks after the start of the experiment revealed that unilateral nephrectomy and orchiectomy significantly increased the numbers of preneoplastic and neoplastic lesions as compared with the corresponding sham-operated groups. Partial hepatectomy also enhanced tumor growth, although not significantly. Immunohistochemical studies examining the effect of organ resection on normal bladder urothelium showed that BrdU immunostaining of urothelial cells significantly increased 7 days after unilateral nephrectomy or orchiectomy, while BrdU incorporation was minimum after partial hepatectomy or sham operation. C-met expression in the bladder urothelium was evident following unilateral nephrectomy or partial hepatectomy, while increased immunoreactivity of androgen receptor was noted following unilateral orchiectomy. Further study is needed to determine the exact mechanism of the bladder tumor growth-enhancing effect associated with organ restriction.

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Introduction

In clinical practice, urologists frequently observe rapid development of bladder cancer in patients who have undergone nephroureterectomy for transitional cell carcinoma of the upper urinary tract [9]. Two mechanisms have been postulated to explain this phenomenon: one is the seeding of cancer cells onto the bladder mucosa, and the other is the theory of "field cancerization" of the urothelium [7]. In addition to these explanations, we hypothesized a third mechanism, that unilateral nephrectomy itself may promote bladder cancer development regardless of the presence or absence of upper urinary tract cancer, and have reported the enhancing effect of unilateral nephrectomy on BBN-induced rat bladder carcinogenesis [13].

Although the exact mechanism of this proliferative response of the bladder epithelium is unknown, it is assumed that tumor growth was stimulated by some endocrine humoral factors associated with the mechanism of the compensatory hypertrophy of the contralateral kidney. If this assumption is correct, it may be possible that a similar phenomenon could happen following the resection of other organs such as the testis or liver, which also have the function of compensatory hyperplasia or hypertrophy after partial or unilateral resection.

In the present study, we therefore compared the effect of surgical resection of three different organs (kidney, testis and liver) on chemically-initiated neoplastic lesions in rat urinary bladder. We also conducted a short-term immunohistochemical study to examine changes in the normal urothelial cells following organ resection in terms of immunoreactivity for bromodeoxyuridine (BrdU), c-met and androgen receptor (AR).

Materials and methods

Chemicals and animals

BBN was obtained from Nacalai Tesque (Kyoto, Japan) and was administered in a 0.05% solution in drinking water. A total of 131 6-week-old male F344 rats (CLEA Japan, Tokyo, Japan) were used. Two or three rats were housed in each plastic disposable cage. Rats were given a conventional diet and water *ad libitum* throughout the experiment. They were kept under standard laboratory conditions at 24 °C and 60% relative humidity, with 12 h of artificial light during the daytime and darkness for the remaining time in the experimental animal laboratory at Fukui Medical University. The study protocol was approved by the Animal Ethics Committee, Fukui Medical University.

Experiment 1

First, the rats were divided into eight groups as shown in Fig. 1. Groups 1 to 7 were given drinking water containing 0.05% BBN for the first 5 weeks. Group 8 was given no carcinogen. At 7 weeks after the start of the experiment, the animals in groups 1 to 7 were anesthetized with ethyl ether. Group 1: a left flank incision was made and the left kidney was removed. Group 2 (sham operation of group 1): a left flank incision was made, and the kidney was manipulated with forceps. Group 3: a lower abdominal incision was made, and the left testis was removed with care taken not to touch the urinary bladder. Group 4 (sham operation of group 3): a lower abdominal incision was made, and the left testis was manipulated with forceps. Group 5: an upper abdominal incision was made, and the left lobe and right inner lobe of the liver were resected. This was equivalent to a 70% partially hepatectomized state. Group 6 (sham operation of group 5): an upper abdominal incision was made, and the liver was manipulated. Group 7: no surgical procedures were performed.

At 24 weeks after the start of the experiment, all the animals were killed, and the bladders were removed. The urinary bladder was inflated by intraluminal injection of 10% phosphate-buffered formalin solution and divided along the midline in the sagittal direction after fixation. The bladder was then cut into eight longitudinal strips and stained with H&E for histological examination. For microscopic quantitative analysis, the number of urinary bladder lesions was counted under light microscopy, and the total length of the basement membrane was measured with a color video image processor. For comparison among the experimental groups, the numbers of lesions were expressed per 10 cm of mucosal basement membrane [3].

Experiment 2

Thirty-five rats were divided into seven groups, as shown in Fig. 2. They were kept under the same conditions as those of experiment 1 for 7 weeks without BBN treatment. They underwent the same type of anesthesia and operations (unilateral nephrectomy, orchiectomy, partial hepatectomy or sham operations) as in experiment 1. No surgical procedures were performed on the rats in group 7 (control group). After 1 week, all the animals were sacrificed, and the bladders were removed. 5-Bromo-2′-deoxyuridine (BrdU, 100 mg/kg; Sigma Chemical, St. Louis, Mo., USA) was injected intraperitoneally 1 h before sacrifice. The urinary bladder was inflated and fixed with chilled 70% ethanol for 24 h and then processed for immunohistochemical examination in a routine manner.

Immunohistochemistry

Tissue sections (5 μm thick) from paraffin-embedded tissue were deparaffinized in xylene, rehydrated through a down-graded alcohol series and washed with phosphate-buffered saline (PBS). For BrdU staining, DNA was denatured in 2 N hydrochloric acid for 60 min at room temperature and neutralized with 0.1 N $Na_2B_4O_7$. For c-met and androgen receptor (AR) staining, the slides were placed in 10 mM citrate buffer and microwaved for 15 min and 60 min, respectively. Thereafter, endogenous peroxidase was reduced in 3% H_2O_2 in methanol, then 1% non-fat milk powder in PBS was used to block non-specific antibody binding for 30 min. The sections were incubated with primary antibody for 60 min at room temperature (BrdU) or overnight at 4 °C (c-met and AR). Antibodies used in this study were monoclonal mouse anti-BrdU (Becton Dickinson, San Jose, Calif., USA), polyclonal rabbit anti-c-met antibody (Santa Cruz Biotechnology, Santa Cruz, Calif., USA) and monoclonal mouse anti-AR (Becton Dickinsson). The anti-BrdU, anti-c-met and anti-AR antibodies were diluted 1:100, 1:400 and 1:100 with 1% bovine serum albumin in PBS, respectively, according to the manufacturer's instructions. After washing with PBS, the sections were incubated with biotin-conjugated rabbit antimouse antibody (gout anti-rabbit antibody for c-met) for 10 min at room temperature followed by incubation with peroxidase-conjugated streptoavidin for 5 min. After washing with PBS, the enzyme reaction was initiated by adding substrate solution containing diaminobenzidine for 5 min, and counterstained for 10 s with Meyer's hematoxylin, dehydrated through an up-graded alcohol series, cleared in xylene and mounted. The immunohistochemical sections were examined under light microscopy by two investigators (S.S. and S.N.). The numbers of BrdU-labeled urothelium were counted, and labeling indices were expressed as positive cell count per 1000 epithelium. C-met and AR staining were also evaluated.

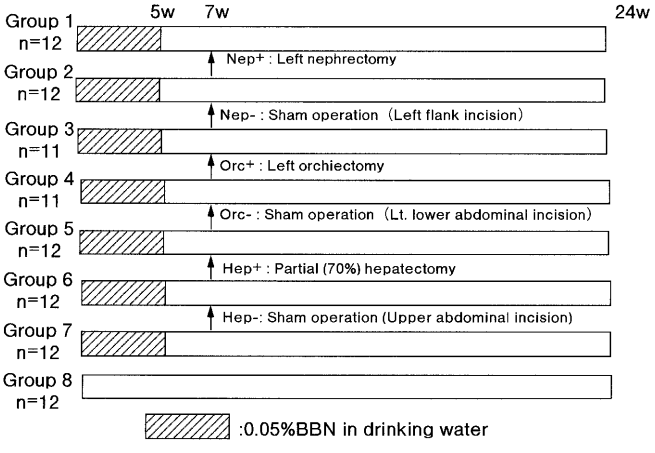

Fig. 1 Experiment 1

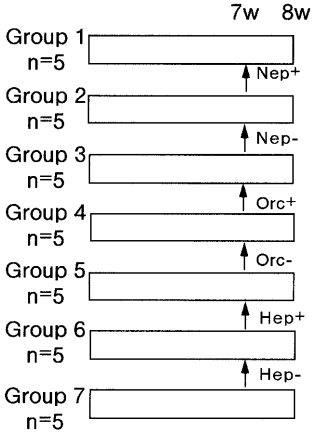

Fig. 2 Experiment 2

Data evaluation

Data are expressed as means ± SD. Statistical comparisons were performed using Welch's *t*-test.

Results

Experiment 1

Data on water consumption and *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BBN) intake are shown in Table 1. There was no difference in average water consumption or BBN intake between groups.

Histopathological lesions of the urinary bladder observed in rats are summarized in Table 2. As described previously [3, 4], the lesions found in the urinary bladder epithelium were classified into four types: simple hyperplasia, papillonodular (PN) hyperplasia, papilloma and cancer. The numbers of neoplastic lesions and cancer per 10 cm of mucosal basement membrane were increased in organ resection groups (groups 1, 3 and 5) compared with those in the corresponding sham groups (groups 2, 4 and 6), while statistically significant increases were seen only in the nephrectomy and orchiectomy groups. No significant differences were seen among sham operation groups and the no-surgery group (group 7). No significant pathological changes were observed in the no-treatment group (group 8).

Experiment 2

BrdU labeling indices for urinary bladder epithelium are shown in Table 3. Significant BrdU incorporation was present 7 days after nephrectomy or orchiectomy. As shown in Fig. 3, BrdU-positive urothelial cells were distributed throughout all the mucosal layers. On the other hand, little BrdU staining was present in the bladder epithelium after hepatectomy or sham operation. No BrdU incorporation was observed in the bladder epithelium in the no-treatment group.

As shown in Fig. 4, c-met immunopositivity was present in the cytoplasm of the urothelium and smooth muscle cells of the bladders of unilaterally nephrectom-

Table 3 BrdU labeling indices for urinary bladder epithelium of rats in experiment 2. Means ± SD

Group	Treatment	No. of rats	Labeling index (positive cell count/1000 epithelium)
1	Nep+	5	39.5 ± 48.2*
2	Nep−	5	7.0 ± 4.0
3	Orc+	5	42.4 ± 31.0*
4	Orc−	5	7.5 ± 4.6
5	Hep+	5	5.4 ± 1.3
6	Hep−	5	7.0 ± 4.8
7	No treatment	5	0

* Significantly different from sham group at $P < 0.01$

Table 1 Water consumption and BBN intake

Group	Treatment	No. of rats	Body weight (g)		Water consumption (ml/rat per day)	Total BBN intake (mg/rat)
			Initial	Final		
1	BBN + Nep+	12	69	312	24.8	347.2
2	BBN + Nep−	12	69	316	25.0	350.0
3	BBN + Orc+	11	69	316	24.7	345.8
4	BBN + Orc−	11	69	325	24.6	344.4
5	BBN + Hep+	12	69	310	24.8	347.2
6	BBN + Hep−	12	69	317	24.5	343.0
7	BBN only	12	69	310	25.1	351.4
8	No treatment	12	69	308	25.3	0

Table 2 Microscopic changes in the urinary bladder (*BM* basement membrane). Numbers *in parentheses* are percentages; means ± SD

Group	Treatment	No. of rats	Simple hyperplasia incidence	PN hyperplasia		Papilloma		Cancer	
				Incidence	No./10 cm of BM	Incidence	No./10 cm of BM	Incidence	No./10 cm of BM
1	BBN + Nep+	12	12 (100)	12 (100)	12.7 ± 4.8*	12 (100)	8.6 ± 5.8**	12 (100)	2.0 ± 1.2**
2	BBN + Nep−	12	12 (100)	12 (100)	7.8 ± 2.7	12 (100)	3.9 ± 2.3	8 (67)	1.0 ± 1.1
3	BBN + Orc+	11	11 (100)	11 (100)	11.7 ± 4.8**	11 (100)	7.7 ± 4.6**	11 (100)	1.9 ± 1.4**
4	BBN + Orc−	11	11 (100)	11 (100)	7.2 ± 3.5	11 (100)	3.8 ± 2.1	8 (73)	0.9 ± 0.7
5	BBN + Hep+	12	12 (100)	12 (100)	10.6 ± 5.3	12 (100)	5.9 ± 5.0	7 (58)	1.6 ± 2.8
6	BBN + Hep−	12	12 (100)	12 (100)	8.7 ± 4.3	12 (100)	5.1 ± 5.0	6 (50)	1.1 ± 1.5
7	BBN only	12	12 (100)	12 (100)	6.9 ± 4.1	11 (92)	2.5 ± 3.5	9 (75)	1.2 ± 1.2
8	No treatment	12	0 (0)	0 (0)	0	0 (0)	0	0 (0)	0

* Significantly different from sham group at $P < 0.01$
** Significantly different from sham group at $P < 0.05$

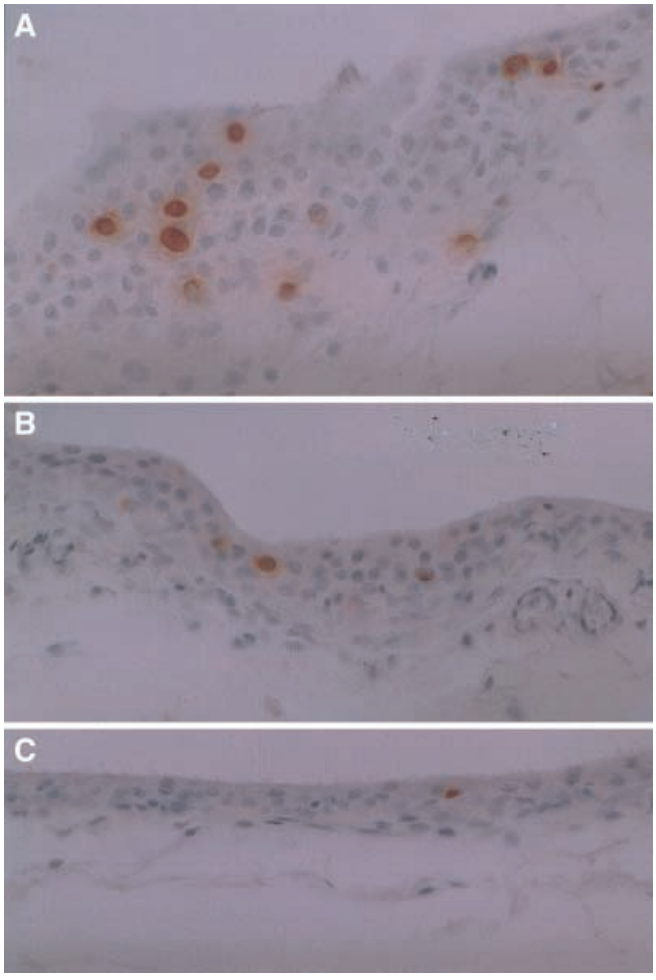

Fig. 3 **A** Anti-BrdU staining of bladder epithelium after 7 days of unilateral nephrectomy, **B** unilateral orchiectomy and **C** partial hepatectomy. Original magnification, ×200

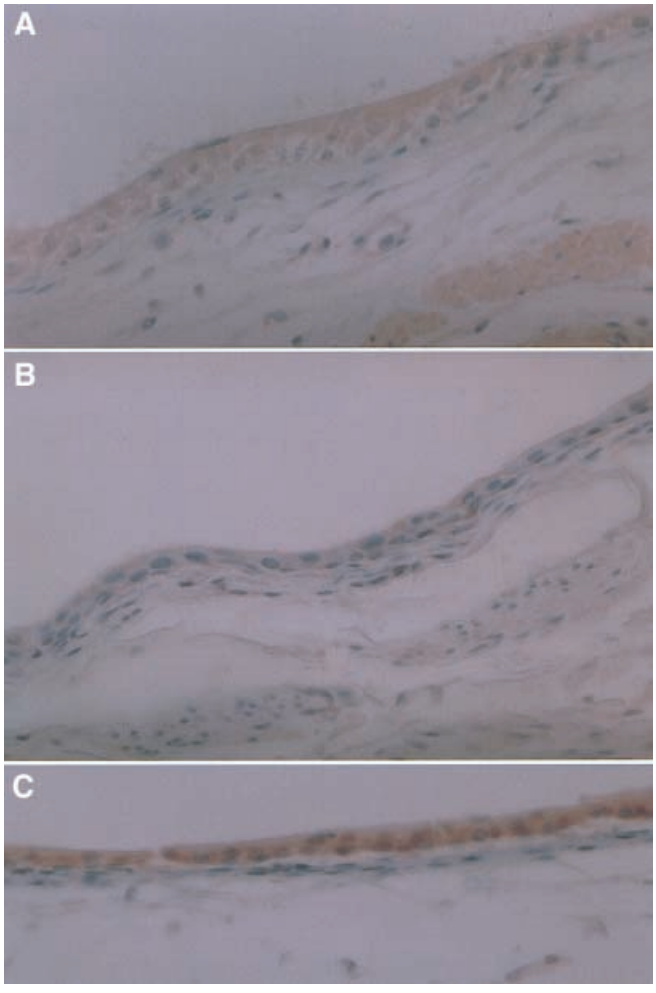

Fig. 4 **A** Immunohistochemical staining of the c-met protein in bladder epithelium after 7 days of unilateral nephrectomy, **B** unilateral orchiectomy and **C** partial hepatectomy. Original magnification, ×200

ized or partially hepatectomized rats, while no c-met immunoreactivity was found in the bladders of unilaterally orchiectomized or sham-operated rats.

As shown in Fig. 5, weak androgen receptor immunoreactivity was found in the urothelial nuclei of the bladders in all experimental groups, and a substantial increase was observed only in the orchiectomy group.

Discussion

In the present study, the surgical resection of each organ (kidney, testis and liver) increased the incidence and the number of BBN-induced rat bladder tumors as compared with those of corresponding sham-operated rats, but statistically significant increases were observed only in the nephrectomy and orchiectomy groups. An additional short-term experiment examining the proliferative response of normal bladder urothelium to organ resection also revealed significantly increased BrdU incorporation in the urothelial cells after unilateral nephrectomy or orchiectomy, while its incorporation was minimal after partial hepatectomy.

In our previous experiment [13], nephrectomy was performed during the BBN administration period, which might have modified the initiative effect of BBN. In the present experiment, therefore, the surgical resection of each organ was performed 2 weeks after the completion of BBN administration so as to separate the initiative step from any promotive procedures. The present results, again, confirmed the promoting effect of unilateral nephrectomy on rat bladder carcinogenesis. Our results also indicated a growth-stimulating effect of unilateral orchiectomy and partial hepatectomy on bladder tumors, although the latter was not substantial. Furthermore, it was evident from the result of the second experiment that such an effect was not tumor specific but rather an unspecific stimulus on cell proliferation.

There are at least two possible explanations for this growth-enhancing effect. One possible explanation is that some humoral factor(s) with growth-promoting activity were produced after organ resection and acted

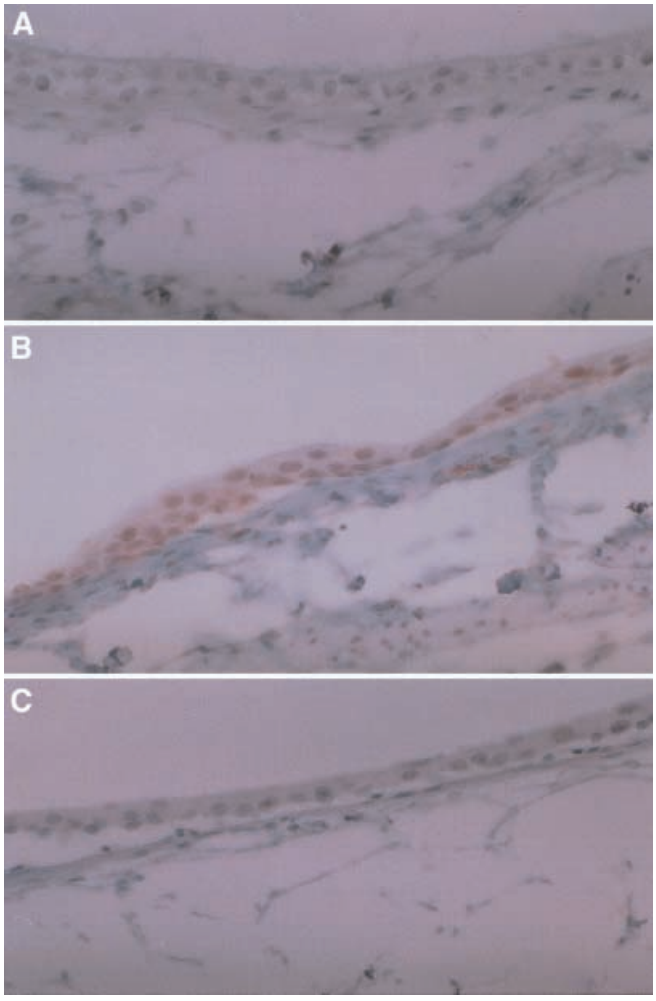

Fig. 5 **A** Immunohistochemical staining of androgen receptor protein in bladder epithelium after 7 days of unilateral nephrectomy, **B** unilateral orchiectomy and **C** partial hepatectomy. Original magnification, ×200

as mitogens on the regenerating organ as well as on the bladder epithelial cells [2, 12]. The presence of this remote growth-enhancing mechanism is supported by the experimental results by others showing that rapid and remarkable proliferation of subcutaneously xenografted tumor was observed following unilateral nephrectomy [15] and 70% partial hepatectomy [5]. Recently, the presence and the role of renotropic and hepatotropic factors after unilateral nephrectomy or partial hepatectomy have been extensively studied. Among these factors, hepatocyte growth factor (HGF) is now considered to be a potent mitogen for the epithelial cells in various organs, not only for the kidney and liver but also for others. HGF mRNA was found to be markedly increased in the non-injured kidneys and spleens of rats after partial hepatectomy or unilateral nephrectomy [10]. In the present study, we found that HGF receptor was consistently detected in the urothelium following unilateral nephrectomy or partial hepatectomy. Thus, HGF might have some role in the growth-enhancing effect on bladder tumors after nephrectomy and partial hepatectomy.

Imada et al. [8] reported that blocking of testosterone production inhibited bladder carcinogenesis and that testosterone itself might have a potent action on bladder carcinogenesis. Furthermore, a rapid increase of follicle stimulating hormone (FSH) from the pituitary gland and subsequent testosterone production from the contralateral testis are known to occur after unilateral orchiectomy [1]. Although we did not measure the serum testosterone levels, the increased expression of androgen receptor in the bladder urothelium following unilateral orchiectomy that was observed in the present study supports the possibility that the transient increase in testosterone level after orchiectomy played a role in urothelial proliferation.

Furthermore, many other growth factors, such as EGF [16], KGF [17] and TGF-β1 [14], have been found to have proliferative effect on the urothelium in regenerating bladder. It is therefore possible that one or more of these growth factors might have also acted as a proliferative stimulus on the urothelium after surgical resection of the organs.

Another possible mechanism is the production of growth-stimulatory signals from the injured sites and their transmission through the genitourinary tracts to the bladder. It has been reported that ureteric ligation alone enhanced normal [6] and neoplastic [11] epithelial proliferation of the bladder. Both the kidney and the testis are connected with the bladder through the ureter and the spermatic duct, respectively. After the ureter or spermatic duct is ligated and cut during nephrectomy or orchiectomy, some growth-enhancing signals might be directly transmitted from the injured cells to the bladder epithelium via cell-to-cell communication. Such signals could then account for the increased urothelial proliferation. In contrast, the liver is a distant organ without direct connection to the bladder. The difference in the anatomical location of the liver compared with the kidney and testis might have resulted in the lesser degree of growth enhancement that was noted in the present study.

Although these explanations for the present experimental results still remain speculative, it is tempting to consider that surgical resection of these organs has some enhancing effect on bladder carcinogenesis in humans, as well as in rats. Further study is needed to determine the mechanism of the promoting effect of organ resection on bladder carcinogenesis in both experimental and clinical settings.

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