

Original article

# Tumor necrosis factor-related apoptosis inducing ligand-R4 decoy receptor expression is correlated with high Gleason scores, prostate-specific antigen recurrence, and decreased survival in patients with prostate carcinoma<sup>☆</sup>

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## Abstract

**Objective:** Tumor necrosis factor-related apoptosis inducing ligand (TRAIL) has recently been investigated because of its ability to selectively kill cancer cells. Despite recent publications mainly focusing on TRAIL resistance in cancer cells, little is known about how TRAIL contributes to the carcinogenesis process. Because the expression patterns of TRAIL and its receptors in patients with prostate carcinoma have recently been reported, this study investigated the significance of TRAIL and TRAIL receptor expression in connection to serum prostate-specific antigen (PSA) and Gleason scoring.

**Materials and methods:** A total of 98 patients were included in the study. Gleason scores, PSA, TRAIL, and TRAIL receptor expressions were used for the comparison purposes. The Spearman rho correlation test was administered to reveal the correlations among the variants. The Kruskal Wallis-Mann Whitney *U* or Friedman-Wilcoxon signed ranks test determined the statistical significance between the pairs. Multinomial and/or multiple binary logistic regression analyses were deployed to test whether TRAIL markers were independent variables to predict the prognosis of prostate cancer. Kaplan-Meier and log-rank tests were used to determine the survival rates.

**Results:** High-serum PSA levels were correlated with higher levels of TRAIL and TRAIL receptor expressions. Patients with high Gleason scores had higher levels of TRAIL-R4 decoy receptor expression but lower levels of TRAIL death ligand expression.

**Conclusions:** TRAIL-R4 decoy receptor expression is strongly correlated with PSA recurrence, which is suggestive of poor prognosis. High levels of TRAIL-R4 expression but low levels of TRAIL death ligand expression are connected to decreased survival. © 2008 Elsevier Inc. All rights reserved.

**Keywords:** Tumor necrosis factor-related apoptosis inducing ligand; Gleason scoring; Prostate-specific antigen recurrence; Prostate cancer

## 1. Introduction

Prostate cancer is 1 of the most frequently diagnosed malignancies among men in the Western world, and 29,900 cases of death are expected this year in the United States alone [1]. The proper assessment of the prostate cancer

progression is a pivotal step when counseling the patient for the curative versus palliative therapy. Stage, Gleason score, and serum prostate-specific antigen (PSA) are all well-established prognostic factors that are routinely used in the clinical decision-making process [2]. Despite the fact that numerous studies linked high-serum PSA levels to the clinically advanced stages of prostate carcinoma [3], in most cases, serum PSA measurement alone does not provide an accurate assessment of the disease progression for a given patient [4]. For example, preoperative evaluation of the serum PSA in patients with prostate cancer is confounded by both the volume of the benign prostate tissue present [5]

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and also the tumor grade [6]. Of the many histologic grading systems introduced to help to predict the pathologic stage and prognosis of prostate cancer, the most commonly used is the Gleason system [7,8]. Despite this fact, even Gleason score alone is insufficient to predict accurately the pathologic stage because of a previously described phenomenon of histologic upgrading from biopsy to prostatectomy specimens [8,9]. Currently, earlier staging systems of prostate carcinoma mainly rely on the digital rectal examination (DRE). However, its relative lack of sensitivity (52%) limits DRE [3]. Thus, traditional prognostic markers (grade, clinical stage, and pretreatment PSA) are of limited predictive value for the pathologic staging of the prostate carcinoma. Consequently, additional markers are strongly needed to define high-risk patients more accurately for both the pathologic staging and forthcoming therapies of prostate carcinoma.

Genes involved in the cellular proliferation such as tumor repressor genes (phosphatase and tensin homolog, etc.) [10,11] and those genes controlling the cell death [12] are very attractive for investigation because of their potential to be used as prognostic markers to predict the disease progression. One such marker is the tumor necrosis factor-related apoptosis inducing ligand (TRAIL), which is an apoptosis-inducing member of the tumor necrosis factor family [13]. Investigations concerning TRAIL have become very popular because of its therapeutic potential as a result of selective apoptosis inducing properties on cancer cells [14]. However, it is still unclear how TRAIL and TRAIL receptor expression profiles influence the carcinogenesis process. TRAIL can interact with 4 distinct receptors. Two of these receptors, DR4 (TRAIL-R1) and DR5 (TRAIL-R2), are membrane-spanning proteins containing intracellular death domains essential for the transmission of the death signal upon TRAIL binding and receptor trimerization. Two other membrane receptors, DcR1 (TRAIL-R3) and DcR2 (TRAIL-R4), can also bind TRAIL but lack death domains and are unable to induce cell death [15]. Because the presence or absence of TRAIL decoy receptors were connected to the sensitivity of cancer cells to apoptotic ligands [16–18], the modulation of TRAIL and TRAIL receptor expression might be essential for the progression of prostate cancer [19]. Therefore, the aim of this study was to investigate the potential connection of TRAIL and its receptors to the currently known prognostic factors (serum PSA and Gleason scoring) for a better assessment of prostate carcinogenesis.

## 2. Materials and methods

### 2.1. Clinical assessment of patients with prostate cancer

A total of 44 patients with benign prostate hyperplasia (BPH), 28 with organ-confined prostate carcinoma and 26 with advanced prostate carcinoma, admitted to the Urology

Clinic of Akdeniz University Hospitals were included in the study. Pretreatment PSA levels were obtained from patient's serum in the Central Laboratory of Akdeniz University Hospitals. Pathologists determined the Gleason score for each patient. Patients with advanced prostate cancer possessed clinical and radiologic evidence of metastatic disease. Prostate tissues were acquired from patients undergoing radical prostatectomy for the organ-confined disease. Other tissues were attained through transurethral resection of prostate (TURP) for patients with BPH and transrectal ultrasound-guided biopsy for patients with advanced prostate carcinoma. Before the TURP operation, patients with BPH received  $\alpha$ -blockers, while no patient with prostate cancer received neoadjuvant or adjuvant therapy, including androgen ablation therapy or chemotherapy/radiation, before surgery. Clinical and pathologic stages were assigned according to the tumor-nodes-metastasis prostate cancer staging system [20]. Disease progression for patients with advanced prostate carcinoma was defined as the appearance of new lesion(s), and/or an increase of  $\geq 25\%$  of measurable metastases, and/or the appearance of new foci on a radio-nuclide bone scan, and/or 3 consecutive increases in PSA concentration at least 1 week apart in the presence of testosterone castrate level ( $< 50$  ng/ml) of patients with metastatic disease. A postoperative total PSA level of  $\geq 0.4$  ng/ml and increasing was considered as evidence of biochemical (PSA) recurrence for patients with organ-confined prostate carcinoma [21].

### 2.2. Histologic grading of the prostate tissue and specimen processing

The processing of prostate tissue samples was performed as previously described [22]. Patients were given a designated Gleason grading score based on the specimens obtained through the radical prostatectomy, transrectal ultrasound-guided biopsy, and TURP [7]. Briefly, the Gleason grading system is based on a low-power microscopic description of the histologic architecture of cancer. A Gleason grade of 1–5 was assigned as a primary grade (pattern occupying in the largest area of the specimen) and as a secondary grade (pattern occupying the second-largest area). Adding the primary and secondary grades determined a Gleason score (2–10). In this study, patients were separated into 2 groups based on Gleason scoring, as those with Gleason score  $\geq 7$  and those with Gleason scores  $< 7$  [23].

### 2.3. Immunohistochemical scoring of TRAIL and TRAIL receptors, and the statistical analysis

The immune staining procedures of prostate sections and the scores were described elsewhere [18,19]. Two independent pathologists who were blinded to the names of the antibodies used for the staining performed immunohistochemical scoring. To explain briefly, both the intensity and the marker distribution (percentage of positively stained

epithelial cells) were used to obtain immune staining scores. The intensity was scored as follows: 0, negative; 1, weak; 2, moderate; and 3, strong staining. The marker distribution was also scored as: 0, less than 10%; 1, between 10% and 40%; 2, between 40% and 70%; and 3, more than 70% of the epithelial cells stained on the specimen. Adding the scores of both the intensity and the marker distribution for a given patient attained the final immune staining score. SPSS 13.0 software for Windows (SPSS Inc., Chicago, IL) was used to perform the statistical analysis, as specifically stated in the Results. Error bars for all data points in all figures represent standard error of the mean ( $\pm$  standard error of the mean).

### 3. Results

#### 3.1. TRAIL and TRAIL receptor expressions are positively correlated with serum PSA levels

Serum PSA levels of 98 patients with prostate problems (44 patients with BPH, 28 with organ-confined prostate carcinoma, and 26 with advanced prostate carcinoma) were determined as described in the Materials and methods. Normality of the patient groups was tested using the Shapiro-Wilk method. Because neither group displayed a gaussian distribution, a nonparametric correlation analysis (Spearman rho correlation test) was applied to document any possible correlation between the serum PSA levels and TRAIL receptor expressions, as revealed by immunohistochemical staining and pathologic analysis of the prostate sections. As shown in Table 1, serum PSA levels were positively correlated with TRAIL and TRAIL receptor expressions in the prostate.

#### 3.2. Comparative analysis of TRAIL and TRAIL receptor expressions in patients with prostate cancer based on serum PSA levels

Although it is not specific for prostate cancer, serum PSA levels were correlated well with the pathologic stage and

Table 1  
Spearman rho correlations (n = 98)

	PSA	R1	R2	R3	R4	L
PSA	1	0.53	0.56	0.47	0.45	0.31
R1	0.53	1	0.64	0.53	0.60	0.45
R2	0.56	0.64	1	0.53	0.56	0.50
R3	0.47	0.53	0.53	1	0.56	0.53
R4	0.45	0.60	0.56	0.56	1	0.43
L	0.31	0.45	0.50	0.53	0.43	1

Nonparametric correlation analysis of 98 patients with prostate ailment in connection with serum PSA, TRAIL, and TRAIL receptors. Correlation coefficients are provided in the table. Since *P* values for all correlations were statistically significant (*P* < 0.05), they were omitted from the table for clarity.

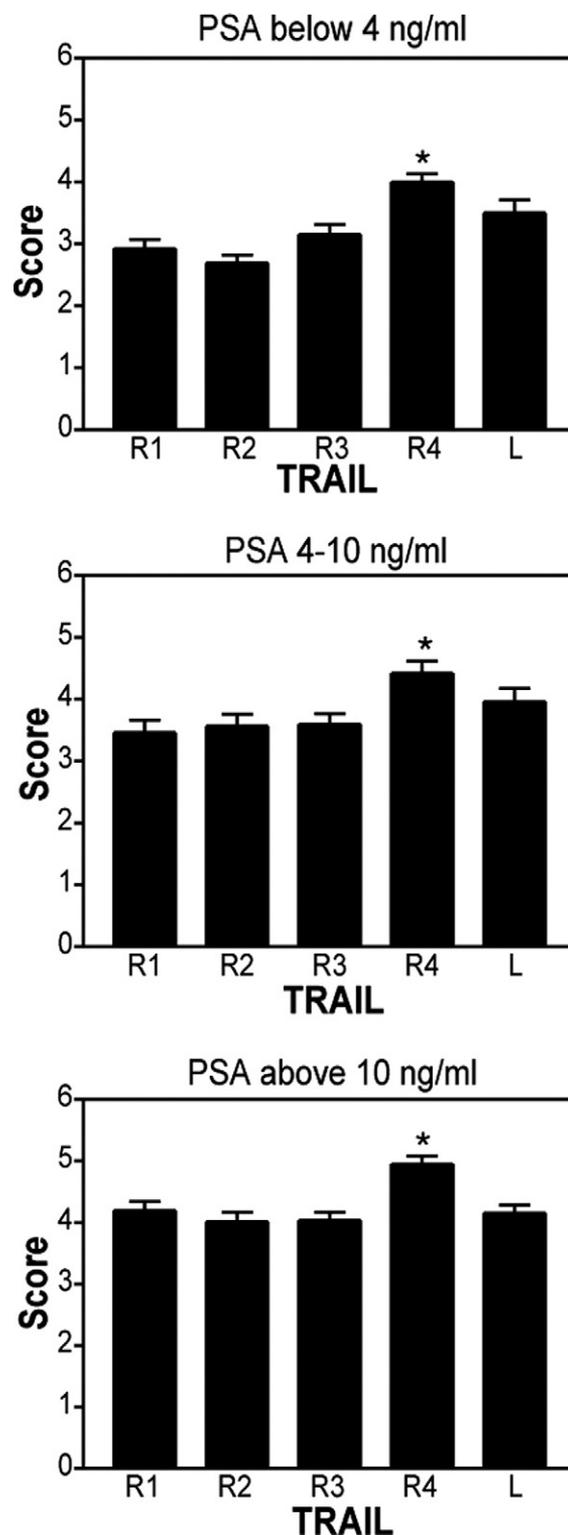


Fig. 1. Comparative analysis of the expression levels of TRAIL and its receptors in connection to serum PSA in patients with prostate carcinoma. Patients were categorized into 3 groups based on PSA values. PSA values of the first group (n = 26) were <4 ng/ml, whereas the PSA values of the second group (n = 28) were within the range of 4–10 ng/ml. The third group (n = 44) consisted of patients whose PSA values were >10 ng/ml, as depicted above each panel.

Table 2  
Descriptive statistics of 3 groups of patients categorized based on serum PSA levels

PSA (ng/ml)	Mean	Standard error	Median	Minimum	Maximum
<4 (n = 26)	1.54	0.21	1.18	0.00	3.60
4–10 (n = 28)	6.82	0.33	7.10	4.13	9.98
>10 (n = 44)	55.52	12.30	23	10.20	500

tumor volume [3]. For example, 80% and 67% of men with PSA values <4 and 4–10 ng/ml have pathologically organ-confined disease, respectively. On the other hand, >50% of men with PSA values >10 ng/ml have disease beyond the prostate [24]. For that reason, patients with high-serum PSA levels are likely to have poor prognosis with primary local therapy alone. In other words, the prognosis of patients with a high-serum PSA level is worse than that of patients with low serum PSA [25]. As depicted in Fig. 1, patients were categorized into 3 groups based on PSA values as those with PSA values: <4 ng/ml (first group: n = 26); 4–10 ng/ml (second group: n = 28); and >10 ng/ml (third group: n = 44). Descriptive statistics of each group are provided in Table 2. None of the groups displayed gaussian distribution as tested by the Shapiro-Wilk method. Thus, the statistical significance among the groups was analyzed using the Friedman test. Next, the Wilcoxon signed ranks test with Bonferroni correction was applied to compare paired groups of 2 (Fig. 1). TRAIL-R4 was the only marker that showed a statistically significant difference compared to other markers in each group. Kruskal-Wallis variance analysis revealed the potential difference among the groups. Later, the Mann-Whitney *U* test with Bonferroni correction was used to compare all groups in pairs. Although the second group manifested higher TRAIL-R2 death receptor expression compared to the first group, the third group displayed higher TRAIL-R1 death receptor expression compared to the second group. Moreover, patients with high PSA values (third group) had increased levels of all TRAIL markers compared to the first group with lower PSA values. Multinomial logistic regression analyses indicated that TRAIL-R2 death receptor and TRAIL-R4 decoy receptor expressions were independent indicators of prognosis based on patients' PSA status (n = 98), as shown in Table 3.

### 3.3. TRAIL and TRAIL-R4 expressions are inversely correlated in patients with high Gleason scores

It has clearly been established that high Gleason scores (Gleason  $\geq 7$ ) were correlated with poor outcomes in patients with prostate carcinoma [23]. To determine whether there was any correlation between Gleason scores of patients and TRAIL receptor expression, the Spearman rho correlation test (n = 54) was administered (Table 4). Although high Gleason scores were positively correlated with TRAIL-R4 expression, a negative correlation was noticed

between TRAIL death ligand expression and patients' Gleason scores. In addition, an inverse correlation was detected between the levels of TRAIL-R4 decoy receptor expression and TRAIL death ligand expression in patients with advanced prostate carcinoma. Finally, there was some degree of correlation between TRAIL-R3 and TRAIL-R4 decoy receptor expressions.

### 3.4. Comparative analysis of TRAIL and TRAIL receptor expressions based on patients' Gleason scores

Based on the fact that high Gleason scores (Gleason  $\geq 7$ ) indicated poor prognosis, patients were categorized into 2 different groups as those with Gleason  $\geq 7$  (n = 36) and those with Gleason <7 (n = 18), as shown in Table 5. Because neither group showed gaussian distribution, the nonparametric Mann-Whitney *U* test was administered to reveal the statistical significance between the 2 advanced prostate carcinoma groups. As shown in Fig. 2, TRAIL-R4 decoy receptor ( $P = 0.002$ ) and TRAIL death ligand ( $P = 0.001$ ) manifested significant differences in patients with Gleason  $\geq 7$  compared to patients with Gleason <7. On the other hand, multiple binary logistic regression analysis based on Gleason scores (n = 54) revealed that compared to TRAIL decoy and death receptors, only TRAIL death ligand expression was an independent predictor of prognosis, just as the patient's Gleason scores (Table 6).

### 3.5. TRAIL-R4 decoy receptor expression is correlated with shortened disease-free survival time

Kaplan-Meier survival analysis and the log-rank test (Mantel-Cox) were administered to determine if TRAIL and TRAIL receptor expression correlated with PSA recurrence, which is indicative of poor prognosis in patients with prostate carcinoma. As shown in Fig. 3, only TRAIL-R4 decoy receptor expression was correlated with shortened disease-free survival time. As TRAIL-R4 immunohistochemical staining scores increased, disease-free survival time significantly decreased.

Table 3  
Multinomial logistic regression analyses based on PSA values (n = 98)

PSA	Univariate	Multivariate
R1	0.00	0.13
R2	0.00	0.01*
R3	0.00	0.55
R4	0.00	0.01*
L	0.01	0.06

Three groups of patients were included in the analysis. Univariate analysis indicates *P* values for Kruskal-Wallis test which are all below  $P < 0.05$ .

\*  $P < 0.05$ .

Table 4  
Spearman rho correlations (n = 54)

	Gleason	R1	R2	R3	R4	L
Gleason						
Correlation coefficient	1	0.12	0.01	0.03	0.40*	-0.49*
Sig (2-tailed)		0.35	0.90	0.81	0.00	0.00
TR1						
Correlation coefficient	0.12	1	0.13	-0.01	0.18	-0.16
Sig (2-tailed)	0.35		0.33	0.89	0.19	0.23
TR2						
Correlation coefficient	0.01	0.13	1	-0.03	-0.04	-0.05
Sig (2-tailed)	0.90	0.33		0.79	0.76	0.70
TR3						
Correlation coefficient	0.03	-0.01	-0.03	1	0.33*	-0.01
Sig (2-tailed)	0.81	0.89	0.79		0.01	0.93
TR4						
Correlation coefficient	0.40*	0.18	-0.04	0.33*	1	-0.34*
Sig (2-tailed)	0.00	0.19	0.76	0.01		0.01
L						
Correlation coefficient	-0.49*	-0.16	-0.05	-0.01	-0.34*	1
Sig (2-tailed)	0.00	0.23	0.70	0.93	0.01	

Nonparametric correlation analysis of 54 patients with prostate carcinoma in conjunction with Gleason scores, TRAIL, and TRAIL receptors.

Sig = significant.

\* Statistically significant correlation coefficients ( $P < 0.05$ ).

### 3.6. TRAIL-R4 and TRAIL expressions, and their connection to patient survival in those with prostate carcinoma

Survival rates and immunohistochemical staining scores of TRAIL and its receptors were compared using Kaplan-Meier survival analysis followed by the log-rank test. Although higher TRAIL-R4 decoy receptor expression was correlated with decreased survival, higher TRAIL expression was correlated with increased survival, as depicted in Figs. 4 and 5, respectively.

## 4. Discussion

Prostate cancer is the most frequently diagnosed cancer type, and it is only the second to lung cancer in cancer-related deaths [1]. The widespread use of the PSA measurement as a diagnostic tool has resulted in a 20% increase in the detection of clinically localized prostate cancer. Despite this result, approximately one third of the newly diagnosed cases are regarded as locally advanced at diagnosis. Locally advanced prostate cancer encompasses a wide spectrum of tumor phenotypes with differing prognoses, and more than

50% of these men are at risk for having tumor recurrence after a local therapy regimen [26]. Therefore, it is important to be able to recognize those patients who are at high risk so that appropriate primary and/or adjuvant treatment can be granted. Although staging accuracy is somewhat enhanced by using the combination of local disease extent on DRE, serum PSA level, and Gleason score [2,3], the prognostic value of clinical criteria to predict tumor extent is still limited for an individual patient. Several biologic and molecular parameters are considered as potential prognostic markers for prostate cancer [11], but tumor Gleason score is currently the most important prognostic variable [27,28].

The expression patterns of TRAIL and its receptors have recently been revealed for patients with prostate carcinoma [19]. However, we still do not know whether these markers

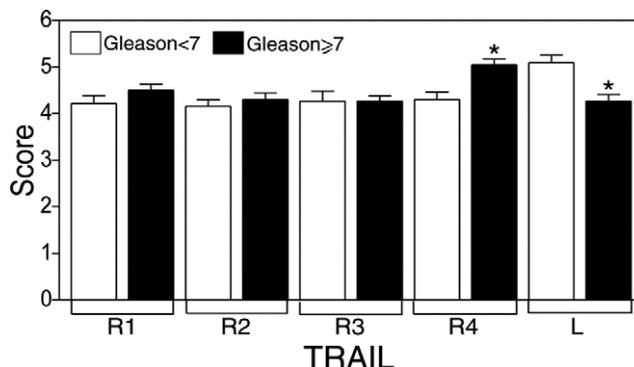


Fig. 2. The expression levels of TRAIL and its receptors in connection to Gleason scores in patients with prostate carcinoma. The Mann-Whitney  $U$  test was applied to determine the statistical significance between the patients with Gleason  $<7$  (n = 18) versus patients with Gleason  $\geq 7$  (n = 36). Only TRAIL-R4 and TRAIL-L showed such a difference ( $*P < 0.05$ ).

Table 5  
Descriptive statistics of patients categorized based on Gleason scores

Gleason score	Mean	Standard error	Median	Minimum	Maximum
$<7$ (n = 18)	5.61	0.14	6.00	4.00	6.00
$>7$ (n = 36)	8.25	0.16	8.00	7.00	10.00

Table 6  
Multiple binary logistic regression analyses based on Gleason scores (n = 54)

Gleason	Univariate	Multivariate
R1	0.244	0.458
R2	0.581	0.733
R3	0.920	0.943
R4	0.002*	0.821
L	0.001*	0.004*

Patients were divided into 2 groups based on Gleason grade: those with Gleason >7 and those with Gleason <7 prior to multiple regression analysis. Mann-Whitney *U* test is used for univariate analysis.

\* *P* < 0.05.

are useful in terms of the assessment of prostate carcinoma in connection to currently used prognostic markers, such as serum PSA and Gleason scoring. In this study, TRAIL and TRAIL receptor expressions were positively correlated with serum PSA levels as deduced from the Spearman rho correlation test. Although TRAIL-R4 decoy receptor expression was the highest TRAIL receptor expressed in all 3 groups of patients categorized based on serum PSA levels, the comparison among the groups suggest that all TRAIL markers were generally high in patients with high-serum PSA levels (third group) compared to patients with low PSA values (first group). On the other hand, multinomial regres-

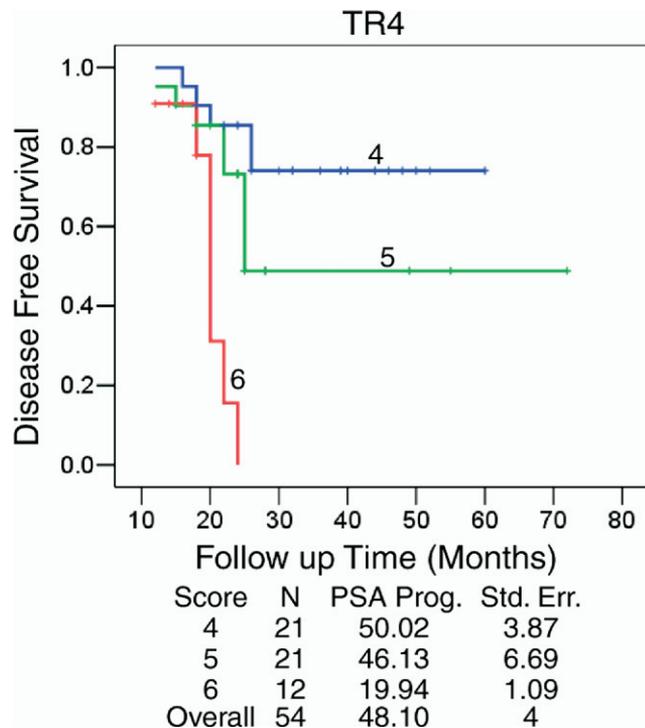


Fig. 3. TRAIL-R4 expression is correlated with PSA recurrence in patients with prostate carcinoma. The numbers (N) above each line represent immunohistochemical staining scores for TRAIL-R4. Follow-up time is provided on the x-axis. Mean PSA progression (Prog) in months is shown below the figure. Std Err, standard error. (Color version of figure is available online.)

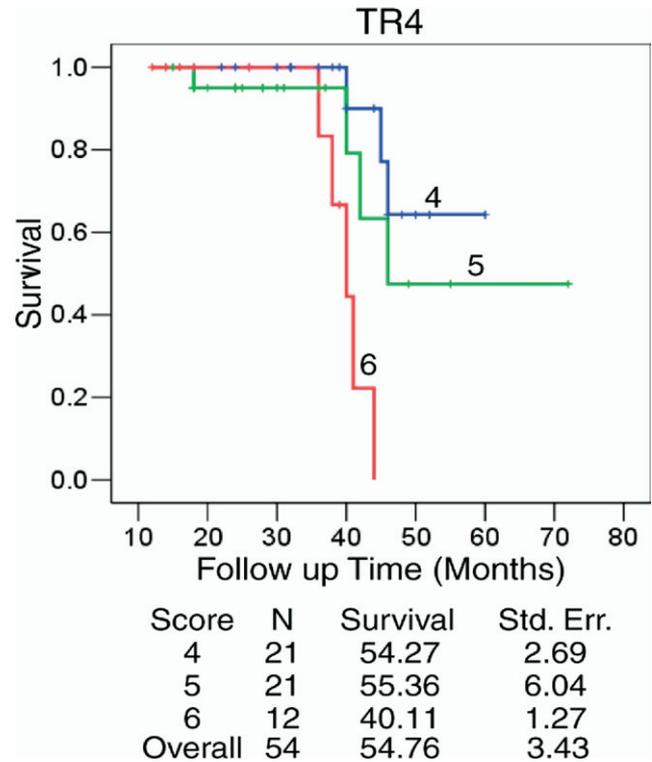


Fig. 4. TRAIL-R4 expression is correlated with decreased survival in patients with prostate carcinoma. The numbers (N) above each line depict TRAIL-R4 staining score. Survival below the figure implies mean survival rates. Std Err, standard error. (Color version of figure is available online.)

sion analyses indicated that TRAIL-R2 death receptor and TRAIL-R4 decoy receptor expressions were the only ones showing an independent correlation with serum PSA levels that might be useful for prognosis.

Numerous multivariate analyses of prognostic criteria concerning the histologic grading of prostate cancer in men with clinically localized disease showed that the Gleason score is a strong predictor of the disease extent [3,28]. The presence of a Gleason grade  $\geq 4$  or a Gleason score  $\geq 7$  is predictive of a poor prognosis [23,28]. In the present study, high Gleason scores correlated with high levels of TRAIL-R4 decoy receptor and low levels of TRAIL death ligand expressions. Interestingly, the presence of high levels of TRAIL-R4 decoy receptor expression has recently played an important role in the development of a resistance mechanism to apoptotic ligands [16,17]. Intriguingly, multiple binary logistic analyses using the Gleason scores of 54 patients suggest that only TRAIL death ligand expression can be used as an independent marker to predict the prognosis of patients with prostate carcinoma, as it is performed with Gleason scoring.

PSA recurrence and survival rates are the other important parameters for prognosis in prostate cancer. In our study, TRAIL-R4 decoy receptor expression was the only marker that displayed a correlation with PSA recurrence, indicating poor prognosis in patients with prostate carcinoma. Con-

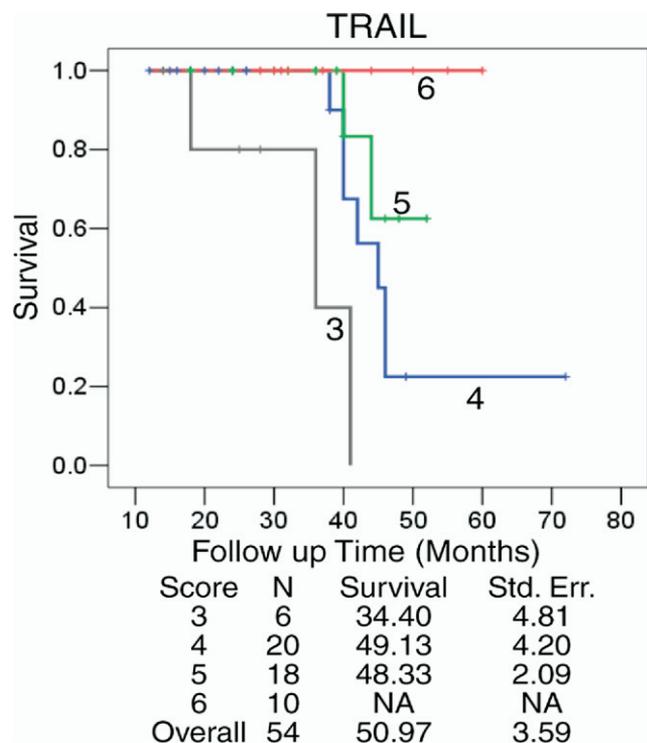


Fig. 5. TRAIL death ligand expression is correlated with increased survival in patients with prostate carcinoma. TRAIL staining scores are provided above each line. Mean survival rates are given below the figure. N, number; Std Err, standard error. (Color version of figure is available online.)

versely, higher levels of TRAIL-R4 decoy receptor expression but lower levels of TRAIL death ligand expression correlated with decreased survival, as shown by Kaplan-Meier survival analysis. Cox regression analysis concerning the PSA recurrence indicated that in addition to Gleason scores, TRAIL-R4 decoy receptor expression was the only valuable parameter for prognosis; however, the use of TRAIL-R4 expression as an independent parameter is not yet advised because of the need for conducting similar tests in a larger population of patients with prostate cancer. In terms of survival rates, TRAIL death ligand expression appeared to be the only important variable in addition to Gleason scores as deduced from the Cox regression analysis. Analysis of archival specimens with a minimum 5-year follow-up after the treatment should be valuable to assess better the prognostic significance of TRAIL markers in prostate carcinogenesis.

## 5. Conclusions

A recombinant soluble form of TRAIL has recently been evaluated as a potential cancer therapeutic agent against a variety of solid tumors and hematologic malignancies in the clinical trials conducted by Genentech (South San Francisco, CA), in collaboration with Amgen (Thousand Oaks, CA). Moreover, a recombinant adenovirus encoding the

human TRAIL complementary deoxyribonucleic acid (Ad5-TRAIL) [29,30] has recently entered into Phase I clinical testing in patients with prostate cancer. Despite these encouraging attempts, the contribution of TRAIL and its receptors to the carcinogenesis process is not known. Thus, the evaluation of TRAIL and TRAIL receptor expressions in connection to other prognostic markers might be useful to evaluate the progression of prostate carcinoma.

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