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Progression to detrusor-muscle invasion in bladder carcinoma is associated with polysomy of chromosomes 1 and 8 in recurrent pTa/pT1 tumours

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Abstract

Transitional cell carcinoma (TCC) provides a unique model of cancer recurrence and progression. Sequential tumours ($n=100$) from 57 patients with an index pTa or pT1 TCC were studied using fluorescence in situ hybridisation (FISH), to determine aberrations of chromosomes 1 and 8. Thirty-seven patients experienced recurrences; eleven developed muscle invasive tumours (pT2+). Polysomy of chromosomes 1 or 8 was associated with pT1 TCC ($P=0.0017$ and $P=0.0037$, respectively), but not with recurrence. Progression was associated with polysomy of chromosomes 1 ($P=0.003$) and 8 ($P=0.011$) in pTa/pT1 recurrences, but not with stage. In conclusion, patients who subsequently developed invasive TCC (pT2+) had significantly higher rates of aneusomy (90%) in their superficial cancers than those patients who did not progress ($P=0.009$). Investigation of sequential tumours in patients with recurrent and progressive TCC showed that polysomy of chromosomes 1 and 8 were linked to subsequent detrusor muscle invasion, but not recurrence *per se*. © 2002 Published by Elsevier Science Ltd.

Keywords: Bladder cancer; FISH; Chromosome 1; Chromosome 8; Recurrence; Progression; Polysomy

1. Introduction

Bladder cancer is the fourth commonest cancer in the Western world. In the West, 95% of bladder cancers are transitional cell carcinomas (TCCs) [1]. At presentation, approximately 80% of TCCs are non-invasive or invasion is limited to the lamina propria, while the remaining 20% have detrusor-muscle-invasion [2]. Fifty to seventy percent of non-detrusor-muscle-invasive TCCs will recur [2] and approximately 20% of these patients will progress to detrusor-muscle-invasion [3], associated with high mortality rates [2,4].

Aneusomy of several chromosomes has been associated with aggressive tumour behaviour. We have previously studied the chromosomes commonly aberrant in TCC by fluorescent *in situ* hybridisation (FISH) and found an association between recurrence and monosomy 9, polysomy 7 and 17 [5,6]. A pilot study of chromosome 8 in this laboratory also showed an association of polysomy 8 with progression [7]. Polysomies of chromosome 1 and genetic aberrations of chromosome 8 have previously been associated with high stage and grade in bladder cancer [8,9]. Polysomy 1 has been associated with recurrence of bladder tumours, but its relationship with progression has not been investigated [9]. Awata and colleagues [10] investigated chromosome 8 in bladder cancer using FISH, and argued that FISH may be an adjunct to methods such as cytology and biopsy for increasing the sensitivity of detection of bladder cancer, but they were unable to show a relationship to stage or grade. To extend these studies, a retrospective analysis of sequential recurrent and progressive TCCs was performed with FISH probes. We wished to test the hypothesis that alterations of chromosomes 1 and 8 are involved in the progression of superficial (pTa/pT1) bladder carcinomas.

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2. Patients and methods

2.1. Patients and tissue specimens

Fifty-seven TCC patients with full clinical histories (mean follow-up = 4 years and 5 months) were selected from an existing database for inclusion in the study. The database comprised patients who presented with pTa or pT1 TCC over the period from 1978 to 1993. Forty-nine patients presented with a single tumour, 8 with multiple tumours. Twelve patients were treated with either doxorubicin (Adriamycin) or Mitomycin C following their primary diagnosis. Twenty patients had no recurrence (NRs), 26 patients had recurrence without progression to detrusor-muscle-invasion (RNPs) and 11 patients had recurrence and progression to detrusor-muscle-invasion (RPs). The TCCs studied were the index (first) lesion for all patients, last documented recurrence (RNPs) and pre- and immediately post-muscle-invasive tumours (RPs). Five micron formalin-fixed paraffin processed tissue sections were cut onto silanised slides and baked at 56°C overnight. All TCCs analysed had a representative section stained with haematoxylin and eosin and were restaged and regraded as outlined below, by a specialist urological pathologist following the International Union of Cancer (UICC) 1997 guidelines [11].

The tumours were graded as G1, G2 or G3 depending on whether they were well, moderately or poorly differentiated. Tumour staging into pTa (non-invasive), pT1 (invasion of the lamina propria) or pT2+ (detrusor muscle invasion) was normally straightforward and the representative slide reviewed. If the tumour invaded to the depth of the biopsy, but detrusor muscle was not present in the slide examined, the true depth of invasion could not be assessed and it was staged at pTx. It would be unlikely for muscle invasion to be present in the slides not reviewed when a definitive staging of pTa or pT1 could be made on the representative slide examined.

2.2. FISH

Together with a control (disomic for both chromosomes 1 and 8) the TCC sections were pretreated on a VP2000 robotic slide processor (Vysis, UK Ltd.) with 0.2 N HCl for 20 min, 8% sodium thiosulphate at 80 °C for 30 min and digested in 0.5% pepsin at 37°C for 26 min. Tissue was assessed for adequate digestion before applying the probe, as previously reported [6]. A SpectrumGreen™ labelled probe for the pericentromere of chromosome 8 and a SpectrumOrange™ labelled pericentromeric probe for chromosome 1 (Vysis, UK) were applied as a double label, co-denatured at 72°C for 5 min and hybridised overnight at 37°C. Following post-hybridisation washes, tissue sections were mounted in 0.5 μg/ml DAPI in antifade (Vectashield, UK) and viewed with a Leica DMLB microsocope at ×400 magnification. A triple band pass filter block spanning the excitation and emission wavelengths of the SpectrumGreen™ and SpectrumOrange™ fluors (Vysis, UK) and DAPI was used in the analysis of the hybridisation. Image capture was achieved using a digital camera mounted on the microscope with the relevant computer software (Leica DC 200, Leica UK).

Evaluation of the results was performed as previously reported [6]; briefly the number of signals (range 0–8) for each chromosome in at least 200 non-overlapping nuclei in the control (normal urothelium) and carcinoma sections were counted at ×1000 magnification. The mean chromosomal copy number was calculated by totalling the number of hybridisation signals and dividing by the total number of nuclei as a measure of overall chromosomal copy number. Control sections from morphologically and genetically normal bladder tissue were included in the analysis. Values for disomy were derived from the analysis of these tissue sections.

2.3. Statistics

ANOVA was used to compare age at diagnosis, follow-up and number of cystoscopies. Chi squared tests were used to compare sex ratios and stage and grade at diagnosis; Chi squared and Fisher's exact tests to compare polysomy with stage and grade, recurrence and progression. Survival curves were plotted according to Kaplan-Meier and log rank tests were used to compare stage or polysomy with recurrence and progression. For survival analysis, patients were censored at the time of their last documented recurrence (RNPs) or pre-invasive recurrence (RPs).

3. Results

3.1. Patients

Thirty-four patients had index pTa carcinomas, 23 had pT1 carcinomas. No significant differences were observed between stage and grade of primary tumours and recurrence or progression (Table 1). Fig. 1 illustrates the percentage of pTa and pT1 tumours included for each patient group.

3.2. FISH

One hundred carcinomas were analysed. All hybridisations were successful apart from one tumour with the probe for chromosome 1 and one tumour with the probe for chromosome 8. The mean copy number per nucleus for normal bladder urothelium was 1.60±0.06 (±S.D.) and 1.59±0.10 (±S.D.) for chromosomes 1 and 8, respectively. The normal range for disomy was defined as the mean±3X S.D. (99% Confidence Inter-

Table 1
Patient data

Patient group	NR ($n=20$)	RNP ($n=26$)	RP ($n=11$)	P value
Age at diagnosis in years, median and range	69 (51–88)	70(45–95)	65(43–88)	0.50
Sex M:F	18:3	17:9	7:4	0.27
Follow-up in days, median and range	1948(1290–2275)	1723(1140–2107)	1410(3604–4646)	0.34
No.of cystoscopies median and range	8 (3–14)	10 (4–20)	8 (3–27)	0.26
Stage at diagnosis				0.81
pTa	12	17	6	
pT1	8	9	5	
Grade at diagnosis				0.02
1	5	14	2	
2	14	8	5	
3	4	4	4	

Table 1 compares clinical and pathological information between patient groups. NR = non-recurrer. These patients did not present with recurrence in the length of time they were followed-up; RNP = recurrer non-progressor. These patients recurred with TCC, but their recurrent tumours did not progress to detrusor muscle invasion. RP = recurrer progressor. These patients had recurrent tumours that progressed to detrusor-muscle-invasion. The range for the variables age, follow-up and cystoscopies are shown in parentheses. M:F, male:female.

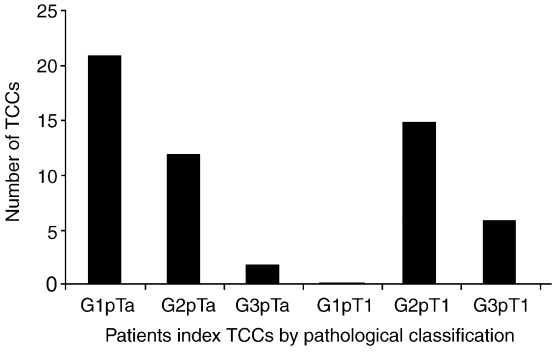

Fig. 1. Number of index TCCs categorised by stage and grade.

nal (CI)), giving a range of 1.42–1.78 mean signals for chromosome 1 and 1.40–1.67 mean signals for chromosome 8 representing disomy (Fig. 2a); <1.42 as monosomy 1 and <1.40 as monosomy 8; >1.78 as polysomy 1; and >1.67 as polysomy 8 (Fig. 2b).

Monosomy of either chromosome was not observed. Heterogeneity of chromosomal copy number was noted in 3 primary tumours, all from the non-recurrent (NR) category. One tumour displayed heterogeneity of chromosome 1 copy number, two displayed heterogeneity of chromosome 8 copy number.

Of *all* the pTa/pT1 tumours analysed, 14 of 57 (25%) pTa tumours were polysomic for chromosome 1 compared with 17 of 27 (63%) pT1 tumours, $P=0.0017$. 19 of 56 (34%) pTa tumours were polysomic for chromosome 8 compared with 19 of 27 (70%) pT1 tumours, $P=0.0037$. However, there was no significant difference between polysomy of either chromosome in pTa or pT1 *index* tumours and risk of progression by univariate analysis nor was there significant risk of progression between pTa and pT1 tumours in premuscle invasive or last documented recurrence events (Fig. 3).

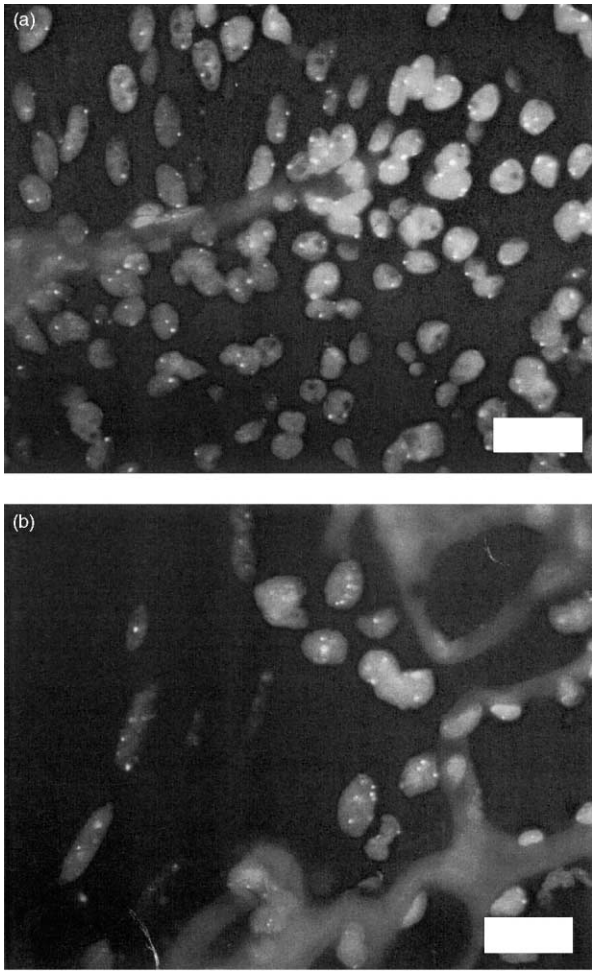

Fig. 2. FISH images depicting chromosome copy number for 1 and 8. Hybridisation signals in orange represent chromosome 1, those in green chromosome 8. Nuclei stained with DAPI fluoresce blue; magnification ×1000, size bars 20 μm. (a) TCC, pTaG1, showing disomic copy number for both chromosomes 1 and 8 as two signals in each nucleus are seen for each chromosome; (b) TCC, pT1G3 exhibiting polysomy for both chromosomes as the majority of nuclei contain 3–4 hybridisation signals for chromosomes 1 and 8.

3.3. Index (first) tumours

3.3.1. Chromosome 1

Twenty of 57 (35%) index TCCs were polysomic, of these 9/35 (26%) were stage pTa, 11/22 (50%) were stage pT1, the rest were disomic ($P = 0.11$, Non significant (NS) Fisher's exact test). Twenty-five per cent (5/20) of index TCCs from NR patients were polysomic for chromosome 1 compared with 31% (8/26) of index TCCs from RNP patients and 64% (7/11) from RP patients, $P = 0.08$, NS (Fig. 4a). There were no significant associations between stage, grade, time to recurrence or relative risk of recurrence and polysomy of chromosome 1 in index TCCs.

3.3.2. Chromosome 8

Twenty-eight of 57 (49%) index TCCs were polysomic, of these 14/35 (40%) were stage pTa, 14/22 (64%) were stage pT1, the remainder of the index TCCs were disomic ($P = 0.33$, NS Fisher's exact test). Fifty-five per cent (11/20) of primary TCCs from NR patients were polysomic for chromosome 8 compared with 38% (10/26) of primary TCCs from RNP patients and 64% (7/11) from RP patients, $P = 0.34$, NS (Fig. 4b). There were no significant associations between stage, grade, time to recurrence or risk of recurrence and polysomy chromosome 8 in index TCCs.

Forty-six per cent (26/57) of primary TCCs were disomic for both chromosomes 1 and 8, 2% (1/57) was polysomic for chromosome 1 *only*, 18% (10/57) were polysomic for chromosome 8 *only* and 32% (18/57) were polysomic for both chromosomes ($P < 0.0001$, Chi square).

3.4. Recurrent tumours

3.4.1. Chromosome 1

Thirty-one per cent (8/26) of last-documented non-detrusor-muscle-invasive TCCs from recurrent non-progressive patients (RNPs) were polysomic for chromosome 1. Four were pTa and 4 were pT1. Fifty-five per cent (6/11) of pre-detrusor-muscle-invasive TCCs in patients who progressed to detrusor-muscle-invasion (RPs) showed polysomy of chromosome 1. Three were pTa and 3 were pT1 ($P = 0.219$, Fisher's exact test, NS). Superficial recurrent TCCs (pTa/pT1) from patients with subsequent progression to detrusor-muscle-invasion (RPs) showed a significantly higher frequency of polysomy 1 than those from patients with documented recurrence, but no evidence of progression (RNP), $P = 0.001$, Fig. 4a.

Ninety-one per cent (10/11) of RPs were polysomic in the detrusor-muscle-invasive tumour. The relative risk of progression was significantly greater for patients with polysomy chromosome 1 in their last documented pTa/pT1

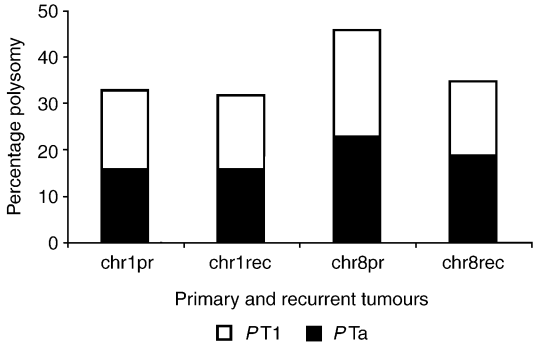

Fig. 3. Percentage of pTa and pT1 TCCs with polysomy of 1 or 8. This figure depicts the percentage of pTa and pT1 tumours in both primary and recurrent tumours, polysomic for chromosomes 1 or 8. Abbreviations: Chr1 pr = percentage of primary tumours polysomic for chromosome 1; Chr1 rec = percentage of recurrent tumours polysomic for chromosome 1; chr8 pr = percentage of primary tumours polysomic for chromosome 8; chr8 rec = percentage of recurrent tumours polysomic for chromosome 8.

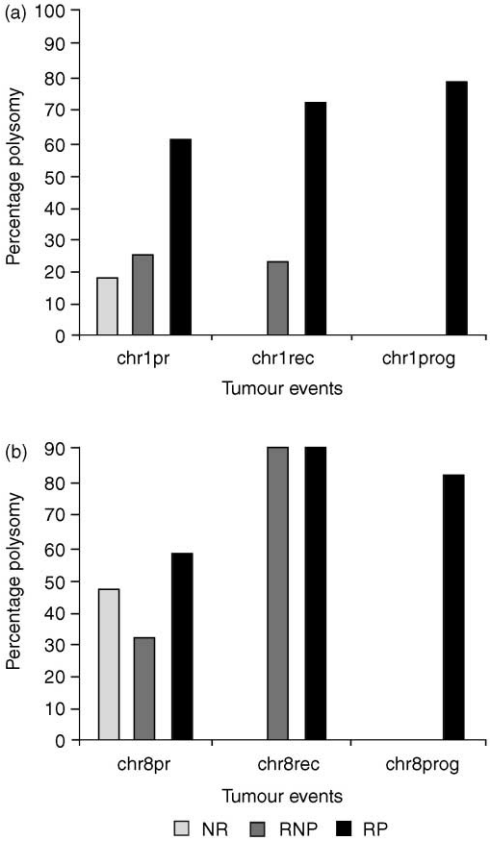

Fig. 4. (a) Percentage of TCCs polysomic for chromosome 1 categorised by disease course. The percentage of index, recurrent and progressive TCCs polysomic for chromosome 1. Patients are divided into 3 groups, NR = non-recurrer, RN*P* = recurrer non-progressor and RP = recurrer progressor. Chr1 pr = primary tumours polysomic for chromosome 1; chr1rec = recurrent tumours polysomic for chromosome 1; chr1prog = -progressed (pT2+) tumours polysomic for chromosome 1; (b) The percentage of index, recurrent and progressive TCCs polysomic for chromosome 8. Patients are divided into 3 groups, NR = non-recurrer, RNP = recurrer non-progressor and RP = recurrer progressor. Chr8 pr = primary tumours polysomic for chromosome 8; chr8rec = recurrent tumours polysomic for chromosome 8; chr8prog = progressed (pT2+) tumours polysomic for chromosome 8.

recurrence than for those patients with no abnormality of chromosome 1 in their tumours ($P = 0.003$), Fig. 5a.

3.4.2. Chromosome 8

Twenty-three per cent (6/26) of last documented non-detrusor-muscle-invasive TCCs from the RNP group were polysomic for chromosome 8. Three were pTa and 3 were pT1. Fifty-five per cent (6/11) of the pre-detrusor-muscle-invasive TCCs in the RP group were polysomic for chromosome 8. Three were pTa and 3 were pT1 ($P = 0.141$, Fisher's exact test, NS). Non-detrusor-muscle-invasive (pTa/ pT1) recurrent tumours from patients with subsequent TCC progression (RP patients) exhibited a significantly higher rate of polysomy 8 than those from patients with documented recurrence, but no subsequent invasion of detrusor muscle (RNPs), $P = 0.007$, Fig. 4b.

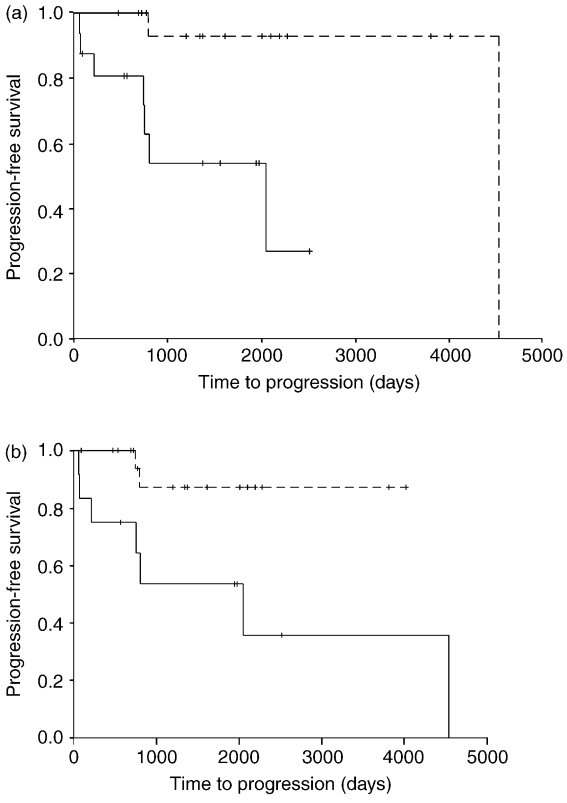

Fig. 5. (a) This survival curve illustrates the relationship between polysomy of chromosome 1 and relative risk of progression to detrusor-muscle-invasion, when polysomy chromosome 1 in the last documented recurrence (pTa/pT1) of patients without progression to detrusor muscle invasion was compared with polysomy chromosome 1 the pre-detrusor-muscle-invasive (pTa/pT1) tumour in patients with clinical and pathological progression, $P = 0.003$. The continuous line represents patients with polysomy 1, the dotted line patients with normal chromosome 1; (b) Polysomy of chromosome 8 and relative risk of progression is illustrated in this survival curve, when the rate of polysomy 8 was compared from patients' last documented non-detrusor-muscle-invasive tumour (pTa/pT1) who had no documented evidence of invasion and patients whose pre-muscle-invasive tumour (pTa/pT1) was followed by detrusor-muscle-invasion, $P = 0.011$. The continuous line represents patients with polysomy 8, the dotted line patients with normal chromosome 8.

Ninety percent (9/10) of patients had polysomy in their muscle-invasive TCC The relative risk of progression was significantly greater for patients with polysomy 8 in their superficial tumours than those patients with no abnormality of chromosome 8, ($P = 0.011$), Fig. 5b.

Overall, patients who subsequently developed pT2+ disease had significantly higher rates of aneusomy 1 or 8 (90%) in their superficial cancers than those patients who did not progress ($P = 0.009$).

3.4.3. Association with other chromosomal abnormalities

The patients included in this study are part of a large database of bladder cancer patients held in the Department of Surgery, Glasgow Royal Infirmary. FISH with probes for chromosomes 9 [5], 10 and 11 [12] and 7 and 17 [6] have been applied to a large number of the tumours reported here. When primary tumours with polysomy of either 1 or 8 or both was compared with previous data, in the NR group, none were abnormal for chromosomes 7, 9, 10, 11, 17. In the RNP group, 3/24 had monosomy 9 and 4/24 had polysomy of any other chromosome. In the RP group, 1/11 had monosomy 9 and 5/11 had polysomy for any other chromosome.

4. Discussion

TCC of the urinary bladder is a complex disease. Patients presenting with non-invasive or locally invasive carcinomas (pTa or pT1) are at significant risk of multiple recurrences (50–70%) and a markedly lesser risk of developing progressive detrusor-muscle-invasive carcinoma. However, pT1 disease carries a higher risk of subsequent progression to muscle invasion. This divergent outcome led to the hypothesis that recurrence and progression were caused by genetically distinct events, supported by recent investigations of sequential patient samples [5,6].

The aim of this study was to explore aberrations of chromosomes 1 and 8 in the context of recurrence and progression in TCC. This was achieved using sequential tumours from patients with repeated recurrences, some of whom progressed to detrusor-muscle-invasion following a primary pTa or pT1 TCC, and comparing the results with those from patients with no history of recurrence.

The data presented here demonstrate that index pTa/pT1 TCCs showed a significant rate of polysomy of chromosomes 1 and 8 (40 and 33%, respectively), but that the frequency of these changes in index carcinomas did not predict patient outcome in terms of either recurrence or progression. Interestingly, no increase in frequency of polysomy of chromosomes 1 and 8 was observed in recurrent tumours from patients who did *not* progress to detrusor muscle invasion (Fig. 4). However, patients with polysomy of either chromosomes 1 or 8 in their recurrent tumours were significantly more likely to experience clinical progression (Figs. 4 and 5).

When all pTa and pT1 tumours were examined separately, polysomy of either 1 or 8 was associated with pT1 disease, but pT1 disease was not associated with either recurrence or progression. That pT1 tumours are more frequently aneusomic than pTa tumours has been previously reported [9,13,14]. However, despite the increase in aneusomy observed in pT1 tumours compared with pTa tumours, the lack of association with stage and recurrence or progression, similar to previous studies carried out within this laboratory [5,6], suggests that it is aneusomy and not pathological classification which is driving progression in pTa or pT1 tumours.

Therefore distinct genetic differences have been identified between pTa or pT1 recurrent tumours from patients in whom progression did occur and patients whose disease remained confined to the superficial layers of the urinary bladder. As illustrated in Fig. 4, there is a trend of increasing aneusomy in patients who will eventually have muscle invasive tumours. This finding suggests that aberrations detected in recurrent, locally confined disease are potentially both predictive for and may be causally related to the subsequent development of progression. This would support the finding by Tsao and colleagues that the majority of genetic alterations in TCC occur prior to progression [15]. They showed that there was an increase of only 10% of genetic aberrations in metastases compared with primary cell lines from two patients reported in their study, and they suggest that most allelic losses occur before progression. Whether this observation relates to all progressive events in TCC (invasion/ metastases) remains to be explored, but TCC is an ideal model to study due to its unique pattern of disease recurrence and progression.

Mutations in cancer are thought to accumulate in a multistep process [16]. The involvement of chromosome 1 in bladder cancer progression has previously been reported [17]; the commonest aberrations appear to be 1q gains which have been associated with pT1 disease [13]. 1q Harbours oncogenes in the region 1q21–24 that include *SKI* and *NTRK1* [13]. The oncogene *SKI* binds DNA and activates transcription [18]. *TRK* is a rearrangement of the *NTRK1* gene that encodes one of the receptors for the nerve growth factors [19]. Both oncogenes are at a region of genetic instability frequently involved in translocations [19,20].

Chromosome 8 aberrations in pTa/pT1 TCC have previously been reported as confined to sub-chromosomal alterations, such as isochromosome 8q and amplification of 8p12 [8]. Candidate oncogenes at 8p12, a region consistently amplified in human breast cancers [21] are the heregulin gene coding for a ligand for the *erbB2* oncogene and *FGFR1* [8]. In a recent study [22] examining amplifications and deletions of *FGFR1* in TCC, genetic aberrations were more frequently observed in pT1–4 and pTaG3 compared with pTaG1 or G2 carcinomas. However, *FGFR1* amplification was not associated with prognosis in pTa or pT1 tumours, and was not thought to be the specific genetic target in TCC [21]. Overexpression of the oncogene *myc* located at 8q24 has been observed in bladder cancer [23]. Gains at 8q24 have been associated with higher stage bladder carcinomas [8]. The protein product of the *myc* oncogene when overexpressed, has been hypothesised to cause degradation of $p27^{kip1}$ leading to activation of cyclinE/ cyclin dependent kinase 2 and cell proliferation [23].

In our patient cohort, all but one of the recurrer progressor patients had polysomy of chromosome 8 in their detrusor-muscle-invasive tumours. In a previous study that included patients with a diagnosis of pT2 and above TCC at first presentation all had polysomy 8 [7]. The evidence appears to be growing that polysomy of chromosome 8 is a late event in bladder cancer, as appears to be the case in prostate cancer [24].

Our observations that polysomy 1 in primary tumours is not predictive of recurrence differ from that of Neuhaus and colleagues [9] who found a significant association with polysomy 1 and recurrence. However, methodological differences may have contributed to this disparity. Neuhaus and colleagues [9] studied genetic aberrations by FISH on dissociated nuclei from 50 μm tissue sections whereas in this study nuclei in 5 μm tissue sections were assessed *in situ*.

The data presented in this study on chromosomes 1 and 8 were compared with previous studies of aneusomy in bladder cancer using the same patient database [5,6] to determine if the rates of polysomy reported here were true non-random aneusomic events or in fact polyploidy. There was no association between the specific chromosomal aberrations reported. This further substantiates the report by Bartlett and colleagues, [12] that showed polysomy in bladder cancer was an independent event rarely associated with polyploidy.

The identification of distinct genetic events, in the context of pTa or pT1 TCC, related to subsequent clinical progression provides both a potential therapeutic and diagnostic benefit to future patients. Ultimately the opportunity exists, following further studies, to identify the genes responsible for progression on these target chromosomes, to exploit this knowledge by developing novel therapeutic targets with the potential to inhibit tumour progression.

In conclusion, abnormalities of chromosomes 1 and 8 are increased in recurrent pTa/pT1 carcinomas from patients whose disease progresses, but not from patients whose disease remains locally confined. These two groups of patients therefore appear to represent distinct genetic pathways in TCC of the urinary bladder.

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