Tuesday 24 June 10.30–11.30
Poster Session 2: Basic Science: Oncology
Chairmen: L. Griffiths and J. Schalken

P010

A high frequency of microsatellite instability is seen at tetranucleotide repeats in TCC of the bladder

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INTRODUCTION

Defects in DNA mismatch repair (MMR) lead to microsatellite instability (MSI) and cancer. MSI is best seen at mono- and dinucleotide repeats, and occurs infrequently in TCC (< 5%). A new form of MSI, primarily affecting tetranucleotide repeat microsatellites (termed 'EMAST') has been described. Its relationship with MMR and MSI at other loci has yet to be defined. We investigated the frequency of EMAST and compared it with our previously published findings of MMR protein expression and MSI at mono- and dinucleotides, in a cohort of TCCs.

MATERIALS AND METHODS

Materials from a cohort of 89 patients with TCCs of various grades and stages were analysed at nine tetranucleotide microsatellite loci. In each case, normal and malignant DNA was amplified using PCR with fluorescence-labelled primers. Instability was judged to be present when new or altered bands appeared in the tumour DNA.

RESULTS

EMAST was detected at one or more loci in 33 (38%) TCCs, of which seven (22%) had EMAST at several loci. We previously reported low rates of MSI at mono- and dinucleotides in these TCC (1/89, 1%). There were no statistically significant relationships between EMAST instability and tumour clinicopathological details, the MMR protein expression or MSI at mono- and dinucleotides.

CONCLUSION

EMAST is a frequent event in TCC carcinogenesis and is unrelated to the expression of MMR proteins or MSI at mono- and dinucleotides. It is unlikely to be caused by loss of MMR function and represents a novel form of genomic instability.

Funding: MRC Clinical research fellowship

P011

The expression of osteoprotegerin in TCC of the bladder reveals an association with p53 accumulation

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INTRODUCTION

Recent studies have shown that osteoprotegerin, an osteoclast regulator, acts as a decoy receptor for TRAIL, enabling tumours to evade apoptosis. Osteoprotegerin expression has been studied in prostate and breast cancers, but has not been evaluated in other tumours. p53 is mutant in most bladder tumours and is important in the induction of apoptosis. We investigated the expression of osteoprotegerin and p53 in a sample of TCCs of the bladder.

MATERIALS AND METHODS

Tumours from 106 patients with TCC of various stages and grades were assessed (median follow-up 5 years). Standard immunohistochemical methods were used to investigate the expression of osteoprotegerin and p53. Two investigators independently scored each section twice, before establishing the final result. Tumours were classified as having normal (1) or increased (2 or 3) levels of osteoprotegerin expression, according to the frequency of positively staining cells.

RESULTS

There was increased osteoprotegerin expression in the tumours of 57 (54%) patients. There was a statistical trend towards

increased osteoprotegerin expression with advanced tumour stage ($P = 0.08$) and grade ($P = 0.09$). Osteoprotegerin expression was unrelated to subsequent tumour relapse ($P = 0.9$), cigarette smoking behaviour or the patients' characteristics. There was a strong association between increased osteoprotegerin and increased p53 expression ($P = 0.006$).

CONCLUSIONS

Increased expression of osteoprotegerin is common in TCC, and may be associated with tumours of advanced stage and poor differentiation. Increased osteoprotegerin expression is associated with increased p53 expression, suggesting a synergistic role in the avoidance of apoptosis.

Funding: MRC Clinical Research Fellowship

P012

Proteomics-based approaches to identifying bladder tumour markers

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OBJECTIVE

To identify proteins expressed in the tumour tissue and urine of specific subsets of patients with TCC and in controls that may have potential to become objective biomarkers for disease prognosis, diagnosis and follow-up. The role of these proteins may increase understanding of TCC pathogenesis and provide novel therapeutic targets.

MATERIALS AND METHODS

Surfaced-enhanced laser desorption/ionisation (SELDI) combines the analytical power of mass spectroscopy with selective affinity capture of different protein-chip surfaces derived from classical chromatological separation moieties. This novel proteomic technique allows rapid characterisation of urinary protein profiles that are associated with disease states within the urinary tract. We developed a strategy using SELDI mass spectrometry, novel peak-detection algorithms and predictive machine-learning models to identify TCC urinary biomarkers from 103 patients with TCC (94 Ta/T1) and 110 controls. The predictive power of this model is presently 80–90%. The biological events that initiate the progression of superficial (Ta) TCC are unclear. We analysed overall protein expression in fresh-frozen biopsies of six high (G3) and six low (G1–2) Ta bladder tumours using two-dimensional gel electrophoresis and mass spectrometry. Seven differentially expressed proteins were selected for further analysis. The expression of these proteins is being validated by immunohistochemistry, western blotting and/or Syber-green real-time PCR in a panel of TCC tumours. Further work includes a functional analysis of differentially expressed tumour-proteins and improvement and validation of the SELDI TCC diagnostic test.

Funding: British Urological Foundation Scholarship 2002/3, British Urological Foundation & Royal College of Surgeons of England.

P013

A targeted radiotherapy/gene therapy strategy for bladder cancer. Transfection of the noradrenaline transporter gene under the control of telomerase promoters: *in vitro* and *in vivo* studies

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INTRODUCTION

Targeted radiotherapy is the selective irradiation of tumours by radionuclides conjugated to tumour-seeking molecules. Meta-iodobenzylguanidine (MIBG) is actively taken up by cells expressing the noradrenaline transporter (NAT). Introduction of the NAT transgene into bladder cancer cells under the control of tumour-specific telomerase promoters should allow them to take up radioiodine-labeled MIBG, with resulting death of the transfected cells and their neighbours (the bystander effect).

MATERIALS AND METHODS

The NAT gene was cloned into a plasmid vector and transfected into a TCC bladder line (EJ 138). Uptake of ^{131}I-MIBG by the transfected cell lines was assessed by γ-counting and cell death by clonogenic assays. Subcutaneous xenografts in nude mice were prepared from untransfected cell lines and cell lines previously transfected *in vitro*. The uptake of ^{131}I-MIBG in tumour and normal organs was measured by γ-counting the excised tissue.

RESULTS

In vitro, the NAT gene transfected cells had a significantly higher uptake of ^{131}I-MIBG, with cell death data which correlated with this. *In vivo*, the biodistribution studies after injection with ^{131}I-MIBG showed preferential uptake of ^{131}I-MIBG in organs rich in noradrenergic innervation, and markedly increased uptake in tumours expressing NAT.

CONCLUSIONS

The expression of a functional NAT after *in vitro* transfection of TCC cells with the NAT gene under the control of a telomerase promoter leads to active uptake of ^{131}I-MIBG and dose-dependent cell death. The *in vivo* tumour xenograft model confirmed the selective biodistribution of ^{131}I-MIBG uptake. A targeted gene- and radiotherapy approach with radioactive MIBG may produce a promising new treatment for bladder cancer.

Funding: British Urological Foundation/ Sanofi-Synthelabo Scholarship (2001/2002)

P014

Urine dendritic cells: a prognostic indicator in BCG-treated patients with bladder cancer?

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INTRODUCTION

Dendritic antigen-presenting cells (DCs) can either initiate or block immune responses and are increasingly used as immunotherapy in patients with cancer. Indeed, DC recruitment and activation is likely to be important in the therapeutic effect of intravesical BCG. We hypothesized that the treatment effect of BCG could be monitored in patients by identifying urinary DCs.

PATIENTS AND METHODS

Voided urine from 12 patients receiving intravesical BCG was collected before therapy and before their sixth and final installation of BCG. Using four-colour flow cytometry, bone marrow-derived cells were discriminated by their expression of CD45, and DCs were identified within this population as lacking the markers of other cell lineages and expressing HLA-DR.

RESULT

DCs were present in all patients, comprising a mean (SD, range) of 2 (1.6, 0.03–11.7)% of the CD45-positive cells. Patients were followed for a mean of 10 months; five of the six patients who later presented with recurrent bladder cancer had a reduction in the percentage of DCs in their urine, the mean decreasing from 3.1% to 1.4%. Patients with no recurrence had increased urinary DCs, from 0.9 to 3.7%.

CONCLUSION

Preliminary studies monitoring urinary DCs in patients with bladder cancer showed that changes in the proportion of these cells could be measured during BCG immunotherapy. Prospective randomized studies are now required to confirm whether decreases in DC percentages in urine during BCG therapy are significantly associated with the early recurrence of bladder cancer.

Funding: BUF/Royal College of Surgeons Fellowship & Ralph Shackman Trust

P015

Can the urinary cytokines interleukin-12, interleukin-18, TNF-α, and interferon-γ predict the response of superficial bladder cancer and carcinoma *in situ* to intravesical BCG?

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INTRODUCTION

BCG is thought to induce a T helper type 1 cell response (TH-1) in the bladder of patients. We aim to show that: (i) the cytokines interleukin-12 and -18, TNF-α and interferon-γ are detectable in the urine of patients receiving BCG at >24 h after each instillation (not previously reported); and (ii) to evaluate these cytokines as potential 'prognostic markers'.

PATIENTS AND METHODS

Twenty patients were treated with BCG (nine carcinoma *in situ*, CIS, and 11 high-grade, G3 superficial bladder TCC). Their urinary levels of the four cytokines were determined before and at specific times after each of the six BCG instillations, using commercially available ELISA kits. These data were standardised to urinary creatinine.

RESULTS

Measurable cytokine levels were detected >24 h after instillation, strengthening the

argument that BCG induces a TH-1 response. There was evidence to suggest a relationship between interferon-γ and the response at 6 months (odds ratio 0.17, 95% CI 0.01–0.65). In patients with higher levels of interferon-γ the treatment was less likely to fail at 6 months. There was no evidence to suggest such a relationship for the other cytokines.

CONCLUSIONS

We report for the first time that these urinary cytokines are detectable 24 h after BCG therapy. This study identified the potential value of using interferon-γ as a 'prognostic marker' to predict freedom from disease. These findings may help in the treatment of patients with superficial TCC and CIS by identifying those with a high risk of disease recurrence after BCG therapy.

P016

MAP kinase kinase-5 over-expression increases the rate of tumour formation and produces ERK5 and MMP-9 over-expression *in vivo*

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INTRODUCTION

MAP kinase kinase-5 (MEK5) is an important cell-signalling protein with a pivotal role in mitogenesis [Nature 1998; 395: 713–6]. It activates the MEK5/ERK5 pathway resulting in increased expression of c-jun [*EMBO J* 1997; **16**: 7054–66]. Previous work by our group showed the over-expression of MEK5 in human prostate cancer tissue, and that MEK5 over-expression in human prostate cancer cells produces increased cellular proliferation, migration, invasion and MMP-9 production. Here we report the action of MEK5 *in vivo*.

MATERIALS AND METHODS

Ponasterone A-inducible MEK5 (constitutively active) was stably transfected into EcR293 cells to produce EcRD cells. Nude mice were implanted with a slow-release Ponasterone A or placebo pellet, then injected with EcRD or EcR293 (control) cells. Developing tumours were measured at regular intervals and growth compared between controls and test mice. Tumours of > 10 mm diameter were removed and analysed immunohistochemically. Ethical approval was obtained for the above experiments.

RESULTS

Test mice developed tumours much more readily (four of five vs three of 10) and quickly (mean 33 vs 70.3 days, $P = 0.02$) than control mice. The mean tumour-specific survival of test mice was 54.5 days and of control mice 82.6 days ($P = 0.003$). Immunohistochemical analysis showed higher expression of MEK5, ERK5 and MMP-9 in the test tumours than in control tumours.

CONCLUSIONS

MEK5 over-expression *in vivo* enhances the tumorigenicity of EcR293 cells. ERK5 and MMP-9 expression were increased, consistent with a role of MEK5/ERK5 pathway in carcinogenesis.

Funding: Newcastle Upon Tyne Hospitals NHS Trust, British Urological Foundation

P017

Interaction with prostate cancer cells leads to altered T-lymphocyte survival

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INTRODUCTION

On histological analysis, prostate tumours are infiltrated by lymphocytes, predominantly CD8+ cytotoxic lymphocytes. This infiltrate is considered to be part of the host's immune response against the tumour. However, despite this infiltrate, tumour progression occurs. We hypothesize that when lymphocytes infiltrate a prostate tumour, they experience a 'hostile' microenvironment, which leads to their functional down-regulation and altered survival.

MATERIALS AND METHODS

PC-3 metastatic prostate cancer cells were cultured to confluence. T-lymphocytes were harvested from peripheral blood of healthy volunteers using CD3 enrichment columns. Cells were then stimulated with interleukin-2 and phytohaemoagglutinin for 15 h, before co-culture with the PC-3 cells for 48 h. Apoptosis and viability were determined by propidium iodide DNA staining and membrane integrity. Fas/CD95 receptor expression was

determined by flow cytometry using CH-11 antibody.

RESULTS

Co-culture (48 h) with PC-3 cells resulted in a mean (SD) decrease of 57.9 (20.2)% in spontaneous rates of T-lymphocyte apoptosis. There was no statistically significant change in viability, at 52.8 (9.2)% in co-cultured lymphocytes and 66.6 (10.3)% in the control. Surface expression of Fas receptor was upregulated in co-cultured lymphocytes, at 128.1 (22.2) LnMCF, vs the control, at 28.1 (19.2) LnMCF ($P < 0.005$, ANOVA).

CONCLUSION

There was a significant decrease in T-lymphocyte spontaneous apoptosis after 48 h of co-culture with PC-3 cells; this was associated with a marked upregulation of the Fas receptor. The ability of prostate cancer cells to alter the apoptotic susceptibility of co-cultured T-lymphocytes suggests that the tumour microenvironment may be capable of influencing the activation and survival of these infiltrating lymphocytes.

Funding: British Urological Foundation

P018

The effect of resveratrol and gefitinib ('Iressa™', ZD1839) on the growth and cell-cycle progression of prostate cancer cells

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BACKGROUND

Resveratrol is a plant polyphenol which prevents breast and colon cancer in rodent models. The experimental antitumour drug, gefitinib ('Iressa™', ZD1839) is an orally active epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI). We tested the hypothesis that these agents in combination would achieve a greater inhibitory effect on the growth of prostate cancer cell lines than either agent on its own.

METHODS

Basal EGFR levels in LNCaP, DU145 and PC3 cells were determined by Western blotting. Cells were incubated with resveratrol (1–25 μmol/L) or gefitinib (0.01–10 μmol/L) and counted at set times. Cells were then exposed to combinations of the two agents. The effects of these agents on cell-cycle progression were determined by FACS analysis.

RESULTS

EGFR expression was highest in DU145 cells, followed by PC3 and LNCaP cells. The IC_{50} values for resveratrol in DU145, PC3 and LNCaP cells were 4.8, 6.6, and 6.9 μmol/L respectively, and for gefitinib were 0.14, 8.2 and 3.8 μmol/L, respectively. The combination was additive or synergistic in DU145 and LNCaP cells ($P < 0.001$), but not in PC3 cells. Resveratrol arrested DU145 cells in the S-phase, and LNCaP cells in the G0/G1 phase. Gefitinib arrested DU145 cells in G0/G1 but had no effect on the cell cycle in LNCaP cells.

CONCLUSIONS

These results show that the combination of resveratrol and gefitinib has a potent growth inhibitory effect on prostate cancer cells, and provide a rationale for testing this combination in murine models.

Funding: British Urological Foundation

'Iressa' is a trademark of the AstraZeneca group of companies.

P019

Silencing of the type 1 insulin-like growth factor receptor (IGF1R) gene by RNA interference inhibits clonogenic survival of human prostate cancer

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INTRODUCTION

The IGF1R mediates tumour cell growth, malignant transformation and protection from apoptosis, and is a potential therapeutic target. The IGF1R is over-expressed in prostate cancer, and IGF1R antisense oligonucleotides (ASOs) induce modest chemosensitivity in DU145 prostate cancer cell lines *in vitro* [*Cancer Res* 2002; **62**: 2942]. Many prostate cancers show PTEN mutation which activates the main anti-apoptotic pathway downstream of the IGF1R.

MATERIALS AND METHODS

We used oligofectamine to transfect small interfering RNAs (siRNAs) homologous to the IGF1R gene, or inverted sequence controls, into three prostate cancer cell lines: DU145 (androgen-resistant, wild-type PTEN), PC3

(androgen resistant, PTEN mutant) and LNCaP (androgen sensitive, PTEN mutant). We measured IGF1R protein levels by western blotting and cell survival by clonogenic assay.

RESULTS

Transfection with 200 nmol/L siRNA inhibited expression of the IGF1R to 15–20% of levels in control-treated cells. Clonogenic survival was reduced to 35% in DU145, 32% in LNCaP and 0.5% in PC3, compared with survival in control-treated cultures. This represented a more profound effect than we had previously achieved with ASOs. Furthermore, survival of control-treated cells was comparable to that in untreated cultures, suggesting that these agents are less toxic than ASOs.

CONCLUSION

IGF1R targeting by siRNA inhibited prostate cancer cell survival, even in cells lacking functional PTEN. This suggests that we were able to block other signalling pathways downstream of the receptor that influence survival. These results support the concept of IGF1R targeting as novel therapy.

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