Low level *STK15* amplification in histologically benign urothelium of patients with bladder cancer adversely predicts patient outcome following cystectomy

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Abstract. The aim of this study was to investigate STK15 amplification in histologically benign urothelium and invasive tumor tissue of urothelial bladder cancer patients in relation to clinicopathologic and molecular characteristics, and to analyze a hypothesized association between the STK15 single nucleotide polymorphism at site T91A (Phe31Ile) and STK15 gene amplification. A tissue microarray (TMA) was constructed and contained formalin-fixed paraffin-embedded tumor tissue and matching histologically benign urothelium of 44 patients who underwent cystectomy for invasive urothelial carcinoma. Expression of TP53, CK20 and MIB1 was evaluated by immunohistochemistry. UroVysion and STK15 fluorescence *in situ* hybridization (FISH) analysis was performed for sensitive detection of polysomy, relative p16 deletion and STK15 amplification, respectively. Genotypes of STK15 at the T91A (Phe31Ile) site were analyzed by PCR-RFLP assay. Low level STK15 amplification was found in 2 of 36 analyzable histologically benign urothelium specimens (5.6%) and in 64% (28/44) of urothelial bladder cancers, whereas 36% (16/44) of cancer lesions showed high level of STK15 amplification. In histologically benign urothelium of bladder cancer patients, low level STK15 amplification was associated with shorter recurrence-free and tumor-specific survival. There was no correlation between allelic variants and high/low level of STK15 gene amplification. Applying STK15 FISH to benign urothelium of bladder cancer patients may help to identify patients at increased risk for adverse clinical outcome. A large randomized prospective study comparing early versus delayed cystectomy in patients with pT1 bladder cancer is currently conducted to validate our findings.

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Introduction

Aneuploidy and genomic instability are common features of tumor cells (1,2). Aneuploidy is often associated with aggressive tumor phenotypes, and it is thus possible that genes regulating chromosome segregation are involved in tumor progression (3). The recently characterized *STK15* gene (Aurora A) on chromosome 20q13 (4) encodes for a centrosome-associated serine/threonine kinase, and is a member of the Aurora/IPL kinase family which has been implicated in the regulation of centrosome duplication. The Aurora-A kinase is specifically associated with the centrosome of interphase cells as well as with the spindle apparatus during mitosis (4). Gene amplification and over-expression of STK15 has been reported frequently in human tumors [e.g. breast and colon cancer (5,6)] and in association with high degrees of genomic instability, suggesting that the STK15 protein may represent a critical regulatory component of chromosomal segregation causing aneuploidy and malignant transformation. Amplification and overexpression of STK15 was indicative of tumor aggressiveness and poor patient outcome (7-9). *STK15* amplification in histologically benign urothelium of bladder cancer patients has not yet been investigated. A functional polymorphism in *STK15* is caused by single nucleotide substitution at gene sequence position 91 (T→A) and leads to replacement of Phe by Ile at amino acid sequence position 31 (10). In colorectal cancer, specific amplification of the 91A-allele was associated with a higher degree of chromosomal imbalances in tumors from individuals homozygous for the 91A genotype. Meta-analysis of the STK15 Phe31Ile polymorphism demonstrated that the

91A-allele is a low penetrance cancer susceptibility allele affecting multiple cancer types (11). To our knowledge, there is no study analyzing the role of the *STK15* Phe31Ile polymorphism in urothelial bladder cancer. The aim of this study was: i) to investigate *STK15* amplification in histologically benign urothelium and invasive tumor tissue of bladder cancer patients following cystectomy relative to clinicopathologic and molecular characteristics; and ii) to analyze an hypothesized association between *STK15* genotypes at the polymorphism T91A (Phe31Ile) and *STK15* amplification in our patient cohort as it has been previously described in colorectal cancer.

Materials and methods

Cystectomy tissue microarray (TMA). A TMA was constructed as described previously (12) and contained formalin-fixed paraffin-embedded tumor tissue and histologically benign urothelium of 44 patients with invasive urothelial bladder cancer. All specimens were selected from a retrospective single center cystectomy study, obtained between 1993 and 2003 from the Institute of Pathology, University of Regensburg, Germany (UICC stage I-IV). In cases of multiple synchronous bladder tumors, the invasive cancer lesion with the worst histological stage and grade was chosen. Two surgical pathologists (A.H. and P.J.W.) evaluated H&E-stained slides of all cases. Tumor stage and grade were assigned according to International Union Against Cancer (UICC) and World Health Organization (WHO) criteria (13). Papillary tumor growth pattern was defined by the presence of a papillary tumor component (≥20% with a histologic grade identical to the invasive tumor). Tumors with both a papillary and a solid component were defined as mixed. All other tumors were considered to have a solid growth pattern. In case of multi-focal synchronous bladder cancer, growth patterns of each invasive lesion were evaluated separately. Median clinical follow-up period was 40 months (range 6-121 months). Time to recurrence and time to tumor-related death were selected as end points. Clinicopathologic data of the cystectomy TMA are summarized in Table I.

Table I. Clinicopathologic characteristics.

Variable	n analyzable	%
Total (n=44)		
Age when cystectomy was performed		
≤70 years	24	54.5
>70 years	20	45.5
Gender		
Female	14	31.8
Male	30	68.2
Tumor stage		
pT1	12	27.3
pT2a/b	13	29.5
pT3a/b	14	31.8
pT4a/b	5	11.4
Histologic grade		
Low grade	3	6.8
High grade	41	93.2
Lymph node status		
pN0	34	77.3
pN1	10	22.7
Residual tumor status		
R0	36	81.8
R1	8	18.2
Adjacent carcinoma *in situ*		
No pTis	18	40.9
pTis	26	59.1
Multifocality		
Unifocal tumor	12	27.3
Multifocal tumor	32	72.7
Growth pattern		
Papillary	8	18.2
Solid	22	50.0
Mixed	14	31.8

Immunohistochemistry (IHC). Immunohistochemical studies for the expression of TP53, CK20 and MIB1 utilized an avidin-biotin peroxidase method with a 3,3'-diaminobenzidine (DAB) chromatogen. After antigen retrieval (microwave oven for 30 min at 250 W), immunohistochemistry was carried out in a NEXES immunostainer (Ventana, Tucson, AZ) following manufacturer's instructions. The following primary antibodies were used: anti-TP53 [mouse monoclonal IgG, clone Bp53-12 (sc-263), Santa Cruz Biotechnology, Inc., Santa Cruz, CA; dilution 1:1,000], anti-CK20 [mouse monoclonal IgG2a, clone IT-Ks20.8 (61026), Progen Biotechnik GmbH, Heidelberg, Germany; dilution 1:10], and anti-Ki-67 [mouse monoclonal IgG1, clone MIB-1 (M7240), Dako, Glostrup, Denmark; dilution 1:50]. One surgical pathologist (A.H.) performed the evaluation of the slides without knowledge of clinical data. Causes of non-interpretable results included lack of tumor tissue and presence of necrosis or crush artifact. Cut-off levels for TP53 and MIB1 were defined at 10%. Cytokeratin 20 (CK20) is a sensitive marker of urothelial differentiation. CK20 staining was defined as normal (superficial staining pattern) or abnormal (negative or ≥10% stained) according to Harnden *et al* (14).

STK15 gene amplification detected by FISH. STK15 FISH analysis of the cystectomy TMA was performed according to Sen *et al* (8). BAC-clones for probe preparation were purchased from RZPD (Deutsches Ressourcenzentrum fuer Genomforschung GmbH, Berlin). After cultivation, harvest and DNA isolation Nick translation was performed, incorporating commercially available (Abbott Laboratories, Abbott Park, IL) Spectrum Orange dye into the gene probe. The spectrum green labeled centromeric probe (chromosome 20) was purchased from Abbott Laboratories. FISH was performed as described below.

UroVysion FISH. Additionally, UroVysion fluorescent probe kits (Abbott Laboratories) were used according to manufacturer's instructions to assess aberrations of chromosomes 3, 7 and 17 by centromeric probes and to detect deletions of p16 on locus 9p21 of specimens on the cystectomy TMA. In brief, 6 μm dewaxed tissue sections of the TMA were treated with proteinase K for 15 min at 37˚C, followed by washing and ethanol dehydration (75, 85 and 100%). Slides were dried at 37˚C and then heat denatured (73˚C for 15 min). For hybridization, 14 μl of the original kit were used per slide. Slides were covered by a cover glass and fixogumm and heated at 96˚C for 9 min. This procedure was followed by an incubation at 37˚C for at least 12 h. After resolving the cover glass using 2x SSC/0.3% NP40 solution, DAPI nuclear counter staining was carried out. For each case 50 nuclei were selected for scoring according to morphological criteria using DAPI staining. Only non-overlapping intact nuclei of urothelial cells were scored.

Scoring of FISH signals. All hybridizations were evaluated independently by two investigators (N.E. and P.J.W.) and the mean of both counts was used. Low level *STK15* amplification was defined if the ratio *STK15*/chromosome 20 was at least >2.0. A sample was considered carrying a high-level *STK15* amplification if the ratio *STK15*/chromosome 20 was >4.0.

UroVysion FISH simultaneously analyzed each cell for centromer signals of chromosomes 3, 7 and 17, and for the p16 locus on 9p21. A cell was considered aberrant if at least one of three centromeric signals was amplified (>2 signals per cell), or if 9p21 was deleted. Polyploid cells (4 signals of all the three probes) were regarded normal (euploid). A relative deletion of the p16 locus was recognized if the signal number of 9p21 was >1 unit lower than the mean value of the centromeric signals. Frequencies of polysomy and deletions of 9p21 in non-tumor associated urothelium (data not shown) defined a cut-off value of three times standard deviation. A case was considered aberrant if >9 cells out of 50 showed polysomy (>18% of the cells). A sample was considered carrying a deletion of p16 if >7 out of 50 cells (>14% of the cells) showed a relative deletion of locus 9p21.

SNP analysis. Genomic DNA of patients on the TMA was isolated from 5 μm tissue paraffin sections. For molecular analysis, DNA was extracted using the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) according to the manufacturer's specifications. Genotypes of STK15 at the T91A (Phe31Ile) site were analyzed by PCR-RFLP assay. The primers for PCR were: sense 5'-CTC AATATATTCATCTTTTGC-3', antisense 5'-AGGACAC AAGACCCGCTG-3'. Amplification was accomplished with a 25 μl reaction mixture containing 100 ng DNA, 0.2 mmol/l dNTPs, 0.3 μmol/l primers, 1.5 mmol/l $MgCl_2$ and 0.5 U GoTaq® DNA polymerase (Promega GmbH, Mannheim, Germany). The reaction mixture was subjected to 3 min of denaturing at 95˚C and 35 cycles of 95˚C for 1 min, specific annealing temperature of 50˚C for 1 min and 72˚C for 1 min, followed by a final extension step at 72˚C for 10 min. PCR conditions were optimized by gradient PCR and were carried out in a MJ Research Thermocycler (PTC100). The 170 bp PCR products were then digested with ApoI (New England BioLabs, Beverly, MA), separated on a 2.5% agarose gel and visualized under UV light by using 0.05% ethidium bromide. The *Phe* allele had no *Apo*I restriction site and resulted in one band (170 bp), whereas the *Ile* allele had one *Apo*I restriction site and thus produced two bands (109+61 bp). Specificity of genotyping using PCR-RFLP assay was confirmed by DNA sequencing of urothelial cell lines with 100% reproducibility (data not shown).

Statistical analysis. Statistical analyses were completed using SPSS version 10.0 (SPSS, Chicago, IL). P-values <0.05 were considered significant. Contingency table analysis and two-sided Fisher's exact tests were used to study the statistical association between clinicopathologic, molecular and immunohistochemical data. Survival curves comparing patients with or without any of the factors were calculated using the Kaplan-Meier method, with significance evaluated by two-sided log-rank statistics. For recurrence-free survival, patients were censored at time of their last tumor-free clinical follow-up appointment. For tumor-specific survival, patients were censored at the time of their last clinical follow-up appointment or at their date of death not related to the tumor. A multivariable Cox regression model concerning recurrence-free survival was adjusted, testing the independent prognostic relevance of STK15 low level amplification in matching histologically benign urothelium of patients with invasive urothelial bladder cancer receiving cystectomy.

Results

Immunohistochemistry. Results of immunohistochemical analyses in tumor tissue and matching histologically benign urothelium are summarized in Table II. A pical expression of the dedifferentiation marker CK20 was seen in 76.5% (26/34) of informative cases; whereas, 23.5% of histologically benign samples (8/34) and 100% (44/44) of invasive carcinomas revealed abnormal CK20 staining pattern.

Invasive tumors (48.8%, 21/43) and 13.2% (5/38) of histologically benign urothelium specimens showed positive TP53 immunoreactivity in at least 10% of nuclei. Representative CK20 and TP53 immunostaining patterns in urothelial carcinomas and matching histologically benign urothelium are shown in Fig. 1. Interestingly, 29.7% (11/37) of histologically benign samples and 100% (44/44) of carcinomas demonstrated a Ki-67 proliferation fraction of at least 10%.

UroVysion FISH. A sample was considered aneuploid, if >18% of the cells displayed polysomy of one or more chromosomes (see Materials and methods). Invasive urothelial carcinomas (90.9%, 40/44) and 2.8% (1/36) of histologically benign samples showed polysomy of at least one chromosome. A relative deletion of 9p21 was found in 3 of 34 (8.8%) histologically benign urothelium specimens and in 45.5% (20/44) of invasive tumors, respectively.

STK15 FISH. Investigation of *STK15* amplification in a series of urothelial bladder cancers and histologically benign urothelium using TMA technology was informative in 100% (44/44) and 82% (36/44) of cases, respectively. Fig. 2 shows

Table II. Results of molecular and immunohistochemical analyses in tumor tissue and matching normal urothelium.

	Normal urothelium		Urothelial cancer	
Variable	n analyzable	%	n analyzable	%
Immunohistochemistry (IHC)				
Ki-67 labeling index				
<10%	26	70.3	0	0
≥10%	11	29.7	44	100
p53 IHC				
<10%	33	86.8	22	51.2
≥10%	5	13.2	21	48.8
CK20 IHC				
Superficial staining pattern	26	76.5	0	0
Negative or ≥10%	8	23.5	44	100
UroVysion FISH				
Polysomy				
Negative	35	97.2	4	9.1
Positive	1	2.8	40	90.9
p16 deletion				
Negative	31	91.2	24	54.5
Positive	3	8.8	20	45.5
STK15 FISH				
No amplification	34	94.4	0	0
Low level amplification	2	5.6	28	63.6
Amplification	0	0	16	36.4

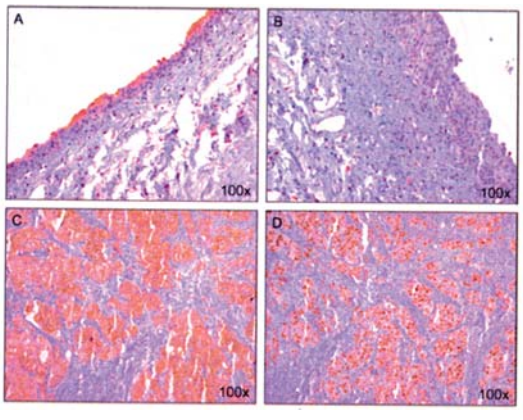

Figure 1. Immunohistochemical staining of histologically benign urothelium and invasive urothelial bladder cancer in the cystectomy tissue microarray-representative examples. Histologically benign urothelium with normal CK20 staining pattern (A) and negative TP53 immunoreactivity (B). Invasive high-grade urothelial bladder cancer with abnormal CK20 staining pattern (C) and positive TP53 immunoreactivity (D).

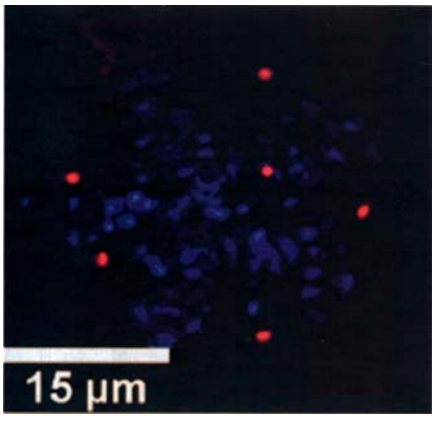

Figure 2. Example of *STK15* FISH analysis. A bladder cancer nucleus with a star-shaped atypical mitosis and six red *STK15* gene signals (DAPI nuclear counterstaining).

a bladder cancer nucleus with a star-shaped atypical mitosis and six red STK15 gene signals. In 2 of 36 (5.6%) histologically benign urothelium samples, low level *STK15* amplification was found. Low level *STK15* amplification was detected in 64% (28/44) of bladder cancers, whereas 36% (16/44) of cancer lesions demonstrated a high level amplification of the *STK15* gene. Results of FISH analysis are summarized in Table II. Descriptive data analysis comparing all relevant variables with STK15 amplification status in tumor tissue failed to show significant associations (Table III).

Prognostic relevance. Tumor-specific and recurrence-free survival was compared between cases with and without *STK15* amplification by univariate log-rank statistics. Low level *STK15* amplification in histologically benign urothelium of patients with urothelial bladder cancer was associated with shorter tumor-specific survival (Fig. 3A).

Table III. *STK15* FISH analysis in urothelial bladder cancer in relation to clinicopathologic, immunohistochemical and FISH data.

Variable	*STK15* FISH analysis			
	n analyzable	Low level amplification	Amplification	P-value[a]
Clinicopathologic data				
Age when cystectomy was performed (median, range)				
≤70 years	24	17	7	0.352
>70 years	20	11	9	
Gender				
Female	14	7	7	0.313
Male	30	21	9	
Tumor stage				
pT1	12	9	3	0.579
pT2	13	9	4	
pT3	14	7	7	
pT4	5	3	2	
Histologic grade				
Low grade	3	3	0	0.290
High grade	41	25	16	
Lymph node status				
pN0	34	20	12	1.000
pN1	10	3	2	
Adjacent carcinoma *in situ*				
No pTis	18	10	8	1.000
pTis	26	18	8	
Multifocality				
Unifocal tumor	12	9	3	0.487
Multifocal tumor	32	19	13	
Growth pattern				
Papillary	8	6	2	0.153
Solid	22	16	6	
Mixed	14	6	8	
Immunohistochemistry (IHC)				
TP53 IHC				
<10%	23	14	8	1.000
≥10%	21	13	8	
UroVysion FISH				
Polysomy				
Negative	4	4	0	0.280
Positive	40	24	16	
p16 deletion				
Negative	24	16	8	0.757
Positive	20	12	8	

[a]Fisher's exact test, two-sided.

Patients with low level *STK15* amplification in histologically benign urothelium had an estimated mean tumor-specific survival of nine months (95% confidence interval 3-15) compared to 99 months (95% confidence interval 84-113) in cases without *STK15* amplification. *STK15* amplification status in histologically benign urothelium of patients with urothelial bladder cancer was also associated with shorter recurrence-free survival (Fig. 3B). In contrast, there was no

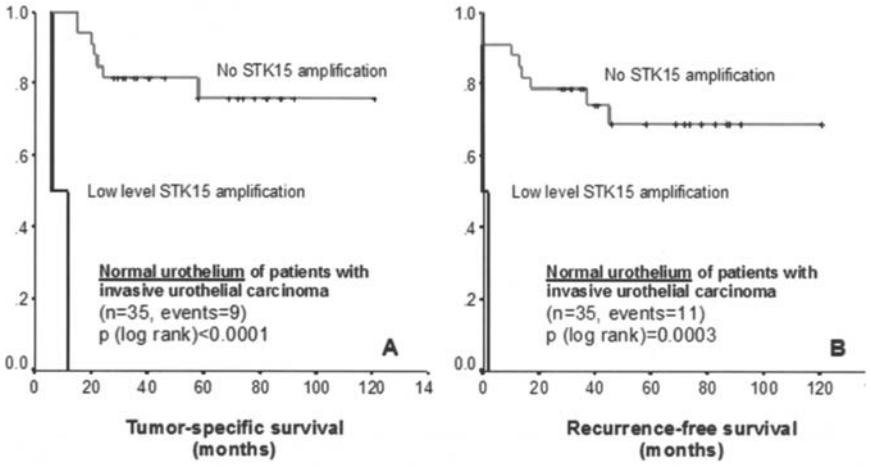

Figure 3. Distributions of time (months) to tumor-related death (A) and tumor recurrence (B) among 35 bladder cancer patients following cystectomy relative to *STK15* gene amplification in histologically benign urothelium adjacent to the tumor, as estimated by the method of Kaplan Meier.

Table IV. Tumor-related death and tumor recurrence.

A. Univariate analysis of factors regarding tumor-related death and tumor recurrence in patients with invasive urothelial bladder cancer treated with cystectomy

	Tumor-related death			Tumor recurrence		
Variable	n	Events	P-value[a]	n	Events	P-value[a]
Clinico-pathologic data						
Age when cystectom was performed						
≤70 years	24	4	**0.0270**	23	6	0.1087
>70 years	20	9		20	9	
Gender						
Female	14	6	0.1039	30	10	0.5413
Male	30	7		13	5	
Tumor stage						
pT1-2	25	3	**0.0020**	25	4	**0.0017**
pT3-4	19	10		18	11	
Histologic grade						
Low grade	3	0	0.2776	3	0	0.2216
High grade	40	13		40	15	
Lymph node status						
pN0	34	8	**0.0253**	32	10	0.0635
pN1	10	3		5	3	
Residual tumor status						
R0	36	11	0.9789	32	11	0.4662
R1	8	2		6	3	
Adjacent carcinoma *in situ*						
No pTis	18	8	0.2166	18	8	0.4814
pTis	26	5		25	7	
Multifocality						
Unifocal tumor	12	4	0.7360	12	6	0.1950
Multifocal tumor	32	9		31	9	
Growth pattern						
Papillary	8	2	0.4541	8	3	0.3688
Solid	21	8		21	9	
Mixed	14	3		14	3	

Table IV. Continued.

B. Univariate analysis of factors regarding tumor-related death and tumor recurrence in tumor tissue

Variable	Tumor-related death n	Tumor-related death Events	Tumor-related death P-value[a]	Tumor recurrence n	Tumor recurrence Events	Tumor recurrence P-value[a]
Invasive urothelial bladder cancer						
Immunohistochemistry (IHC)						
TP53 IHC						
<10%	21	3	**0.0418**	21	5	0.1460
≥10%	21	9		21	9	
UroVysion FISH						
Polysomy						
Negative	4	2	0.4680	4	3	0.1013
Positive	39	11		39	12	
p16 deletion						
Negative	23	6	0.6112	23	6	0.2297
Positive	20	7		20	9	
STK15 FISH						
Low level amplification	27	7	0.5295	27	9	0.9160
Amplification	16	6		16	6	

C. Univariate analysis of factors regarding tumor-related death and tumor recurrence in normal urothelium

Variable	Tumor-related death n	Tumor-related death Events	Tumor-related death P-value[a]	Tumor recurrence n	Tumor recurrence Events	Tumor recurrence P-value[a]
Adjacent normal urothelium						
Immunohistochemistry (IHC)						
Ki-67 labeling index						
<10%	25	6	0.8143	25	7	0.2459
≥10%	11	3		11	5	
TP53 IHC						
<10%	33	8	0.9073	33	10	0.4236
≥10%	4	1		4	2	
CK20 IHC						
Superficial staining pattern	25	8	0.2799	25	11	0.1242
Negative or ≥10%	8	1		8	1	
UroVysion FISH						
Polysomy						
Negative	34	9	0.5621	34	11	0.3135
Positive	1	0		1	1	
p16 deletion						
Negative	30	8	0.9050	30	10	0.9962
Positive	3	1		3	1	
STK15 FISH						
No amplification	33	7	**<0.0001**	33	9	**0.0003**
Low level amplification	2	2		2	2	

[a]Log-rank test, two-sided; bold face represents significant data.

difference in recurrence-free or overall survival in patients with low-level or high-level STK15 amplification in the tumor. Age at diagnosis >70 years, initial tumor stage pT3, positive lymph node status and positive TP53 immuno-reactivity in the tumor were significantly associated with shorter tumor-specific survival. None of the other clinicopathologic, genetic or immunohistochemical factors correlated significantly with tumor-specific survival (Table IV). Only tumor stage

Table V. Multivariate analysis of factors possibly influencing tumor recurrence (n=35).

Variable	Categorization	Stepwise reverse selection		
		Hazard ratio	95% CI	P-value
Tumor stage				
0	pT1-2	4.255	1.059-17.089	**0.041**
1	pT3-4			
STK15 FISH in normal urothelium				
0	No amplification	6.266	0.990-39.668	0.051
1	Low level amplification			

Bold face represents P-values <0.05.

Table VI. *STK15* SNP analysis in relation to *STK15* gene amplification in urothelial bladder cancers and adjacent normal urothelium.

Variable	*STK15* SNP analysis				
	n analyzable	Homozygous (T/T)	Heterozygous (A/T)	Homozygous (A/A)	P-value[a]
Invasive urothelial bladder cancer					
STK15 FISH					
Low level amplification	26	18	7	1	0.248
Amplification	16	8	8	0	
Adjacent normal urothelium					
STK15 FISH					
No amplification	33	23	9	1	0.150
Low level amplification	2	0	2	0	

[a]Fisher's exact test.

≥pT3 and low level STK15 amplification in histologically benign urothelium were significantly associated with recurrence-free survival.

Multivariate analyses utilized a Cox proportional hazards model to assess recurrence-free survival rate relative to tumor stage and *STK15* amplification in histologically benign urothelium (Table V). As expected, higher tumor stages were significantly correlated with shorter recurrence-free survival. The probability of tumor recurrence was four times higher in post-cystectomy patients with stage pT3 or pT4 bladder cancer than that in patients with stage pT1 or pT2 cancers. Low level *STK15* amplification in histologically benign urothelium showed a trend towards significance regarding tumor recurrence in our small patient cohort (n=35).

SNP analysis. The *STK15* T91A polymorphism analysis was informative in 42/44 cases (DNA from two cases showed no amplification products). Polymorphic alleles segregated as TT (62%), AT (36%), and AA (2%). This allelic distribution was compared to large case-control studies in healthy Caucasians (11), which showed polymorphism frequencies similar to those in our series. There was no correlation between allelic variants and high/low level of *STK15* gene amplification (Table VI).

Discussion

One significant risk factor in bladder cancer is DNA aneuploidy of tumor cells. Patients with aneuploid pTa and pT1 urothelial tumors had a higher rate of recurrence and poor clinical outcome (15). Invasive tumors frequently display widespread chromosome aneuploidy and extensive genetic alterations, e.g. defective *TP53* and chromosome 20q amplifications (16,17). Amplification of chromosome 20q may be a critical step in tumorigenesis since it is associated with invasion in urothelial cancers, squamous cell carcinoma of the cervix, and breast cancer (18,19). Recently, several

studies analyzed the role of *STK15* within chromosome 20q in urothelial bladder cancer. Overexpression of *STK15* showed a strong correlation with stage and grade of pTa/pT1 tumors and was an independent predictor of tumor recurrence (7).

This study shows low level *STK15* amplification in 5.6% of histologically benign urothelium of bladder cancer patients. Most associated tumors were classified as stage pT3G3 urothelial bladder cancers with solid growth pattern and TP53 overexpression. One cancer was multifocal with adjacent carcinoma *in situ* (pTis), and another was node positive (pN1) with solitary growth pattern. Most striking is that low level *STK15* amplification in histologically benign urothelium of bladder cancer patients was associated with shorter recurrence-free and tumor-specific survival following cystectomy. Functional studies revealed *STK15* as a key regulatory component of the TP53 pathway, and overexpression of STK15 leads to increased degradation of TP53, which in turn causes downregulation of checkpoint-response pathways and facilitating oncogenic transformation of cells (20). *In vitro* studies showed enhanced chromosomal instability in bladder cancer cell lines overexpressing STK15 (9). Fraizer *et al* suggested that overexpression of *STK15* in bladder tumor cells contributes to tumor progression by promoting chromosomal instability and subsequent aneuploidy (9). *STK15* gene amplification and protein expression have been linked to aneuploidy of bladder cancer and clinical tumor aggressiveness. Tumors with low level *STK15* amplification (3-4 copies) showed minimal deviation in their chromosome copy number and diploid or near-diploid total nuclear DNA content. Tumors with higher levels of *STK15* amplification (>4 copies) had a major increase in both chromosome copy number and total nuclear DNA content; i.e. exhibited pronounced aneuploidy. Overexpression of STK15 was strongly associated with high histologic grade, invasion, increased rate of metastasis, and decreased metastasis-free and overall survival of patients (8). No significant association with TP53 expression, *p16* deletions or polysomy was found in our study (Table III).

The primary indication for cystectomy is muscle-invasive bladder cancer. Other relative indications are high-risk urothelial neoplasia (pT1 high-grade, BCG-resistant pTis), and extensive non-invasive papillary bladder tumor disease (pTa high-grade) that cannot be controlled through conservative measures. Management of stage pT1G3 bladder cancer is still controversial (21), and it is far from certain whether TP53 status can help to select pT1 tumor patients who may benefit from early cystectomy. Recent studies on FGFR3 and TP53 mutations failed to predict, alone or in combination, recurrence or survival of pT1G3 tumor patients (22,23). In our study, TP53 expression (≥10%) was associated with shorter tumor-related survival (Table IV, B).

The allelic distribution of the functional Phe31Ile polymorphism of *STK15* affected cancer risk in several tumor types (11), and most studies found the homozygous 31Ile variant to associate with aggressive and invasive tumors. Our own analysis suggests no association of the Phe31Ile polymorphism with cancer risk or tumor ploidy in this limited sample. The *STK15* Phe31Ile polymorphism may not play a role in tumors of the genitourinary tract, similar to data obtained from renal cell carcinomas (24). Gu *et al* found a reduction of lung cancer risk of the homozygous 31Ile variant in Caucasians (25), underscoring a probably complex and cancer-type specific modification of cancer risk by *STK15* variants. More studies are needed to clarify the role(s) of functional *STK15* variants.

Applying *STK15* FISH to benign urothelium of bladder cancer patients may help to identify patients at increased risk for adverse clinical outcome. A large randomized prospective study comparing early versus delayed cystectomy in patients with pT1 bladder cancer is currently conducted to validate our findings.

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