Differential effects of MAPKs signaling on the growth of invasive bladder cancer cells

BINOD KUMAR1*, JANE SINCLAIR1*, LAKSHMIPATHI KHANDRIKA1, SWEATY KOUL1, SHANDRA WILSON1 and HARI K. KOUL1,2

1Signal Transduction and Molecular Urology Laboratory, Program in Urosciences, Division of Urology, Department of Surgery, School of Medicine and 2University of Colorado Comprehensive Cancer Center, University of Colorado at Denver and Health Sciences Center, Building P15 or RC2, 12700 E 19th Avenue, Aurora, CO 80045, USA

Received September 16, 2008; Accepted November 28, 2008

Abstract. Transitional cell carcinoma (TCC) is the most common form of bladder cancer. In bladder cancer, which in terms of its origins and genetics, is a representative of invasive tumors, the differing clinical course and the limited value of established prognostic markers compelled many researchers to look for new molecular parameters in predicting the prognosis and treatment of patients with bladder cancer. Activation of mitogen activated protein kinase (MAPK) is a frequent event in tumor progression and metastasis. In the current study, we investigated the role of two different MAPKs (ERK1/2 and p38) by using their specific inhibitors PD98059 and SB203580 respectively, on bladder cancer growth in two cell lines derived from different tumor stages. Our preliminary work showed that ERK1/2 and p38 MAP kinase are active during the log phase growth of bladder cancer, and inhibition of these pathways could reduce proliferation and growth. Moreover, treatment with these inhibitors hinders DNA synthesis, and has differential effects on the progression of cell cycle. ERK1/2 inhibitor caused cyclin B1-dependent G2/M arrest in both HTB5 and HTB9 bladder cancer cell lines, where as p38 MAPK inhibitor showed G2/M arrest in HTB9 and G1 arrest in HTB5 cell line. Furthermore, decreased proliferation and growth arrest caused by MAPK inhibitors was found to be a reflection of apoptotic induction by these inhibitors in bladder cancer cells. Thus, these studies establish MAPKs as a molecular target in bladder cancer growth which could provide new molecular modalities in clinical application.

Introduction

Transitional cell carcinoma (TCC) is a type of cancer that originates in the kidney, bladder or ureter. It is the most common type of bladder cancer. It arises from the transitional epithelium found lining these organs. A striking feature of TCC is the existence of two distinct types of tumors with different clinical features and molecular subtypes. More than 70% of TCC tumors at diagnosis are low grade non-invasive papillary lesions that demonstrate relatively benign but recurrent behavior. In contrast ~20% of tumors are muscle invasive with aggressive behavior (1). Each year in the United States, bladder cancer is diagnosed in 38,000 men and 15,000 women. This is the fourth most common type of cancer in men and the eighth most in women. An estimated 68,810 new cases and 14,100 deaths occurred from bladder cancer in the United States in 2008.

Although, there is extensive information from the past about bladder cancer at genetic and molecular level, several questions remain unanswered. Thus, in bladder cancer, there is a need to identify those molecular markers that will predict which superficial bladder tumors will later progress to become invasive (2). To date, no such markers have been detected. Identifying appropriate molecular targets and understanding the molecular basis of these pathways is an important step in creating newer generation chemotherapeutic drugs with improved efficacy and side-effect profiles.

Amplified proliferation is a characteristic of tumor cells, which is frequently caused by enhanced activity of intracellular signal transduction pathways. Over the past years, inhibitors of many signaling pathways, including MAPK inhibitors have been developed, and clinical trials with related compounds have begun. Data from these studies suggest substantial clinical implications in predicting how different stages of TCC may respond to inhibitors of MAPK pathways (3). Mutation of FGF receptor 3 (FGFR3) is the most common genetic event identified in bladder cancer to date (>70% of...
pTa tumors) (4,5). An additional 10-15% of pTa bladder tumors contain mutations in one of the RAS genes (HRAS, NRAS or KRAS4) and these alterations are mutually exclusive with FGFR3 mutation (6). Another factor is the overexpression of epidermal growth factor receptor (EGFR) or ERBB2 occurs in TCC and could result in constitutive MAPK activation, as is commonly observed in squamous carcinomas as a consequence of EGFR overexpression. Recent studies confirmed that all of these events activate the MAPK pathway in urothelial cells and have identified germline mutations in several genes of the MAPK pathway in patients with a range of developmental disorders. These include but are not limited to RAS and BRAF gene mutations outside the normal hotspots found in cancer, mutations in SOS1, PTPNII (SHP2) and MEK 1/2 (7), predicted to activate the MAPK pathways. Moreover, it is also assumed that MAPK pathways drive proliferation of normal uroepithelial (UCE) and urothelial carcinoma (TCC) cells (3).

Activation of MAPK pathways might be important for TCC cells to re-enter the cell cycle from a state of quiescence. However, these cells do not establish a quiescent state easily, as demonstrated by their behavior following serum withdrawal. Since in almost all TCC lines (with the partial exception of BFTC905) SRE luc and AP1 luc activity as well as phosphorylation of MEK, ERK, and ELK1 could be induced by MEKK4 or TPA, suggesting that all the essential components of the MAPK pathways are present in these cells (3). Moreover, the molecular, genetic and cellular changes that occur in TCC of the bladder are numerous and include chromosome alterations, loss of heterogenicity, and loss of cell growth regulation. Several of these genetic alterations have been found within tumor suppressor genes like retinoblastoma (RB) and p53 genes. Thus, bladder cancers display mutations in cell cycle check-point genes that are associated with malignant growth and genetic instability (8). But, the importance of MAPKs in cell cycle control and genetic instability has not been well study so far in the TCC. In this context, our preliminary studies point out the role of MAPKs in cellular proliferation, DNA synthesis, cell cycle, and in death of bladder cancer cell lines. Our effort showed that ERK1/2 and p38 MAP kinase are not only active during the log phase growth of bladder cancer but they are important for maintenance of bladder cancer phenotypes.

Materials and methods

Cell lines and reagents. Human transitional cell carcinoma cell lines HTB5 and HTB9 were procured from ATCC and grown as a monolayer in modified Eagle’s MEM and RPMI-medium respectively supplemented with 10% fetal bovine serum, sodium pyruvate, and antibiotics. Cells were incubated in a humidified atmosphere at 37˚C with 5% CO2. PD98059, a specific inhibitor of MAPK extracellular signaling-regulated kinase (ERK1/2) kinase; SB203580, specific inhibitor of p38 MAPK were obtained from calbiochem (Gibbstown, NJ, USA) and 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT) was obtained from Sigma (St. Louis, MO, USA). Primary antibodies used in this study, against proteins like p21, cyclin B1, caspase-3 and -9 were obtained from Cell Signaling (Danvers, MA, USA).

Measurement of cell proliferation and viability. Log phase HTB9 and HTB5 cells (10,000 cells) were seeded on 96-well plates and allowed to attach overnight followed by treatment with different concentrations of SB203580 or PD98059 for 72 h. Cellular proliferation was quantified with colorimetric methods based on the metabolic reduction of the soluble yellow MTT dye to its insoluble formazan (9). For viability assay, all the cell lines were seeded in a 24-well plate (2x10^4) and were treated as above for 72 h, followed by the addition of 0.4% crystal violet in 0.2 M citrate buffer for 30 min (10). After washing excessive dye in water, incorporated crystal violet was solubilized with ethoxy ethanol and the absorbance was measured at 595 nm.

Inhibition of DNA synthesis. [3H]-thymidine incorporation was used as an index of DNA synthesis and was carried out as described previously (11). Briefly, HTB9 and HTB5 cells were plated in 6-well plates and grown to 60-70% confluence. These cells were then treated with various concentrations of MAPKs inhibitors (PD98059 or SB203580) for ~48 h. During the last 12-14 h of exposure 2 μCi of [3H]-thymidine was added per well. At the end of experimental period, cells were washed with two changes of ice-cold phosphate buffered saline (PBS) and trypsinized at 37˚C. Two ml of cell suspension was combined with 2 ml of 10% trichloroacetic acid, and the acid-insoluble material was collected on Whatman glass fiber filters. Filters were then air-dried, and the incorporated radioactivity was counted using a Beckman Liquid Scintillation Counter.

Cell cycle analysis. Sub-confluent cultures were treated with different concentrations of either SB203580 or PD98059 for ~72 h, harvested, washed with cold PBS and stained overnight at 4˚C with staining solution (RNase; 20 μg/ml, propidium iodide; 50 μg/ml). Cell cycle distribution was then determined using a FACS instrument (Beckman Coulter FC500, Hialeah, FL) in the FACS Core Facility of the University of Colorado Health Sciences System at Denver. ModFit LT 3.0 (Verity House Software, Topsham, ME) cell cycle analysis software was used to determine the percentage of cells in the different phases of cell cycle.

Determination of cellular apoptosis. Cellular apoptosis was measured using Vibrant Apoptosis Assay Kit as recommended by the manufacturer (Molecular Probes-Invitrogen Corp., Carlsbad, CA). Briefly, cells were grown either in presence or absence of either SB203580 (20 μM) or PD98059 (50 μM) for ~36 h. Cells were washed and incubated with Annexin V-FITC and propidium iodide (PI) as per manufacturer’s instructions and were visualized under fluorescence microscope. In another set of experiment, cells were treated for ~72 h with either SB203580 or PD98059, harvested, and stained with DAPI for visualization of nuclei by fluorescence microscopy (Leica RX-DA, Germany).

Western blot analysis. Where indicated, cells treated with either SB203580 or PD98059, were lysed in RIPA buffer, separated on SDS-PAGE and protein bands were transferred to a polyvinylidene difluoride (PVDF) membrane, followed by incubation with appropriate antibodies. Secondary antibody
binding was detected with an enhanced chemiluminescence kit (Thermo Scientific, Rockford, IL) (11).

Statistical analysis. Unless specified, the results were expressed as the mean ± SD of the data collected from at least two independent experiments done in triplicate. Statistical significance was determined by paired, two-tailed Student's t-test wherever necessary. A P<0.05 was considered as significant. The error bars in figures represent standard deviation (SD) of mean.

Results

ERK1/2 and p38 MAPK inhibitor attenuates cell growth and proliferation of bladder cancer cell lines. In order to find out the role of two MAPK inhibitors in bladder cancer cells proliferation/growth, HTB5 and HTB9 cells were treated with different concentrations of PD98059 (an ERK1/2 MAPK inhibitor) and SB203580 (a p38 MAPK inhibitor). We did not find any significant inhibition of cell growth/proliferation after 48 h treatment (data not shown), while 72 h treatment significantly (P<0.01 and P<0.001) decreased proliferation and viability of the both the cell lines (Fig. 1). The effect of PD98059 was concentration-dependent, and 100 μm caused ~50% reduction in proliferation or viability, whereas, 25 and 50 μm showed reasonable effects. Similarly, SB203580 inhibited both proliferation and growth of the cells in a concentration-dependent manner, while HTB5 cells showed resistance towards this activity (Fig. 1C and D). Thus, inhibition of ERK1/2 or p38 MAPK has differential effects on the maintenance of bladder cancer cells.

Inhibition of ERK1/2 and p38 MAPK blocks DNA synthesis in bladder cancer cells. Previous studies and data carry substantial clinical implications in predicting that many TCC may respond to inhibitors of MAPK pathways. However, there is no direct information on the status of the ERK1/2 and p38 MAPK pathways in invasive tumors. Since initial experiments demonstrated that MAPK signaling pathway is selectively activated in bladder cancer, we tested whether DNA synthesis in bladder cancer cells was mediated by MAPK pathway. Results indicated in Fig. 2 suggested that both PD95059 and SB203580, decreased DNA synthesis in a concentration-dependent manner in HTB5 and HTB9 cell lines. Higher concentrations of both MAPK inhibitors restrain ~60-80% of DNA synthesis. Thus, these data imply the importance of MAPKs pathway in the growth of the bladder cancer cells.

Treatment of bladder cancer cells with ERK1/2 and p38 MAPK inhibitors had differential effects on cell cycle. Cell cycle check-points function to maintain genetic stability by providing additional time for repair of DNA damage and completion of events that are necessary for accurate cell division. Because bladder transitional cell carcinomas (TCCs) often contain numerous chromosomal aberrations and appear to have highly unstable genomes, we analyzed cell cycle status with MAPK inhibitors on TCC lines. Our results indicated
Figure 2. Inhibition of ERK1/2 and p38 MAPK reduces DNA synthesis in bladder cancer cells. Log phase growing HTB5 (A) and HTB9 (B) cells were exposed to various doses of either PD98059 (25 and 50 μM) or SB203580 (10 and 20 μM) for 48 h. During the last 12 h of treatments, [3H]-thymidine (2 μCi) was added per well. The radioactivity retained in the trichloroacetic acid precipitate was measured and used as an index of DNA synthesis as described in Materials and methods. Each data point represents mean ± SD of duplicate experiments done in triplicates. Statistical significance is indicated as: *P<0.05, **P<0.01, and ***P<0.001 compared to control.

Figure 3. ERK1/2 and p38 MAPK inhibitors had differential effect on cell cycle progression of bladder cancer cells. HTB5 and HTB9 cells were treated with either PD98059 or SB203580 in culture medium for 72 h and flow cytometric analysis was used to determine the cell cycle distribution as described in Materials and methods. (A), Cell cycle distribution in HTB5 and HTB9 cells after treatment with PD98059 or SB203580. (B and C), Quantitative representation of cell cycle distribution in HTB5 and HTB9 cells, respectively, after treatment with MAPK inhibitors. (D), Effect of PD98059 on G2/M cell cycle regulatory protein (cyclin B1) in bladder cancer cells as detected by Western blotting. (E), Effect of SB203580 on cell cycle regulatory protein p21 (G1 arrest) in HTB5 and cyclin B1 (G2/M arrest) in HTB9, respectively, as detected by Western blot analysis with GAPDH protein as a loading control. Representative gel images from triplicate experiments are shown.
that ERK1/2 inhibitor; PD98059 specifically caused G2/M arrest in both HTB5 and HTB9 cell lines, whereas p38 MAPK inhibitor; SB203580 caused G1 arrest in HTB5, and G2/M arrest in HTB9 cell line (Fig. 3A–C). These results were further confirmed by the expression of different cell cycle regulatory proteins. As indicated in the Fig. 3D, treatment of PD98059 caused downregulation of cyclin B1 in both the cell lines and this effect was more pronounced after 72 h of treatment. Thus, ERK1/2 inhibitor caused cyclin B1-dependent G2/M arrest in the bladder cancer cell lines. Similarly, SB203580 caused downregulation of cyclin B1 in HTB9 cell line where as similar treatment on HTB5 cell line resulted in up-regulation of p21 protein causing G1 arrest (Fig. 3E). These data suggest a differential mode of action of MAPKs on the growth of bladder cancer cell lines.

**ERK1/2 and p38 MAPK pathways are critical for survival of bladder cancer cells.** Earlier in our study we observed that MAPK inhibitors had an impact on cell proliferation, DNA synthesis and cell cycle control in bladder cancer cell lines, HTB5 and HTB9. In an effort to elucidate the fate of these cells, we determined the status of apoptosis markers.

To understand the mechanism of cell loss observed upon exposure of bladder cancer cells to MAPK inhibitors, we evaluated early apoptosis by using Annexin V-FITC after treating HTB5 and HTB9 cells with either 50 μM of PD98059 or 20 μM of SB203580 for ~36 h, and apoptosis was determined by using Annexin V-FITC/PI staining as described in Materials and methods. Representative images from duplicate experiments performed in quadruplicate are shown. These studies revealed that exposure of bladder cancer cells resulted in a marked increase in apoptotic cells compared to untreated cells (Fig. 4A). In order to further confirm the mechanism of cell death, nuclear morphological analysis has been done after long exposure (~72 h) with MAPK inhibitors. Treated cells were fixed, stained with DAPI stain and nuclei were visualized under fluorescence microscope. Results (Fig. 4B) demonstrated nuclear condensation consistent with apoptotic cell death.

---

**Figure 4.** Inhibition of ERK1/2 and p38 MAPK pathways induces morphology of cell death in bladder cancer cells. (A), Bladder cancer cells (HTB5 and HTB9) were treated with either PD98059 or SB203580 for ~36 h, and apoptosis was determined by using Annexin V-FITC/PI staining as described in Materials and methods. Representative images from duplicate experiments performed in quadruplicate are shown. Photographs were taken using fluorescence microscopy. (B), Representative fluorescence microphotographs showing nuclear condensation by DAPI staining in HTB5 and HTB9 cells after MAPKs inhibitor treatment.
Non-malignant cells tend to undergo apoptosis (programmed cell death) although malignant cells develop resistance towards it (12). Since apoptosis can be associated with MAPK inhibition, we also looked for expression of apoptosis-associated proteins in bladder cancer cell lines treated with MAPKs inhibitor for 48-72 h. Fig. 5 suggests that knocking down MAPKs resulted in reduced expression of caspase-3 and -9 (as monitored by loss of the inactive procaspase-3 or -9) after 48 and 72 h; however, the loss of the procaspases was maximum up to 72 h. Surprisingly, we did not find any procaspase cleavage in HTB5 cell line with p38 MAPK inhibitor (Fig. 5B), while PD98059 showed effect in both the cell lines. Thus, these data suggested that ERK1/2 and p38 MAPK are critical for cell survival and inhibition of these pathways could induce apoptosis in bladder cancer cells. 

Taken together these results suggest that inhibition of MAPK pathways in bladder cancer by chemical inhibitors at certain concentrations could result in a significant loss of cell viability, cell cycle arrest and cell death in invasive bladder cancer cell lines.

Discussion

Mitogen activated protein kinase (MAPK) signaling pathways regulate essentially all aspects of the malignant cell phenotype including proliferation, survival, migration and invasion. As such, understanding of the molecular cascades regulated by MAPK signaling in the tumor cells would promote our understanding of cancer biology and provide a novel tool for intervention in cancer treatment (13). Previous studies have demonstrated that Ras-MAPK is constitutively activated in TCC through the activation of FGFR3. Moreover, in invasive TCC, other receptor tyrosine kinases such as epidermal growth factor receptor (EGFR) and ErbB2 are overexpressed and are associated with poor outcome (14-16). To date there are no acceptable and definite treatment options available for patients suffering from invasive bladder cancer. TCC follows the general concept of multistep carcinogenesis and proceed through two distinct pathways responsible for different TCC morphologies and aggressiveness (2). In view of these observations it is necessary to develop new strategies to prevent metastasis of bladder cancer.

In the present study, we sought to evaluate role of MAPKs in invasive bladder cancer cell with special attention to their task in regulation of cell proliferation, cell cycle progression, and survival. There is a growing body of evidence suggesting the activation of MAPK in the pathogenesis of bladder cancer (1). The study focuses on the role of MAPKs (by using two specific chemical inhibitors for ERK1/2 MAPK and p38 MAPK) in bladder cancer proliferation, cell cycle control, and cellular death has been characterized in vitro in two different cell lines, HTB9 (derived from grade 2 bladder cancer) and HTB5 (derived from grade 4 bladder cancer).

We show here, for the first time that MAPKs signaling is active during the log phase growth of bladder cancer cells (Fig. 1). Both ERK1/2 and p38 MAPK inhibitors were able to decrease cellular proliferation of HTB9 and HTB5 cell lines. However, their effects were less intense in HTB5 cells (advance bladder cancer), especially with the p38 MAPK inhibitor. Furthermore, treatment of cells with the inhibitors results in the breakdown of cell cycle machinery, and the subsequent decision to continue the cell cycle or to undergo cell death is an outcome of the balance of promoting factors in which the capacity for DNA repair plays a crucial role (17). To date, however, there is no information available on the possible DNA damage induced by MAPK inhibitors. We made an attempt to study the effect on DNA synthesis capability of bladder cancer cells after PD98059 and SB203580 treatment. When cells were exposed to these MAPKs inhibitors, we observed a dose-dependent decrease in DNA synthesis. The DNA damage was very profound at ~20 μm of SB203580 and ~50 μm of PD98059 in both the cell lines as indicated in Fig. 2. Both DNA synthesis inhibition and incomplete DNA repair induce arrest of cells especially at G2/M check-point. Flow cytometric cell cycle distribution data showed that treatment of cells with ERK1/2 MAPK inhibitor caused G1/M arrest in both the bladder cancer cell lines where as p38 MAPK inhibitor caused G1/M arrest in HTB9 cell line, while in HTB5 cell line, it resulted in G1 arrest (Fig. 3A-C). It is
known that G1/S and G2/M check-point controls prevent the 
asquisition of multiple genetic changes by ensuring that DNA 
is repaired before replication in S-phase and appropriately 
segregated during M phase of the cell cycle, respectively. 
Incompletely replicated and damaged DNA generally signals 
arrrest at G1/M in order to prevent mutations, translocation or 
chromosomal loss (18). Our results presented in Fig. 3D and E 
suggest the loss of cyclin B1 protein expression, a cell 
cycle-regulated transcript, expressed predominantly during 
G2/M phase. Cyclin B1 is the regulatory subunit of M-phase 
promoting factor, and a proper regulation of cyclin B1 is 
esential for the initiation of mitosis (19). However, results 
obtained with p38 MAPK inhibitor in HTB5 cell line suggest 
G1 arrest. This finding is interesting as earlier in our study 
with SB203580 treatment, the proliferation and viability of 
HTB5 cell line was different from HTB9 cell line (Fig. 1C 
and D). Although the exact rationale behind this difference is 
not known, it is possible to reason that it might be due to 
differential signaling mechanisms in different stages of cancer 
progression. The differences in the result also highlights the 
fact that there exists a differential signaling between the 
two cell lines which are derived from different tumor grade, 
thus putting emphasis on the significance of investigating 
individual molecular pathways in multiple cell types or 
tissue. It is known that there are variations in the prognosis 
of patients with stage 2 and 4 bladder tumors, and the 
distinction between organ-defined disease and extravesicle 
extension is important prognostically, and can be used to 
determine the success of preoperative chemotherapy. Thus, 
in the future, a tumor staging system might take relevance of 
tumor biology into account when designating risk.

Since we observed a dose-dependent inhibition in DNA 
synthesis with both PD98059 and SB203580 treatment, we 
also investigated to see whether this mode of treatment 
can induce cell death in bladder cancer cell lines. Our preliminary 
results suggested early apoptosis, nucleus condensation and 
activation of both caspase-3 and -9 (as monitored by loss of 
their enzymatic activity), suggesting that the suppression of 
caspase-3/9 level), a marker of apoptotic cell death, is 
induced in both HTB5 and HTB9 cell lines with SB203580 treatment, the proliferation and viability of 
HTB5 cell line was different from HTB9 cell line (Fig. 1C 
and D). Although the exact rationale behind this difference is 
not known, it is possible to reason that it might be due to 
differential signaling mechanisms in different stages of cancer 
progression. The differences in the result also highlights the 
fact that there exists a differential signaling between the 
two cell lines which are derived from different tumor grade, 
thus putting emphasis on the significance of investigating 
individual molecular pathways in multiple cell types or 
tissue. It is known that there are variations in the prognosis 
of patients with stage 2 and 4 bladder tumors, and the 
distinction between organ-defined disease and extravesicle 
extension is important prognostically, and can be used to 
determine the success of preoperative chemotherapy. Thus, 
in the future, a tumor staging system might take relevance of 
tumor biology into account when designating risk.

Since we observed a dose-dependent inhibition in DNA 
synthesis with both PD98059 and SB203580 treatment, we 
also investigated to see whether this mode of treatment 
can induce cell death in bladder cancer cell lines. Our preliminary 
results suggested early apoptosis, nucleus condensation and 
activation of both caspase-3 and -9 (as monitored by loss of 
their enzymatic activity), suggesting that the suppression of 
caspase-3/9 level), a marker of apoptotic cell death, is 
induced in both HTB5 and HTB9 cell lines with SB203580 treatment, the proliferation and viability of 
HTB5 cell line was different from HTB9 cell line (Fig. 1C 
and D). Although the exact rationale behind this difference is 
not known, it is possible to reason that it might be due to 
differential signaling mechanisms in different stages of cancer 
progression. The differences in the result also highlights the 
fact that there exists a differential signaling between the 
two cell lines which are derived from different tumor grade, 
thus putting emphasis on the significance of investigating 
individual molecular pathways in multiple cell types or 
tissue. It is known that there are variations in the prognosis 
of patients with stage 2 and 4 bladder tumors, and the 
distinction between organ-defined disease and extravesicle 
extension is important prognostically, and can be used to 
determine the success of preoperative chemotherapy. Thus, 
in the future, a tumor staging system might take relevance of 
tumor biology into account when designating risk.

In conclusion, these studies clearly suggest the differential 
effects of MAPK inhibitors on the bladder cancer phenotype. 
We showed that cell cycle check-point may be regulated by 
both ERK1/2 and p38 MAPK, but their mechanism is different, 
and is specific to cell type, stage, and aggressiveness of bladder 
cancer. Hence bladder cancer management strategies now 
need to focus on the molecular alterations involved in each 
individual tumor. Synergism among the agents targeting 
various pathways should be the next step for rational manage- 
ment of TCC, and employment of therapeutic regimens that 
that target multiple molecular pathways may hold the key to 
successful management of bladder cancer.

Acknowledgements

This study was supported in part by NIH/NCI-P20 CA103680 
(H. Koul, Pilot-Project PI). Authors gratefully acknowledge 
service and support provided by University of Colorado Cancer 
Center Flow Cytometry Core.

References

1. Knowles MA: Molecular subtypes of bladder cancer: Jekyll 
and Hyde or chalk and cheese? Carcinogenesis 27: 361-373, 
2006.

2. Al-Sukhun and Hussain M: Current understanding of the 
biology of advanced bladder cancer. Cancer 97: 2064-2075, 
2003.

3. Światkowski S, Seifert HH, Steinhoff C, et al: Activities of 
MAP-kinase pathways in normal uroepithelial cells and 
urothelial carcinoma cell lines. Exp Cell Res 282: 48-57, 
2003.

FGFR3 mutations in papillary non-invasive bladder (pTa) 

5. Sibley K, Cuthbert-Heavens D and Knowles MA: Loss of hetero-
zygosity at 4p16.3 and mutation of FGFR3 in transitional cell 

and Knowles MA: FGFR3 and Ras gene mutations are mutually 
exclusive genetic events in urothelial cell carcinoma. Oncogene 

7. Schubbett S, Shannon K and Bollag G: Hyperactive Ras in 
developmental disorders and cancer. Nat Rev Cancer 7: 295-308, 
2007.

cycle checkpoint function in bladder cancer. J Natl Cancer Inst 

Radiosensitization by diospyrin diethyl ether in MCF-7 breast 

replication-competent oncolytic adenovirus armed with 
improved suicide genes and ADP gene demonstrates greater 
efficacy without increased toxicity. Mol Ther 13: 347-356, 
2006.

activate the p38 mitogen-activated protein kinase signal trans-
duction pathway in renal epithelial cells. J Biol Chem 277: 
36845-36852, 2002.

12. Schmitt CA and Lowe SW: Apoptosis and chemoresistance in 

13. Li SP, Junttila MR, Han J, Kähäri VM and Westermarck J: 
Radiosensitization by diospyrin diethyl ether in MCF-7 breast 

replication-competent oncolytic adenovirus armed with 
improved suicide genes and ADP gene demonstrates greater 
efficacy without increased toxicity. Mol Ther 13: 347-356, 
2006.

epidermal growth factor receptor, T138 and T43 expression in 


