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Predicting Recurrence and Progression of Noninvasive Papillary Bladder Cancer at Initial Presentation Based on Quantitative Gene Expression Profiles

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Abstract

Background—Currently, tumor grade is the best predictor of outcome at first presentation of noninvasive papillary (Ta) bladder cancer. However, reliable predictors of Ta tumor recurrence and progression for individual patients, which could optimize treatment and follow-up schedules based on specific tumor biology, are yet to be identified.

Objective—To identify genes predictive for recurrence and progression in Ta bladder cancer at first presentation using a quantitative, pathway-specific approach.

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Design, setting, and participants—Retrospective study of patients with Ta G2/3 bladder tumors at initial presentation with three distinct clinical outcomes: absence of recurrence ($n = 16$), recurrence without progression ($n = 16$), and progression to carcinoma in situ or invasive disease ($n = 16$).

Measurements—Expressions of 24 genes that feature in relevant pathways that are deregulated in bladder cancer were quantified by real-time polymerase chain reaction on tumor biopsies from the patients at initial presentation.

Results and limitations—*CCND3* ($p = 0.003$) and *HRAS* ($p = 0.01$) were predictive for recurrence by univariate analysis. In a multivariable model based on *CCND3* expression, sensitivity and specificity for recurrence were 97% and 63%, respectively. *HRAS* ($p < 0.001$), *E2F1* ($p = 0.017$), *BIRC5/Survivin* ($p = 0.038$), and *VEGFR2* ($p = 0.047$) were predictive for progression by univariate analysis. Multivariable analysis based on *HRAS*, *VEGFR2*, and *VEGF* identified progression with 81% sensitivity and 94% specificity. Since this is a small retrospective study using medium-throughput profiling, larger confirmatory studies are needed.

Conclusions—Gene expression profiling across relevant cancer pathways appears to be a promising approach for Ta bladder tumor outcome prediction at initial diagnosis. These results could help differentiate between patients who need aggressive versus expectant management.

Keywords

Noninvasive urothelial carcinoma; First presentation; Recurrence; Progression; *CCND3*; *HRAS*; *E2F1*; *Survivin*; *VEGFR2*; *VEGF*

1. Introduction

Urothelial carcinoma (UC) of the urinary bladder is the ninth most common cancer worldwide, accounting for 3% of the global cancer incidence [1]. Most UC cases present at first occurrence as urothelium-confined tumors (noninvasive Ta UC) [2]. After initial diagnosis, some patients with Ta tumors will never have a recurrence, but 50–70% of patients will reexperience a Ta tumor within 5 yr and 10% will progress to invasive disease [3,4]. This diverse biologic behavior compels current guidelines to recommend intense follow-up and invasive treatment [5]. Therefore, it is crucial to determine the recurrence and invasive potential of these tumors. Predicting such behavior is clinically important as invasion bears a significant risk of metastasis and impaired survival [6-8].

While grade and number of foci are the best estimators of subsequent Ta tumor behavior at first diagnosis [4], they are relatively imprecise measures for an individual patient. For monitoring, traditional noninvasive tests have clear limitations and the more reliable invasive techniques such as cystoscopy and biopsy cause patient discomfort and incur substantial costs [9]. Despite efforts to identify molecular markers, no single determinant has changed clinical management of Ta tumors. This study used a pathway-specific approach to profile 24 genes using real-time quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) in primary Ta G2/3 UC tumor biopsies. This unique cohort was composed of frozen primary UC tissues obtained at first presentation from three equally-sized patient groups with noninvasive papillary tumors who (1) did not recur after long-term follow-up, (2) recurred locally without progressing after long-term follow-up, and (3) later progressed to carcinoma in situ (CIS) or higher stage. While we recognize that tumorigenesis involves accumulation of several genetic mutations over time [10], the purpose of this study was to examine if molecular alterations in primary UC tumors at the time of first overt clinical presentation can predict eventual recurrence and/or progression.

2. Patients and methods

2.1. Patient cohort

The entire study population consisted of 177 patients diagnosed with first occurrence of Ta G2/3 UC at Herlev Hospital, University of Copenhagen, Denmark, between March 1993 and November 2004. Tumors were staged and graded according to standard criteria [11,12]. The study cohort included three groups (n = 16 each) based on distinct clinical outcomes: group 1 included patients without recurrence; group 2 included patients with recurrence but without progression; group 3 included patients with progression. Recurrence was defined as one or more relapses of Ta tumor without CIS after initial presentation. Progression was defined as one or more relapses after initial presentation where CIS or invasive disease (T1 or higher) was identified. Follow-up was at least 5 yr in the first two groups (median: 7.9 yr). In addition to first tumor occurrence and the minimum follow-up period, other inclusion criteria were absence of concomitant CIS, and no administration of systemic or intravesical immuno- or chemotherapy at first presentation. Twenty-three, 39, and 16 patients met the inclusion criteria for the three groups, respectively; 16 patients were randomly selected from the first two groups to achieve equally sized subcohorts.

Cold-cup biopsies were taken during white-light cystoscopy at initial tumor occurrence prior to any intervention. Cold-cup biopsies were preferred over transurethral resection of bladder tumor (TURBT) samples for RNA extraction to ensure exclusive tumor content and avoid thermal artifacts that could potentially compromise RNA quality [13]. Samples were frozen, embedded in optimal cutting temperature compound, and stored at −80°C. All patients were treated with TURBT alone at initial presentation. Random quadrant biopsies were taken in all cases; three patients in group 3 had concomitant Ta tumors. Patients were followed by cystoscopy and cytology every 3 mo in the first year. If tumors did not recur, patients were then followed by cystoscopy every 6–12 mo for at least 5 yr.

The study was approved by the respective institutional review boards. Informed consent was obtained in all cases.

2.2. Expression profiling

Twenty-four genes that feature in biologically relevant cellular processes in bladder and other cancer types and that are associated with major pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG) were chosen for analysis (Fig. 1) [14-18]. Gene expression levels were analyzed by qRT-PCR from each biopsy specimen blinded to patient history and clinicopathologic information (Table 1).

Presence of at least 90% UC tissue in each specimen was confirmed on hematoxylin and eosin-stained slides. Embedded tissues were sectioned and RNA extracted using the Bio-Rad PureZOL RNA isolation kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA) following manufacturer s instructions. RNA yield and purity were determined by A_{254} optical density measurements and $A_{254/280}$ and $A_{254/230}$ ratios. After complementary DNA synthesis using the Bio-Rad iScript kit, an RT-PCR for β-actin with product visualization on agarose gel was performed. Quantitative RT-PCR reactions were performed on the Stratagene Mx3000P thermocycler (Stratagene, La Jolla, CA, USA) with TaqMan Expression assays (Biosearch Technologies, Novato, CA, USA). Primers and probes were designed using Primer Express 2.0 (Applied Biosystems, Foster City, CA, USA) or obtained from published literature [19, 20], and crossed at least one intron–exon junction, thereby minimizing amplification of contaminating genomic DNA. Expression levels were normalized against *TBP* and *SDHA*, which are constitutively expressed genes that are appropriate qRT-PCR reference transcripts for UC [19]. All measurements were made in triplicates. For each primer–pair/probe

combination, reaction efficiency estimates were derived from standard curves generated using serial dilutions of RNA from the RT4 cell line. Efficiencies were between 89% and 104% and between 94% and 95% for interrogated and reference genes, respectively. Relative quantification ($\Delta\Delta C_t$) was employed to normalize raw C_t values using the geometric mean of expression of both reference genes [21]. Thus, transcript expressions were reported as fold changes compared to the reference genes, and were quantitative and reproducible.

2.3. Statistics and outcome analysis

Univariate analysis was performed using Wilcoxon rank-sum test in three steps. First, differences in expression levels of each individual gene among the three groups were evaluated. Second, to identify associations with recurrence, patients without recurrence (group 1) were compared to patients with recurrence (without/with progression; groups 2 and 3). Third, to identify associations with progression, patients without progression (without/with recurrence; groups 1 and 2) were compared to patients with progression (group 3) (Fig. 2). The permutation method was used to obtain *p* values adjusted for multiple testing [22]. To identify genes that, in combination, could predict recurrence or progression, a multivariable, nonparametric, recursive partitioning (RP) analysis was performed. Specifically, a classification and regression tree model using RPART, an S-plus function, was constructed [23]. In this process, the entire cohort was divided into subgroups with the greatest dissimilarities in clinical outcome based on gene expression levels. The trees were validated using 100 bootstrap samples.

3. Results

3.1. Clinicopathologic parameters and clinical outcome

The median patient age was 67.5 yr (range: 29–86 yr), and median follow-up was 87.2 mo (range: 10.3–149.6 mo). Median age, tumor grade, and multifocality were comparable across the groups (Table 2). Tumor staging and grading of the subsequent TURBT specimen was identical to biopsy in all cases. Patients in group 2 had a median of four recurrences, with median time to first recurrence of 11.3 mo. Median time to first progression was 31.8 mo (range: 3.3–94.7 mo), including progression to CIS ($n = 3$), T1 ($n = 12$), and T3a ($n = 1$). Median time to first clinically significant event (ie, either recurrence or progression, whichever came first) for group 3 patients was 7.4 mo. At last follow-up, 35 patients were alive, 2 patients in group 3 had died of UC, and 11 patients had died from other causes without evidence of UC.

When clinicopathologic parameters were input into the European Organization for Research and Treatment of Cancer (EORTC) recurrence calculator, all patients scored between 1 and 5, indicating an intermediate risk of recurring (5-yr recurrence probability 46–62%) [4]. When the factors were assessed against the EORTC progression calculator, 37 patients were at low risk for progression while 11 patients were at intermediate-high risk (5-yr progression probability 0.8% vs 6–17%, respectively). The progression calculator labeled four patients who did not eventually progress as candidates at intermediate risk for progression (88% specificity) and nine patients who eventually progressed as low risk candidates (44% sensitivity) (Table 2).

3.2. Comparison of gene expression levels among the three patient groups

Differences in gene expression levels were first evaluated across the individual patient groups. Expression levels were significantly different for *HRAS* ($p = 0.002$), *CCND3* ($p = 0.009$), *BCL2L1* ($p = 0.039$), and *E2F1* ($p = 0.047$) and showed a trend towards significance for *TP53* ($p = 0.051$) by univariate analysis (Fig. 3a, Table 3). These genes could therefore independently differentiate among the three outcome categories.

3.3. Identification of genes predictive for recurrence

Gene expression levels in patients without recurrence (group 1) were compared with those in patients with recurrence (without/with progression; groups 2 and 3). By univariate analysis, *CCND3* ($p = 0.003$) and *HRAS* ($p = 0.01$) were significantly lower in patients with recurrence (Fig. 3b, Table 3). By multivariate RP analysis, *CCND3* was an independent predictor of recurrence. Ninety-seven percent of patients who recurred had low *CCND3* expression (sensitivity), while 63% of patients without recurrence had high expression levels (specificity) (Fig. 4a). The model proved robust in this cohort with *CCND3* appearing in 54% of bootstrap validation samples.

3.4. Identification of genes predictive for progression

Gene expression levels in patients without progression (without/with recurrence; groups 1 and 2) were compared to those in patients with progression (group 3). By univariate analysis, progression was significantly associated with decreased *HRAS* expression ($p < 0.001$), and increased expression of *E2F1* ($p = 0.017$), *BIRC5/Survivin* ($p = 0.038$), and *VEGFR2* ($p = 0.047$) (Fig. 3c, Table 3). In multivariate RP analysis, the first split was based on *HRAS* expression (Fig. 4b). All patients with low *HRAS* developed progression. *HRAS* inclusion in the RP analysis was robust with it being part of 70% of bootstrap samples. Among patients with high *HRAS*, those with low *VEGFR2* had the lowest probability of progression. However, for patients with high *HRAS* and *VEGFR2, VEGF* expression provided another tier of discrimination for progression probabilities. In this subgroup, patients with high *HRAS, VEGFR2*, and *VEGF* had a 75% probability of progressing, compared with 15% probability in patients with high *HRAS* and *VEGFR2*, and low *VEGF* levels. While these tier additions were compelling, *VEGFR2* and *VEGF* appeared in only 19% and 5% of bootstrap samples, respectively. This model correctly identified 81% of patients with progression (sensitivity) and 94% of patients without progression (specificity).

4. Discussion

This study used a quantitative, reproducible, gene expression profiling approach while choosing an efficient case-control patient-selection design to specifically represent distinct and important clinical outcomes after first occurrences of Ta UC. Two genes were identified by univariate analysis (*CCND3, HRAS*), one of which was also identified by multivariable analysis (*CCND3*) to significantly predict recurrence. Four genes were identified by univariate analysis (*HRAS, E2F1, BIRC5, VEGFR2*) to significantly predict progression; three were also identified by multivariable analysis (*HRAS, VEGFR2, VEGF*) for this outcome measure. *CCND3* and *HRAS* were particularly robust predictors of recurrence and progression, respectively. Tumors that progressed displayed molecular characteristics of invasive disease at first presentation (ie, activation of angiogenesis and decreased activity of the Ras–mitogen-activated protein kinase pathway). This study therefore identified genes that could, either individually or in combination, significantly predict recurrence (two genes) and progression (five genes) in patients with noninvasive papillary UC at first presentation with high sensitivity and specificity and better than standard clinicopathologic criteria. Current methods have limited reliability in predicting biological behavior of individual Ta UCs at first presentation. Therefore, all patients, especially those with G2/3 tumors, are treated by TURBT, often followed by intravesical therapy; and frequent, expensive, and invasive surveillance procedures. In our study cohort, EORTC risk calculators performed poorly in predicting recurrence, classifying all patients to the same intermediate risk category. It performed better for progression, but still missed most patients who eventually progressed, resulting in modest specificity but low sensitivity. The clinical implications are clear: Reliable identification of patients who will not recur, and more importantly, those who will not progress, can lead to optimization of follow-up schedules and personalization of adjuvant treatment strategies.

Rather than evaluating expression levels between different tumor stages, we sought to identify genes that predicted outcome in a unique cohort that was very homogenous through defined patient selection. We compared patients with similar clinicopathologic characteristics at initial diagnosis who subsequently experienced distinct clinical courses: no recurrence, recurrence without progression, and progression. Due to the treatment regimen followed at time of diagnosis, no patient received systemic, intravesical, or adjuvant therapy at first tumor occurrence. This implies that the genes may not only predict tumor behavior, but can also identify patients who could benefit from additional therapy (ie, those at risk for recurrence or progression).

T1 tumors were excluded because combined analysis of Ta and T1 UC as “superficial” disease is not a valid approach [6,7]. Furthermore, the cohort’s long follow-up ensured that patients in groups 1 and 2 would very likely never recur or progress, respectively. These results support the view that multiple distinct pathways are responsible for the biological behavior of UC [15]. Ta tumors that became invasive harbored molecular alterations characteristic of aggressive behavior at first presentation. They had decreased *HRAS* and increased *VEGF* and *VEGFR2* expressions. These genetic changes have been associated with invasive tumors [17]. Although the prognostic role of *HRAS* is unclear, activating mutations are more common in low-grade/low-stage disease than invasive disease [17,24,25]. Intriguingly, while *FGFR3* mutations have been documented in Ta tumors [15], we did not observe any significant association of its expression levels with prognosis in our cohort. Activation of proangiogenic factors is a rate-limiting step in neoplastic progression as the tumor develops its own blood supply. In melanoma, ovarian carcinoma, prostate carcinoma, and colon carcinoma, overexpression of VEGF and its receptor VEGFR2 are associated with tumor progression and poor prognosis [26,27]. VEGFR2 is also overexpressed in muscle-invasive and advanced UC [26]. This is also consistent with our findings that *VEGFR2* expression is predictive for nodal metastasis in UC [28]. The significant association of survivin expression with Ta UC outcome is also supported by previous studies that show its ability to predict recurrence without controlling for progression as a distinct outcome parameter [20].

The role of cyclin D3 (encoded by *CCND3*) in Ta/T1 UC progression has been previously demonstrated [29,30]. Our results add an interesting aspect from a biological and mechanistic viewpoint: Decreased *CCND3* expression identified patients with recurrence. As activation of this pathway has been linked with invasive disease, we expected a more important role in progression and *CCND3* to be decreased in nonrecurrent tumors [31,32]. However, when the three groups were compared individually, the transcription factor *E2F1*, which is functionally associated with *CCND3*, was expressed the lowest in nonrecurring tumors ($p = 0.047$). Thus, although *CCND3* is upregulated in nonrecurring tumors, decreased *E2F1* may potentially subvert *CCND3* activity, making it functionally ineffective. We postulate that in recurring tumors, other cyclin proteins may be overexpressed and compensate for decreased *CCND3* expression; additional studies are needed to test this hypothesis.

Our findings are based on a relatively small cohort. However, only a fraction of Ta tumors progress to more aggressive disease. In fact, within our entire clinical cohort of 177 patients, about 10% cases showed progression, which is consistent with other reports [3,4]. This low percentage naturally limited their sample size in our study cohort. Nevertheless, these patients are indeed those who justify the rigid follow-up schedule currently implemented for all Ta cases. Also, while patients with identifiable concomitant CIS were excluded from our cohort, there exists a probability that concomitant CIS foci may have been missed by white-light cystoscopy. While the recent advent of hexyl aminolevulinate fluorescence cystoscopy allows more focused sampling [33], and this is superior to random biopsy [34], it should be noted that none of the significant genes in this study are part of any established CIS-gene expression signature classifier [35], thereby suggesting that these markers are truly prognostic for outcome

and not representative of concomitant CIS. Furthermore, while our medium-throughput expression profiling approach was limited in its discovery potential compared with oligonucleotide microarray technology, it is nevertheless more hypothesis-driven, quantitative, and reproducible [36,37].

Given the low rate of Ta UC progression, it is important to identify patients at impending risk. This could also identify the large proportion of patients who do not require aggressive treatment and surveillance.

5. Conclusions

Using biopsies from initial occurrences of noninvasive papillary UC, we quantified expressions of relevant genes in a reproducible fashion and identified a set of transcripts that can predict recurrence and progression at first presentation better than standard clinicopathologic criteria. The multivariable modeling showed promise with high sensitivity and specificity. These findings could affect Ta tumor management, including surveillance frequency, administration of adjuvant treatment, and selection of candidates for expectant approach. While these findings are preliminary and need further validation, this study indicates that the identified genes and their associated pathways may be critical for noninvasive UC prognosis.

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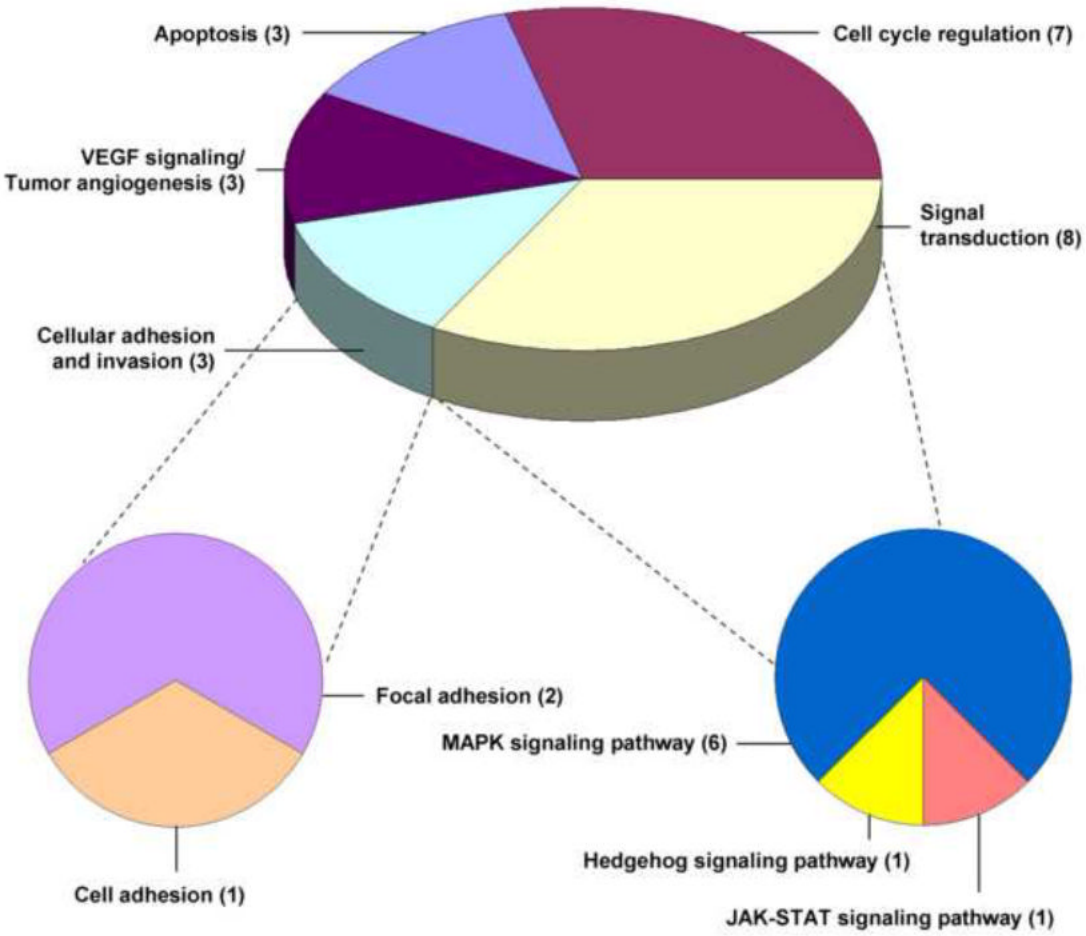

Fig. 1. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways associated with genes included in the analysis

Signal transduction includes the mitogen-activated protein kinase (MAPK), Hedgehog, and Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signaling pathways. Cellular adhesion and invasion includes the focal and cell adhesion pathways. Numbers in parentheses indicate the number of genes associated with each major pathway. VEGF = vascular endothelial growth factor.

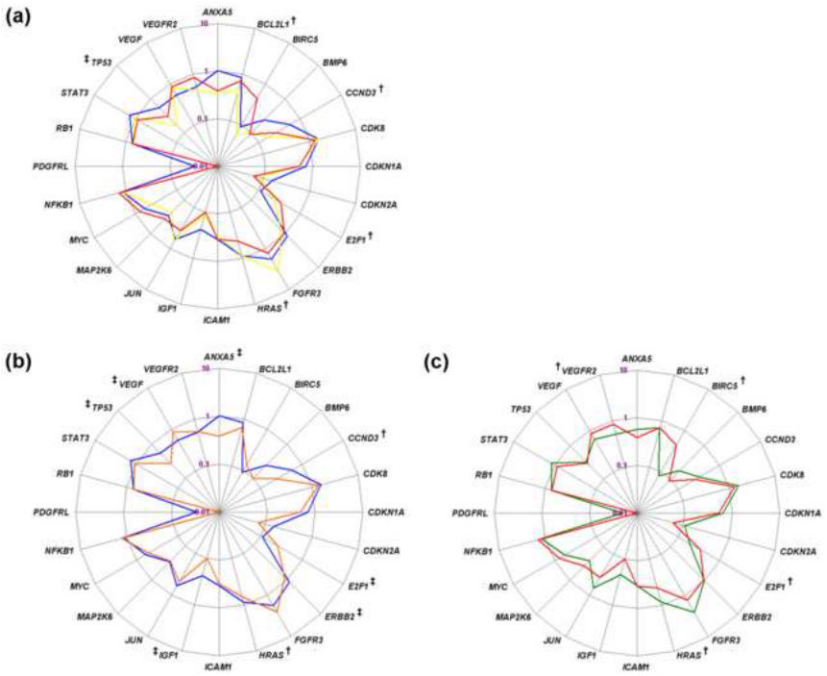

Fig. 2. Normalized expression levels of 24 genes in the prognostic subgroups

Radar plots show the median relative quantification ($\Delta\Delta C_t$) values of all interrogated genes on a logarithmic scale. Comparisons were made between (a) the individual groups (group 1, blue; group 2, yellow; group 3, red), (b) patients without recurrence (group 1, blue) and with recurrence (groups 2 and 3, orange), and (c) patients without progression (groups 1 and 2, green) and with progression (group 3, red).

†$p \leq 0.050$.

‡ $0.050 < p \leq 0.100$.

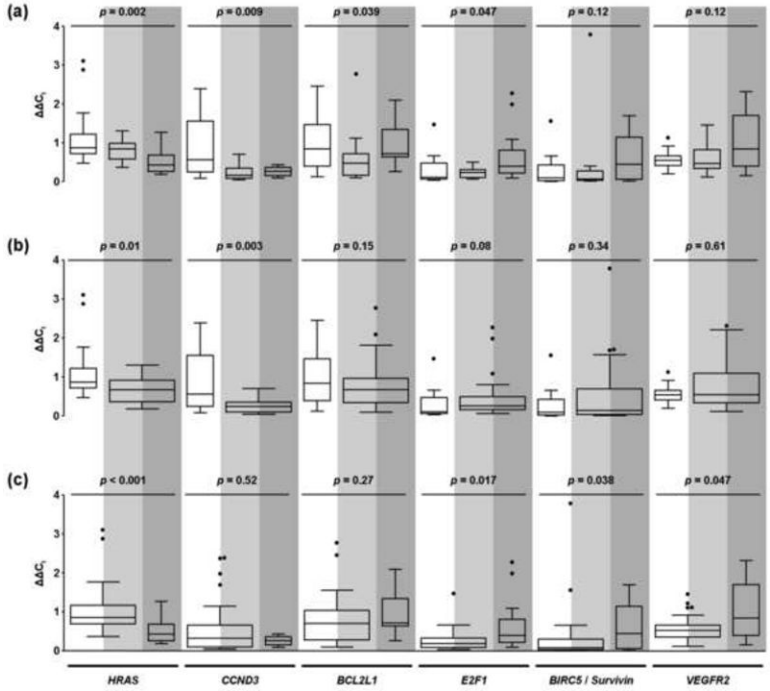

Fig. 3. Distribution of *HRAS, CCND3, BCL2L1, E2F1, BIRC5/Survivin*, and *VEGFR2* relative expression levels in the prognostic subgroups

The white, light grey, and dark grey areas denote patient groups 1, 2, and 3, respectively. Tukey boxplots for the six genes are shown (a) across each of the individual prognostic subgroups; (b) in patients with no recurrence (group 1, white area) versus patients with recurrence, without or with progression (groups 2 and 3, light and dark grey areas); and (c) in patients with no progression, without or with recurrence (groups 1 and 2, white and light grey areas) versus patients with progression (group 3, dark grey area). The boxes represent median with interquartile range; whiskers go 1.5 times the interquartile distance or to the highest or lowest point, whichever is shorter. Dots represent outliers.

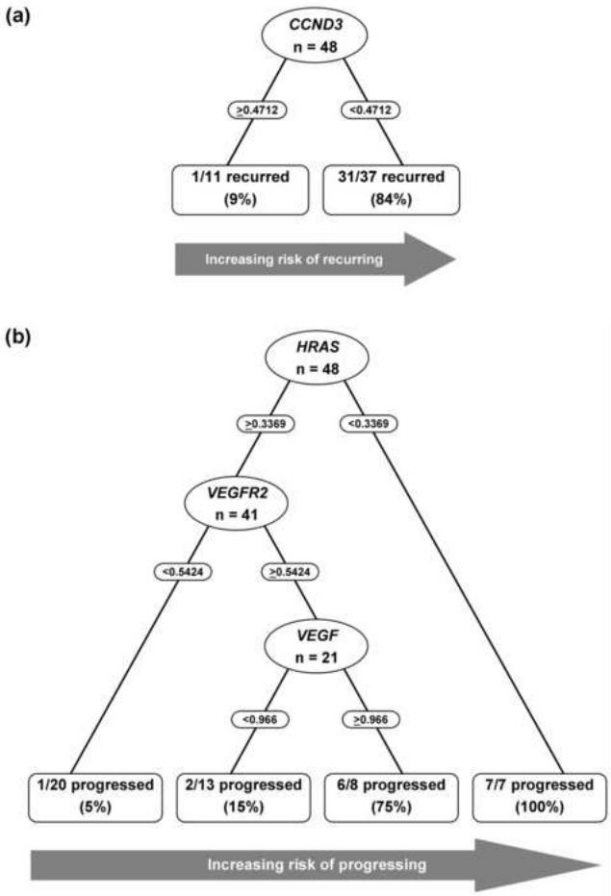

Fig. 4. Recursive partitioning analysis for outcome prediction

(a) *CCND3* expression level with a cut-off value of 0.4712 was identified as a predictor for recurrence. Thirty-one of 32 patients with recurrence and 10 of 16 patients without recurrence were correctly identified. (b) Expression levels of *HRAS*, *VEGFR2*, and *VEGF* were identified as predictors for progression, with cut-off values of 0.3369, 0.5424, and 0.966, respectively. Thirteen of 16 patients with progression and 30 of 32 patients without progression were correctly identified.

Table 1

Genes profiled in study cohort

Gene (and associated major KEGG pathway)	Full name	GeneID
ANXA5[a]	annexin A5	308
BCL2L1[a]	B-cell CLL/lymphoma 2-like 1	598
BIRC5 / *Survivin*[a]	baculoviral IAP repeat-containing 5	332
BMP6[b]	bone morphogenetic protein 6	654
CCND3[c]	cyclin D3	896
CDK8[c]	cyclin-dependent kinase 8	1024
CDKN1A[c]	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	1026
CDKN2A[c]	cyclin-dependent kinase inhibitor 2A	1029
E2F1[c]	E2F transcription factor 1	1869
ERBB2[d]	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2	2064
FGFR3[e]	fibroblast growth factor receptor 3	2261
HRAS[e]	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	3265
ICAM1[f]	intercellular adhesion molecule 1	3383
IGF1[d]	insulin-like growth factor 1	3479
JUN[e]	jun oncogene	3725
MAP2K6[e]	mitogen-activated protein kinase kinase 6	5608
MYC[e]	v-myc myelocytomatosis viral oncogene homolog	4609
NFKB1[e]	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	4790
PDGFRL[g]	platelet-derived growth factor receptor-like	5157
RB1[c]	retinoblastoma 1	5925
STAT3[h]	signal transducer and activator of transcription 3	6774
TP53[c]	tumor protein p53	7157
VEGF[g]	vascular endothelial growth factor A	7422
VEGFR2[g]	kinase insert domain receptor	3791
TBP[i,k]	TATA box binding protein	6908
SDHA[j,k]	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	6389

KEGG = Kyoto Encyclopedia of Genes and Genomes; MAPK = mitogen-activated protein kinase.

Major KEGG pathway:

[a] Apoptosis.

[b] Hedgehog signaling pathway.

[c] Cell-cycle regulation.

[d] Focal adhesion.

[e] MAPK signaling pathway.

[f] Cell adhesion.

[g] VEGF signaling/tumor angiogenesis.

[h] Jak-STAT signaling pathway.

[i] Basal transcription factor.

[j] Citrate cycle.

[k] Reference gene.

Table 2

Clinicopathologic characteristics of patient groups in the study cohort[†]

	No Recurrence	Recurrence		
	Group 1 (*n* = 16)	Without progression Group 2 (*n* = 16)	With progression Group 3 (*n* = 16)	*p* value
Gender (%)				0.025[a]
Female	1 (6)	7 (44)	7 (44)	
Male	15 (94)	9 (56)	9 (56)	
Median age, yr (range)	66.5 (29–83)	64.5 (39–76)	67 (62–86)	0.38[b]
Tumor grade (%)				0.47[a]
G2	14 (88)	15 (94)	12 (75)	
G3	2 (12)	1 (6)	4 (25)	
Multifocality (%)				0.11[a]
Multifocal	1 (6)	0 (0)	4 (25)	
Single tumor	15 (94)	16 (100)	12 (75)	
Median follow-up, yr (range)	7.9 (5.1–12.0)	8.0 (5.5–12.5)	5.7 (0.9–10.0)	0.017[b]
Median no. of recurrences before progression (range)	–	4 (1–9)	2.5 (0–7)	0.035[b]
Median time to first clinically significant event, mo[*] (95% CI)	–	11.3 (8.6–43.8)	7.4 (4.2–12.1)	0.012[c]
Clinical probability of recurrence[§] (%)				–
Intermediate	16 (100)	16 (100)	16 (100)	
Clinical probability of progression[§] (%)				0.051[a]
Low	13 (81)	15 (94)	9 (56)	
Intermediate to high	3 (19)	1 (6)	7 (44)	

[*] Event is recurrence for group 2, and recurrence or progression (whichever occurred first) for group 3.

[§] Based on European Organization for Research and Treatment of Cancer Ta T1 bladder cancer risk tables [4].

[a] Fisher exact test.

[b] Wilcoxon rank-sum test.

[c] Log-rank test.

[†] Except for a higher proportion of males in group 1, all groups had comparable demographic and pathologic features.

Table 3

Differential gene expressions between the individual prognostic groups[†]

		Individual groups (1 vs 2 vs 3)		No recurrence vs recurrence (1 vs 2 + 3)		No progression vs progression (1 + 2 vs 3)	
		median (range)	***p* value**	**median (range)**	***p* value**	**median (range)**	***p* value**
HRAS	1	0.87 (0.47–3.11)	0.002[*]	0.87 (0.47–3.11)	0.01	0.85 (0.37–3.11)	<0.001[*]
	2	0.84 (0.37–1.31)		0.67 (0.18–1.31)			
	3	0.43 (0.18–1.27)				0.43 (0.18–1.27)	
CCND3	1	0.56 (0.09–2.39)	0.009	0.56 (0.09–2.39)	0.003[*]	0.32 (0.05–2.39)	0.52
	2	0.16 (0.05–0.70)		0.24 (0.05–0.70)			
	3	0.26 (0.09–0.43)				0.26 (0.09–0.43)	
BCL2L1	1	0.84 (0.12–2.45)	0.039	0.84 (0.12–2.45)	0.15	0.71 (0.10–2.77)	0.27
	2	0.47 (0.10–2.77)		0.67 (0.10–2.77)			
	3	0.71 (0.26–2.09)				0.71 (0.26–2.09)	
E2F1	1	0.11 (0.04–1.47)	0.047	0.11 (0.04–1.47)	0.08	0.19 (0.04–1.47)	0.017
	2	0.23 (0.07–0.50)		0.25 (0.00–2.27)			
	3	0.34 (0.00–2.27)				0.34 (0.00–2.27)	
BIRC5/Survivin	1	0.09 (0.00–1.56)	0.12	0.09 (0.00–1.56)	0.34	0.08 (0.00–3.78)	0.038
	2	0.06 (0.01–3.78)		0.14 (0.01–3.78)			
	3	0.44 (0.01–1.69)				0.44 (0.01–1.69)	
VEGFR2	1	0.54 (0.20–1.13)	0.12	0.54 (0.20–1.13)	0.61	0.52 (0.12–1.45)	0.047
	2	0.46 (0.12–1.45)		0.55 (0.12–2.31)			
	3	0.84 (0.15–2.31)				0.84 (0.15–2.31)	

[†]Values for median and range are relative quantification ($\Delta\Delta C_t$) values; *p* values given for univariate analysis with Wilcoxon rank-sum test: Group 1, no recurrence; Group 2, recurrence without progression; Group 3, progression without or with recurrence.

[*]Adjusted *p* values were significant for *HRAS* between the individual groups ($p = 0.029$) and no progression versus progression groups ($p = 0.010$) and for *CCND3* between no recurrence versus recurrence groups ($p = 0.053$).