REVIEW

Prevention of Urinary Bladder Cancer: The Interface Between Experimental and Human Studies

Shoji Fukushima, Hideki Wanibuchi

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1. Introduction

Urinary bladder carcinomas have attracted major attention as one of the occupational cancers strongly linked to industrial chemical exposure (Case et al., 1954), with several dye stuffs found to be carcinogens, and contamination of the environment with arsenic carcinogens (see Shirai et al., 1995; Fukushima et al., 1999, for reviews). Recently a great deal of interest has been generated by the finding of urinary bladder lesions in association with radioactive contamination after the Chernobyl disaster (Vozianov et al., 1996). However, in the majority of cases of urinary bladder cancers in the Western world, there is no obvious explanation for the tumor development, although factors such as smoking, saccharine or analgesic consumption and coffee drinking have been speculated as playing causal roles (Yu et al., 1997; IARC, 1980; 1991). Work with experimental animal models has greatly increased our knowledge of possible etiologic factors, the processes involved in urinary bladder carcinogenesis and the molecular events responsible for development of cancers. This review will concentrate on providing a comprehensive coverage of the present state of our knowledge in this area with emphasis on possible

Department of Pathology, OsakaÅ City University Medical School, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Fax 81-6-646-3093 Email fukuchan@med.osaka-cu.ac.jp

avenues for cancer proevention. Bladder cancer is generally used as an inclusive term for tumors of the renal pelvis, ureter, bladder and urethra and has been employed here in this sense. TCCs of the renal pelvis and ureter, associated with kidney and ureter stones (Chow et al., 1997), are linked with bladder cancers (Curtis et al., 1985; Levi et al., 1993, Moore et al., 1999) (see Fig 1). Indeed, there appears to be a general relationship among genito-urinary sites (Fukagai et al., 1996; Koyama et al., 1995; Moore et al., 1999).

2. Geographical Variation and Linked Experimental Findings

As shown in Fig 2, bladder cancer is more prevalent in males than females with considerable inter-country variation. Within the developed world, Northern Europe and especially Scotland and Denmark have high rates, while Australasia and the USA have intermediate, and Japan relatively low incidences (Parkin et al., 1997). Highest levels are however, found in association with particular environmental factors, as detailed below.

Schistosomiasis

The largest incidences of bladder cancer in man are found in populations with high levels of infestation with schistosomes, where is most common cancer, accounting for as many as 30% of all cancers ((see Badawi et al., 1995 for review). There is a geographical correlation with level of endemicity of schistosomiasis (Chen and Mott, 1989), and as well as in Egypt ; (El-Bokainy et al., 1981) high levels of both are found in Iraq (Aladnani and Saleh, 1983), Kuwait (Al-Shukri et al., 1987), Sudan (Malik et al., 1975), Zambia (Bhagdaween, 1976, Elem and Purohit, 1983), Malawi (Lucas, 1982) and) (Zimbabwe (Gelfand et al., 1967). Parasite-associated bladder tumors are much more prevealent in males who work the fields and therefore are exposed to the parasite, relative incidence rates being 4-6: 1 rather than the about 3:1 ratio which is usual for male to female incidence rates. They are characterised by early development, arising in individuals in their 40s to 50s instead of 60s to 70s (Badawi et al., 1995; Koriatim et al (1995) and of squamous cell carcinoma (SCC) rather than the typical transitional cell carcinoma (TCC) type which predominates elsewhere (Silverman et al., 1992). In hamsters infection causes squamous cell metaplasia (El-Morsi et al., 1974) and the change in histopathological type is typical of situations in which pronounced elevation of proliferation or high exposure to carcinogens occur. For example, severe long-standing cystitis may be associated with SCCs (Polsky et al., 1976; Dolin et al., 1994). The reason for the relatively high proportion of squamous as opposed to transitional cell lesions in the black population in the United States remains to be clarified (Parkin et al., 1997).

Bladder cancers in fact develop in many different types of animal infected with schistosomes and given carcinogen, including baboons (Hicks et al., 1980). Evaluation of the carcinogenic potential of the parasite alone showed that early lesions are reversible on removal of the stimulus (Cheever et al., 1988), very similar to the lesions induced by urinary calculi in rodents (Shirai et al, 1986). However, hyperplastic lesions go on to produce carcinomas especially when linked to sub-carcinogenic doses of carcinogen (Hicks et al., 1980).

With regard to the aetiology, egg-induced local inflammation appears to be particularly important, and the associated chronic fibrotic reaction (Rosin et al., 1994; Badawi et al., 1995). Cancers often arise with a collar of epithelium containing high numbers of eggs (Christie et al., 1986) and preneoplastic lesions in one monkey model were preferentially located near foci of egg deposition (Cheever et al., 1988). Fibrosis-induced urinary retention could play a role, with factors in the urine promoting carcinogenesis (Oyasu et al.,). Furthermore, it is well established that the inflammatory state caused by schistosomiasis is associated with increased generation of nitroso compounds which are well known to be active as carcinogens in the bladder (El-Merzabani et al., 1979; Badawi et al., 1992a; Abdel-Mohsen et al., 1999). Chronic exposure would be expected to cause cancer and adducts formed by such carcinogens may be present in infected tissue (Badawi et al., 1992b). It is clear that the presence of parasites within the tisuue can itself bring about changes in metabolising enzymes and itslef lead to DNA damage (Badawi et al., 1993). Elevated levels of carcinogenic tryptophan metabolites may also contribute (Abdel-Tawab et al., 1986).

A similar situation if less pronounced in influence may prevail with other infections of the urinary tract. For example, enhancement of urinary bladder carcinogenesis has also been reported with other urinary tract diseases in man (Kantor et al., 1984; La Vecchia et al., 1991). It should here be noted that excretion of N-nitorsamines has also been described for patients with bacterial bladder infections or diversions of the urinary tract (Tricker, 1996).While massive calculi in the urinary bladder unequivocally cause papillomatosis and eventually cancer development in rats and mice (Fukushima et al., 1992), kidney or urinary stones as they occur in man appear to have limited or no clear effects (Wynder et al., 1963; Kjaer et al., 1989; Gonzales et al., 1991; Sturgeon et al., 1994; Chow et al., 1997; Tavani et al., 1998).

Arsenic Poisoning

Epidemiological investigations have revealed that arsenicals are carcinogenic to man, especially affecting the skin and lung. In the blackfoot disease endemic area of Taiwan, because of the ingestion of a high concentration of arsenicals in the drinking water, an elevated mortality

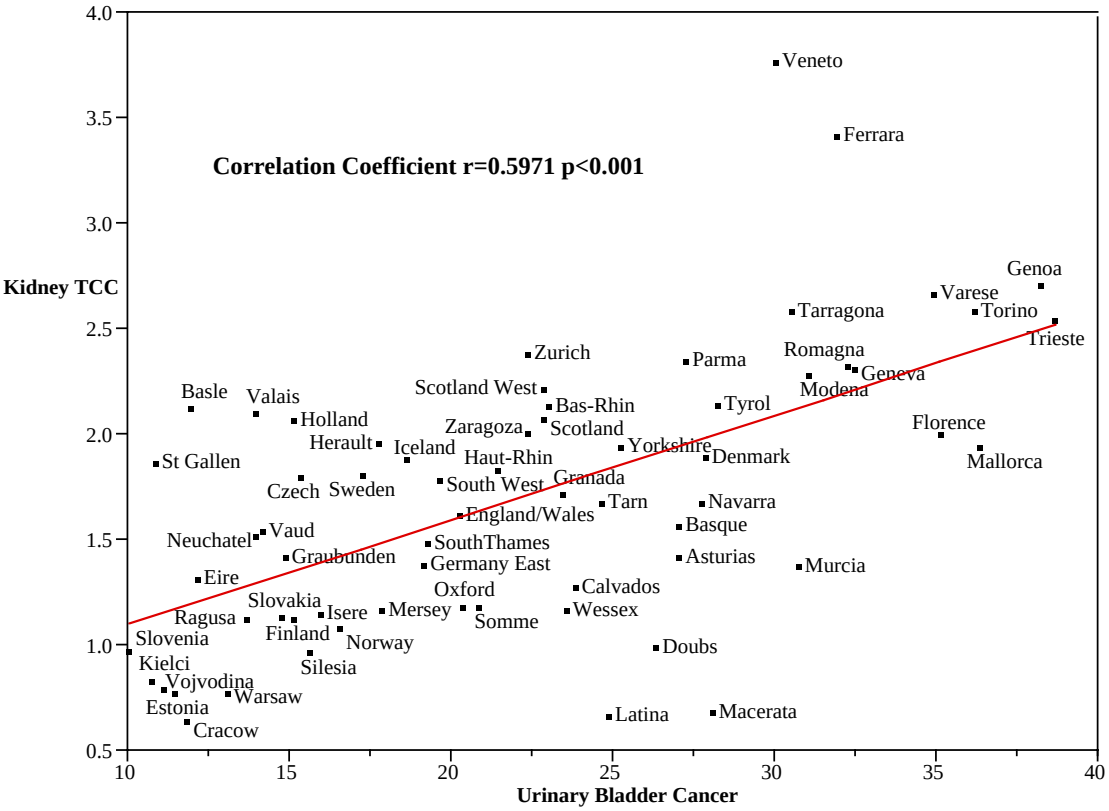

Figure 1. Relation between TCC Incidences in the Urinary Bladder and Kidney in Europe

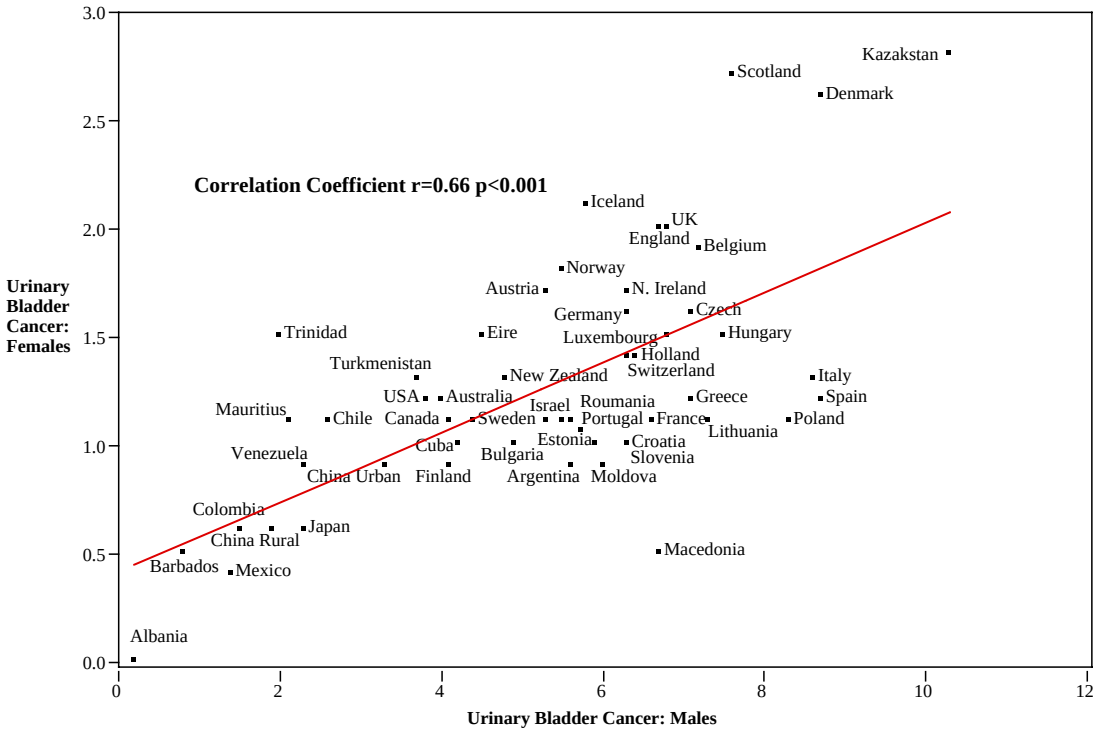

Figure 2. Relation between Urinary Bladder Cancer Incidences in Males and Females

from internal cancers, especially those of the urinary bladder, kidney, liver and lung, as well as skin cancer, has been reported (Chen et al., 1985). The tumor incidence was found to be dependent on the concentration of arsenicals in the water. In experimental animals, promotion potential was revealed for dimethylarsinic acid (DMA), a major metabolite of inorganic arsenic in most animals, in the urinary bladder, kidney, liver and thyroid gland using a multiorgan carcinogenesis bioassay in rats (Yamamoto et al., 1995; see Yamamoto et al., 1997 for review). In a two stage rat urinary bladder model, the development of preneoplastic lesions and tumors (papillomas and carcinomas) was also enhanced by treatment with DMA in a dose-dependent manner from 10ppm (Wanibuchi et al., 1996). The promoting potential of DMA at this low dose thus provides support for the epidemiological data pointing to development of urinary bladder cancer due to arsenics. Indeed, very recently, an arsenical exerted complete carcinogenicity on long-term application to Fr44 rats (Wei et al.,1999). The toxicity of DMA to rats is very characteristic, most animals aged 6 weeks receiving 200ppm dying within 4 weeks, in contrast to very few animals aged 8 or 10 weeks at the commencement. Thus chronic DMA administration is primarily toxic for young rats. Since DMA induces chromosomal alterations and DNA damage, such as DNA single-strand breaks and DNA-protein crosslinks (Endo et al., 1992), clastogenic effects might be important for rat carcinogenesis. On the other hand, it was recently reported that sodium arsenite increases rat hepatic ODC activity and hepatic heme oxygenase activity, but causes no DNA damage, indicating that it might be a promoter rather than an initiator of carcinogenesis (Brown and Kitchin, 1996). Isothiocyanates are also known to promote in the bladder (Hirose et al., 1998).

Chernobyl

As noted above, urinary bladder cancer has been linked to the radiation exposure following Chernobyl, presumably because of cesium (Rundo et al., 1963). Thus, in the 11 years between 1986 and 1996, the incidence in the Ukraine population increased from 26.2 to 36.1 per 100,000 (Vozianov et al., 1996). In addition to moderate or severe dysplasia or carcinoma in situ, along with two small TCCs, one study demonstrated nuclear accumulation of p53 and the proliferation-associated PCNA and cyclin D1 in urothelial cells of individuals living in the most-highly contaminated areas (Romanenko et al.,1999), along with increased levels of oxidative stress (Romanenko et al., 2000). These findings are all suggestive of pre-malignant or malignant change. Furthermore, specific p53 gene mutations have been found in the same tissues, with a relative hot spot at codon 245, again pointing to a future elevated occurrence of bladder cancers (Yamamoto et al., 1999).

3.Analytical Epidemiological and Linked Experimental Findings

Smoking

Descriptive epidemiology (Cartwright et al., 1993), case-control (Ishcovic et al., 1987; Burch et al., 1989; Clavel et al., 1989; De Stefani et al., 1991; Gremy et al., Hartge et al., 1993; Momas et al., 1994a,b; McCarthy et al., 1995) and cohort studies (Steineck et al., 1988; Chyou et al., 1993) have all pointed to cigarette smoking as a risk factor for urinary bladder cancer. Although there are exceptions (Yu et al., 1997), the association has been observed for both males and females, and the risk correlates with numbers of cigarettes smoked, the duration of smoking, and the degree of inhalation of the smoke (Morrison et al., 1984). While no unequivocal experimental data have so far been published to support the epidemiological findings, cigarette smoke is well known to contain a number of carcinogenic nitroso compounds and arylamines (Patrianakos and Hoffmann, 1979). Relatively rrecently it was reported that one component, acrolein, can initiate rat urinary bladder carcinogenesis (Cohen et al., 1992). Two known bladder carcinogens found in tobacco smoke, the aromatic amines 4-aminobiphenyl and 2-naphylamine, are likely from biochemical evidence to be smoke-related causative agents for bladder cancer (Vineis and Terracini, 1990). Barbiturate protection in smokers (Habel et al., 1998), could be explained by altered aromatic amine metabolism (Wallin et al., 1995). Also the contribution of polymorphisms in GSTM1, GSTT1 and N-acetyltransferase genes are in line with a role for smoking (Cartwright et al., 1982; Abdel-Rahman et al., 1998; Peluso et al., 1998). In addition, since cigarette smokers exhibit increased cell proliferation in the urinary bladder as evidenced by epithelial hyperplasia (Auerbach and Garefinkel, 1989), cigarette smoke may exert enhancing effects on urinary bladder carcinogenesis. In this context the finding that there may be a particular link with non-transition al cell carcinomas, especially SCCs, is of interest (Bedwani et al., 1997; Kantor et al., 1988; Fortuny et al., 1999), as well as the association with invasion (Jensen et al., 1987).

Analgesic abuse

Analgesic therapy has long been hypothesized to be a risk factor (Hultenen et al., 1968; McCredie et al., 1982; 1986; Piper et al., 1986; Linet et al., 1995; Thon et al., 1995; Rosenberg et al., 1998). Thus, phenacetin, used alone or in combination with aspirin, was first found to be associated with an increased level of renal pelvic in Sweden (Hultenen et al., 1968). Long exposure was also found to increase the risk of urinary bladder cancer development and finally phenacetin was evaluated to be carcinogenic for the human urinary tract (IARC, 1987).

Experimental studies similarly revealed carcinogenic effects on the kidneys of (C57BL/6Å~Çb3H) Fl mice, and the nasal cavity and urinary bladder of SD rats (Isaka et al., 1979; Johansson, 1981; Nakanishi et al., 1982). An increase of the labeling index of renal pelvic and renal papillary epithelium in rats fed phenacetin and a promoting effect on urinary tract carcinogenesis have also been reported (Kunze and Mohlmann, 1983; Johansson et al., 1989). Examination of the complete carcinogenicity of phenacetin in spontaneous hydronephrosis-bearing rats further demonstrated induction of both ureteral and urinary bladder carcinomas (Murai et al., 1993).

Chemotherapeutic agents

Since the first report of urinary bladder cancers related to cyclophosphamide therapy in 1971 (Worth, 1971), a large number of cases have been described and it has been calculated that patients undergoing this type of treatment have a ninefold increased risk of bladder tumour development. IARC has evaluated cyclophosphamide to be a human carcinogen (IARC, 1987). In animal models, Schmähl and Habs (1983) reported that oral administration of cyclophosphamide induced transitional cell carcinomas of the urinary bladder in rats and Hicks et al. (1975) described cyclophosphamide to act as cocarcinogen. Acrolein, which is considered to be a toxic metabolite of cyclophosphamide, binds to DNA and Cohen et al. (1992) reported that it initiates urinary bladder carcinogenesis in the rat. It is also an important industrial chemical and is present in cigarette smoke(10Å‘140mg/cigarette).

There is experimental evidence that other chemotherapeutic agents are also dangerous in this context (Ohtani et al., 1984) and another compound, N,N-bis(2-chloroethyl)-2-naphthylamine(chlornaphazine) has been used for therapy of polycythemia but 13 urinary bladder carcinomas developed among 61 treated patients and IARC has thus evaluated this compound as a human carcinogen (IARC 1987), although there is no literature regarding any carcinogenicity in rodents.

Saccharine and sweeteners

Since the first report in 1970 (Bryan et al., 1970), sodium saccharin has attracted considerable attention as a urinary bladder carcinogen in rodents, but the conclusion of complete carcinogenic potential is controversial (Ellwein and Cohen, 1990). Hicks et al. (1975) demonstrated that prior instillation of a subcarcinogenic dose of MNU, resulted in a high incidence of urinary bladder carcinomas when followed by oral administration of sodium saccharin. Subsequently, Cohen et al. (32) confirmed that sodium saccharin is a strong promoter in rats. Its effects are dose dependent (43) but are not shared by the parent acid (West et al., 1983). Sodium cyclamate has also been shown to act as a promoter of urinary bladder carcinogenesis in rats (Hicks et al., 1975).

In nonhuman primates no carcinogenicity could be detected (Takayama et al., 1998) althought the conclusions drawn were contested (Jacobsen et al., 1998).

Dietary Factors

Dietary fruits and vegetables may play aprotective role against development of bladder cancer (Mettlin and Graham, 1979; La Vecchia, 1989; Mills et al., 1991; Nomura et al., 1991; Vena et al., 1992b; Chyou et al., 1993; Momas et al., 1994) although some studies have provided no evidence for this (Steineck et al., 1988;Riboli et al., 1991). Total fruit intake and fuit and vegetable intake in males were found to inversely correlate with risk in two case-control studies (Claude et al., 1986; Bruemmer et al., 1996). In one recent study only high cruciferous vegetable consumption was protective (Michaud et al., 1999). Fruit intake was positively linked to protection in two cohort studies (Shibata et al., 1992; Chyou et al., 1993).

With regard to other dietary components, an ecological study pointed to positive link between cancer mortality and consumption of fats and oils (Armstrong and Doll, 1975) but neither saturated nor unsaturated fat had any promoting effect in an animal model (Kitano et al., 1995).

4. Histogenesis of Bladder Cancers

Histopathology

The histopathologic lesions observed in the urinary bladder epithelium of rats treated with BBN have been classified into four types: simple hyperplasia, papillary or nodular hyperplasia, papilloma, and carcinoma (Fukushima et al., 1982) (see Figs 3-6). Simple hyperplasia consists of diffuse or focal thickening of the epithelium with four to eight layers of transitional epithelial cells. In papillary or nodular hyperplasia, the epithelium is six to eight cells thick, and in most cases the changes are strictly localized. Cellular atypia and mitotic figures are only rarely observed in areas of hyperplasia. Areas of hyperplasia demonstrate either exophytic growth, with a delicate fibrovascular core and protrusion into the lumen of the urinary bladder, or endophytic growth. Papillomas are defined as benign epithelial tumors in which the transitional epithelium cells are arranged in branched finger-like processes surrounding a delicate fibrovascular core. They are generally exophytic but may show an endophytic growth pattern. Cellular irregularity is slight and few mitotic figures are present. Carcinomas have morphologic characteristics of atypia, invade the muscularis and demonstrate a high degree of mitotic activity.

Papillary or nodular hyperplasia of the urinary bladder in rats treated with 0.05% BBN develops before the

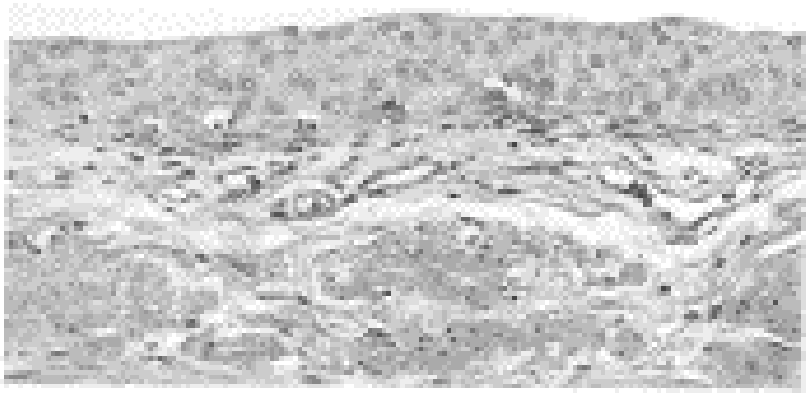

Fig 1. Simple hyperplasia induced by BBN in a rat.

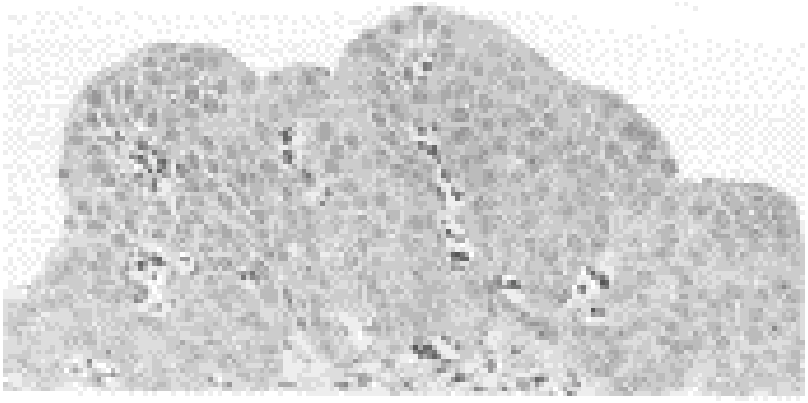

Fig 2. PN Hyperplaisa induced by BBN.

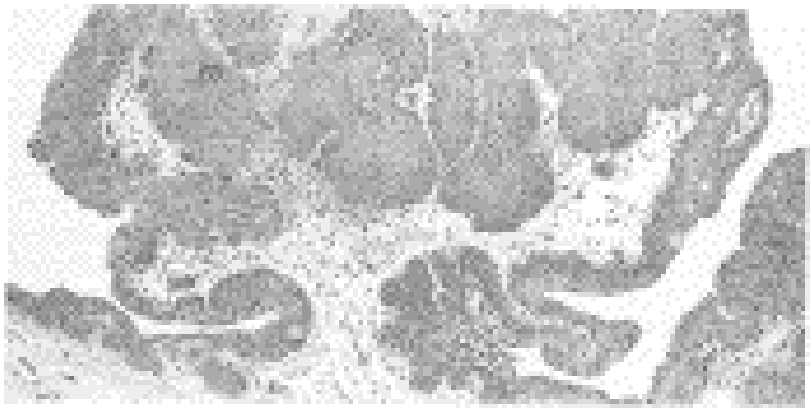

Fig 3. Papilloma induced by BBN

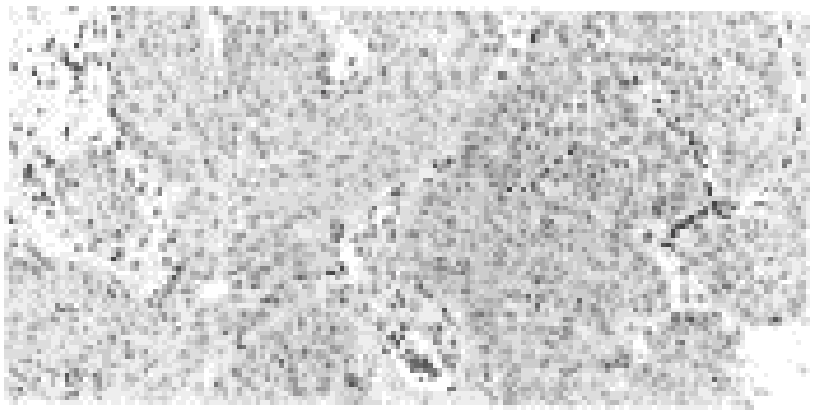

Fig 4. Carcinoma TCC) induced by BBN

induction of papillomas or carcinomas (Fukushima et al., 1982), and can be induced at high incidence by large doses of BBN within a short period. It is characterized by increased proliferation (Fig 7) and a dose-response relationship has been observed. The period of carcinogen exposure also plays a role. Moreover, a good correlation exists between the degree of development and the numbers of eventual carcinomas. Thus, it is considered that papillary or nodular hyperplasia is a pre-neoplastic lesion in the rat urinary bladder.

In man, three categories of TCCs of the urinary bladder can be distinguished on the basis of phenotype and biological behaviour: 1) papillary, non-invasive; 2) nonpapillary, invasive; and 3) carcinoma in situ. Most patients present with superficial, low grade disease (Gilbert et al., 1978; Heney et al., 1983; Herr et al., 1987). However, after initial treatment, recurrence as a higher grade lesion is relatively common (Kaye and Lange, 1982). While invasive bladder cancers may occasionally arise from superficial papillary lesions, more often there is no such previous history (Brawn, 1982; Heney et al., 1982). Therefore carcinoma in situ, whcih can involve extensive areas of mucosa is important (Utz and Farrow, 1984. The data from animal experimentation point to a similar situation in rodents. Thus in two-stage experimental protocols, non-invasive papillary lesions predominate, with features similar to those in man (Fukushima et al., 1983). If carcinogen is administered continuously to mice, however, carcinoma in situ usually precede the appearance of advanced lesions like TCCs and SCCs (Ito et al, 1969; Tamano et al., 1991).

Molecular pathology

It is generally accepted that tumor development occurs as the result of accumulation of genetic alterations and this has also been argued for bladder cancer (Schulte 1988; Lee et al., 1998). A schematic illustration of possible pathways to invasive TCCs with possible roles for specific changes at the gene level is given in Fig 8. Putative candidate genes for urinary bladder carcinogenesis include the retinoblastoma (Rb) (Presti et al., 1991) and p53 (Sidransky et al., 1991) tumor suppressor genes and several oncogenes, H-ras (Bos, 1989), c-myc (Perucca et al., 1990), and c-erb B-2 (Fujimoto et al., 1992).

Particular attention has been concentrated on the possible participation of p53 gene alterations in urinary bladder carcinogenesis. It has been reported that mutations in this tumor suppressor gene are common in invasive and/or high-grade urinary bladder carcinomas and roles in differentiation or tumor progression have therefore been speculated (Fujimoto et al., 1992; Spruck et al., 1994). Another molecular marker of advanced lesions is inactivation of the Retinoblastoma tumor suppressor gene, by mutations and LOH, this being associated with aggressive behaviour (Ishikawa et al., 1991; Cordon-Cardo et al, 1992; Logothetic et al., 1992). The changes in cadherin-catenin complexes that have been described to play a role in progression may be of interest in this context (Giroldi et al., 1999). Early rat lesions, characterized by papillary structures and infrequent metastasis, only demonstrate p53 mutations in rare cases with an initiation-promotion protocol (Lee et al., 1997b), although continued exposure to carcinogen in one series

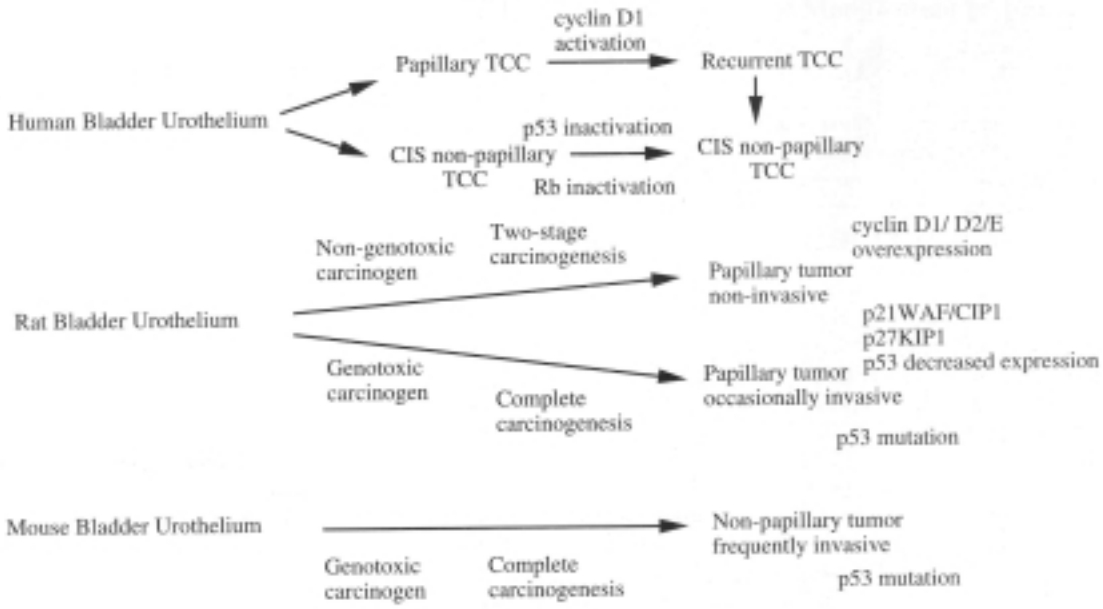

Fig 8. Pathways to Transitional Cell Carcinoma (TCC) Development

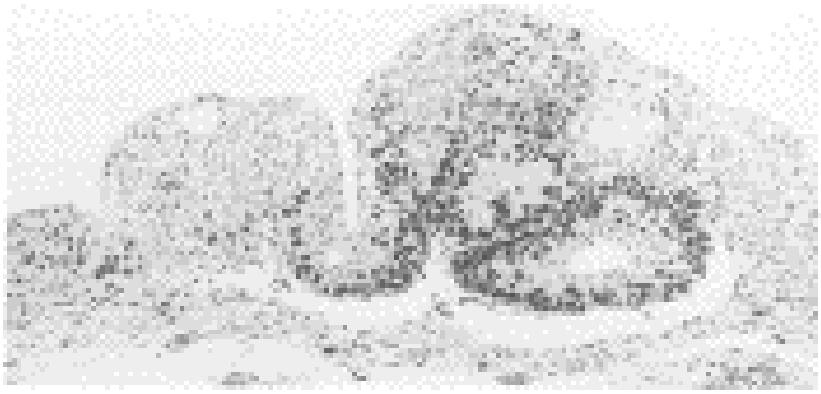

Fig 5. Brd U labeled cells in a PN hyperplasia

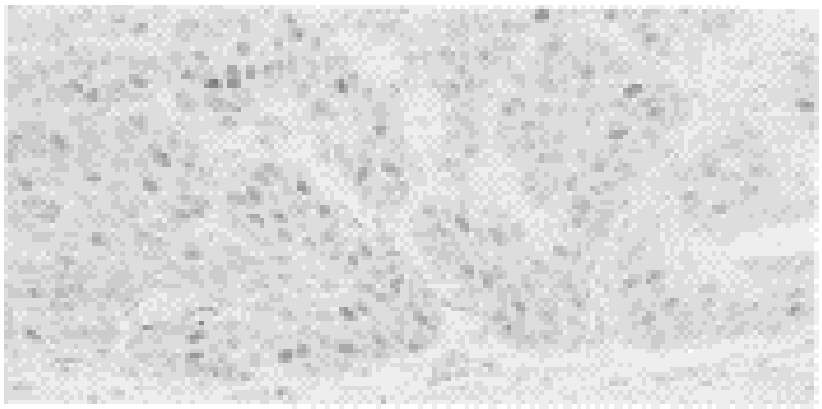

Fig 6. Cyclin D1 positive cells in a TCC induced by BBN + Na-AsA

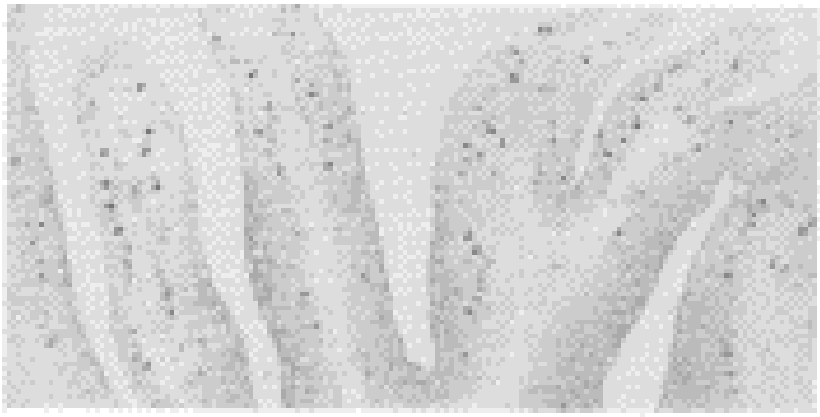

Fig 7. PCNA positive cells in papillomatosis induced by uracil.

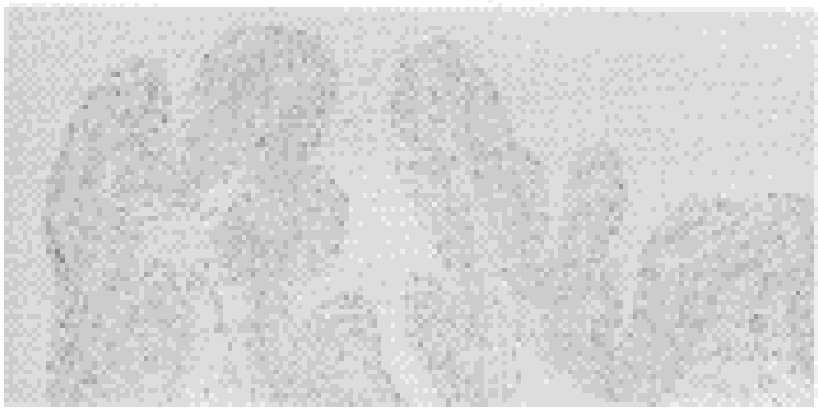

Fig 8. Apoptotic cells in papillomatosis induced by uracil (one week after withdrawal of insult).

about half of the cases were positive (Masui et al., 1994). whereas the figure is about 80% for mouse urinary bladder carcinomas induced by BBN, these typically of flat type and rapidly progressing to invasion and metastasis (Yamamoto et al., 1995). Alterations appear to be associated with invasive character and genetic instability in mouse carcinomas (Yamamoto et al., 1997; Morimura et al., 1999). The role of wild-type p53 as a critical regulator of a G1 cell cycle checkpoint means that its loss might accelerate the accumulation of genetic alterations and this has been regarded as one of the most important indicators of likely further progression and a poor prognosis. In fact both nullizygous and heterozygous knockout mice demonstrate increased susceptibility to BBN bladder carcinogenicity (Ozaki et al., 1998; Yamamoto et al., 1999).

Generally, the spectrum of mutations within p53 gene in human urinary bladder carcinomas does not demonstrate any hot-spot, although, Shibata et al. (1994) reported a distinct pattern in lesions from the endemic area of black foot disease in Taiwan, which might be related to high arsenic levels in artesian well water. Such a phenomenon is indicative of preferential interaction of a carcinogen with certain bases of DNA and is helpful for aetiological assessment. Contrary to expected, studies using BBN did not reveal any mutational hot-spot, although mutations were found to be relatively concentrated within p53 gene exons 5 and 7. Mutational defects in either p53 allele are often accompanied by the loss of the remaining allele (Sidransky et al., 1991). Presence of loss of heterozygosity (LOH) on p53 allele in experimentally induced urinary bladder carcinomas was evaluated using F1 hybrid mice and a microdissection procedure (unpublished data). Invasive urinary bladder carcinomas were induced after 12 weeks administration of BBN in male (NON/Shi X C3H/HeN/Shi) F1 offspring within a 21 week total experimental period. Abnormal band-shifts suggesting p53 mutations and loss of either allele were observed in 57% and 29% of carcinomas, respectively. Of four carcinomas with allelic losses, three had p53 mutations in the remaining allele.

H-ras and K-ras mutations are rare in urinary bladder lesions in both animals (Masui et al., 1992; Lee et al., 1997b) and humans (Knowles and Williamson, 1993; Nagata et al., 1990). Significant participation of this tumor suppressor gene in progression has, however, been indicated by a good correlation with malignant potential observed with various animal urinary bladder carcinomas. The incidence of p53 alterations in mouse primary carcinomas without metastasis is similar to those for human invasive carcinomas reported previously (Fujimoto etal., 1992; Spruck et al., 1994), while rat early neoplastic lesions ppear to be more likely to have mutations than their corresponding human papillary low-grade tumors. c-erbB-2/neu , another candidate as a gene responsible for urinary bladder cancer progression (Underwood et al., 1995), was not reported to play a significant role in N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide-induction of rat urinary bladder carcinomas or 2-amino-4-(5-nitro-2-furyl)thiazole transformation of rat bladder epithelial cells (Mann et al., 1994).

Microsatellite instability (MSI), due to DNA replication errors in repetitive nucleotide sequences, has been reported to occur at a low rate in human urinary bladder carcinomas (Gonzalez-Zulueta et al., 1993; Rosin et al., 1995). At least in animal models, microsatellite instability is relatively infrequent as is associated loss of heterozygosity (Chen et al., 1998; 1999). MSI was thus present in 9 of 28 (32%) invasive urinary bladder carcinomas which were also used for p53 mutational analysis. The significance of this must be considered low, however, since 8 of the 9 cases harbored p53 mutations and a relationship between MSI and p53 defects is suspected.

Of the members of the cyclin family, cyclin D1 has been reported to be amplified, with mRNA and protein overexpression in TCCs of the human bladder (Bringuier et al., 1996; Lee et al., 1997a) and in an animal model (Lee et al., 1997). In man it appears to be associated with early recurrence (Shin et al., 1997). On the other hand, p27Kip1, a member of the protein family responsible for negatively regulating cyclin kinases, is reduced in rat carcinomas (Lee et al., 1999; Ogawa et al., 2000. Similarly, $p21^{WAF1/CIP1}$ expression has been found to be down-regulated (Lee et al., 1997b), with a relation to progression (Stein et al., 1998), and novel mutations have also been described in this oncogene in superficial and invasive transitional carcinomas (Malkowicz et al., 1996).

5. Carcinogens and Modification of Tumour Development

Carcinogens

The existence of evidence pointing to occupational links to bladder cancer (Vineis and Simonato, 1986; Barbone et al., 1994) suggests that environmental carcinogens might be of importance. Experimentally, nitrosamines such as butyl-N-(4-hydroxybutyl)- nitrosamine(BBN) (Druckrey et al., 1964; Iot et al., 1969; Okajima et al., 1981), N-ethyl-N-(4-hydroxybutyl)nitrosamine(EHEN) (Hashimoto et al., 1974) and N-methyl-N-nitrosourea(MNU) (Hicks and Wakefield, 1972) are well known to induce urinary bladder carcinomas in rodents and dogs. It was reported that endogenous nitrosation can be mediated by bacteria and macrophages in infected organs (Leaf et al., 1989) and N-nitroso compounds have been found in infected urinary bladders, in particular in patients having the *Schistosoma hematobium* which is significantly associated with neoplasia in the urinary bladder (El-Merzabani et al., 1979).

Recently a great deal of attention has been drawn to the significance of of heterocyclic amines generated by cooking processes in proteinaceous foods (Sugimura,

Table 2 . Classification of Urinary Bladder Cancer Promoters

1. Sodium or potassium salts
Sodium saccharin
Sodium *L*-ascorbate
Sodium *o*-phenylphenate
Sodium bicarbonate
Sodium citrate
Sodium phenobarbital
Potassium carbonate with or without ascorbic acid
2. Urolithiasis-inducing chemicals
Uracil
Diphenyl
3. Antioxidants
Butylated hydroxyanisole
Butylated hydroxytoluene
Ethoxyquin
t-Butylhydroxyquinone
2-*t*-Butyl-4-methylphenol
4. Anticancer agents
Adriamycin
Mitomycin C
5. Amino acids
DL-Tryptophan
L-Leucine
L-Isoleucine
6. Others
Urinary components (fractions I and II)
Allopurinol (?)

1997). One of the series tested, 3-amino-1-methyl-5H-pyridol[4,3-b]indole, otherwise known as Trp-P-2, was found to be carcinogenic in rat urinary bladder as well as the liver (Takahashi et al., 1993). Cigarette smoke contains carcinogenic heterocyclic amines and Trp-P-2 was reported to be present at levels of 0.95 ng per cigarette.

Promoting Agents

Many researchers have directed their experimental efforts to detecting environmental promoters of urinary bladder carcinogenesis and it has been established that there are several kinds of agents which can exert promoting potential in rats, as shown in Table 2 (see Fukushima, 1991, for an earlier review), all apparently causing increase in proliferation in the urinary bladder.

1. Sodium or potassium salts

In the light of findings for sodium saccharin we examined promoting effects of sodium L-ascorbate(Na-AsA) and found that oral administration of a 5% dietary dose clearly enhanced urinary bladder carcinogenesis (Fukushima et al., 1983), whereas L-ascorbic acid(AsA) did not. In subsequent studies, attention was focused on the roles played by urinary pH and Na ion concentration in such promotion (Fukushima et al., 1986). Like Na-AsA, AsA plus NaHCO3 brings about marked enhancement of neoplastic lesion induction, this being reduced by NH4Cl. The lack of increase in urinary pH observed after combined treatment with NH4Cl and Na-AsA provides further evidence of important roles for urinary Na ion concentration and pH in modulation of urinary bladder carcinogenesis. AsA was also found to act as a copromoter (an amplifier) under conditions of increased urinary pH and Na ion concentration (Fukushima et al., 1988; Iwata et al., 1997). The promoting activity of NaHCO3 was confirmed by Lina and Woutersen (1989). It has been demonstrated that administration of sodium hippurate and 1% NaCl was not associated with any promotion, increased urinary Na ion concentration in this case not being accompanied by elevation of pH (Ito and Fukushima 1989). Examination of ions other than Na revealed dietary administration of AsA plus K2CO3 to clearly promote the development of bladder carcinomas while inducing changes in urinary parameters: elevation of pH, increased K ion concentration and increase of AsA (Fukushima et al., 1987). K2CO3 alone also exerted weak promoting activity. In this case increases of urinary pH and K ion concentration were observed without change in AsA. Other researchers (Lina and Woutersen, 1989) have recently stressed that the K ion is as potent as the Na ion regarding potential for promotion of urinary bladder cancer development under conditions of elevated urinary pH. Thus, elevated K ion concentration together with pH are also associated with promoting activity, especially when acting in concert with the co-promoter AsA. Although Ca ion or Mg ion concentrations were elevated in groups given CaCO3 or MgCO3, and an increase in pH was found for CaCO3, these treatments did not exert any promoting activity.

2. Antioxidants

Antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), Éø-tocopherol, and propyl gallate, have been widely used as additives in various processed foods. Their application in this way has generally been thought to be without hazard and, indeed, antioxidants have been demonstrated to have anticarcinogenic activities when given before and/or together with carcinogens (Wattenberg, 1978). However, several recent reports have demonstrated their enhancement of tumor formation in animals. In our two-stage urinary bladder carcinogenesis model, BHA and BHT exerted strong, and ethoxyquin and tertiary butylhydroquinone (TBHQ) weak promoting activities (Imaida et al., 1983). However,Éø-tocopherol and propyl gallate administration did not result in any enhancement of urinary bladder carcinogenesis (Tamano et al., 1987). No specific relations between urinary components and the promoting activity of BHA, BHT, ethoxyquin or TBHQ could be established and it appears that the potency of antioxidant action also does not correlate with the

demonstrated enhancing effects. Recently it was suggested that toxicity of glutathione conjugates of TBHQ to the urinary bladder may contribute to the promoting activity of BHA and TBHQ (Peters et al., 1996).

3. Amino acids

Cohen et al. (32) found that *DL*-tryptophan demonstrated promoting activity for bladder carcinogenesis in male rats initiated with FANFT, although the effects were less marked than with sodium saccharin. However, *L*-tryptophan, which is the biologically more important form, did not exert any significant promoting activity. It is thus interesting that allopurinol, which is widely used in the treatment of gout and is known to inhibit tryptophan oxygenase and may thus alter the pattern of urinary tryptophan metabolites can also promote the induction of bladder carcinomas by FANFT treatment (Wang et al., 1976). Further studies, however, are required to confirm this result, because no promoting effect was demonstrated after BBN initiation (Ito and Fukushima, 1989).

Other amino acids found to exert promoting activities on urinary bladder carcinogenesis of rats are L-isoleucine and L-leucine (Nishio et al., 1986). This suggests a possible relation between the high incidence of urinary bladder cancers in western countries, where the diet is rich in protein.

4. Others

Oyasu et al. (1978) developed a heterotopically transplanted rat bladder (HTB) model to investigate the role of urinary factors in urinary bladder carcinogenesis. Exposure of the HTB to test fluids first revealed that normal rat urine exerts promoting activity after initiation with BBN or MNU (Oyasu et al., 1981). Urine fractions designated as fraction I (molecular weight, 37,000) and II (molecular weight, 4,300), and particularly the former were subsequently found to strongly promote development of tumors. It is now thought that the main constituents of fractions I and II are epidermal growth factor and a related molecule (Matsui-Yuasa et al., 1992).

Evidence for an integral role for proliferation is provided by the finding that freeze ulceration, linked to regeneration, plus sodium saccharin promotion is sufficient to cause neoplasia (Hasegawa et al., 1985). Unilateral nephrectomy or ureteral ligation results in growth and promotes neoplasia (Ito et al., 1971; Saikawa et al., 1996) and in a large series of studies Fukushima's group have demonstrated a link between induction of proliferation by exogenous chemicals and promotion potential (Fukushima et al., 1991; Shibata et al., 1992). The same rat bladder promoters were given to mice after BBN initiation neither increase cell turnover nor enhance neoplasia (Tamano et al., 1993). Urinary components capable of increasing ODC activity exert a positive effect on urothelial tumorigenicity (Babaya et al., 1983)

In experimental animals, it has long been known that chronic irritation of the urinary bladder can lead to tumor development. Uracil, which induces papillomatosis due to the formation of urinary bladder stones, strongly enhances bladder carcinogenesis by BBN and also causes tumors when given alone for a long period (Fukushima et al., 1988). The same is the case for melamine, with a good correlation observed between tumor induction, and urolith formation/ papillomatosis (Ogasawara et al., 1995).

Inhibitory Agents

In line with the positive link between proliferation and urinary bladder cancer development, the specific inhibitors of ornithine decarboxylase difluoromethylornithine and 1,3-diamonpropane demonstrate protective influence (Homma et al., 1987; Salim et al., 2000). Elevation of the activity of ornithine decarboxylase and spermidine/ spermine N'-acetyltransferase, enzymes related to polyamine metabolism, is caused in the rat urinary bladder epithelium by treatment with promoters (Babaya et al., 1983; Matsui-Yuasa et al., 1992). Increased levels of prostaglandins, especially prostaglandin E2, have also been found in urinary bladder epithelium following application of tumor promoters (Ito and Fukushima, 1989) to rats and non steroid antiinflammatory agents have been found to exert protective influence in a number of animal models (Murasaki et al., 1984; Shibata et al., 1992; 1993; Grubbs et al., 1993; Klän et al., 1993; Moon et al., 1993; Rao et al., 1996), including a specific C0X-2 inhibitor, nimesulide (Okajima et al., 1998) Furthermore, inhibition of promotion has been described for dehydro-epiandrosterone (Shibata et al., 1993) and in humans serum levels of this hormone and its sulphate negatively correlate with risk of bladder cancer (Gordon et al., 1991).

6. Prevention of Urinary Bladder Cancer

Primary Prevention

Since any condition associated with chronically elevated proliferation may be positively linked to development of cancer in the transitional cell epithelium of the ureter and bladder, affected individuals present as a high risk group. For these, other than surgical intervention or clinical treatment to remove the source of the stimulus to growth, chemopreventive agents like NSAID's might find application. Clearly this is an area which will reward further exploration.

Secondary Prevention

Although major risk factors other than parasites in some parts of the world have not been described, even for the general population over 50, regular hematuria testing appears to significantly decrease cancer morbidity and mortality in a cost-effective fashion (Kryger and Messing, 1996). Occult blood approaches for urological cancers,

mostly in the bladder, have been found to have a positive predictive value of 0.41 in those aged over 40 (Bintinx and Wauters, 1997). The reliability is supported by finding of a better predictive power than cystoscopy (Friedman et al., 1996). However, single dipstick urinalysis for microhematuria, was found in one study to demonstrate a sensitivity within 3 years of only 3%, a specificity of 96.7% and a positive predictive value as low as 0.5%, and therefore may not be recommended (Hiatt and Ordonnez, 1994). Assessment of microsatellite instability in the urine has more recently been proposed as a detection method (Mourah et al., 1998).

As positive intervention BCG has been reported to be highly efficacious, with repeated application for resistant cases (Okamura et al?), in addition to an array of agents for intravesical apoplication (Herr et al., 1987).

Conclusions

In conclusion, a great deal of information is available regarding neoplasia of the lower urinary tract, gleaned from experimental animals as well as the clinic. Overall the findings from rodent and other animal models are consistent with what is known about the human situation, underlining their applicability for research to provide mechanistic insights and facilitate development of new preventive strategies.

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Personal Profile: **Shoji Fukushima**

Dr Shoji Fukushima graduated from Nagoya City University in 1967 and received his D.M.Sc. degree in 1973. In 1974 he became Assistant Professor at Fujita Gakuen University, School of Technology and Nursing before moving back to Nagoya City University Medical School with the same title in 1979. Since 1990, he has been Professor and Chairman in the Department of pathology, Osaka City University Medical School. His research areas are chemical carcinogenesis, carcinogenic risk assessment, cancer chemoprevention and toxicologic pathology.

Dr Fukushima has published over 370 articles. In addition to his activities as an Editorial Team member of the APJCP he also serves on tje Editorial Boards of Cancer Letters, the Japanese Journal of Cancer research, Pathology International, Journal of Toxicological Pathology and the Journal of Toxicological Sciences.

Famed for his seemingly insatiable appetite for ramen (Chinese noodles), some of his colleagues have conjectured on whether he might be a morphological wonder, with an extra stomach or a cheek pouch like the hamster.