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### Nuclear transcription factors in bladder cancer: *c-myc* is associated with apoptosis

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**Introduction:** In Ta and T1 bladder carcinoma (TCC), 60–70% of patients develop recurrence and 10–20% develop progression; high *p53* and epidermal growth factor receptor (EGFr) levels are associated with progression. High mitotic rates are associated with high grade, but apoptosis has not been well studied. Mechanisms underlying apoptosis, *p53* and EGFr immuno-reactivity are unclear although *in vitro* studies suggest that c-*myc* can induce *p53*-mediated apoptosis. The aims were to assess i) expression of c-*myc*, c-*fos* and c-*jun*; ii) associations with apoptotic/mitotic ratios (AMR), *p53*, EGFr and Ki67 staining; iii) potential clinical value.
**Materials and methods:** Tumours from 89 patients with newly-diagnosed TCC (33 Ta, 34 T1, 22 muscle-invasive) were collected. Frozen sections (c-*myc*, c-*fos*, c-*jun*, EGFr) and paraffin sections (*p53* [DO-7 antibody], Ki67) were assessed by immuno-histochemistry. Apoptosis and mitosis per 1000 cells were measured.
**Results:** c-*myc* and c-*fos* were more commonly found in superficial and low-grade TCC, but c-*jun* was associated with high-grade TCC. Positive associations were detected in superficial TCC between i) c-*myc* and high AMR ($P < 0{\cdot}01$) ii) c-*jun* and EGFr staining ($P < 0{\cdot}001$). At short-term follow-up (median of 18 months; CI = 15 to 26), c-*myc*, c-*fos* and c-*jun* did not predict tumour recurrence; two patients progressed to muscle invasion (both were c-*myc*-negative; AMR < 1).
**Conclusion:** These findings are consistent with previous *in vitro* studies showing that c-*myc* can induce apoptosis; c-*myc* expression and high AMR may have prognostic value in terms of tumour progression.

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### Is recurrent TCC derived from the same clone of cells?

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**Introduction:** It has always been debated whether recurrent TCCs of the bladder are derived from the original tumour cell or a new tumour cell line. To test these hypotheses *p53* mutations within TCC were studied.
**Patients and methods:** Five patients with *p53* mutations in their primary tumours were identified. Seventeen recurrent tumours from these patients, occurring over 6·5 years, were then analysed for the presence of identical mutations.
**Results:** Sixteen of the 17 recurrences were found to be monoclonal with respect to the *p53* mutations observed in the primary tumour.
**Conclusions:** These results indicate that recurrences of G2/G3 TCC are clonally derived from an initial population of neoplastic cells. The results also show that tumour cell clones can survive for long periods within the bladder. It also suggests that further research is needed to define the most advantageous time to give adjuvant therapies.

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### Does the accumulation of genetic mutations in bladder cancer precede pathological progression?

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**Introduction:** Tumour progression in TCC of the bladder is believed to correlate with the multistep accumulation of genetic change. Combinations of mutations may define clinical subsets of tumours and provide a framework alongside histopathology with which to identify patients at risk of invasive disease. Histochemical and genetic events have been investigated in cohorts of patients with non-recurrent, recurrent non-progressive and recurrent progressive bladder cancer.
**Materials and methods:** Archival samples from more than 100 patients were analysed for genetic changes at specific loci on chromosomes 17 (c-*erb*B2 and *p53*) and nine microsatellite markers using molecular genetic methods and immunohistochemistry.
**Results:** Multivariate regression analysis of the chromosome 17 risk factors showed that alterations are not useful as independent molecular markers of bladder cancer. Two of 10 chromosome 9 markers (D9S15 and the major tumour-suppressor gene *CDKN2*) showed high frequencies of genetic change in tumours from each category of patient, with an accumulation of areas of change within individual tumours as the disease progressed. Genetic changes at some other markers were more frequent in recurrent and progressive tumours. Genetic microheterogeneity occurred within tumours and between tumours removed on separate occasions from the same patient. Pathologically normal areas rarely showed genetic change.
**Conclusion:** Chromosome 9 microsatellite markers have potential as clinically useful diagnostic and prognostic markers of bladder cancer. Genetic microheterogeneity within and between tumours has implications for the aetiology of bladder cancer and the theory of tumour clonality. Genetic events can precede observable pathological change. This study suggests that genetic profiles of individual tumours will contribute valuable diagnostic and prognostic information when used as an adjunct to routine histopathology.

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### The roles of interstitial collagenase and tissue inhibitor of metalloproteinases in bladder tumours

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**Introduction:** Invasion by malignant tumours requires proteolysis of extracellular matrix (ECM) barriers. There is increasing evidence of a correlation between matrix metalloproteinase (MMP) activity and the invasive behaviour of tumours. Interstitial collagenase (MMP-1) cleaves collagen helices of the ECM. Tissue inhibitor of metalloproteinase-1 (TIMP-1) is an inhibitor of the MMPs. MMP-1 and TIMP-1 levels are increased in several human tumours. The role played by the MMPs in tumour invasion and metastasis is probably the result of an interaction between metalloproteinases and their inhibitors. We studied the roles and interactions of MMP-1 and TIMP-1 in tumours of the bladder.
**Materials and methods:** Bladder tumours and normal urothelium were collected prospectively from 114 patients and immediately snap frozen. Tissue extracts were prepared and assayed for the concentrations of total MMP-1, total TIMP-1 and MMP-1/TIMP-1 complex (a measure of activated MMP-1) using ELISA assays.
**Results:** There were significant increases in MMP-1, TIMP-1 and MMP-1/TIMP-1 complex concentrations in bladder tumours when compared with levels in normal urothelium. MMP-1 levels increased only slightly with increasing tumour grade but no differences were detected between MMP-1 levels in superficial and invasive tumours. TIMP-1 levels show significant increases with increasing tumour grade and also increasing tumour stage. MMP-1/TIMP-1 complex concentrations showed increases with tumour grade but not stage.
**Conclusion:** In common with other human tumours, it appears that bladder tumours have increased concentrations of MMP-1, TIMP-1 and MMP-1/TIMP-1 complex when compared to levels in normal urothelium. The increases most closely related to tumour grade are those of TIMP-1. These findings point to a role for both interstitial collagenase and its inhibitor in bladder tumour invasion and suggest that TIMP-1 levels may assist in determining those tumours of more aggressive potential.

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### Determination and reversal of resistance to intravesical epirubicin

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**Introduction:** The use of intravesical epirubicin in the treatment of superficial bladder cancer is well established. Resistance to this agent implies the presence of multidrug resistance (MDR). Decreased cellular levels of the drug characterize MDR. We have used sensitive and resistant MGH-U1 bladder tumour cells to investigate the potential for improving therapy by manipulating extracellular pH and incorporating MDR-reversing agents into an *in vitro* model.
**Materials and methods:** Sensitive and resistant tumour cells were grown as monolayers. Procedures were performed *in-situ* for confocal microscopy and cells were monodispersed in suspension for flow cytometry. Cells were exposed to epirubicin (10 μg/mL) for 2 h.
**Results:** Resistant cells took up eight times less drug than did sensitive cells (assessed by flow cytometry; $P < 0{\cdot}001$). Confocal microscopy showed drug localization to be punctuate and cytoplasmic in resistant cells and nuclear in sensitive cells. Drug accumulation in resistant cells can be increased to sensitive levels using 100 μg/mL of the MDR-reversing agent, verapamil ($P < 0{\cdot}001$) or 5 μg/mL of the non-immunosuppressive Cyclosporin A analogue, PSC 833 ($P < 0{\cdot}001$). Increasing extracellular alkalinity from pH 6·0 to 7·6 also increased epirubicin uptake in sensitive cells and resistant cells treated with resistance-reversing agents ($P < 0{\cdot}001$). Untreated resistant cells showed no such effect.
**Conclusions:** These results suggest that alkalinization of the epirubicin instillate and/or addition of resistance-reversing agents may be effective in overcoming MDR. Furthermore, application of these techniques to primary bladder tumour cultures may enable the differentiation of resistance from sensitive cells and the evaluation of different MDR-reversing agents in individual patients.

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### A prospective, randomized, multi-centre, phase III clinical trial of the effect of different initial therapy regimens and maintenance prophylaxis in superficial bladder cancer, using Mitomycin C

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**Introduction:** In the use of intravesical chemo-prophylaxis in superficial bladder cancer, doubts still exist as to the most appropriate initial therapy and the length of maintenance necessary to achieve the best results.
**Patients and methods:** In this study, 1287 patients with multiple or recurrent superficial bladder carcinoma, after resection of their tumours, were randomized into four different treatment groups. Group A received eight weekly instillations with 40 mg Mitomycin C and 50 mL 0·9% saline, followed by 4 months of monthly prophylaxis with the same dose. Group B received four weekly instillations as initial therapy followed by 5 months of monthly prophylaxis. Group C received the same therapy as Group A but the prophylaxis was continued to 12 months, and Group D the same initial regimen as Group B but again with maintenance continued to 12 months. The individual groups of patients were evenly matched for stage and grade of their tumours and there was no significant sex difference among the four groups. All patients were treated for a minimum of 18 months.
**Results:** The side-effects were similar in Groups A and B, and Groups C and D, but almost twice as many patients in Group C and D missed one or more instillations due to side-effects, compared with the two groups with the shorter maintenance regimen.
**Conclusions:** With regard to efficacy, patients with recurrent, multiple Ta and T1 tumours appear to enjoy longer recurrence-free periods when treated with an intensive start regimen and long-term maintenance.

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### Radical transurethral resection and chemotherapy in the treatment of muscle invasive bladder cancer – long-term follow-up

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**Introduction:** Radical transurethral resection (TUR) and chemotherapy has been reported as an alternative to cystectomy in patients with muscle-invasive bladder cancer, with the benefit of bladder conservation. We report the long-term follow-up (median 3·98 years) for a group of 50 patients with muscle-invasive bladder cancer treated by radical local TUR and systemic chemotherapy.
**Methods:** All patients had TCC with no evidence of nodal or metastatic disease (nine with T2, 36 with T3, and five with T4a); 70% of tumours were < 4 cm in diameter. The technique involved resecting all tumours 'completely', through muscle out to perivesical fat. All patients subsequently received two or more cycles of cisplatin (70 mg/m$^2$) and methotrexate (40-mg/m$^2$).
**Results:** Using Kaplan–Meier survival analysis, disease-specific survival at 5 years was 68% and the overall 5-year survival was 52%; 16 (32%) patients progressed to metastatic disease at a median time of 22·6 months. Thirteen (26%) patients had superficial local recurrence at a median interval of 19·1 months; 14 (28%) patients developed invasive locally recurrent disease at a median interval of 14·1 months and these patients were treated with either radical cystectomy ($n = 3$) or radical radiotherapy ($n = 11$).
**Conclusions:** These results compare favourably with survival rates achieved with conventional treatments such as immediate radical cystectomy or radical radiotherapy. The combination of radical TUR and combination chemotherapy in selected patients resulted in bladder conservation, with 28% of patients requiring salvage therapy. A randomized study is required, comparing this method of treatment with conventional treatments for muscle-invasive bladder cancer.

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### A meticulous lymphadenectomy may improve survival in invasive bladder cancer – the influence of location of nodal metastasis

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**Introduction:** Lymph node metastases in invasive bladder cancer generally imply a poor prognosis. However, Skinner suggested in 1982 that a meticulous lymphadenectomy improved survival, but did not discuss the influence of the site of pelvic node metastasis on survival.
**Patients and methods:** Since 1984, 320 patients, radiologically and scintigraphically M0 and having radical cystectomy for invasive bladder cancer underwent a meticulous lymphadenectomy and the internal iliac, external iliac and obturator nodes were sent for separate pathological examination. We report on 44 consecutive pN+ patients.
**Results:** The median age was 65 years (range 38–88), with tumour stages pT2 (6), pT3 (29), pT4 (9). Platinum-based chemotherapy was given to one patient pre-operatively and 16 post-operatively. The median total number of nodes resected per patient was 14 (range 8–32) and 50% of patients had a single nodal metastasis (range 1–8). The median overall survival was 15 months, the overall 3-year survival was 30% and the 5-year survival was 10%. The 11 survivors have a median follow-up of 39 months; eight survivors had a single involved node (five internal iliac, three external iliac) and three survivors had multiple nodes. Median survival has not been reached for patients with a single involved internal iliac node. Chemotherapy did not affect outcome significantly.
**Conclusion:** These data support Skinner's results, suggesting that after meticulous lymphadenectomy, patients with disease restricted to a single involved internal iliac node have a chance, albeit small, of long-term survival. It warrants a meticulous lymph node dissection, particularly in patients with apparently uninvolved nodes.