

Laboratory-Prostate cancer

TRIM24 as an independent prognostic biomarker for prostate cancer

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Abstract

Introduction: Simply applicable biomarkers for prostate cancer patients predicting the clinical course are urgently needed. Recently, TRIM24 has been identified to promote androgen receptor signaling and to correlate with an aggressive prostate cancer phenotype. Based on these data, we proofed TRIM24 as a prognostic biomarker for risk stratification.

Materials and Methods: We performed TRIM24 immunohistochemistry on 2 independent cohorts including a total of 806 primary tumors, 26 locally advanced/recurrent tumors, 30 lymph node metastases, 30 distant metastases, and 129 benign prostatic samples from 497 patients as well as on 246 prostate needle biopsies. Expression data were correlated with clinic-pathological data including biochemical recurrence-free survival (bRFS) as endpoint.

Results: Benign samples show no or low TRIM24 expression in 94%, while tumor tissues demonstrate significant higher levels. Strongest expression is observed in advanced and metastatic tumors. In multivariate analyses, TRIM24 up-regulation on radical prostatectomy specimens correlates with shorter bRFS independent of other prognostic parameters. 5-(10-) year bRFS rates for TRIM24 negative, low, medium and high expressing tumors are 93.1(93.1)%, 75.4(68.5)%, 54.9(47.5)% and 43.1(32.3)%, respectively. Of interest, tumors diagnosed as indolent disease, TRIM24 expression stratifies patients into specific risk groups. Increased TRIM24 expression associates with higher grade group, positive nodal status and extraprostatic tumor growth. TRIM24 assessment on prostate needle biopsies taken prior to treatment decision at time of initial diagnosis significantly correlates with recurrence after surgery.

Conclusion: Using 2 large independent radical prostatectomy specimen cohorts, we found that TRIM24 expression predicts patients' risk to develop disease recurrence with high accuracy and independent from other established biomarkers. Further, this is the first study exploring TRIM24 expression on prostate needle biopsies which represents the clinically relevant tissue type on which biomarkers guide treatment decisions. Thus, we strongly suggest introducing TRIM24 evaluation in prostate needle biopsies in clinical routine as an inexpensive and simple immunohistochemical test. © 2019 Elsevier Inc. All rights reserved.

Keywords: Prostate cancer; TRIM24; Biomarker; Disease recurrence; Immunohistochemical test

Abbreviations: CaP, prostate cancer; bRFS, biochemical recurrence-free survival; GG, grade group; TRIM24, tripartite motif containing 24; CRPC, castration resistant prostate cancer; RPE, radical prostatectomy; TMA, tissue microarray

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1. Introduction

Prostate cancer (CaP) is the most common cancer type in men in western countries and accounts for more than 20% of all diagnosed cancers [1]. Due to its heterogeneous clinical course, decisions for optimal individual therapy regimes remain challenging. Efforts are being made to select patients with indolent CaP for active surveillance or watchful waiting in order to avoid overtreatment, and to identify patients with high-risk disease who benefit from intensive pre- and postoperative treatment approaches [2].

At time of diagnosis, determining tumor grade group (GG) in biopsies is used to stratify tumors into low- or high-risk cancer. Based on GG, prostate-specific antigen (PSA), tumor stage and individual factors, urologists recommend either radical treatment, such as surgery or radiation, or conservative treatment regimens such as active surveillance, hormonal therapy or watchful waiting [3]. Among others, these parameters are used for post-treatment risk stratification to predict local tumor recurrence, biochemical failure, and probability to develop distant metastases [4]. Aggressive intervention is recommended for patients with GG 4 and 5 on biopsy. For patients with GG 1 to 3 tumors, regarded as low to intermediate risk, no parameters predicting disease recurrence have been established yet [5]. Thus, identifying patients who are diagnosed with low-risk CaP but will develop an aggressive course remains a major challenge [6].

Tripartite motif containing 24 (TRIM24), also known as Tif1alpha, is a member of the tripartite motif (TRIM) family [7]. In humans, more than 80 different TRIM proteins regulate multiple cellular functions, mainly through ubiquitin ligase-mediated post-translational protein modifications. Aberrant expression and activity of TRIM proteins have been associated with several human diseases, including diverse cancer types [7]. A number of studies investigated TRIM24 expression in cancer tissues and suggested an oncogenic role of TRIM24 in carcinogenesis [8–11]. Functionally, TRIM24 emerged as a negative regulator of p53 [12], as chromatin regulator in breast cancer [13] as well as a co-activator of different nuclear receptors [14–16]. In 2009, TRIM24 has been identified as androgen receptor co-activator which physically binds to the androgen receptor and interacts with TIP60 to promote transcription of androgen receptor target genes [17]. Later, Theurillat et al. revealed TRIM24 as a Prostate cancer-associated mutations in speckle-type POZ protein (SPOP) substrate, and to be up-regulated in CaP due to SPOP mutations [18]. Importantly, TRIM24 mediates androgen receptor signaling both androgen-dependent as well as in a ligand-independent manner [16,17]. Functional studies revealed that TRIM24 drives castration-resistant CaP by mediating oncogenic androgen receptor activity under androgen deprivation [16]. The same study reported highest TRIM24 levels in castration-resistant CaP tissues in comparison to benign and primary tumors, and also correlation with tumor recurrence after surgery, Gleason score, tumor size, and the proliferation marker Ki67 [16].

The aim of this study was to validate that assessment of TRIM24 expression on CaP tissue predicts biochemical recurrence after surgery. Subgroup analyses including patients with presumed intermediate and indolent CaP were performed to investigate TRIM24 as a parameter with additive prognostic significance, thus allowing risk stratification in intermediate GG. Further, we aimed to develop TRIM24 as prognostic marker at time of initial diagnosis thus proofed its prognostic value on prostate needle biopsies which were taken prior to treatment decision.

2. Materials and methods

2.1. Cohorts

For developing TRIM24 as prognostic marker, we used 2 large independent cohorts, one from Örebro, Sweden and the other one from Goepingen, Germany.

The Örebro cohort includes 288 radical prostatectomy specimens (RPE) and 83 benign prostatic samples from 201 patients who underwent surgery at the University Hospital of Örebro, Sweden between 1989 and 2005. Survival analysis was performed including all patients with available follow-up data ($n=201$). Clinicopathological data of patients are presented in Table 1.

The Goepingen cohort includes 518 RPE specimens, 26 locally advanced or recurrent tumors obtained from transurethral resection of the prostate in a palliative setting (pTUR-P), 30 lymph node metastases, and 46 benign prostatic samples from patients who underwent surgery in the Hospital of Göppingen, Germany between 2002 and 2014. Survival analysis was performed considering RPE specimens of all patients with available follow-up data ($n=256$). Subset analysis was performed for patients with GG 1 to 3 at RPE specimen ($n=210$; postoperative model) known to represent a patient group with variable clinical course. Additionally, patients with GG 1 tumors on diagnostic prostate biopsy as well as preoperative PSA <10 ng/ml ($n=79$, preoperative model), according to the selection criteria for active surveillance, were selected for further subset analysis. Additional 30 distant metastases of patients who were treated at the University Hospital Schleswig-Holstein, Campus Luebeck, Germany were included in the present study. Clinicopathological data of patients are presented in Table 1.

In addition, TRIM24 was assessed on a prostate biopsy cohort including 246 patients. For each patient, the needle biopsy with highest GG was selected for immunohistochemistry (IHC) staining. In cases with consistent GGs between biopsies, the core with highest tumor volume was selected for IHC. For 224 patients, IHC was technically successful, in 22 cases there was no further tumor tissue on deeper sections.

Biochemical recurrence defined as PSA increase above the postoperative nadir was used as endpoint for survival analysis.

Table 1
Patients' characteristics of the Örebro and Göppingen cohort

Characteristic		Örebro cohort	Göppingen cohort
Samples <i>n</i>	Benign prostatic tissue	83	46
	RPE	288	518
	pTUR-P	–	26
	Lymph nodes	–	30
	Distant metastases	–	30
Patients <i>n</i>		201	296
pT stage <i>n</i> (%)	1	119 (58.9)	
	2a-c	75 (37.1)	162 (67.2)
	3a + b	8 (4.0)	77 (32.0)
	4		2 (0.8)
N stage <i>n</i> (%)	0	n.a.	233 (91.0)
	1	n.a.	23 (9.0)
M stage <i>n</i> (%)	0	n.a.	256 (100)
	1	n.a.	0 (0)
R status <i>n</i> (%)	0	n.a.	181 (73.6)
	1	n.a.	65 (26.4)
ISUP/WHO Grade Group <i>n</i> (%)	1	46 (21.9)	92 (35.9)
	2	47 (22.4)	97 (37.9)
	3	86 (41.0)	37 (14.5)
	4	22 (10.5)	18 (7.0)
	5	9 (4.3)	12 (4.7)
preoperative PSA <i>n</i> (%)	<10 ng/ml	119 (60.4)	184/256
	>10 ng/ml	78 (39.6)	72/256
Biochemical recurrence <i>n</i> (%)		65 (32.3)	77 (30.1)
CaP specific death <i>n</i> (%)		14 (7.0)	3 (1.2)
TRIM24 expression RPE <i>n</i> (%)	No expression	83 (41.3)	30 (11.7)
	Low expression	73 (36.3)	113 (44.1)
	Medium expression	36 (17.9)	83 (32.4)
	High expression	9 (4.5)	30 (11.7)
TRIM24 status RPE <i>n</i> (%)	TRIM24 negative	156 (77.6)	143 (55.9)
	TRIM24 positive	45 (22.4)	113 (44.1)

Approval for the present study was obtained from the Internal Review Board of the University Hospital of Luebeck, Germany.

2.2. Tissue microarrays

Tissue microarrays from paraffin-embedded prostatic tissue were constructed as described earlier [19]. IHC on needle biopsies was performed in whole slides of paraffin blocks.

2.3. Immunohistochemistry

Immunohistochemical staining was performed using the Ventana Discovery automated staining system (Ventana Medical System). In brief, slides were incubated at room temperature with primary antibody: anti-TRIM24 rabbit polyclonal 1:75, 14208-1-AP, Proteintech. Primary antibody was detected with the ultraView Universal DAB Detection Kit (Ventana Medical System). Samples with a lack of tissue in all cores present on the tissue microarrays were excluded from further analysis.

2.4. IHC analysis

Stained slides were scanned (Panoramic Desk, 3DHistech) and evaluation of the staining intensity was performed by Definiens Tissue Studio 2.1 (Definiens Inc.), a semiautomated Image Software, as reported before [19–22]. Nuclear mean brown intensity of each core was automatically calculated, resulting in continuous arbitrary variables. Based on re-evaluation of a subset of IHC staining by 2 independent pathologists, samples were categorized into 5 groups: no (<15), low (15–25), medium (25–40), and high expression (>40). To dichotomize samples into positive and negative staining, no/low (<25) expression was defined as negative and medium/high staining (>25) was defined as positive. For tumors with multiple distinct foci within the prostate or for patients with multiple lymph node metastases, we calculated the average TRIM24 expression. For statistical analyses, we used the continuous TRIM24 variable, TRIM24 expression groups, and dichotomized groups. Nuclear TRIM24 expression on needle biopsies was categorized according to its intensity per eye balling and scored as the following: 0 (no expression), 1 (low expression), 2 (medium expression), and 3 (high expression).

2.5. Statistics

Univariate Cox analysis was performed to investigate the association between TRIM24 expression in RPE specimens (low, medium, and high vs. no expression or dichotomized categories) and biochemical recurrence. For multivariate analysis, TRIM24 expression was adjusted to T-stage and preoperative PSA in the Örebro cohort, and to T-stage, preoperative PSA, R-Status, N-Status, and GG in the Göppingen cohort. In subset analysis for GG 1 to 3 tumors (postoperative model), TRIM24 expression was adjusted to T-stage, preoperative PSA, R-Status, and N-Status. Kaplan-Meier curves were used to illustrate biochemical recurrence-free survival (bRFS) and statistically proved by log-rank test. Fisher's exact test was performed to investigate association between TRIM24 expression groups and GGs. Means of the continuous TRIM24 score between lymph node metastasized and nonmetastasized as well as between tumors with extraprostatic growth (pT3/pT4) and tumors restricted to the prostate (pT2) was compared using unpaired *t* test. Continuous TRIM24 scores of benign samples, primary tumors, locally advanced tumors, lymph node metastases, and distant metastases were subjected to Kruskal-Wallis test to determine significance. Chi-square test was used to associate TRIM24 expression with tissue type.

All statistical analyses were performed using Microsoft Excel and SPSS 20.

3. Results

3.1. TRIM24 highlights CaP cells and lacks expression in benign prostatic glands

We compared TRIM24 expression between benign prostatic tissue and CaP during progression and metastasis

(Fig. 1a). TRIM24 is significantly higher expressed in CaP (28.3) compared to benign prostatic tissue (16.1) (*t* test, $P < 0.001$; Fig. 1b). The median of TRIM24 expression increases from benign tissue to localized tumors and shows highest expression in locally advanced and metastatic CaP (Kruskal-Wallis test $P < 0.001$; Fig. 1c). While CaP tissue harbor a wide range of TRIM24 levels ranking from no to high expressing samples, nonmalignant glands are negative for TRIM24 in 44% or show low expression (50%; Fig. 1d). In regional lymph node and distant metastases, TRIM24 was highly expressed in 30% and 50%, respectively (Fig. 1d).

3.2. TRIM24 independently predicts bRFS

In order to assess TRIM24 as prognostic biomarker, we analyzed TRIM24 on 518 RPE specimens from 256 patients (Table 1). Increasing TRIM24 expression was strongly associated with patients' risk to develop biochemical recurrent disease. Mean TRIM24 expression in tumors of patients who developed biochemical recurrence (29.18) was significantly higher compared to patients who remained disease free after surgery (24.26) (unpaired *t* test, $P = 0.001$). Kaplan-Meier curves illustrate significant association between TRIM24 expression and bRFS (log-rank test $P < 0.001$; Fig. 2a). The 5-year-bRFS rate consistently decreases with increasing TRIM24 expression with 93.1% for no, 75.4% for low, 54.9% for medium, and 43.1% for high TRIM24 expression (Fig. 2a). In multivariate analysis, the risk for biochemical failure of patients with tumors harboring low-, medium-, and high TRIM24 expression is 4.87-, 8.05-, and 9.83-fold higher compared to TRIM24 negative tumors, respectively (Multivariate Cox regression, $P = 0.34, 0.005, \text{ and } 0.004$) when adjusting TRIM24 to other clinic-pathological parameters (Fig. 2b). By stratifying samples into TRIM24 negative and positive tumors, Kaplan-Meier curve allows risk stratification of patients over time (log-rank test $P < 0.001$; Fig. 2c). The 5-year-bRFS rates are 79.6% and 53.1% for patients with TRIM24 negative and positive tumors, respectively (Fig. 2c). After 10 years, 74.2% of patients with TRIM24 negative tumors are still biochemical recurrence free, while 55% of patients harboring tumoral TRIM24 expression developed disease recurrence (Fig. 2c). TRIM24 positive tumors are associated with higher risk for biochemical failure after surgery in multivariate analysis independent from other prognostic markers ($P = 0.004$; Fig. 2d).

3.3. TRIM24 independently predicts bRFS: results from an independent validation cohort

To validate our results highlighting TRIM24 as prognostic marker, analysis of TRIM24 expression on additional RPE specimens from 201 patients from an independent cohort revealed significant association between high TRIM24 expression and risk of biochemical recurrence after surgery (univariate Cox regression, $P = 0.036$).

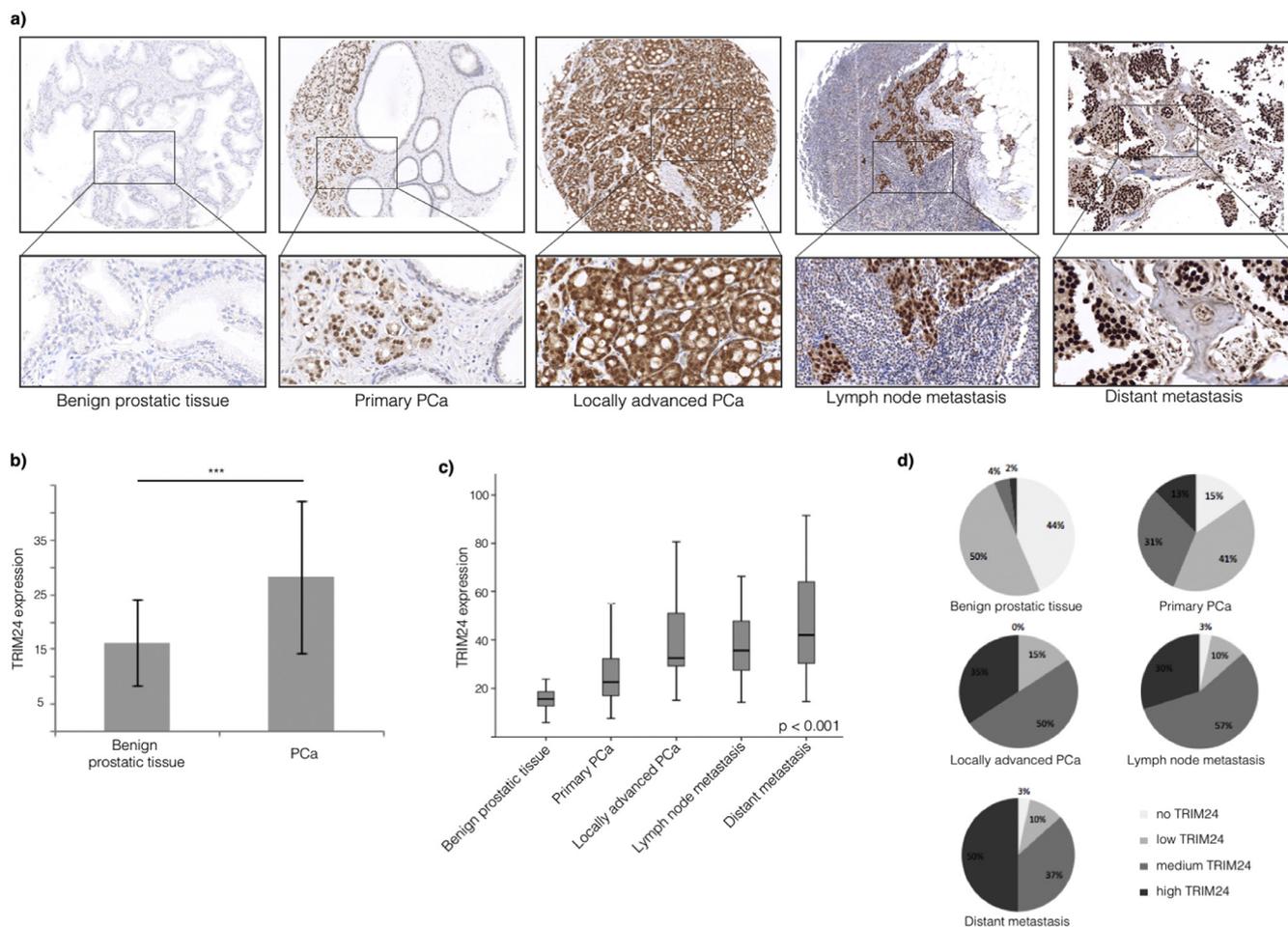


Fig. 1. TRIM24 expression during CaP progression and compared to benign prostatic tissue. (a) Immunohistochemical staining for TRIM24 in benign prostatic glands, primary CaP, locally advanced CaP, regional lymph node metastases, and distant metastases, 10x (upper image) and 40x (lower image) objective magnification. (b) Mean of continuous TRIM24 variables in benign prostatic tissue compared to CaP. Standard deviation, nonpaired sample *t* test $P < 0.001$. (c) Median of continuous TRIM24 variables in benign prostatic glands, primary CaP, locally advanced CaP, lymph node metastases, and distant metastases, 95% confidence interval. Kruskal-Wallis test $P < 0.001$. (d) TRIM24 distribution in benign prostatic tissue compared to primary CaP, locally advanced CaP, lymph node metastases, and distant metastases.

Dichotomized TRIM24 expression revealed 1.96-fold hazard for biochemical recurrence in TRIM24 positive tumors compared to TRIM24 negative tumors (univariate Cox regression, $P = 0.014$). In multivariate analysis, patients with TRIM24 positive tumors had a 2.18-fold higher hazard for biochemical failure compared to patients with TRIM24 negative tumors independent of T-stage and PSA (multivariate Cox regression, $P = 0.005$). 5-year-bRFS rates of patients with TRIM24 negative-, low-, medium-, and high expressing tumors were 78%, 82%, 64%, and 47%, respectively (log-rank test $P = 0.045$). 5-year-bRFS rates of patients with TRIM24 negative and positive tumors were 79.1% and 62.1%, respectively (log-rank test $P = 0.012$). TRIM24 score associates with Gleason score (Fisher's exact test $P = 0.032$), while there was no significant association with T-stage (Fisher's exact test $P = 0.117$) or preoperative PSA level (Fisher's exact test $P = 0.407$).

3.4. TRIM24 allows risk stratification of presumed intermediate and indolent CaP

Next, we performed subgroup analysis of patients who have been diagnosed with GG 1 to 3 in RPE specimens which is associated with variable risk for disease progression. Increasing TRIM24 was significantly associated with lower bRFS rates considering all TRIM24 expression groups (log-rank test $P = 0.001$; Fig. 3a) as well as by comparing TRIM24 positive and negative tumors (log-rank test $P = 0.003$; Fig. 3c). The 5-year-bRFS rate for patients harboring tumors with low-, medium-, and high TRIM24 expression was 78.1%, 62.3%, and 50.9%, respectively, compared to TRIM24 negative tumors (96.3%; Fig. 2a). Multivariate analysis revealed strongly increasing risk for the development of recurrent disease in TRIM24 expressing tumors independent of other prognostic factors (Fig. 3b

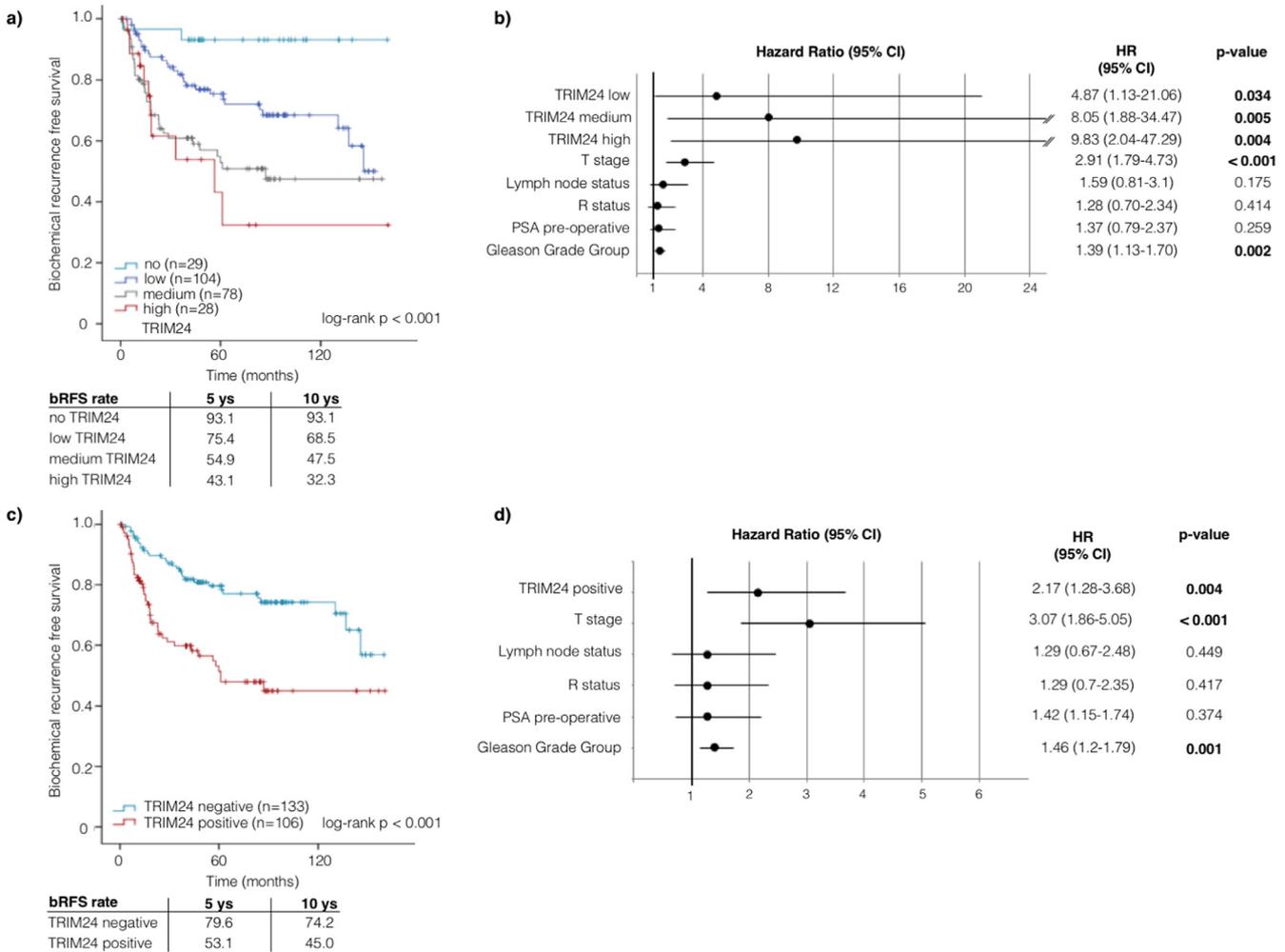


Fig. 2. Biochemical recurrence-free survival of 256 patients undergoing radical prostatectomy. (a) Kaplan-Meier biochemical recurrence-free survival (bRFS) curve and 5(10)-year-bRFS rates stratified by TRIM24 expression. (b) Forest plot of hazard ratio (HR) with 95% confident interval (CI) of TRIM24 expression in multivariate analyses adjusted to other parameters. (c) Kaplan-Meier bRFS curve and 5(10)-year-bRFS rates stratified by dichotomized TRIM24 expression (no/low vs. medium/high). (d) Forest plot of hazard ratio (HR) with 95% confident interval (CI) of dichotomized TRIM24 expression in multivariate analyses adjusted to other parameters.

and d). Mean TRIM24 expression was 29.1 in tumors of patients who developed biochemical recurrence compared to tumors of patients staying disease free after surgery (23.9) (t test, $P = 0.002$).

To determine whether TRIM24 might stratify patients with presumed indolent disease, we selected patients at low risk for disease progression (preoperative PSA < 10 ng/ml at diagnostic biopsy) for subgroup analysis. Within this group, we observed reduced 5-year-bRFS rates in TRIM24 low and medium tumors compared to TRIM24 negative tumors (log-rank test $P = 0.022$; Fig. 4a) while TRIM24 high expressing tumors ($n = 9$) were not associated with shorter bRFS (Fig. 4a). Dichotomizing tumors into TRIM24 negative and positive allow significant risk stratification in Kaplan-Meier curves (log-rank test $P = 0.007$; Fig. 4b). After 10 years follow-up, 96% of patients with TRIM24 negative tumors remained disease free, while patients with TRIM24 expressing tumors developed

biochemical recurrence in 31.2% (Fig. 4b) and had a 10.68-fold higher risk for biochemical recurrence in univariate analysis (univariate Cox regression, $P = 0.029$).

3.5. TRIM24 as prognostic biomarker at time of initial diagnosis

To establish TRIM24 as biomarker at time of initial diagnosis, we assessed TRIM24 expression on tumor tissues obtained by prostate needle biopsies ($n = 224$). Kaplan-Meier analysis revealed 5-year-bRFS rates for patients harboring tumors with no-, low-, medium-, and high TRIM24 expression of 76.8%, 74.9%, and 66.7% and 54.9%, respectively (log-rank $P = 0.015$, Fig. 4c). In addition, TRIM24 expression associates with the GG on needle biopsies (Chi-quadrat $P = 0.002$) revealing that tumors with high TRIM24 expression are GG 4/5-tumors in 37.7%

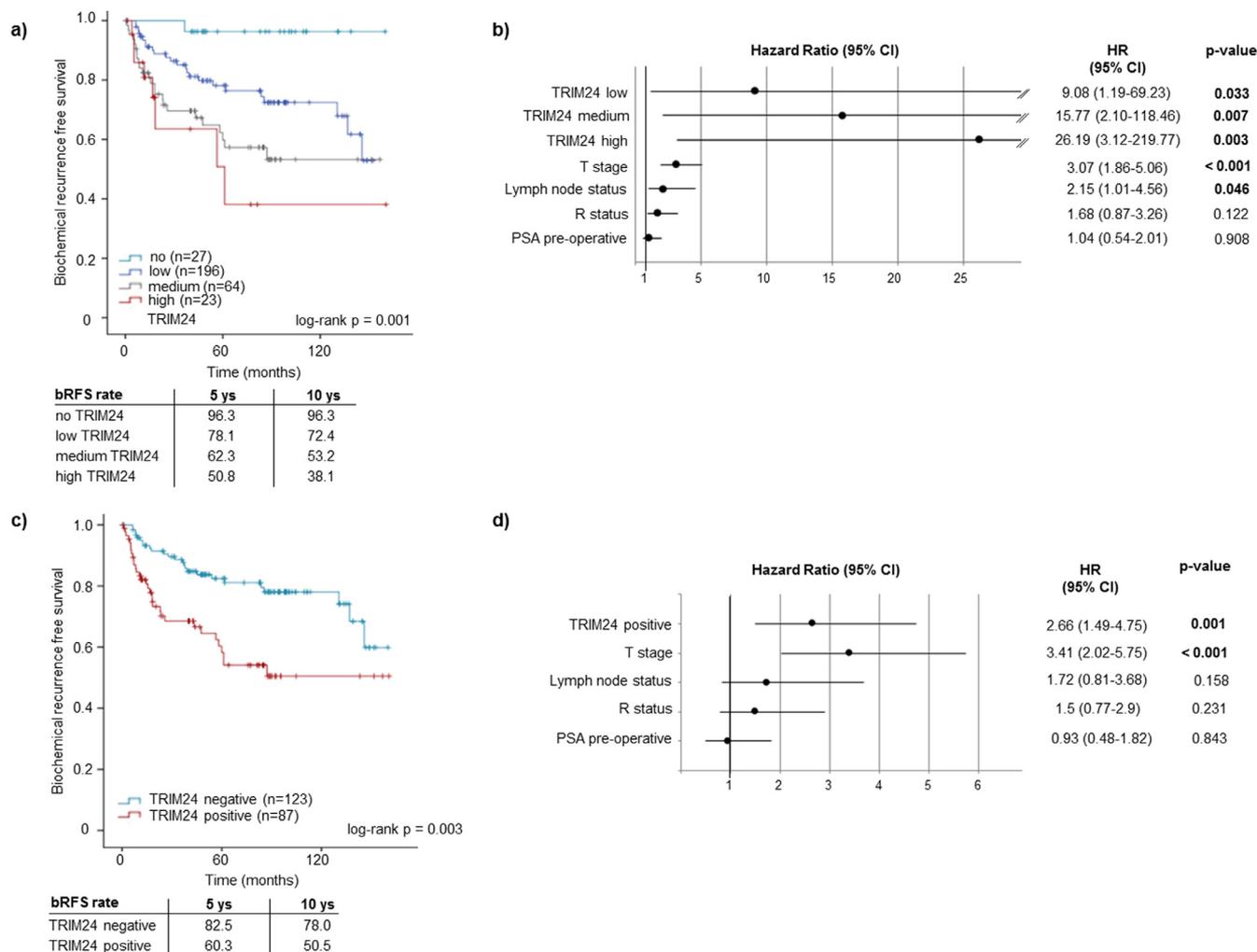


Fig. 3. Biochemical recurrence-free survival of 210 patients with diagnosed Grade Group 1 to 3 tumors at RPE specimens. (a) Kaplan-Meier biochemical recurrence-free survival (bRFS) curve and 5(10)-year-bRFS rates stratified by TRIM24 expression. (b) Forest plot of hazard ratio (HR) with 95% confident interval (CI) of TRIM24 expression in multivariate analyses adjusted to other parameters. (c) Kaplan-Meier bRFS curve and 5(10)-year-bRFS rates stratified by dichotomized TRIM24 expression (no/low vs. medium/high). (d) Forest plot of hazard ratio (HR) with 95% confident interval (CI) of dichotomized TRIM24 expression in multivariate analyses adjusted to other parameters.

while TRIM24-negative tumors are GG 4/5-tumors in only 10.8% (Fig. 4d).

3.6. Correlation between TRIM24 and other clinic-pathological data

TRIM24 expression significantly associates with increasing GGs evaluated on RPE specimens (Chi square test, $P=0.001$). TRIM24 expression is significantly higher in tumors with extraprostatic growth (T3/T4; 27.9) compared to localized tumors (T2; 24.6) (t test $P=0.045$). Regional lymph node metastasized tumors show higher mean TRIM24 expression (30.5) compared to nonmetastasized tumors (25.3; t test $P=0.028$). There was no significant correlation between TRIM24 expression and preoperative PSA level (Pearson correlation $P=0.649$).

4. Discussion

Metastatic and castration-resistant CaP is characterized by a higher frequency of molecular alterations in comparison to localized primary tumors [23]. Thus, their detection cannot be used as prognostic marker in early-stage disease or as guidance for treatment decisions at time of diagnosis. This is particularly important as CaP is a clinically variable disease ranging from indolent to widespread metastatic tumors. The major challenge is to identify features of the primary tumor that predict progressive disease and thus to separate patients who benefit from immediate and aggressive therapy from patients whose tumors will likely remain indolent. Gleason grading is the best pathological parameter routinely used as prognostic marker on both diagnostic biopsy as well as RPE specimen [2,3]. While having strong prognostic value by differentiating GG 1 to 3 from GG 4 to

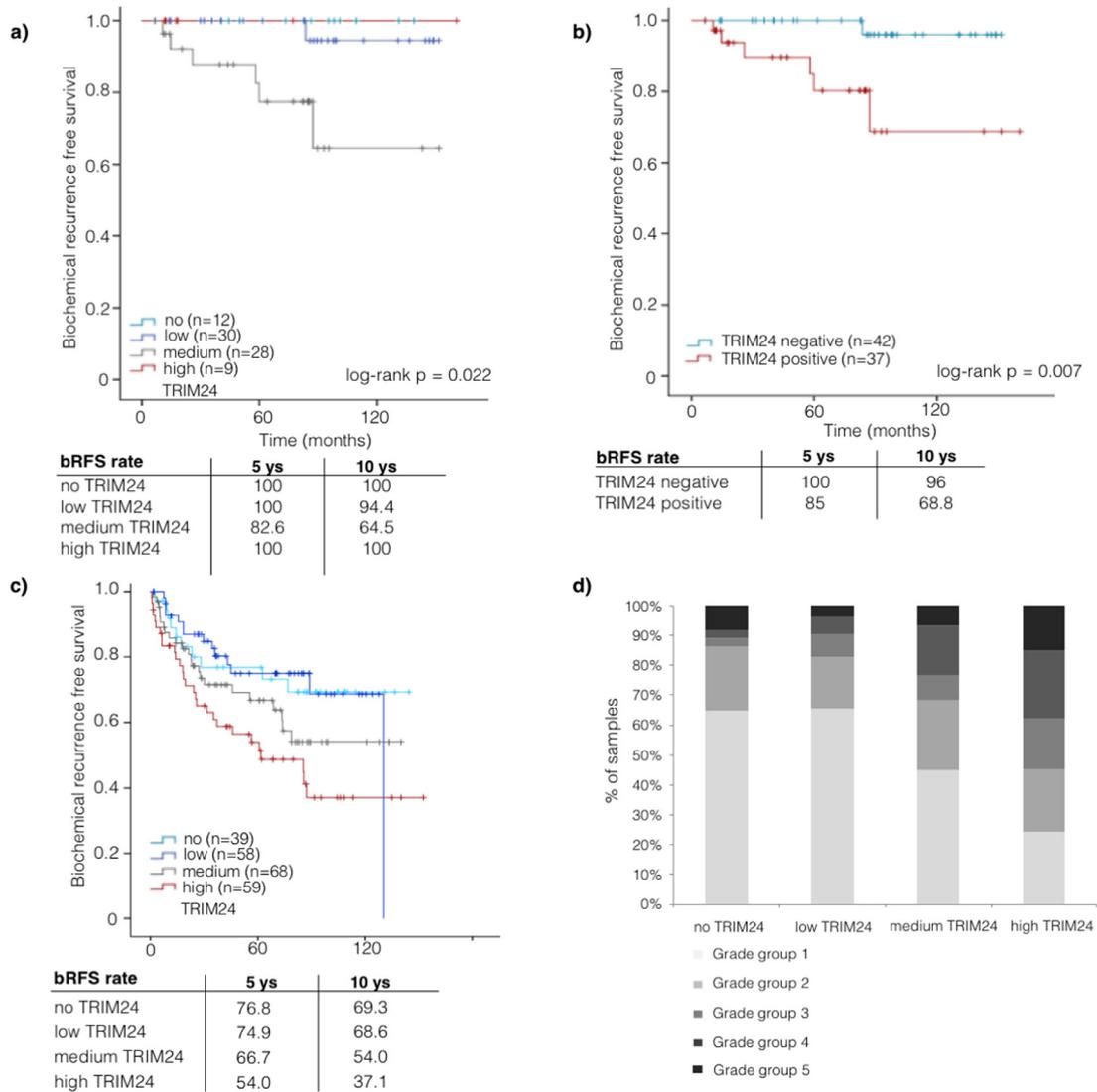


Fig. 4. Biochemical recurrence-free survival of 79 patients with diagnosed Grade Group 1 tumors at diagnostic biopsy and simultaneous preoperative PSA < 10 ng/ml as well as on prostate needle biopsies. (a) Kaplan-Meier biochemical recurrence-free survival (bRFS) curve and 5(10)-year-bRFS rates stratified by TRIM24 expression. (b) Kaplan-Meier bRFS curve and 5(10)-year-bRFS rates stratified by dichotomized TRIM24 expression (no/low vs. medium/high). (c) Kaplan-Meier biochemical recurrence-free survival (bRFS) curve and 5(10)-year-bRFS rates stratified by TRIM24 expression on prostate needle biopsies. (d) Bar graphs showing the distribution of grade groups on needle biopsies according to the corresponding TRIM24 expression.

5 tumors, prognostic significance in stratifying low to intermediate risk tumors with GG 1 to 3 is limited [2,3]. Thus, multiple studies reported biomarkers, gene panels or genetic constellation with prognostic value independent from Gleason grading and other clinic-pathological parameters. However, so far there are no routinely used biomarkers due to the lack of independence or reproducibility on independent cohorts as well as low sensitivity and specificity. In the present study, we observed that TRIM24 assessment stratifies patients into distinct risk groups on 2 independent RPE cohorts. Increasing TRIM24 staining significantly associates with shorter BFS after surgery independent of other clinically used parameters. To simplify, we further dichotomized tumors into TRIM24 negative

tumors with absent or low TRIM24 expression and TRIM24 positive tumors with medium to high expression revealing highly significant correlation with disease recurrence.

As information about the GG on corresponding diagnostic biopsies was available for the Göttingen cohort, we performed further subgroup analysis in certain risk groups. We observed that TRIM24 assessment identifies patients with high-risk disease who have been diagnosed with indolent to intermediate CaP based on currently used parameters. TRIM24 offers additional information in GG 1 to 3 tumors with uncertain aggressive potential, in which Gleason grading exhibits limitations and is also associated with high interobserver variability. We hypothesized that TRIM24

expression status could improve preoperative risk assessment. We therefore performed subgroup analysis including presumed low-risk group defined as GG 1 on diagnostic biopsy and preoperative PSA <10 ng/ml, corresponding to selection criteria for active surveillance. The 5(10)-year bRFS rate of patients with TRIM24 negative and positive tumors was 100%(85%) and 96%(68.8)%, respectively. However, no patient with high expression developed disease recurrence, likely due to low sample size in this subset of patients ($n = 9$). Another limitation is that TRIM24 assessment has been conducted on RPE specimen but interpretation refers to a preoperative model. Although diagnostic biopsies are biologically equivalent to tumors resected by RPE and allow indirect conclusions, further studies investigating tissues from biopsies are needed to prove whether TRIM24 can serve as preoperative prognostic biomarker. Multivariate analyses were not performed as eligible preoperative prognostic parameters comprise PSA and GG, which were used as selection criteria for subset analysis, as well as clinical T-stage which was not available. Collectively, our results give evidence that TRIM24 assessment on diagnostic biopsies might help to decide whether immediate radical treatment is needed or alternative strategies such as active surveillance should be taken into account.

Importantly, most retrospective biomarker studies use RPE as tool to perform experiments. Giving valuable information about the disease course after surgery, this approach is clinically limited as the treatment decision is made at time of initial diagnosis based on the serum PSA, the clinical T-stage as well as the GG on tumor tissue observed by needle biopsies. Therefore, it is essential to validate biomarkers on tumor tissues from initial needle biopsies aiming to translate results into clinical routine. Here, we observed high prognostic accuracy of TRIM24 evaluation on prostate needle biopsies allowing risk stratification of patients at time of initial diagnosis. These results strongly support TRIM24 as valuable biomarker which has the potential to be translated into clinical routine.

Previous studies reported that the AR co-activator TRIM24 is up-regulated in CaP and promotes disease progression [16]. Concordantly, we observed higher TRIM24 expression in locally advanced and recurrent tumors compared to primary tumors, and highest levels in regional lymph node and distant metastases. In contrast, 94% of benign prostatic glands included in the present study were TRIM24 negative or expressed low TRIM24, highlighting its oncogenic role in CaP. Efforts are being made to characterize lethal CaP in order to understand mechanisms promoting disease recurrence, metastasis, and castration-resistance. The identification of novel therapeutic targets requires a profound understanding of molecular alterations in highly aggressive CaP cells. Our results support previous observations that TRIM24 associates with an aggressive phenotype of CaP and suggest TRIM24 as candidate for therapeutic inhibition for advanced CaP.

In the present study, we support TRIM24 as an independent prognostic biomarker for CaP and were also able to validate its prognostic significance on 2 large independent cohorts. Further, we established TRIM24 as prognostic biomarker on prostate needle biopsies which presents the virtual tool to establish biomarkers. Together with data published by Groner et al. [16] we strongly suggest to prove TRIM24 as independent, reproducible, specific, and sensitive biomarker in further prospective cohorts in order to improve individual treatment management of CaP patients.

Authors' contribution

SP and AO performed study design. VL, RK, OA, and MS provided tissue and patients data. AO, FB, FS, WV, KC, JRI, and SH performed experiments. AO, MH, and JC performed statistical analysis. SP, AO, MH, VS, AM, VL, SD, JK, LT, and RK analyzed data. SP and AO wrote the manuscript. All authors reviewed the final manuscript. SP supervised the study.

Conflict of interest

The authors declare no conflict of interest.

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