Mechanisms of recurrence of Ta/T1 bladder cancer

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ABSTRACT

INTRODUCTION Bladder cancer recurrence occurs via four mechanisms – incomplete resection, tumour cell re-implantation, growth of microscopic tumours, and new tumour formation. The first two mechanisms are influenced by clinicians before and immediately after resection; the remaining mechanisms have the potential to be influenced by chemopreventive agents. However, the relative importance and timing of these mechanisms is currently unknown. Our objective was to postulate the incidence and timing of these mechanisms by investigating the location of bladder cancer recurrences over time.

PATIENTS AND METHODS The topographical locations of tumours and their recurrences were analysed retrospectively for 169 patients newly-diagnosed with Ta/T1 bladder cancer, with median follow-up of 33.8 months. Tumours were assigned to one or more of six bladder sectors, and time to recurrence and location of recurrences were recorded.

RESULTS Median time to first tumour recurrence was 40 months. Median times between subsequent recurrences were 6.6, 7.9, 8.0 and 6.6 months for recurrences 1 to 2, 2 to 3, 3 to 4, and 4 to 5, respectively. The risk of first tumour recurrence in any given bladder sector increased by nearly 4-fold if the primary tumour was resected from that sector ($P < 0.001$); this association was not significant for subsequent recurrences. The proportion of tumour recurrences in multiple bladder sectors increased from 13% for the first recurrence to 100% for recurrence seven onwards.

CONCLUSIONS First tumour recurrence appears different to subsequent recurrences; incomplete resection and tumour cell re-implantation may dominate at this time-point. Only later does genuine new tumour formation appear to increase in importance. This has important implications for clinical trials, especially those involving chemopreventive agents.

KEYWORDS

Bladder cancer – Mechanisms – Recurrence

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Bladder cancer is the fifth most common malignancy in the UK with 10,200 new cases and 5000 deaths per year.¹ In Europe and North America, 80–90% of bladder cancers are transitional cell carcinomas of urothelial origin (urothelial cancer) with rates of recurrence and progression of 50–60% and 15–25% at 5 years, respectively (excluding solitary grade 1 pTa tumours).^{2–5} In developed countries, at any one time there are around one million patients with urothelial cancer who are at risk of recurrence (70,000–80,000 in the UK).

Bladder cancer has the highest cost per patient from diagnosis to death among all cancers in the US Medicare system.^{6,7} More effective approaches to treatment would, therefore, benefit patients in terms of quality of life, as well as bring about healthcare savings, and so a thorough understanding of the mechanisms of occurrence and recurrence of non-muscle-invasive (Ta/T1) bladder cancer are essential.

Urothelial cancer behaves as a multifocal disease, often with multiple primary tumours and frequent recurrences.

Two biological theories are proposed to explain this behaviour.⁸ The traditional theory of 'field cancerisation' suggests that the whole urothelium is exposed to the same urinary carcinogens, transforming topographically separate urothelial cells and resulting in tumours developing independently and metachronously in multiple sites (such tumours being genetically unrelated). The theory of 'clonality' is considered a more accurate model, suggesting that urothelial cancers arise as a result of a single carcinogenic insult to a single cell or group of cells.⁹⁻¹² The progeny of these cells spread throughout the urothelium, leading to multiple synchronous and metachronous tumours. These tumours are topographically distinct and genetically related. In reality, both processes probably occur simultaneously to produce the well-characterised genetic alterations associated with urothelial cancers.¹³⁻¹⁵

Four mechanisms of bladder urothelial cancer recurrence are described:¹⁶ (i) incomplete resection of the primary urothelial cancer; (ii) tumour cell re-implantation; (iii) growth of microscopic tumours present at the time of the previous resection; and (iv) new tumour formation. All four processes contribute to what is termed 'recurrence', but only new tumour formation can be considered to result from sporadic biological instability of the urothelium, a process potentially modifiable by chemopreventive agents. New tumour formation is included within the blanket term 'recurrence', but its incidence and timing following primary resection are currently unknown, especially as there is no way to distinguish these new tumours at the time of transurethral resection (TURBT). The objective of this study was to investigate the topographical location of recurrences over time in a group of bladder cancer patients in an attempt to elucidate the incidence and timing of each of these mechanisms.

Patients and Methods

All patients newly-diagnosed with Ta/T1 bladder cancer treated at The County Hospital, Hereford, UK between January 1999 and April 2004 were identified retrospectively from the unit's BAUS registry. Date of diagnosis, stage, grade and topographical location of primary urothelial cancers and all recurrences occurring until 1 September 2004 were extracted from patient notes. To record location, the bladder was divided into six mutually exclusive sectors that were considered appropriate subdivisions by the lead urologists (DMAW, GMS; Fig. 1). The unit's standardised bladder tumour charts were used to determine which of these sectors contained tumour, all notes being reviewed consecutively by one investigator who had not been present at the resections (MCD). Patients received a single dose of intravesical mitomycin-C within 24-h post-TURBT (unless contraindicated), with further intravesical treatment determined

Figure 1 Topographical definition of the six bladder sectors.

by clinicopathological factors. Elective re-resection of pT1 urothelial cancers was not routine unless the original specimen failed to include a sample of detrusor muscle; instead, patients with pT1 disease underwent their first check cystoscopy at 3 months in the operating theatre. Other patients were followed-up according to standard protocols. No patients died prior to the end of follow-up.

It was possible for a tumour to be present in more than one sector: two or more distinct tumours could be present in different sectors, or one tumour could straddle the division between two or more sectors. We did not attempt to distinguish these scenarios, nor did we attempt to distinguish the occurrence of multiple tumours in the same sector from the occurrence of single tumours.

Two types of analyses were undertaken – patient-based and bladder sector-based. In the patient-based analysis, time to first recurrence was measured from date of primary TURBT to date of resection of first recurrence. Time to subsequent recurrences was measured as the interval between resections for each recurrence.

In the sector-based analysis, each patient contributed six times to recurrence, one for each of the six bladder sectors. For each sector, time to first recurrence was measured from date of resection of primary urothelial cancer until date of resection of first recurrence within that sector. Time to recurrence was measured as the interval between resections for recurrences in that sector (recurrences in another sector were not counted as recurrences, *e.g.* the first recurrence in a sector may correspond to the third recurrence in that patient overall).

Estimates of cumulative recurrence-free survival were obtained using the Kaplan–Meier method. In patient-based analyses, Cox regression models were used to estimate hazard ratios; in bladder sector-based analyses, robust Cox regression models were used to estimate hazard ratios,

Table 1 Tumour stage at baseline and for each recurrence

	Stage				Total
	Ta	T1	T2+	N/A	
Primary UC	133	36	–	–	169
Recurrence 1	67	12	–	–	79
Recurrence 2	47	7	–	–	54
Recurrence 3	26	1	1	1	29
Recurrence 4	14	1	1	–	16
Recurrence 5	8	1	1	–	10
Recurrence 6	5	–	–	–	5
Recurrence 7	2	–	–	–	2
Recurrence 8	1	–	–	–	1
Recurrence 9	1	–	–	–	1
Recurrence 10	1	–	–	–	1

UC, urothelial cancer

stratified by sector and allowing for the fact that each subject contributed six times-to-event. Estimates of hazard ratios (HRs) and 95% confidence intervals were obtained from estimated coefficients and their standard errors. Tests of statistical significance were performed using the likelihood ratio test in patient-based analyses, and the robust score (log-rank) test in sector-based analyses. All tests of statistical significance were conducted at the 5% two-sided level.

Results

A total of 169 patients were included in the analysis. Of these, 79% of patients received intravesical mitomycin-C within 24 h of TURBT. Median follow-up was 33.8 months (range, 4.5–67.8 months). Seventy-nine patients (47%) experienced at least one recurrence. The grade and stage details for primary and recurrent tumours are shown in Tables 1 and 2. Median time to first recurrence was 40 months; median times for recurrences 1 to 2, 2 to 3, 3 to 4, and 4 to 5 were 6.6, 7.9, 8.0 and 6.6 months, respectively.

The 169 primary tumours arose in 190 bladder sectors: 156 patients had urothelial cancers in a single sector, and 13 (8%) had urothelial cancers in multiple sectors. The proportion of recurrences in multiple sectors increased with recurrence number, rising from 13% for recurrence one to 100% for recurrence seven onwards. The sector locations of primary tumours and recurrences are shown in Table 3. The risk of a first recurrence in a bladder sector was significantly increased by nearly 4-fold if the primary diagnosis was made in that sector, compared to if it was not (HR 3.77; 95% CI 2.76–5.15, chi squared 39.2; 1df; $P < 0.001$). The risk of the first recurrence also differed from the risks of all sub-

Table 2 Tumour grade at baseline and for each recurrence

	Grade			Total
	1	2	3	
Primary urothelial cancer	94	61	14	169
Recurrence 1	43	26	10	79
Recurrence 2	29	20	5	54
Recurrence 3	17	8	4	29
Recurrence 4	9	5	2	16
Recurrence 5	5	4	1	10
Recurrence 6	3	2	–	5
Recurrence 7	1	1	–	2
Recurrence 8	–	1	–	1
Recurrence 9	–	1	–	1
Recurrence 10	–	1	–	1

sequent recurrences (Figs 2 and 3). Controlling for sector instead of performing a stratified analysis, or not accounting for sector at all, made very little difference to the estimated risk: the increased risk associated with the location of the primary was seen in all sectors.

The risk of a second recurrence in a bladder sector was increased if the primary diagnosis was made in that sector, but the increase was small and no longer statistically significant (HR 1.28; 95% CI 0.81–2.01; chi squared 1.03; 1df; $P = 0.31$; Fig. 4). The risk of a third recurrence in a bladder sector did not differ according to whether or not the primary

Table 3 Location of diagnosis of primary tumour and all recurrences

	Bladder Sector					Cancers	
	1	2	3	4	5	6	(n)
Primary UC	55	28	28	53	13	13	169
Recurrence 1	24	8	11	30	10	9	79
Recurrence 2	15	5	13	18	8	10	54
Recurrence 3	3	4	11	7	6	8	29
Recurrence 4	3	8	7	4	5	3	16
Recurrence 5	0	5	4	4	2	4	10
Recurrence 6	2	3	2	1	1	2	5
Recurrence 7	1	2	2	1	1	1	2
Recurrence 8	0	1	1	0	0	1	1
Recurrence 9	0	1	1	0	1	1	1
Recurrence 10	0	1	0	0	0	1	1

UC, urothelial cancer

Note that a tumour can be diagnosed in more than one sector

Figure 2 Recurrence-free survival following diagnosis of primary, following recurrences one to four inclusive. In this figure, n is the number of patients in the analysis, and e the number of patients experiencing a recurrence. Small sample size prevents display of recurrences beyond number 5.

resection took place in that sector and was comparable to the risk for the second recurrence (HR 1.05; 95% CI 0.44–2.49; chi squared 0.01; 1df; $P = 0.92$).

Discussion

Evidence suggests that a high proportion of early urothelial cancer recurrences occur at the site of the original resection, resulting from a combination of incomplete resection of the primary tumour and tumour cell re-implanta-

tion.^{4,17–21} Early repeat resection or the use of new optical technologies (*e.g.* photodynamic diagnosis [PDD] or narrow band imaging [NBI]) to enable a more thorough initial TURBT are, therefore, advocated, but studies consistently demonstrate a residual tumour rate of 25–39% following conventional white light resection.^{4,19–21} Tumour cell re-implantation is reduced by instillation of intravesical chemotherapy immediately post-TURBT, with an improvement in recurrence-free survival after a single instillation of mitomycin-C of 13–18% (maximal at 24 months).^{22–25} In

Figure 3 Time to first recurrence in a sector for all bladder sectors combined, by whether the location of the primary was in that sector. n is the number of patients in the analysis, and e the number of patients experiencing a recurrence.

addition, up to 12% of patients with Ta/T1 urothelial cancers will have malignant changes when random mucosal biopsies are taken.^{26,27} These changes may represent microscopic tumours present at the time of resection of the primary or previous urothelial cancer, which subsequently develop into macroscopic ‘recurrences’. Once again, new technologies may enable better visualisation of such lesions.²⁸ This evidence implies that genuine new tumour formation will initially be outweighed by the other mechanisms of recurrence following the primary resection, and investigating the topographical location of recurrences over time may shed light on these phenomena. This was our approach: recurrences in the same location as the initial urothelial cancer probably result from residual disease (mechanisms i and ii); distant recurrences probably result from the growth of microscopic tumours present at the time of the previous resection and/or genuine new tumour formation (mechanisms iii and iv).

We have demonstrated that the behaviour of the first recurrence following primary TURBT appears to be very different to all subsequent recurrences: median time to first recurrence was 40 months, compared with 6.6–8.0 months for subsequent recurrences. In addition, the risk of a first recurrence in a bladder sector was increased nearly 4-fold if the primary resection took place in that sector. The risk of a second recurrence was also increased if the primary TURBT took place in that sector, but the increase was small and no longer statistically significant. The analyses suggested attenuation in risk between recurrences one to two and two to three, not just a result of decreasing sample size and the consequent reduction in statistical significance.

Our results imply that factors related to the initial resection

are responsible for the first recurrence, most likely incomplete resection and tumour cell re-implantation (the high proportion of patients treated with mitomycin-C immediately after TURBT will have reduced the impact of these mechanisms). Subsequently, the influence of the initial resection on the location of recurrences diminishes, suggesting that the early dominance of these mechanisms also diminishes and that other mechanisms then become more important. The proportion of diagnoses in multiple bladder sectors also increases with later recurrences, emphasising the growing importance of ‘sporadic’ urothelial transformation in multiple sites (*i.e.* genuine new tumour formation) as time progresses after initial resection.

This study was not intended to be definitive, but was exploratory in its aims in recognition of the limitations of the design. For example, we have assumed that recurrence in the same sector as the primary resection occurs at the same site of the primary resection, and this may not be the case in all patients. However, we believe that our findings contribute to the better understanding of Ta/T1 bladder cancer and hope that they will stimulate further discussion. Inevitably, a prospective study could provide more compelling data: molecular-genetic or proteomic analyses of resected urothelial cancers and subsequent recurrences may shed more light on the mechanisms of interest (*i.e.* do recurrences carry identical or different mutations or peptide profiles to the primary tumour?), although such studies could be difficult to interpret in an environment where clonality and field cancerisation are occurring simultaneously.^{11,12,14}

Nevertheless, factors associated with the primary TURBT (incomplete resection and tumour cell re-implantation) appear to dominate as the cause of early recurrence,

and genuine new tumour formation only seems to become important some time later. These findings have significant implications for the design of clinical trials, especially those involving chemopreventive agents, as the first recurrence seems to be very different from subsequent recurrences. Primary chemopreventive agents aim to 'protect' normal cells from the effects of initiators and promoters of malignancy, thus preventing cellular transformation and subsequent development of a macroscopic tumour. Following cancer diagnosis, chemopreventive agents aim to reduce the risk of new tumour formation and impair the progression of existing tumours. Clearly, these agents will be less effective against the mechanisms of recurrence that dominate early post-TURBT and which seem to be directly related to surgical 'failure'. Therefore, only after a more prolonged interval can the effects of chemopreventive agents on genuine new tumour formation be accurately assessed, and early recurrences should possibly be ignored for this purpose. Combining chemoprevention with 'best practice' for resecting the primary tumour (reviewed by Wilby *et al.*²⁹) may represent the optimal approach for reducing the high incidence of bladder cancer 'recurrence'.³⁰

Conclusions

Incomplete resection and tumour cell re-implantation seem to dominate as the cause of early bladder urothelial cancer recurrence, with genuine new tumour formation only becoming an important mechanism some time later. These findings have important implications for the design of clinical trials and for optimising treatment.

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