Multiple Mechanisms of Telomere Maintenance Exist and Differentially Affect Clinical Outcome in Diffuse Malignant Peritoneal Mesothelioma

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Abstract

Purpose: This study aims to investigate the prevalence of the two known telomere maintenance mechanisms, telomerase activity (TA) and alternative lengthening of telomeres (ALT), and to assess their prognostic relevance in diffuse malignant peritoneal mesothelioma (DMPM).

Experimental Design: In 44 DMPM specimens obtained from 38 patients, TA was determined using the telomeric repeat amplification protocol and ALT was detected by assaying ALT-associated promyelocytic leukemia nuclear bodies. The prognostic significance of telomere maintenance mechanisms was analyzed by Cox regression in the overall series and in a subset of 29 patients who underwent a uniform treatment regimen consisting of cytoreductive surgery and hyperthermic i.p. chemotherapy.

Results: Telomere maintenance mechanisms were detectable in 86.4% of DMPM: ALT or TA alone was found in 18.2% or 63.6% of lesions, respectively, whereas two cases (4.6%) were ALT+/TA+. TA and ALT proved to be inversely associated ($P = 0.002$). In the overall series, TA was prognostic for 4-year relapse (TA+ versus TA-, hazard ratio, 3.30; 95% confidence interval, 1.23-8.86; $P = 0.018$) and cancer-related death (TA+ versus TA-, hazard ratio, 3.56; 95% confidence interval, 1.03-12.51; $P = 0.045$), whereas ALT failed to significantly affect clinical outcome. These results held true also in the subset of patients submitted to uniform treatment with cytoreductive surgery and hyperthermic i.p. chemotherapy.

Conclusions: Our results indicate that both known telomere maintenance mechanisms, TA and ALT, are present in DMPM and differentially affect patient prognosis.

Diffuse malignant peritoneal mesothelioma (DMPM) is an uncommon malignancy that develops from the mesothelial cells lining the peritoneal cavity, and it accounts for 10% to 15% of all malignant mesotheliomas (1). Although locally invasive rather than metastatic, DMPM is a rapidly fatal disease (2), with a median survival that generally does not exceed 1 year (3) notwithstanding the different treatment options proposed thus far. Recently, an integrated approach has been developed combining aggressive cytoreductive surgery with hyperthermic i.p. chemotherapy (4) and has given encouraging results in selected DMPM patients. However, although this therapeutic strategy improved median patient survival from 12 months to 34 to 92 months according to the different studies, 50% to 70% of the patients did not benefit from treatment and died within 5 years (5).

The biology of peritoneal mesothelioma is largely unknown, and the cellular and molecular mechanisms responsible for its proliferative potential and biological aggressiveness have not yet been elucidated. One of the hallmarks of cancer cells is their limitless replicative potential (6). In a high percentage of human tumors (>85%), the attainment of immortality is due to the reactivation of telomerase (7), a RNA-dependent DNA polymerase that stabilizes telomeres, allows cells to avoid the senescence checkpoint, and may therefore contribute to tumorigenesis and neoplastic progression (8). The core enzyme consists of a RNA component (hTR) that provides the template for the *de novo* synthesis of telomeric DNA (9) and a catalytic subunit, human telomerase reverse transcriptase (hTERT), with reverse transcriptase activity (10). Recent evidence suggests that the catalytically active telomerase exists as a complex of two molecules each of hTERT, hTR, and dyskerin (11). In addition to its role in maintaining chromosome ends, telomerase activation has recently been

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Received 1/11/08; revised 3/5/08; accepted 3/10/08.

Grant support: Associazione Italiana per la Ricerca sul Cancro, European Community grant LSHC-CT-2004-502943, and Istituto Superiore Sanità grant I-07-005.

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doi:10.1158/1078-0432.CCR-08-0099

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implicated in providing growth-promoting properties to tumor cells (12).

Some tumors, however, do not have telomerase activity and maintain their telomeres by one or more mechanisms referred to as alternative lengthening of telomeres (ALT; ref. 13). Telomere dynamics in ALT cells are consistent with a recombination-mediated DNA replication mechanism, and the characteristics of ALT cells usually include long and heterogeneous telomeres and subnuclear structures termed ALT-associated promyelocytic leukemia (PML) bodies (APB) containing telomeric DNA, telomere-specific binding proteins, and proteins involved in DNA recombination and replication (14). Available information indicates that ALT is more frequently present in tumors of mesenchymal and neuroepithelial origin than in those of epithelial origin, possibly because of a tighter repression of telomerase in normal mesenchymal and neuroepithelial cells than in epithelial cells (15). Recently, it was shown that a significant proportion of osteosarcomas (16, 17), soft tissue sarcomas (18–20), and glioblastoma multiforme (17, 21) use ALT for telomere maintenance. Moreover, in sarcomas, ALT is more frequently activated in the subtypes with complex karyotypes, which are evidence of chromosomal instability (18, 20, 22). Results in animal models suggest that ALT is less potent than telomerase activity (TA) in generating fully malignant tumors (13, 22, 23). However, ALT+ spontaneously immortalized Li-Fraumeni syndrome cells transfected with mutant H-Ras formed tumors in athymic nude mice (15). Moreover, ALT+ human SV40-immortalized fibroblasts were found to require only oncogenic H-Ras to be converted to a fully tumorigenic state and metastasize to lung after implantation in the kidney capsule of an immunodeficient mouse (24).

It is known that TA is expressed in more than 90% of malignant pleural mesotheliomas (25), and the enzyme activity and hTERT expression have been proposed for differential diagnosis in mesothelial lesions (26). However, no information is available thus far concerning the presence of telomere maintenance mechanisms in DMPM. Taking advantage of a monoinstitutional series of DMPM patients with a long follow-up, we investigated the prevalence and the prognostic role of the two known telomere maintenance mechanisms, TA and ALT.

Materials and Methods

Study population. A total of 44 DMPM lesions from 38 adult patients (median age, 60 years; range, 25-78 years) treated at the Istituto Nazionale dei Tumori, Milan, from September 1999 to May 2006 were available for telomere maintenance mechanism analysis. The H&E-stained slides of all cases were reviewed, and the tumors were classified as epithelial, sarcomatoid, and biphasic (mixed epithelial and sarcomatoid) according to the WHO classification (27). The epithelial type was further subdivided into tubulopapillary and solid groups based on the predominant pattern. The histologic diagnosis of DMPM was confirmed using calretinin and WT-1 as positive mesothelial markers and polyclonal carcinoembryonic antigen and Ber-EP4 as negative markers (28). Eight normal peritoneum specimens were also obtained from patients who underwent surgery for nononcologic diseases.

The specimens were consecutive with respect to the availability of frozen tissue for telomere maintenance mechanism studies and adequate clinicopathologic and follow-up information. Patient and tumor characteristics are summarized in Table 1. According to Istituto Nazionale dei Tumori treatment guidelines, patients presenting with resectable tumors (29 cases) were submitted to cytoreductive surgery and hyperthermic i.p. chemotherapy with cisplatin plus mitomycin C or doxorubicin at 42.5°C (29), whereas the remaining nine patients, with a disease not amenable to radical cytoreduction, underwent debulking surgery with or without systemic chemotherapy. The median follow-up of the entire series was 38 mo (range, 2-94 mo). At 48 mo from surgery, 24 patients had developed new disease manifestations and 17 had died from cancer-related disease. A total of 18 relapses and 11 deaths occurred in the subset of patients subjected to cytoreductive and hyperthermic i.p. chemotherapy.

Table 1. Patient and tumor characteristics

	No. of patients (%)
Total	38
Gender	
Female	18 (47.4)
Male	20 (52.6)
Histologic subtype	
Epithelial, tubulopapillary/solid	5/30 (92.1)
Biphasic	3 (7.9)
Preoperative systemic chemotherapy	
Yes	15 (39.4)
No	23 (60.6)
Treatment	
CRS + HIPEC	29 (76.3)
Debulking ± systemic chemotherapy	9 (23.7)
Extent of carcinomatosis	
PCI ≤ 25%	9 (23.7)
PCI > 25%	29 (76.3)

Abbreviations: CRS + HIPEC, cytoreductive surgery + hyperthermic i.p. chemotherapy; PCI, peritoneal cancer index (54).

This study was approved by the institutional review board of the Istituto Nazionale dei Tumori, and each patient provided written informed consent to donate to the Istituto Nazionale dei Tumori the tissues left over after diagnostic procedures.

Molecular studies. Normal and tumor tissues were sampled by a pathologist at the time of surgery, flash-frozen in liquid nitrogen, and stored at -80°C. On H&E-stained slides, diagnosis and sampling adequacy were pathologically confirmed at the time of telomere maintenance mechanism analyses. A fragment of 70 to 100 mg was cut from each lesion and further subdivided into a long central portion and two ends. APBs were detected on frozen sections obtained from each end to control for intratumor clonal heterogeneity with respect to telomere maintenance mechanisms. Protein (for TA assay) and RNA (for hTERT mRNA expression) were extracted from the central part of the sample.

Detection of APB. Frozen sections were cut to 5 to 7 μm thickness, fixed in 1:1 methanol/acetone, processed to detect APB by combined PML immunofluorescence and telomere fluorescence *in situ* hybridization (17), and independently scored by two observers. Images were captured on a Nikon Eclipse E600 fluorescence microscope using ACT-1 (Nikon) image analysis. APB status was determined according to previously defined criteria (17). The presence of an APB was defined by the localization of a telomeric DNA focus within a nuclear PML body; sections were scored as APB positive if they contained APB in ≥0.5% of tumor cells; and a tumor was considered ALT+ when at least one section was APB positive. To avoid false positives, an APB was considered to be present only when the telomeric DNA fluorescence within a PML body was more intense than that of telomeres, and a cell was not considered to contain APB if >25% of the colocalized foci occurred outside the nucleus. To avoid false negatives, at least 2,000 tumor nuclei were examined, and the assay was repeated in the presence of negative results. ALT+ (IIICF/c-EJ-*ras*; ref. 14) or TA+ (HeLa) cell lines were used as positive or negative controls, respectively.

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Telomerase activity detection assay. TA was measured on 0.6 and 6 μg of protein by the telomeric repeat amplification protocol (TRAP; ref. 30), with the TRAPeze kit (Intergen Company). A tumor was scored as TA+ when positive TRAP results were obtained for at least one protein concentration. In the case of tumors negative at both protein concentrations, the TRAP assay was repeated to avoid false negatives.

RNA extraction and reverse transcription-PCR analysis of hTERT. Total cellular RNA was extracted from frozen samples with the TRIzol reagent (Life Technologies). Total RNA (0.5 μg) from each sample was used for cDNA production using the reverse transcription-PCR Core kit (Applied Biosystems) with random hexamers. Amplification of hTERT cDNA was obtained as previously described (31) using TERT-2164S (5′-GCCTGAGCTGTACTTTGTCAA-3′) and TERT-2620AS (5′-CGCAAACAGCTTGTTCTCCATGTC-3′) primers that flank regions encompassing the alternative splicing sites. Amplification for β-actin as a constitutive gene was done using 774S and 775AS primers (31). PCR products were resolved on a polyacrylamide gel, which was then dried and autoradiographed. Amplified products were analyzed by a ScanJET IIcx/T scanner (Hewlett Packard).

Statistical analysis. Telomere maintenance mechanism measurements were done by personnel blinded to patient data (R.V., R.M., and L.V.), whereas clinical data were collected by personnel blinded to telomere maintenance mechanism results (M.D. and S.K.). The clinical end points of this study were disease-free and cancer-related survival, and the time of their occurrence was computed from the date of surgery (debulking or cytoreductive) to the time of relapse (for disease-free) or of death, or censored at the date of the last recorded follow-up for living patients or for those who died from cancer-unrelated conditions. Survival curves were estimated by means of the Kaplan-Meier product limit method (32), and the Cox proportional hazards model (33) was used to calculate the hazard ratios (HR) and their 95% confidence interval (95% CI). All P values were two-sided, and values ≤0.05 were considered statistically significant.

Results

We tested 44 frozen tumor samples obtained from 38 patients with DMPM for the presence of telomere maintenance mechanisms. TA was detected by the TRAP assay (Fig. 1A), and tumors were defined as ALT+ on the basis of the presence of APB in at least 10 (0.5%) of 2,000 tumor cells (Fig. 1B). Thirty-eight lesions (86.4%) expressed at least one telomere maintenance mechanism. Specifically, 28 lesions (63.6%) were TA+/ALT-, 8 (18.2%) were TA-/ALT+, and 2 (4.6%) were defined as TA+/ALT+ due to the concomitant expression of APB and TA (Table 2). The remaining six lesions (13.6%) did not express any known telomere maintenance mechanism. ALT and TA proved to be inversely associated (χ^2 13.85; P = 0.002). APB was observed in a variable but always limited fraction of cells in the ALT+ tumors, ranging from 0.5% to 3.9%, with a median value of 2.6%. Two separate sections of each tumor were assayed for APB, and the results indicated minimal intratumor spatial heterogeneity for ALT: We found one site to be APB+ and the other APB- in only 2 of 44 (4.5%) lesions. In the six patients for whom APB and TA were assayed on different lesions at the time of cytoreductive surgery (two cases) or during disease progression (four cases, in an interval ranging from 8 to 28 months), we observed that telomere maintenance mechanisms were stable among lesions and over time. In fact, all the lesions analyzed for these six cases were TA+/ALT-. Neither TA (Fig. 1A) nor APB (data not shown) was detected in the eight normal peritoneum specimens.

Most of the lesions (40 of 44) were epithelial, tubulopapillary/solid DMPM, and the prevalence of TA and ALT in this subset of cases was superimposable to that observed in the overall series. Among the four biphasic peritoneal mesotheliomas, three were TA+ and one did not express any telomere maintenance mechanism (Table 2). Age at diagnosis was significantly lower in patients with ALT+ than in those with ALT- tumors (mean age, 40 versus 57 years; P = 0.0037), whereas it was higher in patients with TA+ than in those with TA- tumors (mean age, 58 versus 46 years; P = 0.02). There was no significant association of presence or type of telomere maintenance mechanisms with gender, preoperative systemic chemotherapy, or extent of carcinomatosis (data not shown).

The expression of hTERT was evaluated in 37 DMPM specimens. Because alternative splicing of hTERT is thought to be involved in the regulation of telomerase activity (31), we analyzed the presence of different hTERT transcripts by reverse transcription-PCR using primers that amplify the mRNA encoding the full-length, enzymatically active hTERT (457 bp) as well as smaller alternatively spliced variants (α^-, 421 bp; β^-, 275 bp; and $\alpha^-\beta^-$, 239 bp) that are not able to maintain the telomeric array (Fig. 1C). In the 23 TA+ specimens, the

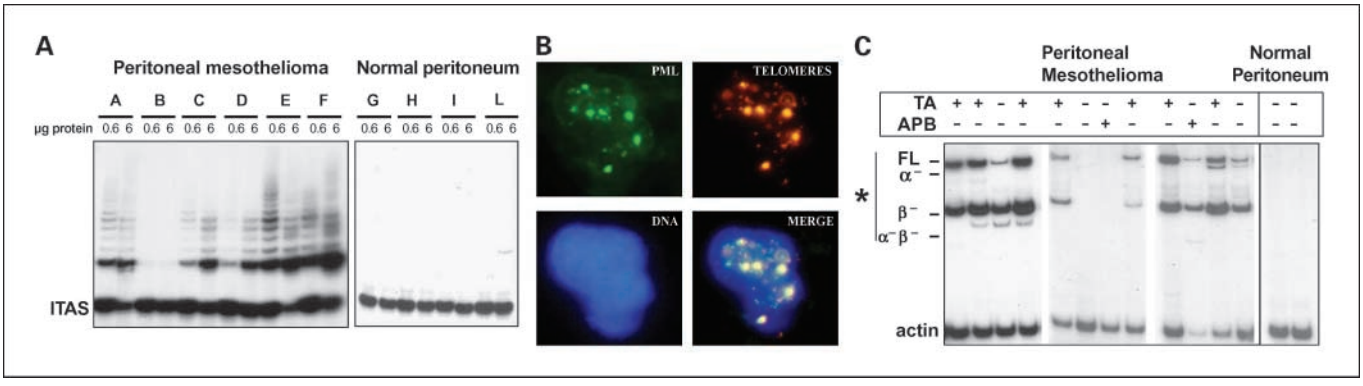

Fig. 1. *A,* representative series of DMPM and normal peritoneum specimens in which telomerase activity was assayed by TRAP using 0.6 and 6 μg of protein. The location of the internal amplification standard (*ITAS*) is indicated. *B,* APB assay: Combined PML immunofluorescence and telomere fluorescence *in situ* hybridization in a frozen section of an ABP-positive DMPM. Indirect immunofluorescence was used for the PML protein (FITC label, *green stain*); telomere fluorescence *in situ* hybridization was done using a Cy3-conjugated telomeric peptide nucleic acid probe (*red stain*); nuclei were counterstained with 4′,6-diamidino-2-phenylindole (*blue stain*); the foci of telomeric DNA that colocalize with PML represent APBs. *C,* expression of TERT transcripts: full-length (*FL*), and alternative spliced variants α^-, β^-, $\alpha^-\beta^-$, as detected by RT-PCR in a representative series of DMPM and normal peritoneum specimens.

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Table 2. Telomere maintenance mechanisms in individual patients

Patient	Histologic subtype	
1	Epithelial, tubulopapillary	TA-/ALT-
2	Epithelial, solid	TA+/ALT-
3	Epithelial, solid	TA-/ALT+
4*	Epithelial, tubulopapillary	TA+/ALT-
5*	Epithelial, tubulopapillary	TA+/ALT-
6	Epithelial, solid	TA+/ALT-
7	Epithelial, solid	TA-/ALT+
8	Biphasic, solid	TA+/ALT-
9*	Epithelial, solid	TA+/ALT-
10	Epithelial, solid	TA+/ALT-
11	Epithelial, solid	TA+/ALT-
12	Epithelial, solid	TA+/ALT+
13	Epithelial, solid	TA+/ALT-
14	Epithelial, solid	TA+/ALT-
15	Epithelial, solid	TA+/ALT-
16	Epithelial, solid	TA-/ALT+
17	Epithelial, solid	TA-/ALT+
18	Epithelial, solid	TA+/ALT-
19*	Biphasic, solid	TA+/ALT-
20	Epithelial, solid	TA-/ALT+
21	Epithelial, solid	TA-/ALT+
22	Epithelial, tubulopapillary	TA-/ALT+
23	Epithelial, solid	TA-/ALT+
24	Epithelial, solid	TA-/ALT-
25	Epithelial, solid	TA+/ALT+
26	Epithelial, solid	TA-/ALT-
27	Epithelial, solid	TA-/ALT-
28	Biphasic, solid	TA-/ALT-
29	Epithelial, solid	TA+/ALT-
30	Epithelial, solid	TA+/ALT-
31	Epithelial, solid	TA+/ALT-
32	Epithelial, solid	TA+/ALT-
33	Epithelial, solid	TA-/ALT-
34	Epithelial, solid	TA+/ALT-
35	Epithelial, solid	TA+/ALT-
36	Epithelial, solid	TA+/ALT-
37*	Epithelial, solid	TA+/ALT-
38*	Epithelial, tubulopapillary	TA+/ALT-

*For these patients, two different lesions were assayed. All the lesions were TA+/ALT-.

full-length transcript was always present in combination with one or more splice variants (Table 3). As regards the 14 TA- specimens, no hTERT transcript was observed in five cases, and only two cases expressed the β^- splice variant. The remaining seven specimens expressed the full-length transcript together with one or more splice variants. However, conversely to that observed in TA+ specimens, these tumors generally showed a lower expression of the full-length transcript than that of the alternatively spliced variants (Fig. 1C). No hTERT transcript was consistently observed in normal peritoneum specimens (Fig. 1C).

The prognostic significance of telomere maintenance mechanism expression was initially analyzed on the overall series of patients. At 4 years of follow-up, TA proved to be prognostic for both disease-free (TA+ versus TA-, 10% versus 64%; HR, 3.30; 95% CI, 1.23-8.86; $P = 0.018$) and cancer-related survival (TA+ versus TA-, 32% versus 79%; HR, 3.56; 95% CI, 1.03-12.51; $P = 0.045$), whereas ALT failed to significantly affect clinical outcome (APB+ versus APB-: disease-free survival, 60% versus 16%; HR, 0.40; 95% CI, 0.14-1.19; $P = 0.10$; cancer-related survival: 69% versus 39%; HR, 0.45; 95% CI, 0.13-1.56; $P = 0.21$). These results held true also for the 29 patients with resectable tumors, who underwent cytoreductive surgery and hyperthermic i.p. chemotherapy. Specifically, patients with TA+ tumors (16 cases) had a significantly lower probability of being disease-free than patients with TA- tumors (13 cases; HR, 3.32; 95% CI, 1.09-10.12; $P = 0.03$; Fig. 2A) and showed a trend in favor of a lower although not statistically significant probability of being alive (HR, 3.69; 95% CI, 0.79-17.13; $P = 0.09$; Fig. 2B). Conversely, patients with ALT+ tumors (10 cases) did not show a significantly different probability of being disease-free (HR, 0.53; 95% CI, 0.17-1.62; $P = 0.26$; Fig. 3A) and surviving (HR, 0.72; 95% CI, 0.19-2.73; $P = 0.63$; Fig. 3B) compared with patients with ALT- tumors (19 cases).

Discussion

This is the first report of a comparative analysis of the expression and clinical significance of the two currently known telomere maintenance mechanisms, ALT and TA, in DMPM. TA proved to be the more frequently activated telomere maintenance mechanism in the disease, although the frequency of TA+ cases (63.6%) was lower than that previously reported for pleural mesothelioma (25). Eighteen percent of DMPM specimens were ALT+. The ALT phenotype was previously observed by Foddis et al. (34) in a pleural mesothelioma cell line, which was characterized by long and heterogeneous telomeres and exhibited colocalization of TRF2 with the PML nuclear body. Coexistence of two telomere maintenance mechanisms in the same tumor, which we observed in two DMPM specimens, was previously shown also in other human tumor types, including osteosarcoma (16), liposarcoma (18–20), and glioblastoma multiforme (21). However, it is not known at present whether TA and ALT can coexist within the same tumor cell or whether a given tumor lesion may contain distinct ALT+ and TA+ subpopulations, although experimental evidence obtained in ALT cells genetically engineered to express hTERT suggests that the two telomere maintenance mechanisms can function concurrently in most (35–38) but not all (39) cases. We found

Table 3. Expression of the different hTERT transcripts in DMPM lesions

	TA+/ALT- (21 cases)	TA-/ALT+ (8 cases)	TA+/ALT+ (2 cases)	TA-/ALT- (6 cases)
No transcript	—	3	—	2
Full length				
$+\beta^-$ splice variant	16	3	2	1
$+\alpha^- + \beta^-$ splice variants	1	—	—	1
$+\beta^- + (\alpha^-\beta^-)$ splice variants	4	—	—	2
β^- splice variant	—	2	—	—

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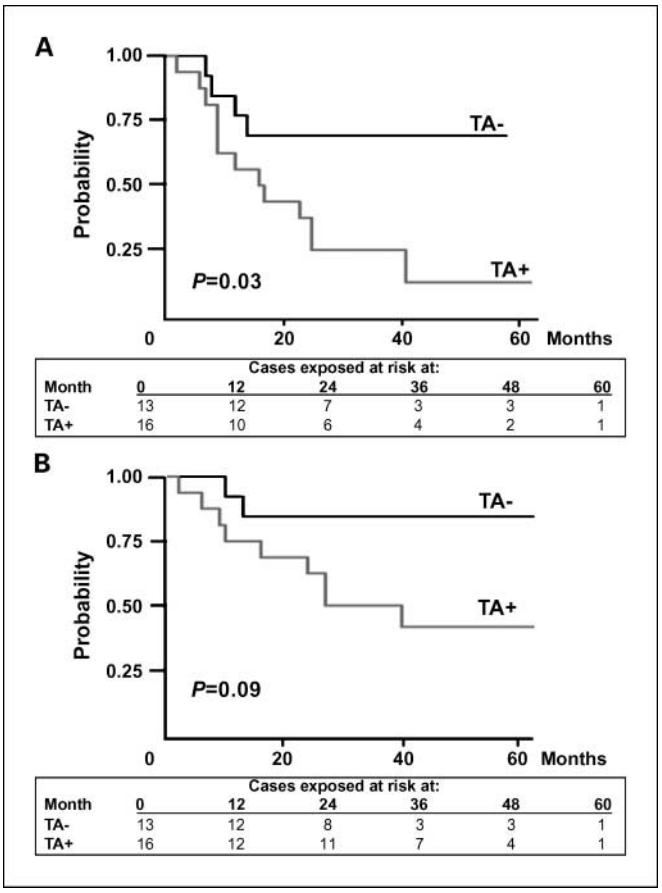

Fig. 2. Probability of disease-free survival (*A*) and cancer-related survival (*B*) as a function of TA in DMPM patients. TA negative: 13 cases (for disease-free survival analysis: 4 failures and 4 cases exposed at risk at 48 mo; for survival analysis: 2 failures and 3 cases exposed at risk at 48 mo). TA positive: 16 cases (for disease-free survival analysis: 14 failures and 2 cases exposed at risk at 48 mo; for survival analysis: 9 failures and 4 cases exposed at risk at 48 mo).

that ~14% of DMPM possessed no apparent telomere maintenance mechanisms, despite being informative for the different assays, suggesting that the presence of a constitutively active telomere maintenance mechanism is not a stringent requirement for a subset of DMPM or, alternatively, that these tumors use a mechanism that has not yet been identified. Moreover, such a lack of any known telomere maintenance mechanism, previously observed also in subsets of other tumor types (16, 20, 21), is in accord with experimental data suggesting that telomere maintenance mechanism acquisition is not always required for malignant transformation of normal human cells (40).

We found that ALT was associated with a younger age at diagnosis in DMPM patients. This has been seen before in glioblastoma multiforme (21), where the association seems to result from ALT occurring predominantly in the tumors that arise from lower-grade lesions that preferentially occur in younger patients. As far as DMPM is concerned, it is only possible to speculate that ALT+ DMPM has a different set of molecular changes with respect to the TA+ tumors.

TA+ DMPM was characterized by the presence of the full-length, catalytically active hTERT transcript, together with one or more alternatively spliced variants. TA- DMPM did not express any hTERT transcript or, alternatively, displayed the presence of the β^- splice variant alone or different combinations of full-length and deletion transcripts, with a generally higher level of alternatively spliced variants compared with that of the complete hTERT transcript. These data would suggest that the presence of the full-length hTERT transcript is necessary but not sufficient to activate the enzyme in DMPM and that not only hTERT transcription but also alternative splicing can contribute to the regulation of TA in this disease, as already reported for melanomas and lung carcinoids (41, 42). In this context, we previously showed that forced accumulation of alternatively spliced transcripts with a concomitant decrease of the full-length hTERT, accomplished through the use of 2′-*O*-methyl-RNA phosphorothioate oligonucleotides targeting the splicing site in the hTERT pre-mRNA, induced a decline of TA in prostate cancer cells (43).

Our retrospective study carried out in a consecutive, although limited, series of DMPM patients showed that TA correlates with prognosis because patients with a TA+ tumor experienced a significantly worse clinical outcome than patients with a TA- tumor. Results available in the literature suggest that the association between prognosis and telomerase expression is not so straightforward in human tumors. In fact, increased telomerase activity/expression was related to a poor prognosis

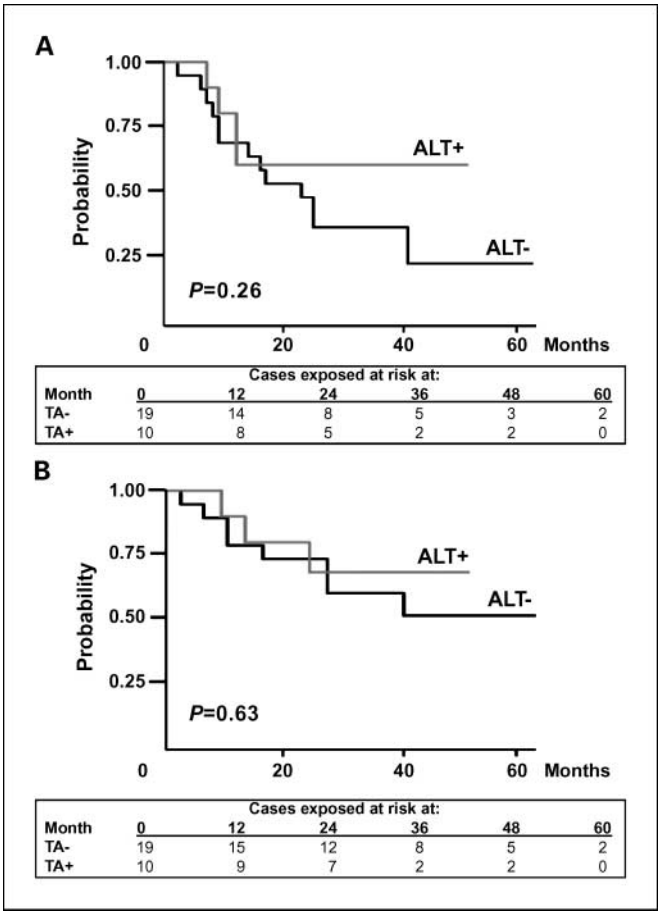

Fig. 3. Probability of disease-free survival (*A*) and cancer-related survival (*B*) as a function of ALT in DMPM patients. ALT negative: 19 cases (for disease-free survival analysis: 14 failures and 3 cases exposed at risk at 48 mo; for survival analysis: 8 failures and 5 cases exposed at risk at 48 mo). ALT positive: 10 cases (for disease-free survival analysis: 4 failures and 2 cases exposed at risk at 48 mo; for survival analysis: 3 failures and 2 cases exposed at risk at 48 mo).

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in different human tumor types, including breast, non–small–cell lung, gastric, and colorectal cancers and neuroblastoma (44). However, other studies did not confirm such an association, also due to differences in assay conditions, data analysis, and patient selection, thus making it difficult to draw a conclusion regarding the role of telomerase in cancer prognosis.

In our small series of DMPM patients, ALT did not significantly affect clinical outcome, although patients with an ALT+ tumor displayed slightly higher probabilities to be disease-free and survive than patients with an ALT- tumor. The association of ALT with patient prognosis seems to be disease related. In fact, in glioblastoma multiforme, a better survival for patients with an ALT+ tumor than for those with an ALT- tumor was consistently observed in two studies in which ALT was detected by terminal restriction fragment analysis or the presence of APB (17, 21). In addition, in this tumor type, an association between the mutant *p53* gene and the ALT mechanism was recently reported, suggesting that *p53* gene deficiency plays a permissive role in the activation of ALT (45). In this context, it is worthy of note that *p53* mutations are rarely seen in mesothelioma (46). In contrast, there was no significant difference in survival between ALT+ and ALT- osteosarcoma patients (16, 17). At the other end of the spectrum, we recently found that in liposarcoma patients, ALT proved to be a stronger prognostic discriminant of increased mortality than TA (18). A possible explanation for these different outcomes may be that activation of ALT results from sets of genetic changes that are tumor-type specific, with those present in ALT+ glioblastoma or in ALT+ liposarcoma causing a better or worse prognosis, respectively, than those present in the corresponding TA+ tumors.

Statistical projections of mesothelioma-related deaths predict continuing increases in many countries (47), mainly as a late consequence of the widespread use of asbestos (48). Although combined cytoreductive surgery plus hyperthermic i.p. chemotherapy has resulted in a significant improvement in survival, there is still a substantial subset of DMPM with poor prognosis (5, 49, 50). These considerations make it mandatory to identify new therapeutic targets and strategies. Preclinical data generated in experimental tumor models indicate that inhibition of telomere maintenance mechanisms and interference with telomere dynamics results in cell senescence and apoptosis (51, 52), thus suggesting telomere maintenance mechanisms as potential new targets for cancer treatment. Tumor types, such as DMPM, which are refractory to conventional therapies and express telomere maintenance mechanisms in most of the cases, could likely benefit from such new therapeutic interventions. To date, no therapeutic strategies have been developed to specifically inhibit ALT, although the recent identification of genes required for APB formation (53) should open new possibilities to directly target this mechanism. On the other hand, telomerase inhibitors are already entering clinical trials, but they are likely to be ineffective against ALT+ tumors. As a consequence, for tumor types like DMPM where both TA and ALT are expressed, telomere maintenance mechanism status should be characterized in individual tumors before starting treatment with antitelomerase agents.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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