Tumoral Expression of TXR1 and TSP1 Predicts Overall Survival of Patients with Lung Adenocarcinoma Treated with First-line Docetaxel-Gemcitabine Regimen

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Abstract **Purpose:** *In vitro* data suggest that down-regulation of thrombospondin 1 (*TSP1*) expression from *TXR1* is associated with resistance to taxane-based chemotherapy. The prognostic and predictive value of tumoral expression of both genes was evaluated in patients with lung adenocarcinoma treated with first-line docetaxel and gemcitabine.
Experimental Design: Tumor samples from 96 patients, with stage IIIB (with pleural effusion) or IV lung adenocarcinomas, were analyzed for *TXR1* and *TSP1* mRNA levels by quantitative real-time PCR, from microdissected cells derived from patients' primary tumors.
Results: The mRNA levels of the two genes were inversely correlated (Spearman's test = -0.49; $P < 0.0001$). Patients with low TXR1 mRNA levels experienced a longer median time to tumor progression (TTP; $P < 0.0001$) and median overall survival (mOS; $P = 0.001$) when compared with patients with high TXR1 expression. Patients with high TSP1 expression presented longer TTP ($P = 0.002$) and mOS ($P < 0.0001$) when compared with patients with low TSP1 expression. Moreover, patients with high TSP1 and low TXR1 expression ($n = 36$) presented higher prolonged TTP ($P = 0.009$) and mOS ($P < 0.0001$) compared with patients with high TXR1 and low TSP1 expression. Multivariate analysis showed that high TXR1/low TSP1 expression was an independent prognostic factor for decreased TTP (hazard ratio, 1.7; 95% confidence interval, 1.1-3.27; $P = 0.016$) and mOS (hazard ratio, 2.55; 95% confidence interval, 1.57-4.15; $P < 0.0001$).
Conclusion: These data confirm the *in vitro* model of TSP1 and TXR1 effect on taxane resistance in lung adenocarcinomas and merit further evaluation.

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Non–small cell lung cancer (NSCLC) remains the leading cause of cancer death worldwide despite the fact that significant treatment improvements have been made over the last decade (1). Combinations of "third-generation" cytotoxic agents, such as taxanes, vinorelbine, and gemcitabine with cisplatin, have emerged as new standards, producing higher response rates and, in some cases, prolonged survival. In phase III clinical trials conducted in patients with advanced NSCLC, treatment with the combination of a platinum compound and a taxane extended the median survival time to 8 to 11 months and the 1-year survival rate to 31% to 46% (2–4). As an alternative option, non–cisplatin-containing doublets have also been tested in several randomized phase III studies (5–9). The majority of them have reported that the non–platinum-containing combinations have substantial efficacy against advanced/metastatic NSCLC with a more favorable toxicity profile than the corresponding cisplatin-based regimens. Therefore, non–platinum-containing combinations are considered as alternative options to platinum-based regimens in the first-line setting, especially for patients who have a contraindication for platinum administration (10).

The antitumor activity of taxanes (paclitaxel and docetaxel) is the result of their binding to the β subunits of tubulin, which causes the stabilization of tubulin polymerization. This stabilization results in cell cycle arrest at the G_2-M phase, thus inhibiting mitosis (11). At present, the widely studied mechanism of resistance to anti-tubulin agents is the multidrug-resistant (MDR) phenotype, which is mediated by the ATP-dependent cell membrane–bound P-glycoprotein encoded by the *MDR1* gene (12, 13).

A recently reported *in vitro* study proposed another mechanism of resistance to taxanes (14). Using function-based screens, the investigators identified a previously unknown gene,

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Translational Relevance

Taxanes are among the most active antitumor agents in the treatment of non–small cell lung cancer and against a wide spectrum of human neoplastic malignancies. However, an increasing number of patients being treated with taxanes develop resistance that finally limits chemotherapeutic efficacy. Thus, the elucidation of the mechanisms underlying taxane resistance and their evaluation in clinical samples are of pivotal importance for the development of therapeutic strategies taking into account these mechanisms. Recently, a novel mechanism of taxane resistance has been suggested involving TXR1 protein that impedes taxane-induced apoptosis by down-regulation of thrombospondin 1 (*TSP1*). This finding has not been tested in clinical level thus far. Our results confirm the *in vitro* evidence that overexpression of *TXR1* suppresses the expression of *TSP1* and is significantly correlated with resistance to taxane-based chemotherapy. This finding underscores the potential of *TXR1/TSP1* expression as predictive biomarker for resistance to taxane-based chemotherapy.

TXR1 (15), whose protein overexpression conferred resistance to paclitaxel. They also showed that *TXR1* down-regulates mRNA expression of an antiangiogenic and proapoptotic glycoprotein, thrombospondin 1 (encoded by the *TSP1* gene). This was proven to prevent taxane-induced apoptosis in human prostate cancer cell lines. Moreover, the cytotoxicity of taxanes is increased in cancer cells by either inactivation of *TXR1* using small interfering RNA or activation of integrin-associated protein (also known as CD47; ref. 14).

In a previous randomized study comparing docetaxel/cisplatin with docetaxel/gemcitabine in patients with advanced NSCLC, we have observed that the incidence of objective responses induced by the docetaxel-gemcitabine regimen was significantly higher in patients with adenocarcinoma histology than in patients with nonadenocarcinoma histology (6). To investigate this observation, we have initiated a pharmacogenomic project studying several markers related with taxane and/or gemcitabine resistance in adenocarcinomas (16, 17). In this context, we investigated whether the tumoral expression of *TXR1* and *TSP1* is clinically relevant and correlates with taxane resistance in patients with lung adenocarcinomas treated with the docetaxel-gemcitabine regimen.

Materials and Methods

Patients. Tumoral samples from patients with histologically confirmed inoperable stage IIIB (with pleural effusion) and IV adenocarcinoma of the lung, who were treaded with docetaxel-gemcitabine as first-line treatment, in the context of two randomized trials conducted by the Hellenic Oncology Research Group, where the docetaxel-gemcitabine regimen was the investigational arm in both of them, were retrospectively collected and analyzed. The main eligibility criteria were identical in both studies (6, 7). The studies were approved by the Ethics and Scientific Committees of the participating hospitals and conducted according to the Declaration of Helsinki. All patients gave written informed consent before study entry and approved the use of their biological material for translational research studies. Gemcitabine (Gemzar; 1,000 mg/m^2 on days 1 and 8; Eli Lilly) and docetaxel (Taxotere; 100 mg/m^2 on day 8; Sanofi-Aventis) with human granulocyte colony-stimulating factor support were administered every 3 wk as previously described (6). The doses of anticancer drugs were adjusted depending on the hematologic and nonhematologic toxicity. Patients' evaluation was done at baseline and every three cycles of chemotherapy thereafter (6, 7).

Specimen characteristics and assay methods. All paraffin-embedded tumors were reviewed by two independent pathologists (A.K. and E.S.) to ensure the validity of the specimen and define the most appropriate tumoral area for microdissection. From each paraffin block of representative tumoral areas, serial sections with a thickness of 5 μm were prepared and then stained with nuclear Fast Red (Sigma-Aldrich). Malignant cells were procured using a piezoelectric microdissector (Eppendorf).

The pellet of microdissected cells was resuspended in 400 μL RNA lysis buffer (18) supplemented with 300 mg proteinase K (Qiagen) and incubated at 60°C for 16 h until the tissue was completely solubilized. RNA was purified by Trizol LS (Invitrogen) and subsequently treated with DNase (DNÁ-free; Ambion) to avoid genomic DNA contamination. The SuperScript III Reverse Transcriptase (Invitrogen) was used to prepare cDNA from 300 ng of total RNA. The reverse transcription-PCR was carried out using 10 ng of RNA input included in 2.5 μL of cDNA template and 6.25 μL of Taqman Universal Master Mix (Applied Biosystems) with the addition of specific primers and probe for each gene and adjusted with diethyl pyrocarbonate water to a final volume of 12.5 μL per reaction. Relative cDNA quantification for *TXR1*, *TSP1*, and *β-actin* as an internal reference gene was done using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). The primers and probe sets were designed using Primer Express 2.0 Software (Applied Biosystems).

Primers and probes were designed according to the RefSeq NM_001005355 for *TXR1* and NM 003246 for *TSP1*. The primers and 5′-labeled fluorescent reporter dye (6FAM) probe were as follows: *TXR1*, 5′-GCAGAAGAAAATGAAGAAAGCTCATAA-3′ (forward), 5′-GGAATGCTTGCCATGCTTGT-3′ (reverse), and 5′-ATGCACAAGCACCAAAAGCACCACAAGTAC-3′ (probe); *TSP1*, 5′-TGGTGTGCGTGGCCAAT-3′ (forward), 5′-TGCCCTGAGTTGGGAAGGT-3′ (reverse), and 5′-CGACTTACCACTGCAAAAAGGATAATTGCCC-3′ (probe).

Relative gene expression quantification was done according to the comparative C_T method using β-actin as an endogenous control and commercial RNA controls (Stratagene) as calibrators. Final results were determined as follows: $2^{-(\Delta CT\ \mathrm{sample}\ -\ \Delta CT\ \mathrm{calibrator})}$, where ΔC_T values of the calibrator and sample were determined by subtracting the C_T value of the target gene from the value of the reference gene. In all experiments, only triplicates with a SD of the C_T value <0.25 were accepted. In addition, genomic DNA contamination of each sample has been excluded by non–reverse transcription of RNA.

Study design and statistics. The present study was a retrospective analysis aiming to explore the prognostic and predictive value of TXR1 and TSP1 mRNA expression in patients with lung adenocarcinomas treated with first-line docetaxel-gemcitabine regimen. All available biopsies of the primary tumor with >100 cells per section were included in the analysis. Objective responses were recorded according to the Response Evaluation Criteria in Solid Tumors criteria (19). All efficacy and toxicity results were assessed on an intention-to-treat basis. Median time to tumor progression (TTP) and overall survival (OS) were calculated from the start of treatment to the first documented disease progression or death, respectively. Quantitative PCR analyses yielded values that were expressed as ratios between two absolute measurements (gene of interest/internal reference gene). Cutoff points were calculated according to the median value for the mRNA expression of each gene. Samples with mRNA expression above or equal to the median were considered as samples with high expression, whereas those with value

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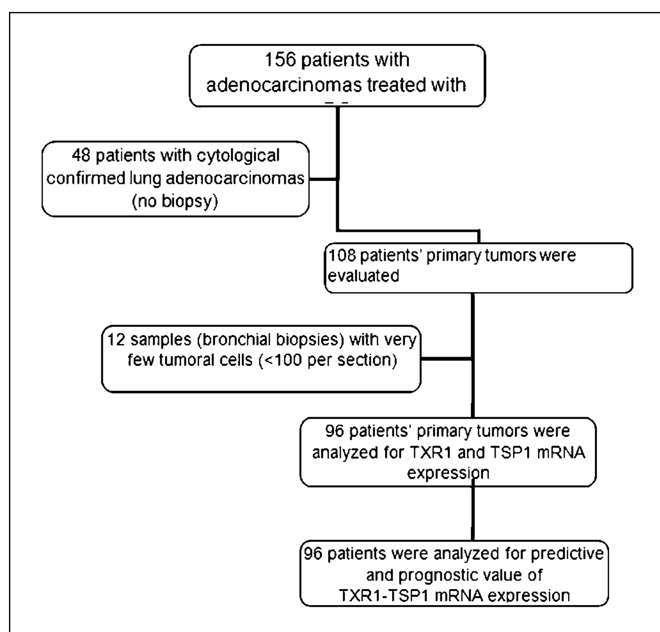

Fig. 1. Flow chart showing the process of obtaining archival paraffin-embedded tumor biopsies for the assessment of *TXR1* and *TSP1* mRNA expression.

below the median as samples with low expression. In addition, the mRNA expression values of the genes were divided in terciles (T1-T3) and comparisons were done between all three groups. All the laboratory analysis was done blinding to the clinical data.

The potential association between baseline characteristics, response, and gene expression levels were compared with either the two-sided Fisher's exact test or the χ^2 test for categorical variables and the Kruskal-Wallis test for continuous variables. The normality of continuous variables was verified with a Kolmogorov-Smirnov test. Spearman's exact test was used to evaluate the correlation between *TXR1* and *TSP1* mRNA expression. The association of risk factors with time-to-event end points was analyzed with the log-rank test, and the Kaplan-Meier method was used to plot the corresponding time to progression and survival curves. A univariate Cox regression analysis, with hazard ratios (HR) and 95% confidence intervals (95% CI), was used to assess the association between each potential prognostic factor and survival and time to progression. These factors were then included in a multivariate Cox proportional hazards regression model with a stepwise procedure (both forward and backward) to evaluate the independent significance of different variables on survival and time to progression. Statistical significance was set at $P = 0.05$.

Results

Patients' characteristics. Clinical data and representative samples from the primary tumors were available from 96 patients (group A) for molecular analysis representing the 61.5% of the 156 patients (group B) with lung adenocarcinomas treated with the docetaxel-gemcitabine regimen in both trials. There was no significant difference in the main patients' characteristics between the two groups of patients. Among the 156 patients treated with the docetaxel-gemcitabine regimen, 48 had a cytologic diagnosis (Fig. 1). From the remaining 108 patients' specimens that were evaluated microscopically, 12 specimens were considered inappropriate for further analysis due to the small

Table 1. Patients' characteristics and gene expression

	Patients treated with docetaxel-gemcitabine		Patients analyzed		*P*
	n	%	*n*	%	
Gender					
Male	138	89	90	94	0.6
Female	18	11	6	6	0.2
Age (y)					
Median	63		62		0.8
Range	34-78		38-77		
Performance status (Eastern Cooperative Oncology Group)					
0	53	34	34	36	0.8
1	91	58	58	60	
2	12	8	4	4	
Stage					
IIIB (wet)	55	35	32	33	0.9
IV	101	65	64	67	
Response rate (CR + PR)	50	32	26	27	0.4
TTP (mo)	4.0		3.6		0.7
mOS (mo)	9.6		10.2		0.5
TXR1 mRNA expression					
Samples analyzed			96		
Median (range)	1.02 (0.01-27.5)				
High expression			47	49	
Low expression			49	61	
TSP1 mRNA expression					
Samples analyzed			96		
Median (range)	0.16 (0.01-1.11)				
High expression			48	50	
Low expression			48	50	

Abbreviations: CR, complete response; PR, partial response.

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number of cancer cells (Fig. 1). Successful amplification of both genes was achieved in all of the remaining 96 specimens.

Clinical outcome. In an intention-to-treat analysis, complete response was observed in 3 (3%) and partial response in 23 (24%) patients (overall response rate, 27%; 95% CI, 21.4-38.4%). After a median follow-up period of 8.6 months (range, 1.2-71.7), the median TTP was 3.6 months (range, 0.7-52.4) and the median OS (mOS) was 10.2 months (range, 1.2-71.7); the 1-year survival rate was 41.3%. The clinical outcome of the 96 analyzed patients was similar with that reported for the whole patient population treated with the docetaxel-gemcitabine regimen in either of the randomized trials.

TXR1 and TSP1 mRNA expression levels and response to treatment. Clinical characteristics and gene mRNA levels are summarized in Table 1. The median mRNA expression levels were 1.02 (range, 0.01-27.5) for *TXR1* and 0.16 (range, 0.01-1.11) for *TSP1*. There was no correlation between age ($P = 0.81$), gender ($P = 0.24$), performance status ($P = 0.49$), or stage of disease ($P = 0.32$) and TXR1 or TSP1 ($P = 0.59$, 0.17, 0.28, and 0.23, respectively) mRNA levels. A highly significant inverse correlation was observed for *TXR1* and *TSP1* expression levels (Spearman's test = -0.49; $P < 0.0001$). By adopting cutoff values according to median expression levels, high (above the median) tumoral *TXR1* mRNA expression was observed in 47 (49%) and low (below or equal to the median) in 49 (51%) patients; an equal number of patients with high and low tumoral *TSP1* mRNA expression was observed (48 patients in each group).

Table 2 summarizes the treatment outcomes according to *TXR1* and *TSP1* mRNA expression. Patients with low tumoral *TXR1* expression experienced a longer TTP (8.5 versus 2.3 months; $P < 0.0001$; Fig. 2A) and OS (18.6 versus 6.7 months; $P = 0.001$; Fig. 3A) and disease control rate (DCR; objective response and disease stabilization; 71% versus 36%; $P = 0.001$) but not objective response rate (ORR; 35.0% versus 19%; $P = 0.1$) compared with patients whose tumors had high *TXR1* mRNA expression. In addition, patients with high tumoral *TSP1* expression presented longer TTP (6.1 versus 2.3 months; $P = 0.002$; Fig. 2B), OS (19.1 versus 7.4 months; $P < 0.0001$; Fig. 3B), and DCR (67% versus 32%; $P < 0.0001$) without any difference in ORR (31% versus 22%; $P = 0.5$) compared with patients with low tumoral *TSP1* mRNA expression. Nevertheless, analysis of the mRNA expression of the genes as continuous variables revealed that as *TXR1* mRNA levels increased, the probability of response significantly decreased (odds ratio, 0.74; 95% CI, 0.47-0.89; $P = 0.001$); conversely, as the *TSP1* mRNA levels increased, the probability of response significantly increased (odds ratio, 1.76; 95% CI, 1.38-3.61; $P < 0.0001$).

Analysis according to terciles of expression levels revealed that patients with tumoral *TXR1* expression in the lowest tercile (T1 expression value ≤0.75; $n = 32$) experienced significantly higher ORR ($P = 0.02$) and DCR and longer TTP and OS compared with patients whose tumors had *TXR1* mRNA expression in the middle (T2 expression value >0.73 and <1.23; $n = 32$) and top (T3 expression value ≥1.23; $n = 32$) terciles. Additionally, DCR, TTP, and OS (Table 2) were significantly higher in patients with tumoral *TSP1* mRNA expression in the top tercile (T3, expression value ≥0.32; $n = 32$) compared with those in the lowest tercile (T1, expression value ≤0.09; $n = 32$) or middle tercile (T2, expression value >0.09 and <0.32; $n = 32$), whereas ORR was not different between the three groups ($P = 0.3$).

The combined analysis of tumoral expression levels for both *TXR1* and *TSP1* showed that DCR (84% versus 30%; $P < 0.0001$), TTP (8.6 versus 2.3 months; $P = 0.009$), and OS (20.1 versus 4.2; $P < 0.0001$) were significantly increased for patients with low *TXR1*/high *TSP1* mRNA expression levels compared with patients with high *TXR1*/low *TSP1* mRNA

Table 2. Tumoral expression of TXR1 and TSP1 mRNA and treatment efficacy

		TTP (mo)		OS (mo)		DCR, *n* (%)		
		Median (95% CI)	*P*	Median (95% CI)	*P*	CR + PR + SD (%)	PD	*P*
TXR1 low	49 (51)	8.5 (4.3-12.6)	<0.0001	18.6 (10.8-26.5)	0.001	71	29	0.001
TXR1 high	47 (49)	2.3 (1.8-2.7)		6.7 (4.8-8.5)		36	64	
TXR1 (terciles)								
T1*	32	8.5 (3.6-13.3)	<0.0001†	19.1 (12.9-25.2)	0.001†	81	19	<0.0001†
T2*	32	4.2 (2.2-6.1)	0.006‡	11.5 (4.0-21.0)	0.03‡	59	41	0.004‡
T3*	32	2.0 (1.5-2.5)	5.9 (2.1-9.7)	22	78			
TSP1 low	48 (50)	2.3 (1.8-2.7)	0.002	7.4 (5.9-8.8)	<0.0001	32	68	<0.0001
TSP1 high	48 (50)	6.1 (3.5-9.6)		19.1 (15.9-22.2)		67	33	
TSP1 (terciles)								
T1*	32	2.3 (2.1-2.5)	7.4 (1.6-910.3)	34	66			
T2*	32	2.7 (1.3-4.0)	0.003‡	7.4 (5.1-9.6)	0.008‡	43	57	0.009‡
T3*	32	8.6 (4.8-12.3)	<0.0001†	19.4 (17.3-21.5)	<0.0001†	84	16	<0.0001†
TXR1 low/TSP1 high	36	8.6 (6.3-10.9)	20.1 (16.0-24.3)	84	16			
TXR1 high/TSP1 low	40	2.3 (1.8-2.7)	0.009§	4.2 (3.6-4.8)	<0.0001§	30	70	<0.0001§
TXR1 low/TSP1 low	11	3.6 (1.6-5.6)	0.07‖	9.8 (4.9-15.0)	0.004‖	41	59	0.012‖
TXR1 high/TSP1 high	9	2.7 (1.4-3.9)	0.009¶	7.4 (5.9-8.8)	0.028¶	34	66	0.002¶

Abbreviations: SD, stable disease; PD, progressive disease.
*T1, lower tercile; T2, middle tercile; T3, higher tercile.
†T1 versus T3.
‡T1 versus T2.
§TXR1 low and TSP1 high versus TXR1 high and TSP1 low.
‖TXR1 low and TSP1 high versus both low.
¶TXR1 low and TSP1 high versus both high.

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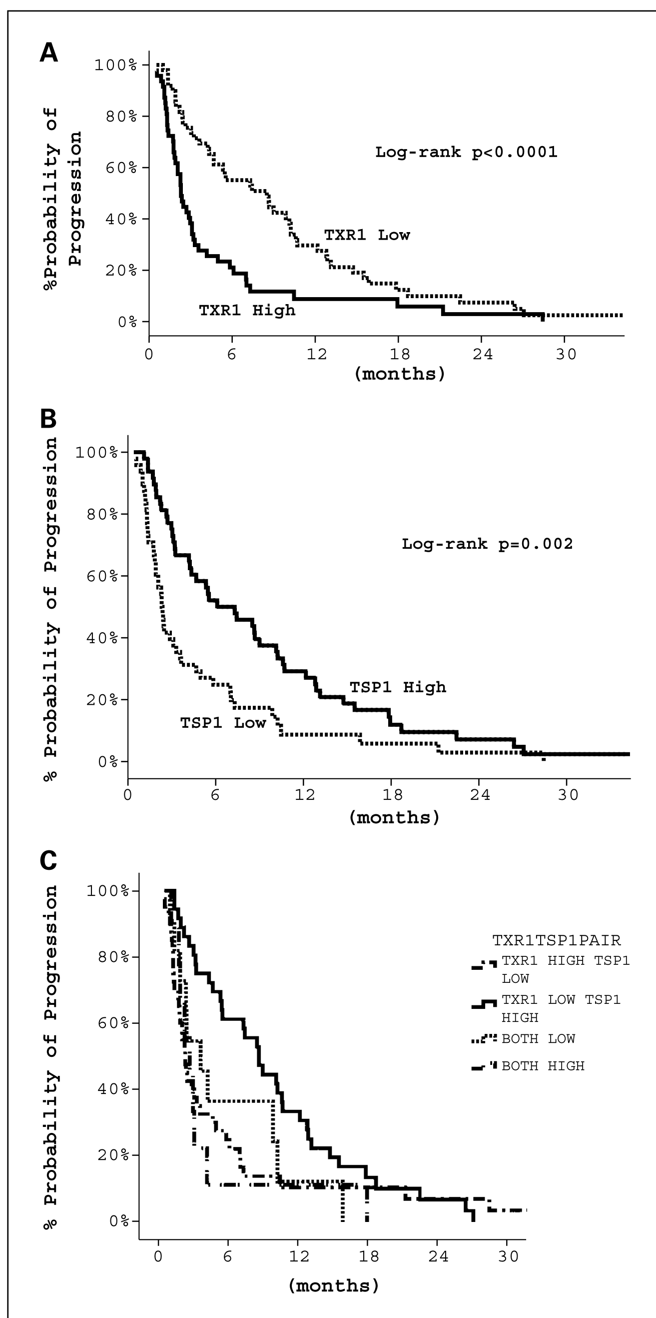

Fig. 2. TTP according to the mRNA expression of *TXR1* (*A*), *TSP1* (*B*), and both genes (*C*). Higher levels of *TXR1* (*A*) and lower levels of *TSP1* (*B*) were associated with significantly decreased TTP. Patients were classified in four groups according to the combined mRNA expression of the genes. Patients with the favorable genotype (high TSP1/low TXR1) presented significantly higher TTP in comparison with those with any other combination of expression (for more details, see Table 2).

expression or patients with high or low mRNA expression of both genes (Table 2; Figs. 2C and 3C).

Univariate and multivariate analysis. Univariate analysis showed that factors significantly correlated with decreased TTP was high *TXR1* ($P = 0.001$) and low *TSP1* ($P = 0.003$) mRNA expression as well as stage ($P = 0.004$), whereas performance status of 2 ($P = 0.028$) and high *TXR1* ($P = 0.001$) and low *TSP1* ($P < 0.0001$) mRNA expression were significantly associated with decreased OS (Table 3A). The logistic regression analysis revealed that combined tumoral expression of *TXR1* and *TSP1* (high *TXR1*/low *TSP1* expression) emerged as an independent prognostic factor for decreased TTP (HR, 1.7; 95% CI, 1.1-3.27; $P = 0.016$) and OS (HR, 2.55; 95% CI, 1.57-4.15; $P < 0.0001$; Table 3B). Stage at diagnosis was also an independent prognostic factor for decreased TTP (HR, 2.02; 95% CI, 1.26-3.27; $P = 0.004$) and OS (HR, 4.59; 95% CI, 1.53-13.22; $P = 0.006$).

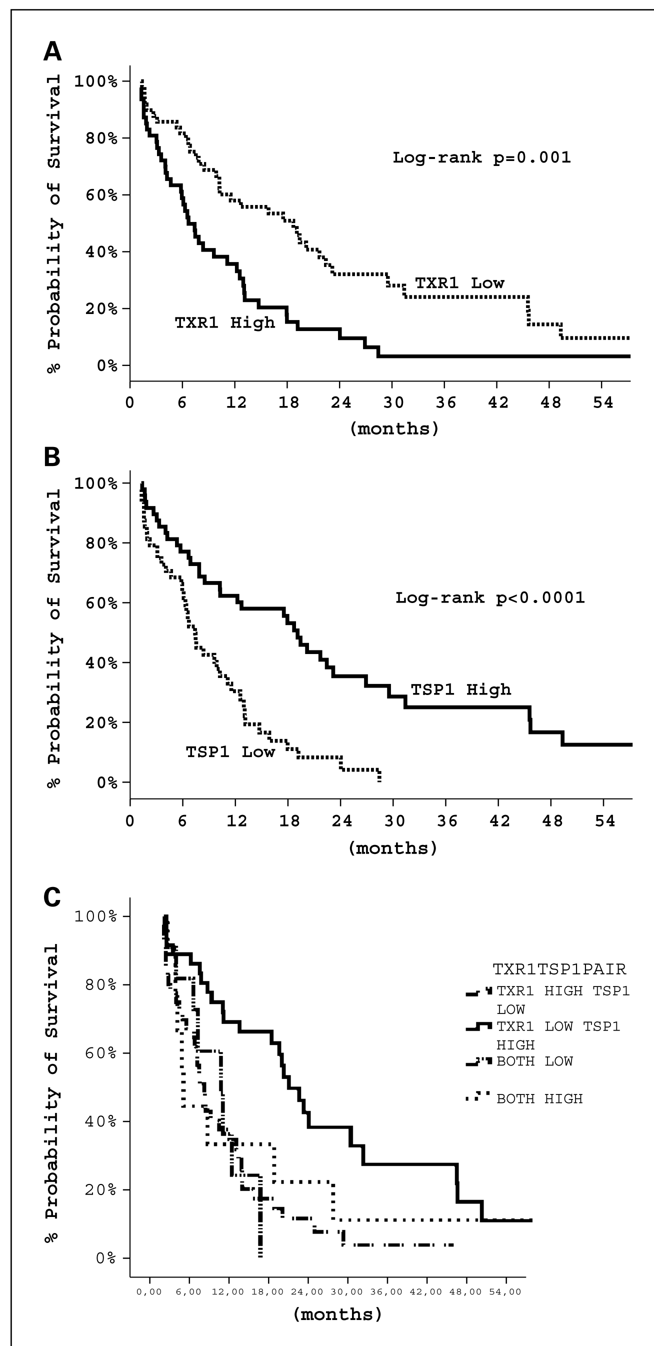

Fig. 3. mOS according to the mRNA expression of *TXR1* (*A*), *TSP1* (*B*), and both genes (*C*). Higher levels of *TXR1* (*A*) and lower levels of *TSP1* (*B*) were associated with significantly decreased OS. Patients were classified in four groups according to the combined mRNA expression of the genes. Patients with the favorable genotype (high TSP1/low TXR1) presented significantly higher OS in comparison with those with any other combination of expression (for more details, see Table 2).

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Table 3. Univariate and multivariate analysis for TTP and median OS for patients with lung adenocarcinoma treated with docetaxel-gemcitabine

	HR (95% CI)	*P*
A. Univariate analysis for TTP and OS		
TTP		
TXR1 expression (high vs low)	2. 1 (1.37-3.2)	0.001
TSP1 expression (low vs high)	1.9 (1.25-2.89)	0.003
TXR1-TSP1 expression (TXR high/TSP low vs others)	1.69 (1.1-2.59)	0.01
PS (0-1 vs 2)	2.33 (0.83-6.53)	0.1
Age (<70 vs >70 y)	1.15 (0.74-1.78)	0.53
Gender (male vs female)	1.78 (0.77-4.12)	0.17
Stage (IV vs IIIB)	2.03 (1.25-3.28)	0.004
OS		
TXR1 expression (high vs low)	2.16 (1.36-3.43)	0.001
TSP1 expression (low vs high)	2.74 (1.67-4. 48)	<0.0001
TXR1-TSP1 expression (TXR high/TSP low vs others)	2.38 (1.48-3.81)	<0.0001
PS (0-1 vs 2)	3.19 (1.13-9.0)	0.028
Age (≤65 vs >65 y)	1.074 (0.622-1.854)	0.797
Gender (male vs female)	1.07 (0.46-2.48)	0.87
Stage (IV vs IIIB)	1.71 (1.03-2.83)	0.037
B. Multivariate analysis for TTP and OS		
TTP		
Stage (IV vs IIIB)	2.02 (1.26-3.27)	0.004
TXR1-TSP1 expression (TXR high/TSP low vs others)	1.7 (1.1-3.27)	0.016
OS		
Stage (IV vs IIIB)	4.49 (1.53-13.22)	0.006
TXR1-TSP1 expression (TXR high/TSP low vs others)	2.55 (1.57-4.15)	<0.0001
PS (0-1 vs 2)	1.53 (0.92-2.57)	0.1

Abbreviation: PS, performance status.

Discussion

In the present study, we evaluated in tumoral samples from patients with lung adenocarcinomas treated with the docetaxel/gemcitabine combination a novel mechanism that has been suggested to modulate the cellular cytotoxicity of taxanes *in vitro* (14). Our results confirmed the *in vitro* evidence that overexpression of *TXR1* was significantly correlated with down-regulation of *TSP1* expression ($P < 0.0001$). Moreover, the expression of both genes was significantly correlated with treatment outcome. More importantly, patients with the favorable genotype (low *TXR1*/high *TSP1* expression) presented significantly higher DCR ($P < 0.0001$), TTP ($P = 0.009$), and mOS ($P < 0.0001$) in comparison with patients with the unfavorable genotype (high *TXR1*/low *TSP1* expression), although these two groups represented the 79% of the study population. Furthermore, multivariate analysis revealed that the unfavorable expression pattern was an independent prognostic factor for TTP ($P = 0.016$) and survival ($P < 0.0001$) and was associated with a 1.7 and 2.5 times higher risk for progression and death, respectively. The only other factor that was significant for time to progression was the stage of the disease.

The mechanisms involved in taxane resistance are not fully elucidated, and most of our current knowledge is based on *in vitro* data. The best-described mechanism of taxane resistance is the efflux of taxanes (and other drugs) from tumor cells by up-regulation of the ATP-dependent cell membrane glycoproteins (MDR phenotype; refs. 11, 12). The effect of *TXR1* and/or *TSP1* mRNA expression on taxane cytotoxicity seems to be independent of the MDR phenotype because the altered expression of *TXR1* did not affect the cellular accumulation of ^{3}H-labeled paclitaxel in the resistant cells (14), as it has been observed in MDR-overexpressing cell lines (12); it is of note that the addition of a MDR inhibitor had no effect on paclitaxel resistance. In addition, *TXR1* overexpression was not associated with reduced sensitivity to other drugs that are also expelled from tumor cells by the MDR.

Another extensively studied mechanism of taxane resistance proposed that mutations in *β-tubulin* gene may interfere with the taxane binding sites to microtubules, thus causing resistance to paclitaxel and/or docetaxel (20, 21). Despite an initial report (22), this mechanism of resistance has never been confirmed in tumoral samples from patients with NSCLC. Moreover, quantitative biochemical analysis of resistant to taxane cell lines has shown the same tubulin dynamics as that of the parental cells; in addition, there was no increase of microtubule isoforms that are commonly up-regulated in tubulin-related resistance to taxanes (14).

The proposed mechanism involves that *TSP1* is an effector of the apoptotic response to taxane treatment and repression of *TSP1* following up-regulation of *TXR1* induces resistance to taxane in lung adenocarcinomas treated with the docetaxel-gemcitabine regimen. To elucidate the biological mechanism behind paclitaxel-induced cytotoxicity through TSP1, Lih et al. (14) focused their research in two cell surface receptors known to mediate the TSP1 signal: CD36 and CD47 (integrin-associated protein). Because only the CD47 was expressed on the cells, they assumed that TSP1 was most likely to act via this receptor. Indeed, when different TSP1 mimetic peptides were added to the cells, only 4N1K, which binds to CD47, but not the mutated peptides 4NGG or ABT-510, which bind to CD36, decreased cell survival in the presence of 10 nmol/L paclitaxel. In addition, the inhibition of CD47 signaling (by an anti-CD47 antibody) increased cell viability in the presence of paclitaxel

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(14). The TSP1-mediated CD47 activation has previously been shown to result in caspase-independent apoptosis in hematopoietic cells (23). However, signaling through CD47 was also reported to augment Fas/CD95-mediated apoptosis (24), which proceeds through a classic, caspase-dependent pathway.

As we mentioned above, all clinical data and tumor specimen were retrospectively collected from patients treated in the context of two randomized trials conducted by our group (6, 7). All patients who were not treated with taxanes received a vinorelbine-containing regimen, which shares the same cellular target with the taxanes (mitotic spindle assembling). Due to the lack of a non–taxane-treated control group for comparison in our study, we are not able to show with certainty that the effect of *TSP1* expression was a taxane-specific effect and not a more generic predictive marker for response to chemotherapy. However, the preclinical data support the hypothesis that the effect of *TSP1* is taxane specific. When the resistant cells were treated with a combination of TSP1 and 10 nmol/L paclitaxel, there was an increase in the number of apoptotic cells compared with treatment with either TSP1 or paclitaxel alone. More importantly, the observed effect was specific for taxanes because the addition of TSP1 left the sensitivity of the cells to other anticancer drugs unaffected (14). The finding that *TSP1* is involved in taxane cytotoxicity agrees with a previous observation reporting a strong induction of *TSP1* in docetaxel-treated head and neck squamous cell carcinoma cell lines using cDNA microarrays (25).

It is interesting to note that metronomic chemotherapy exerts its antiangiogenic effects by causing the up-regulation of endogenous angiogenesis inhibitors, and one of the best-studied targets in this concept is TSP1 (26). Moreover, TSP1 levels are elevated following low-dose treatment with various different cytotoxic agents in addition to taxanes, suggesting that a general stress- or damage-related signaling pathway may result in increased *TSP1* gene expression.

Despite the fact that the interpretation of results from retrospective studies, with the heterogeneous second-line treatment administered to patients, should be cautious, our data suggest that the molecular profile of the primary tumor could be used as a predictive marker for response to the docetaxel-gemcitabine combination and clinical outcome. We selected patients with lung adenocarcinomas treated with the docetaxel-gemcitabine regimen to investigate the potential prognostic and predictive value of the *TXR1*/*TSP1* in one specific subtype of NSCLC rather than in tumors with mixed histology. However, the clinical relevance of the tumoral *TXR1*/*TSP1* expression should be validated prospectively in an adequate, statistically powered, and independent set of patients, including patients with squamous cell carcinomas as well as in patients treated with taxane-platinum combinations. In addition, the role of *TXR1*/*TSP1* expression in taxane chemosensitivity should be investigated in other tumor types, such as breast, ovarian, or prostate cancer, in which taxanes are often used in the daily clinical practice.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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