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Effects of multi and selective targeted tyrosine kinase inhibitors on function and signaling of different bladder cancer cells

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ABSTRACT

Background: Signaling of receptor tyrosine kinases (RTK) is dysregulated in various malignancies including bladder cancer. RTKs trigger pro-proliferative, anti-apoptotic and metastatic signaling pathways. Here, we assessed the effects of a selective tyrosine kinase inhibitor (TKI) (BGJ398) targeting fibroblast growth factor receptor (FGFR) and a pan-TKI (TKI258) targeting (FGFR), platelet derived growth factor receptor (PDGFR) and vascular endothelial growth factor receptor (VEGFR) in bladder cancer cells.

Methods: Levels of mRNA transcripts were measured in nine human cell lines by quantitative RT-PCR. Cell function was assessed for viability, colony formation, migration, apoptosis and proliferation. Protein mediators of signal transduction were measured by Western-blot.

Results: mRNA transcripts encoding RTK-related components, transcription factors, epithelial and mesenchymal transition (EMT) markers as well as cell cycle and apoptotic factors were determined in the cell lines. Principal component analysis ordered one epithelial-like cell cluster (5637, BFTC-905, MGHU4, RT112) and one mesenchymal-like cell cluster (T24, UMUC3, HU456, TCC-SUP). Cell response scores towards TKI258 and BGJ398 treatment were heterogeneous between cell lines and correlated with certain transcript levels. Analysis of signal transduction pathways revealed inhibition of fibroblast growth factor receptor (FGFR) signaling and induction of cell cycle dependent kinase (CDKN1A, p21) in epithelial-like cells differing in this regard from responses to mesenchymal-like cells that exhibited inhibition of mitogen-activated protein kinase (MAPK).

Conclusion: RTK and EMT related transcript analysis separate bladder cancer cells in two clusters. Functional responses towards TKI258 and BGJ398 treatment of bladder Fcancer cells were heterogeneous with deviating effects on signaling and possibly different therapeutic outcome.

1. Introduction

Receptor tyrosine kinases (RTKs) comprise manifold subtypes with oncogenic characteristics that can be targeted by tyrosine kinase inhibitors (TKIs) for anti-cancer treatment [1,2]. RTK signaling is enhanced due to high expression of certain components or by gain of function mutations in various malignancies such as bladder cancer, breast cancer, lung cancer and myeloma [3,4]. Apart from cancer cells, TKIs can also target endothelial cells which are responsible for tumor neo-vascularization [5].

In bladder cancer, three major dysregulated pathways of cell cycle regulation (p53, p21), chromatin remodelling as well as RTKs with downstream signaling mediators (Ras/PI3K) were identified with high frequency offering as druggable targets [6,7]. Among RTKs epidermal growth factor receptor (EGFR), fibroblast growth factor receptor (FGFR), hepatocyte growth factor receptor (MET), platelet derived growth factor receptor (PDGFR), and vascular endothelial growth factor receptor (VEGFR) represent therapeutic relevant targets [1,8,9]. Particularly, FGFRs and their ligands play a critical role in the pathogenesis of urothelial carcinoma. FGFR3 mutations are common but also overexpression of FGFR1, FGFR3 and of FGFs have been observed [10–13]. These aberrations and downstream Ras mutations are described with higher frequency in low grade non muscle invasive forms than in high grade muscle invasive forms. Aberrant p53 and p21 may be critical for progression in higher tumor stages [1]. Induction of extracellular matrix remodelling genes causing epithelial, mesenchymal transition (EMT) can be observed during tumor progression [10,14–17]. Dependence of EMT components on FGFR signaling haven been described [11,18].

TKI258 and BGJ398 are two TKIs with different target selectivity. TKI258 is a multi-targeted inhibitor of PDGF, VEGFR, FGFR [19] and BGJ398 represents a selective FGFR inhibitor [20]. Dissecting the

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Table 1
Acronyms and full names of genes of transcript (mRNA) analysis.

Gene Acronym	Gene meaning
ACTB	Cytoplasmic b-actin
BAX	Bcl-2 Associated X
BCL2	B-Cell Lymphoma 2
CCND1	Cyclin D1
CDH1	Cadherin1 (epithelial ECDH)
CDH2	Cadherin 2 (neuronal NCDH)
CDKN1A	CDKN1A, Protein 21, p21
CDKN1B	CDKN1B, Protein 27, p27
CJUN	Jun proto-oncogene
E2F3	Transcription factor E2F3
EGFR1	Epidermal Growth Factor Receptor 1
FGF1	Fibroblast Growth Factor 1
FGF2	Fibroblast Growth Factor 2
FGFR1	Fibroblast Growth Factor Receptor 1
FGFR2	Fibroblast Growth Factor Receptor 2
FGFR3	Fibroblast Growth Factor Receptor 3
FGFR4	Fibroblast Growth Factor Receptor 4
FLT1	Fms Related Tyrosine Kinase 1
FLT3	Fms Related Tyrosine Kinase 3
FRS2	Factor Receptor Substate 2
HBEGF	Heparin-binding EGF-like growth factor
HER2	Human Epithelial Growth Factor Receptor 2
HER3	Human Epithelial Growth Factor Receptor 3
KIT	Stem Cell Growth Factor Receptor (c-kit)
MET	Hepatocyte Growth Factor Receptor (c-Met)
MKI67	Cellular marker for proliferation (Ki-67)
MYC	Avian Myelocytomatosis Virus Oncogene Cell Homolog (c-Myc)
PDGFRA	Plateled derived Growth Factor Receptor A
PLAU	Urokinase Plasminogen Activator (UPA1)
PTGS2	Cyclooxygenase 2 (COX2)
RAC1	Ras Related C3 Botulinum Toxin
TBP	TATA-Box Binding Protein
VEGFA	Vascular Endothelial Growth Factor A
VIM	Vimentin

cellular effects of these TKIs may enable to deepen the rationale for targeted therapies of patients affected by bladder cancer. Here, we analyzed the expression of RTK components, markers of proliferation, apoptosis and EMT in bladder cancer cell lines. In parallel, we analyzed the effects BGJ398 versus TKI258 on cell viability, proliferation, apoptosis, and migration and tested biochemical responses. We identified two different classes of cell lines with different EMT status and TKI responsiveness.

2. Materials and methods

2.1. Cell culture

Human bladder cancer cell lines T24, HT1376, BFTC-905, 5637, HU456, UMUC3, RT112, TCC-SUP, MGHU4 [21–31] were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum, 1% stable glutamine and 1% Penicillin/Streptomycin solutions (PAA Laboratories, Pasching, Austria) at 37 °C with 5% CO_2 in humidified air. NVP-BGJ398 (BGJ398) [32] and Dovitinib (TKI-258) was kindly provided by Novartis Pharma AG (Basel, Switzerland).

2.2. RNA and protein extraction

RNA extraction was performed with Trifast (Peqlab, Erlangen, Germany) and protein extraction with RIPA buffer (Cell signaling Technology). Extraction procedures were according to the manufacturers' protocols.

2.3. Quantitative real time RT-PCR

The transcript mRNA targets with names and acronyms are listed (Table 1). 1 µg RNA from cell lines was used as template for cDNA synthesis after digest of genomic DNA with RNase-free DNase (RevertAid First Strand cDNA synthesis Kit, Fermentas Life Science, St. Leon-Rot, Germany). Realtime RT-PCR was performed with SYBR Green Fluorescein Mix (ABgene UK, Epsom, UK). Cycling conditions were 95 °C for 15 min, followed by 45 cycles of 95 °C for 15 s, 60 °C for 15 s, 72 °C for 30 s. Relative levels of mRNA are displayed as ΔCt values (log-2-scale) with the mean of β-actin and TBP as reference mRNA. Changes of mRNA levels (treatment versus control) are indicated as ΔΔCt values. The sequences of primer sets (Supplementary Table S1) were commercially synthesized (Biomers GmbH, Ulm, Germany).

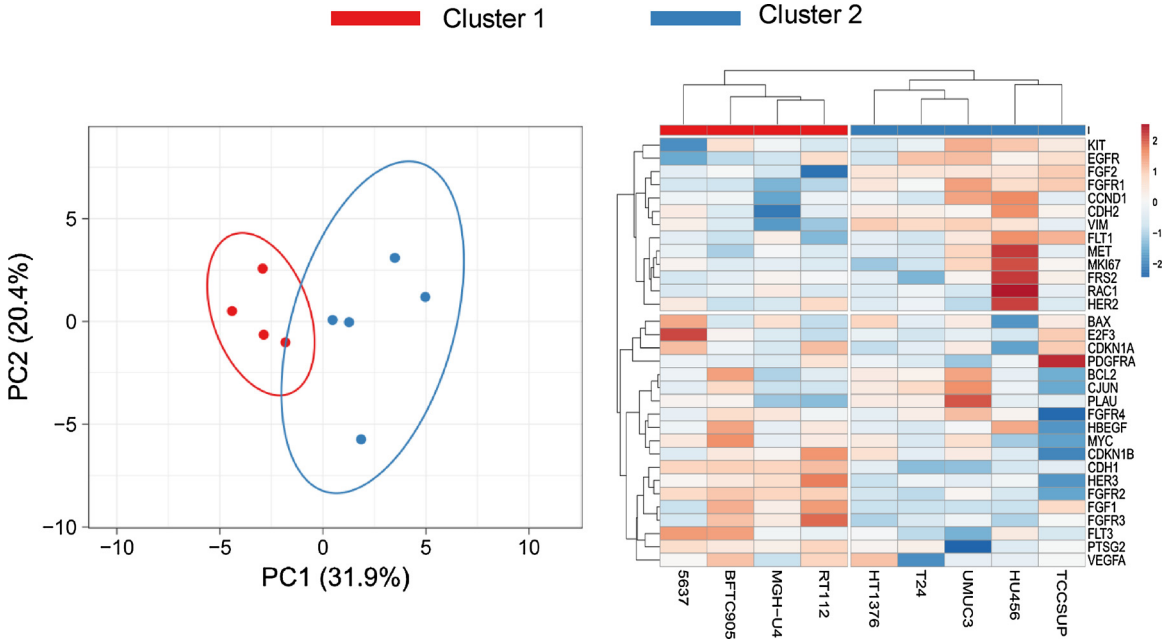

Fig. 1. *Principal Component Analysis* (PCA) of mRNA transcript levels (n = 32) in bladder cancer cell lines (n = 9). Left: Principle components (PC1 and PC2) are displayed that explain 31.9% and 20.4% of the total variance. Right: Heatmap output displaying hierarchical clustering of transcript mRNA levels and cell lines. Colors correspond to epithelial-like cells (cluster 1, red) and mesenchymal-like cells (cluster 2, blue).