SHORT COMMUNICATION

High level expression of the multidrug resistance (MDR1) gene in the normal bladder urothelium: a potential involvement in protection against carcinogens?

Steven C.Chipford1, David E.Neal2 and John Lunec1,3

1Cancer Research Unit and 2Department of Surgery, University of Newcastle-upon-Tyne, The Medical School, Framlington Place, Newcastle-upon-Tyne NE2 4HH, UK
2To whom correspondence should be addressed

It has been suggested that expression of the P-glycoprotein transmembrane efflux pump (PGP), encoded by the multidrug resistance (MDR1) gene, may play a role in the protection of epithelial tissues from a variety of local and systemic toxins. We report that in ~50% (6/11) of the population, MDR1 messenger RNA levels in the normal urinary epithelium are comparable to those found in the highest expressing tissues in the body, and suggest a role for PGP in the normal bladder urothelium. MDR1 mRNA levels in the normal urothelium do, however, vary over a 60-fold range between individuals, and furthermore are uniformly significantly lower (~6-fold, \( P < 0.01 \)) in all low-grade bladder carcinoma samples. On the basis of these observations we conclude that low MDR1 mRNA levels are a uniformly consistent characteristic of low-grade bladder tumours, and hypothesize that high MDR1 expression plays a role in protection of the normal bladder from carcinogen exposure, and that individuals with low normal bladder MDR1 mRNA levels may consequently be at an increased risk of developing bladder cancer. Furthermore, the low level of MDR1 expression generally found in low grade superficial tumours may predispose them to additional carcinogen exposure and in this way contribute to possible tumour progression. In addition, MDR1 mRNA levels were observed to be elevated in a significant proportion (~25%, 8/30, \( P = 0.015 \)) of high grade tumours compared to low grade samples, and may therefore represent a marker of bladder tumour progression.

Cancer of the urinary bladder represents the fifth most common group of neoplasia in Western populations (1-3), with an annual incidence of 29 cases per 100 000 males per annum in the UK (4). The development of transitional cell carcinoma (TCC*) from the normal urinary epithelium has been most compellingly associated with cigarette smoking and exposure to industrial carcinogens, which together reportedly account for up to 50% of all bladder neoplasia (3,5). Bladder carcinogenesis results from exposure to specifically implicated carcinogens including the nitrosamines, aniline dyes, nitrodiphenyl and the aromatic amines (3,5,6), many of which have been detected in human urine (7,8).

About 75% of TCC present as well/moderately differentiated (G1/G2; low-grade) and non-muscle invasive (Ta/T1) tumours, and have an excellent 5-year survival rate of 80% following treatment by local resection (9,10). However the prognosis for patients (~25%) who present with muscle invasive (T2/T3/ T4), poorly differentiated (G3) tumours is poor, with typical 5-year survival rates of less than 50% following radiotherapy, chemotherapy or surgery. Only 10-15% of all invasive TCC have a previous history of superficial disease (9,10).

The MDR1 gene encodes P-glycoprotein (PGP) (11,12), a 170 kDa protein which functions as an ATP-dependent transmembrane transporter, first discovered almost 20 years ago by researchers investigating the phenomenon of multidrug resistance in drug-selected tumour cell lines (13). The presence of PGP has been demonstrated to reduce the intracellular concentration of a diversity of compounds in the cell, which although generally lipophilic in nature, share no common chemical structure or chemical target (14). Subsequent gene transfection studies have conclusively shown that transfer of the MDR1 gene into cells confers drug resistance via active drug efflux, and results in a distinctive pattern of cross-resistance to many ‘natural product’ drugs currently used in the chemotherapy of human tumours, including the vinca alkaloids, epipodophyllotoxins and anthracyclines (15). Recent clinical studies have begun to clarify and support a role for PGP in the clinical resistance of a variety of human tumour types to chemotherapy (16).

Aside from its role in cancer chemotherapy, little is known regarding the function of MDR1/PGP in normal tissues. Several large-scale studies have revealed a close accordance between MDR1 mRNA levels and PGP expression in normal tissues; the highest expression was detected in the adrenal cortex and kidney (proximal tubule epithelium), with readily detectable levels also found in the liver (luminal surfaces of the hepatocyte and biliary ductules), jejunum, colon and rectum (mucosal surface), pancreas (exocrine collecting ducts), lung (bronchus), brain and testis (capillary endothelium) (17-19). This distribution of MDR1/PGP expression has led to the proposal that PGP may be involved in the protection of epithelial tissues against hydrophobic natural product toxins (jejunum, colon, rectum, lung), and may furthermore play a role in the exclusion of toxins as part of the blood-brain and blood-testis barriers. The high levels found at luminal surfaces of excretory organs (liver, kidney) also suggest a normal excretory role in some tissues (20). However, few studies have directly investigated the role of PGP in normal epithelial tissues or considered substrates other than chemotherapeutic agents.

None of the previously mentioned large scale surveys of MDR1 mRNA expression in normal tissues (17,20) have included bladder samples. Similarly, with the exception of an earlier report from our group (21), no studies have systematically investigated MDR1 mRNA levels in the neoplastic bladder. We presently report the determination of MDR1 mRNA levels for a series of normal and previously untreated neoplastic bladder samples (all TCC). These results extend our previously reported cohort (21) of samples by a further 25 TCC samples (to 53 in total) and now include 11 normal, non-neoplastic bladder samples for comparison.

Tumour resection, assessment, handling and storage tech-

© Oxford University Press
niques have previously been described (21). Normal tissue samples were taken from patients undergoing cystectomy for non-neoplastic disorders, obtained as either bulk bladder wall or as pure urothelium (which was carefully stripped away from the underlying sub-mucosa and muscle), and handled identically to tumour samples. Using a sensitive quantitative gene transcript assay based on reverse transcription and the polymerase chain reaction (RT-PCR), which we have described elsewhere and extensively validated (21), MDR1 mRNA levels were determined relative to those of 18S ribosomal RNA as an endogenous internal reference standard. At least three replicate determinations were performed for each sample, and individual results are thus expressed as a mean MDR1 mRNA/18S rRNA ratio (± SE). Results are summarized in Figure 1.

High MDR1 mRNA (>1×10⁻⁵) levels were detected in approximately half of all normal samples (3/5 urothelium, 3/6 bulk), in contrast to the uniformly low levels (<1×10⁻⁵) found in all (23/23) low grade tumours. The distribution of MDR1 mRNA levels within the study group showed no sex or treatment bias (data not shown), with no differences apparent between the level and pattern of MDR1 expression in groups of stripped urothelium and bulk bladder wall samples. The mean MDR1 mRNA level detected in the normal bladder (2.36 ± 2.08×10⁻⁵, mean ± SD) was only 8.7 and 2.4 times lower than those levels we have detected in two adrenal samples (2.00 ± 0.46×10⁻⁴ and 5.68 ± 1.10×10⁻⁵, mean ± SE), which has been previously reported in several studies as the highest expressing tissue in the body (17,20,22). Indeed on an individual basis, 45% (5/11) of the normal bladder samples analysed had MDR1 mRNA levels that were greater than 67% of the lower adrenal value. These results therefore suggest that the MDR1 mRNA levels found in the normal bladder are comparable to other high expressing normal tissues in the body. The pooled mean and median levels of MDR1 mRNA expression in the normal bladder were significantly higher (−6-fold) than those in superficial or low/moderate grade groups of tumours (P ≤ 0.01; by Welch’s adjusted ‘t’ and Mann–Whitney tests).

The high MDR1 mRNA levels observed in the normal bladder are thus consistent with a putative role for PGP in the protection of the normal urothelium from toxins. The 60-fold variation in normal urothelial MDR1 mRNA levels observed between individuals suggest considerable differences in the level of protection provided. Two alternative hypotheses are therefore suggested to explain the striking differences between the high MDR1 mRNA levels observed in approximately half of the normal urothelium samples compared with the uniformly low MDR1 mRNA levels observed in the low-grade group of TCC.

The first hypothesis is that the development of low-grade TCC may preferentially occur in individuals whose normal urothelium express low MDR1 mRNA levels, and that these low levels of expression are maintained in the early stage tumours. This assumes that low MDR1 mRNA levels in the normal urothelium lead to the selection of a sub-population of individuals who are predisposed to the development of low and moderate grade TCC, as a result of enhanced intracellular carcinogen exposure. Alternatively, MDR1 gene expression may be universally down-regulated in the transformation of normal urothelial cells into low-grade TCC. However, such a hypothesis seems unlikely, since all of the early stage genetic events so far associated with low-grade bladder tumours (e.g. chromosome 9p and 9q deletion, 11q13 amplification, mutation of the c-H-ras oncogene) have only been observed in subsets of cases (23–25). Also, in vitro studies have shown that ras transfection leads to increased MDR1 expression (26) rather than a reduction. Based on such ideas, the observed down-regulation of the MDR1 gene in low-grade tumours would have to be associated with either a global transformation and de-differentiation switch that can be brought about by many genetic alterations, or a single as yet unidentified alteration.

Thus, the first hypothesis would seem more plausible, and is lent support by two recent reports (27,28) showing that some carcinogens are indeed substrates for the PGP efflux pump. These studies have demonstrated that MDR1/PGP overexpressing tumour cells are cross-resistant to the environmental/dietary carcinogens benzo[a]pyrene and 1,12-dimethylbenz[a]anthracene, which are also effluxed from these cells at a higher rate than from their parental cells which do not overexpress MDR1/PGP. Although these compounds are not directly implicated in bladder carcinogenesis, these observations nevertheless set an important initial precedent for a role for PGP expression in the protection of cells from carcinogens which is worthy of further investigation focusing specifically on bladder-related carcinogens.

The pattern of MDR1 mRNA expression in high-grade tumours is markedly different from that observed in low-grade tumours (see Figure 1). These data show that MDR1 mRNA levels are elevated in a significant proportion of high grade tumours (27%, 8/30, P = 0.015 by Fisher’s exact test) compared to low-grade tumours. The pooled mean MDR1 mRNA level for high grade tumours is consequently significantly higher (2-fold, P = 0.031, by Welch’s adjusted ‘t’-test) than for low grade tumours, with a greater level of inter-patient variation in MDR1 mRNA levels observed in the high-grade group of tumours (113-fold, 115% coefficient of variation versus 15-fold, 60% COV) than in the low-grade group. These results therefore confirm our previously reported and discussed association between elevated MDR1 mRNA levels and high-grade carcinoma (21) in an extended cohort of patients. On the basis of our hypotheses, and irrespective of their mechanism of origin, the uniformly low MDR1 mRNA levels observed in low-grade TCC suggest that these low-grade tumours are...
susceptible to further mutagenic events from continued carcinogen exposure. The probability of progression to high-grade carcinoma may therefore be higher as a consequence of the low MDR1 expression in superficial tumours.

In those cases where stage and grade progression of initially superficial tumours has occurred, an increase in MDR1 mRNA may be a consequence of the further genetic alterations which are associated with progression. It is known, for instance, that p53 mutations are late events in the progression of bladder cancer (23) and that some mutant forms of p53 directly upregulate the transcription of the MDR1 gene (26).

This report has added the urinary epithelium to the list of epithelial tissues which express high levels of MDR1 mRNA, and gives rise to several potential clinical implications. Firstly, low expression in the normal epithelium may identify a proportion (~50%) of the population who are predisposed to the development of TCC. On the basis of the TCC incidence in the general population (28.7 per 100 000 males per annum in the UK (4), corresponding to an approximate 1 in 50 lifetime risk), it is clear that not all of these individuals will develop TCC, and that other factors such as population differences in carcinogen exposure and metabolism/detoxification (e.g. the glutathione S-transferase M1 and N-acetyl transferase genotypes) (29–34) also contribute to the aetiology of bladder cancer. However, this is the first suggestion that MDR1 expression may play an important role.

One of the strategies aimed at improving the efficacy of PGP-substrate drugs is the reversal of multidrug resistance using agents which inhibit the function of PGP (e.g. verapamil, cyclosporin A) (35). Such agents are beginning to enter clinical trials, and there is evidence to suggest that such inhibitors may also inhibit PGP function in normal tissues (36). This may have consequences for MDR1-expressing epithelial tissues such as the bladder, since the inhibitors may potentiate an enhanced exposure of these tissues to carcinogens during the course of treatment. Such problems may be of particular future relevance to the use of PGP inhibitors in the treatment of paediatric malignancies, where PGP-substrate drugs including vincristine and etoposide are widely used over long therapeutic periods (up to 3 years), and where time may permit the development of further malignancies in later life. The question of whether patients treated with PGP inhibitors develop further malignancies in later life at a greater rate than patients treated without PGP-inhibitors is therefore a distinct and perhaps overlooked potential consequence associated with the development and clinical use of PGP inhibitors, and is worthy of further investigation.

Although in vitro studies do suggest that even small variations (<10-fold) in MDR1 expression are causal of increased substrate efflux (37), it remains to be directly established whether the 60-fold variations in MDR1 mRNA levels observed between different normal urothelia are significant in terms of protection from carcinogens. This could be approached by testing the effect of inhibitors of PGP inhibition (e.g. by verapamil) and/or down-regulation of the MDR1 gene (e.g. by ribozyme or antisense approaches) on the uptake of relevant carcinogens using short-term cultures of normal urothelial cells.

In addition, the relationship between MDR1 mRNA levels in the normal urothelium and bladder tumours could be examined by taking paired tumour and stripped epithelium samples from the same bladder. This would help to establish whether MDR1 mRNA in TCC samples (particularly low-grade TCC) are reflective of those found in the normal bladder on an individual basis. This would test whether low and moderate grade tumours do indeed arise exclusively from urothelia with low MDR1 mRNA levels, or whether their universal down-regulation in the transition to low-grade TCC is a more viable hypothesis.

Acknowledgement
This work is supported by the North of England Cancer Research Campaign.

References


