Altered mRNA Expression of Telomere-Associated Genes in Monoclonal Gammopathy of Undetermined Significance and Multiple Myeloma

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In this study, we explored changes in the expression of the telomere maintenance genes, TRF1, TRF2 and TANK1 in patients with monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM). Results were correlated with human telomerase reverse transcriptase (hTERT) expression, telomere length (TL) and clinicopathological characteristics. Bone marrow (BM) samples from 132 patients, 64 with MGUS and 68 with MM, were studied. Real-time quantitative reverse transcription–polymerase chain reaction was used to quantify gene expression. TL was evaluated by terminal restriction fragment length analysis. MGUS patients showed increased TRF1 levels ($P = 0.006$) and lower expression of TRF2 ($P = 0.005$) and TANK1 ($P = 0.003$) compared with MM patients. For hTERT analysis, patients were divided into three groups by use of receiver operating characteristics: low (group I [G1]), intermediate (group II [GII]) and high (group III [GIII]) expression. We observed increasing expression of TRF2 and TANK1 from G1 to GIII in MGUS and MM, with differences for both genes in MM ($P < 0.01$) and for TRF2 in MGUS ($P < 0.01$). GIII patients with the highest telomerase expression had the shortest TL. In both entities, a positive association between TRF2-TANK1, TRF2-hTERT and TANK1-hTERT ($P < 0.01$) was observed. In MM, the percentage of BM infiltration and Ki-67 index were positively associated with TRF2, TANK1 and hTERT expression ($P < 0.03$) and negatively with TL ($P = 0.02$), whereas lactate dehydrogenase was significantly correlated with TRF2 mRNA ($P = 0.008$). Our findings provide the first evidence of a modification in the expression of telomeric proteins in plasma cell disorders, and suggest that mechanisms other than telomerase activation are involved in TL maintenance in these pathologies.

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INTRODUCTION

Monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM) are the two most common plasma cell disorders characterized by the presence of clonal bone marrow (BM) plasma cells and of a monoclonal protein in serum and/or urine. MM constitutes approximately 10 to 15% of all hematologic malignancies and about 1% of all forms of cancer. Clinical manifestations that include osteolytic lesions, anemia, hypercalcemia, immunodeficiency and renal abnormalities can be attributed to the underlying plasma cell proliferation (1). The natural course of the disease may progress from MGUS, a presymptomatic phase, to MM. MGUS is characterized by serum M protein levels less than 3 mg/dL, BM plasma cell infiltration (BMPCI) less than 10% and no clinical manifestations related to monoclonal gammopathy (2). This entity is one of the most common premalignant disorders in Western countries, with a prevalence of 3.2% in the population of white individuals age 50 years and older. The transformation rate of MGUS to MM is about 1% per year, with an actuarial probability of malignant evolution of 30% at 25 years. After a median of 10 years, about one-quarter of MGUS patients develop MM. Recent studies have identified markers that can be used to identify patients with high risk of progression: higher levels of monoclonal protein, non-IgG protein iso-type and abnormal ratio of free light chains (3).

Human telomeres comprise tandem repeats of the noncodificant DNA sequence TTAGGG and are involved in the maintenance of chromosomal stability and genome integrity by DNA-binding proteins, which associate with other
proteins/complexes to achieve telomere-end protection and length control (4). Because of the end-replication problem, telomeres progressively shorten with repeated cell division, a process that leads to telomere dysfunction and, ultimately, contributes to tumorigenesis. In cancer cells, telomere length (TL) is maintained by the enzyme telomerase, a ribonucleoprotein complex that compensates for telomere reduction by adding new repeats to chromosome ends. Telomerase is composed of two subunits: human telomerase reverse transcriptase (hTERT), which has catalytic activity, and the RNA component (hTERC), which provides the template for telomeric synthesis. Activation of telomerase may therefore be a critical step in human cancer development because telomerase activity is absent in most normal somatic cells, but it is present in most malignant tissues and immortal human cell lines (5,6).

Telomerase activity is regulated in cis by the shelterin hexa-protein complex (TRF1, TRF2, POT1, RAP1, Tin2 and TPP1) and epigenetic factors (7,8). In particular, TRF1 and TRF2 bind to DNA as preformed homodimers, and despite the similarities in their sequence and architecture, TRF1 and TRF2 have different functions. TRF1 is involved in a negative feedback mechanism that allows telomere shortening by inhibiting the activity of telomerase (9). Although TRF2 is also involved in negative TL regulation, it participates in t-loop formation, capping and protecting the 3' single-strand overhang. Like TRF1, increased expression of TRF2 shortens telomeres, but loss of its activity leads to telomere-telomere fusion events, suggesting a protective role for TRF2 in the maintenance of telomere structure and function (10).

Nonshelterin proteins at chromosome ends may also play important roles at telomeres. Among them, the enzyme TANK1 (tankyrase-like protein 1) is a member of the growing family of poly (ADP-ribose) polymerases (PARPs) that interacts with and ADP-ribosylates the telomere-binding protein TRF1 (11). In vitro, ribosylation by TANK1 displaces TRF1 from telomeric DNA, a process that suggests that TANK1 might be a positive regulator of TL in telomerase-expressing cells (9). Evidence has also confirmed this role in vivo (12). Tankyrase overexpression in human cells induces a progressive elongation of telomeres and is expected to be upregulated in all human tumors.

Widmann et al. (13) recently reported their finding of significantly short telomeres in both myeloid and lymphoid non-neoplastic cells of patients with aggressive non-Hodgkin lymphoma. Furthermore, telomere shortening in peripheral blood lymphocytes in patients suffering from various solid tumors has also been reported (14). The results of these studies suggest that chromosome instability associated with telomere dysfunction is an early event in tumorigenesis.

A number of reported studies have evaluated TL and telomerase activity in MM patients (15–19). However, information is very scarce regarding telomere dysfunction in patients with MGUS (15,17) (five and two patients, respectively), and no studies about changes in the expression of shelterin proteins in plasma cell disorders have been reported. Thus, the aim of this study was to investigate mRNA expression of a set of genes that encode the telomere-binding proteins TRF1, TRF2 and TANK1 in patients with MGUS and MM, to determine the role of telomere dysfunction as one of the steps involved in the progression of MGUS to MM. Results were correlated with hTERT expression, TL and clinicopathological characteristics of patients.

MATERIALS AND METHODS

Patients

The present study included 132 patients with plasma cell disorders: 64 patients with MGUS and 68 with MM. The diagnoses were based on standard criteria (1,2). MM staging was made according to the criteria proposed by Durie and Salmon (20) and the recently developed International Staging System (21). Patients were studied at diagnosis and all individuals provided their informed consent according to institutional guidelines. The study was approved by the ethics committee of our institution. Median follow-up was 17 months (range: 2–48 months). Detailed data about sex, age, disease stage and clinicopathological characteristics of all patients are summarized in Table 1.

RNA Extraction, Reverse Transcription and Real-Time Quantitative Reverse Transcription–Polymerase Chain Reaction

Total RNA was extracted with the Trizol reagent (Invitrogen, Buenos Aires, Argentina) from mononuclear cells isolated from BM samples from patients and peripheral blood of controls and from the K-562 cell line. Reverse transcription–polymerase chain reaction (RT-PCR) was carried out using 1× RT Buffer (Promega, Madison, WI, USA), 200 U/μL of Moloney murine leukemia virus RT (Promega), 250 ng/μL random primer (Promega) and 10 mmol/L each deoxynucleoside-5’triphosphate (Invitrogen). The cDNA synthesis was performed in a total volume of 20 μL, containing 1 μg of the total RNA, for 10 min at 95°C, for 60 min at 37°C and 10 min at 95°C to inactivate the enzyme. cDNA was stored at −20°C until use.

The analyses of TRF1, TRF2, TANK1 and hTERT mRNAs were performed using quantitative real-time PCR (QRT-PCR) in a LightCycler system (Roche Diagnostics, Mannheim, Germany), based on TaqMan methods. The mRNA expression levels were measured by using gene-specific fluorescent-labeled probes. 5-Carboxyfluorescein was added as the 5’-fluorescent reporter, and 5-carboxytetramethylrhodamine was added to the 3’ end as a quencher. Primers and probe sequences have been previously described (22,23). The housekeeping gene GAPDH was used to normalize sample-to-sample differences in cDNA input, RNA quality and RT efficiency, and it was amplified by using the primers and probe described by Hu
et al. (23). The PCR reaction was performed by using 4 μL of each RT reaction, 1 × TaqMan master mix (Roche Diagnostics), 200 nmol/L of the probe and 300 nmol/L of TRF1 primers and 500 nmol/L of TRF2, TANK1, hTERT and GAPDH primers in a 20-μL final volume. For all targets, the thermal cycling conditions were 10 min at 95°C to activate the enzyme, followed by 45 cycles at 95°C for 15 s and 60°C for 1 min. All measurements included a determination of the standards and the use of a no-template as a negative control, in which water was substituted for the cDNA. Standard curves were constructed with five-fold serial dilutions of the cDNA from the K562 cell line (0.15–5 μg total RNA). The ratio between copy numbers of GAPDH and TRF1, TRF2, TANK1 or hTERT and GAPDH primers in a 20-μL final volume. Telomere length was evaluated by use of terminal restriction fragments (TRF) assay as previously described (17). Briefly, for terminal restriction analysis, high molecular weight DNA was extracted from BM samples, followed by double digestion of 10 μg DNA with HindIII and RsaI restriction enzymes at 37°C, overnight. The digested products were separated by electrophoresis in 0.8% agarose gels during a 20-h period at 35 V. Gels were first depurinated, then denatured and neutralized and finally transferred to nylon membranes by Southern blotting. The telomeric fragments were detected through hybridization with a 5’ end-labeled telomeric probe (TTAGGG)7. The hybridized membranes were exposed to films for 10–15 d at –70°C before the film was developed. Hybridization signals were evaluated in the autoradiograph by densitometric scanning in each lane with respect to a λ/HindIII molecular weight standard and were analyzed using the Gel Pro software (Media Cybernetics, Bethesda, MD, USA). Telomere mean values were measured by estimating the band size corresponding to the point with the highest intensity. For comparative analysis, peripheral blood mononuclear cells from 18 normal controls (7 males and 11 females; median age 65 years, range: 33–86 years) defined as healthy individuals with no personal or family history of cancer and matched by sex and age, were also evaluated. All of the study participants who were controls provided their informed consent. In addition, as internal controls for telomere shortening we used the K-562 cell line (positive control of telomere shortening), and cord blood cells (negative control) as a very young tissue without telomere reduction.

### Immunohistochemical Analysis of Ki67

BM biopsies were fixed and decalcified for 72 h in Bouin's solution and embedded in paraffin. Tissue sections of 4-μm thickness were stained with hematoxylin-eosin, reticulin and Giemsa. For immunohistochemical analysis, tissue sections were deparaffinized and brought to phosphate-buffered saline solution, pH 7.2. After blocking endogenous peroxidase with 3% H2O2 in methanol and antigen retrieval pretreatment with citrate buffer in a microwave
oven, slides were incubated 1 h with monoclonal antibody against the Ki67 antigen (Dako, Carpinteria, CA, USA). Tissue sections were subsequently post-treated with a biotinylated antimouse immunoglobulin antiserum (Biogenex, San Ramon, CA, USA), followed by peroxidase-labeled avidin, and revealed with diaminobenzidine as chromogen. Counterstaining was then performed by using hematoxylin. Specificity tests, performed by omission of the specific antibody and incubation with nonimmune mouse serum, produced negative results.

**Statistical Evaluation**

The comparison of data from patients and controls and between subgroups was performed by using the Mann–Whitney test. Groupwise comparison of the distribution of clinical and laboratory variables was performed with the Student t test (for quantitative variables) and the χ² or Fisher exact test (for categorical variables). Correlations between gene expression and TL or clinical variables were assessed by using the Kendall coefficient. The cutoff point for hTERT expression was selected according to receiver operating characteristic (ROC) analysis. Overall survival was estimated by use of the Kaplan–Meier method and compared with the log-rank test. For all tests, P < 0.05 was regarded as statistically significant.

**RESULTS**

*Expression of Telomere-Binding Proteins by QRT-PCR*

The mRNA expression of TRF1, TRF2, TANK1 and hTERT was evaluated in 64 patients with MGUS and 68 with MM. A differential expression pattern with lower levels for TRF1 and higher for TRF2, TANK1 and hTERT in patients with respect to controls were observed (Figure 1). When both pathologies were compared, a significant increase of TRF1 mRNA levels (P = 0.006) and a lower expression of TRF2 (P = 0.005) and TANK1 (P = 0.003) in MGUS with respect to MM patients were found. The mean hTERT mRNA level in MGUS patients was lower than that in MM, with no significant difference between both entities was observed.

**Table 2.** Analysis of telomeric gene expression and mean TL according to groups of hTERT expression in patients with MM and MGUS.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Telomeric genes (X ± ES)</th>
<th>TRF1</th>
<th>TRF2</th>
<th>TANK1</th>
<th>TL, kb (X ± ES)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGUS</td>
<td>hTERT</td>
<td>0.27 ± 0.03</td>
<td>0.38 ± 0.08</td>
<td>0.04 ± 0.004</td>
<td>0.24 ± 0.01</td>
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<td></td>
<td>TRF1</td>
<td>2.53 ± 0.28</td>
<td>0.65 ± 0.28</td>
<td>0.05 ± 0.02</td>
<td>0.26 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>TRF2</td>
<td>8.05 ± 1.28</td>
<td>0.23 ± 0.06</td>
<td>0.10 ± 0.02</td>
<td>0.39 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>TANK1</td>
<td>0.30 ± 0.04</td>
<td>0.18 ± 0.04</td>
<td>0.06 ± 0.01</td>
<td>0.28 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>TL, kb (X ± ES)</td>
<td>2.17 ± 0.24</td>
<td>0.21 ± 0.03</td>
<td>0.07 ± 0.01</td>
<td>0.39 ± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.10 ± 1.52</td>
<td>0.34 ± 0.08</td>
<td>0.22 ± 0.03</td>
<td>0.79 ± 0.16</td>
</tr>
</tbody>
</table>

*Table 2.** Analysis of telomeric gene expression and mean TL according to groups of hTERT expression in patients with MM and MGUS.

Analysis of data showed that hTERT expression varied considerably in both pathologies, with cases that overexpressed hTERT and many others with low mRNA transcripts (Figure 2). For a better analysis, patients were divided into three groups according to hTERT levels by using ROC curves: low (≤1.08) (group I [GI]), intermediate (1.08 to ≤5.0) (group II [GII]) and high (>5.0) (group III [GIII]) mRNA expression (Table 2). MM and MGUS both demonstrated a similar distribution of patients per group, with most of the cases in GI (50 MGUS and 44 MM) and the least in GIII (5 MGUS and 7 MM). The evaluation of remaining genes according to hTERT groups showed an increased expression of TRF2 and TANK1 from GI to GIII in MGUS and MM. Significant differences for both genes in MM (GI versus GI and GI: P < 0.01) and for TRF2 in MGUS (GI versus GI: P < 0.01) were observed. In addition, the comparison between GIII patients showed that both genes had lower mRNA expression in MGUS than MM, with significant differences for TRF2 (P = 0.01). As for TRF1, a nonsignificant increase from GI to GIII in MM was observed. However, we observed heterogeneous behavior in MGUS, with higher values in GI and GII with respect to GIII, probably due to the presence of patients with elevated TRF1 expression.
mRNA expression in both groups (two cases in GII and five cases in GI).

In addition, we explored the relationship among mRNA expression of different genes. Both entities showed a positive association between TRF2-TANK1 ($P < 0.0001$), TRF2-hTERT ($P < 0.0001$) and TANK1-hTERT genes ($P < 0.0001$ for MM and $P = 0.01$ for MGUS). The correlation between genes is shown in Figure 3.

**TL Evaluation**

TL measured by TRF assay was performed in 43 patients with MGUS and 47 with MM. As we have previously reported (17), all patients showed significant telomere shortening (MGUS: $6.55 \pm 0.45$ kb and MM: $6.39 \pm 0.52$ kb) compared with controls ($8.12 \pm 0.26$ kb) ($P = 0.003$ and $P = 0.004$, respectively) (Figure 1). However, when the analysis of TL was performed according to hTERT groups, we found that patients from GIII, with the highest telomerase expression, had the shortest TL (Table 2). Significant differences in patients from GIII with respect to GII (MGUS: $P = 0.03$; MM: $P = 0.04$) and GI (MM: $P = 0.04$) were observed. Moreover, in MM the GIII mean TRF value was also significantly reduced compared with that for the K-562 cell line ($5.02 \pm 0.3$ kb) ($P = 0.04$).

**Correlation of Gene Expression and TL with Clinical Parameters and Ki67 Index**

The correlations among gene expression and TL with known prognostic factors are shown in Figure 4. In patients with MM, the percentage of BMPCI was positively associated with TRF2 ($P = 0.0002$), TANK1 ($P = 0.005$) and hTERT ($P = 0.003$) expression and negatively with TL ($P = 0.02$), whereas lactate dehydrogenase (LDH) was significantly correlated with TRF2 mRNA ($P = 0.008$). The Ki-67 index (Figure 5) was positively associated with TRF2 ($P = 0.006$), TANK1 ($P = 0.03$) and hTERT ($P = 0.03$) mRNA levels and negatively with TL ($P = 0.02$). When patients were distributed according to hTERT expression groups, significant differences in the percentage of...
BMPCI ($P = 0.04$) and albumin levels ($P = 0.03$) in GII/GIII with respect to GI were observed (Figure 6). The median overall survival was 17 months (range: 2–48 months). Although the number of patients was too small to allow valid statistical analysis, MM patients in GIII showed a shorter overall survival than GI patients (13.5 and 24.5 months, respectively), and similar results were observed when progression-free survival was evaluated (8 and 17 months, respectively). No association among genes and clinical parameters was found in MGUS.

**DISCUSSION**

The role of TL and telomerase activity in the development of human cancer has been extensively investigated in recent years. In contrast, there are few reports of evaluations of the participation of telomere-binding proteins in tumorigenesis. In the present study, mRNA expression of TRF1, TRF2 and TANK1 genes was analyzed in a similar number of patients with MGUS and MM, and compared with hTERT expression and TL. As a whole, both entities showed shorter TL and higher hTERT expression in patients with MGUS and MM than in healthy controls, but no differences between MGUS and MM patients were observed. Analysis of data revealed that for both pathologies patients had a great heterogeneity in hTERT levels, with some cases in which this gene was overexpressed and others in which hTERT mRNA expression was low. The use of ROC curves permitted us to divide patients into three different groups of hTERT expression. MGUS and MM exhibited a similar distribution of patients per group. In agreement with our data, heterogeneity in telomerase activity has been previously reported for MM patients (15,18). However, to our knowledge, this is the first study with results demonstrating a similar hTERT behavior in MGUS cases. As for TL, an inverse correlation with hTERT levels was observed in both MGUS and MM, with shortest telomeres for patients in GIII who had the highest hTERT expression. Our results showing a group of MGUS patients with very short telomeres and high hTERT mRNA levels suggest a role for these parameters in the progression from MGUS to MM. Supporting this idea, one MGUS patient of our series with high hTERT expression (6.87) and very short TL (4.28 kb) but without markers of high-risk disease, suffered progression to MM after 20 months of follow-up. More cases must be studied to confirm these data.

There have been a limited number of investigations evaluating the expression profile of telomere-binding factors in lymphoid malignancies. A number of studies have demonstrated increased mRNA levels of TANK1, TRF2 and hPif1 in high-grade non-Hodgkin lymphomas (22,24), and of TRF1, TRF2 and TIN2 in adult T-cell leukemia (25). In another reported study, investigators detected a low expression of many components involved in capping and telomere elongation in chronic lymphocytic leukemia (26). In our study, a nonsignificant decrease of TRF1 and a significant increase of TRF2 and TANK1 levels in patients with respect to controls and in MM compared with MGUS patients were observed. TRF1 was identified as a negative length regulator that limits telomere elongation and results in stable TL. According to a model proposed by Smogorzewska et al. (27), an unsuitably long telomere would recruit a large amount of TRF1 protein, blocking the
telomerase-mediated elongation. In addition, van Steensel et al. (9) reported that long-term overexpression of TRF1 in the telomerase-positive tumor-cell line HT1080 resulted in a gradual and progressive telomere shortening, supporting the role of TRF1 as a suppressor of telomere elongation. In MM patients, we have observed an increase of TRF1 associated with a decrease of TL from GI to GIII, suggesting the possible participation of TRF1 in the maintenance of short telomeres. In MGUS, we have detected heterogeneous behavior, with higher values in GI and GII compared with GIII. One explanation for these results is that patients in GI and GII had elevated TRF1 mRNA expression that raised mean values. More studies will be necessary to improve our understanding of TRF1 function in MGUS.

As previously mentioned, our patients showed overexpression of TRF2 and TANK1 with significant differences between MGUS and MM. Both genes had a direct correlation with hTERT mRNA levels. The upregulation of TANK1 and its association with telomerase expression was previously observed by Xu et al. (15) in a small number of MM and plasma cell leukemia patients, but no studies of TANK1 expression in MGUS have been reported. Thus, our data confirm a previous observation in MM and, interestingly, showed similar behavior for MGUS patients. Simultaneously, our study results provide the first evidence of TRF2 upregulation in MGUS and MM. In addition, we were able to demonstrate that TRF2 and TANK1 levels increased from GI to GIII in both MM and MGUS, showing a direct correlation between TRF2 and TANK1 expression. These findings indicate an interaction between positive and negative factors in the maintenance of TL in plasma cell disorders and support a role for TRF2 and TANK1 upregulation in disease progression. In a related study of gastric cancer, a significantly higher expression of TRF2 in tumor tissue and in lymph-node metastasis compared with precancerous lesions and normal gastric mucosa was found (28). Changes in telomeric gene expression and their negative correlations with telomere reduction, observed in our series, suggest that mechanisms other than telomerase activation are involved in TL maintenance in plasma cell disorders.

The present study is the first to correlate mRNA expression of telomere-associated genes and known prognostic factors in plasma cell disorders. In MM, we found that the percentage of BMPCI was positively correlated with the expression levels of TRF2, TANK1 and hTERT, and negatively with TL, whereas LDH was associated with TRF2, suggesting these genes as probable prognostic markers in this entity. Furthermore, Ki67 was also positively associated with these genes and negatively with TL, supporting a high degree of telomere dysfunction in those myeloma cells with increased proliferative capacity. This finding supports the recent observation of Gastinne et al. (29), who found correlations between Ki67 and markers of intrinsic malignancy and tumor burden in MM patients, and supports previous data showing a strong correlation between telomerase activity and Ki67 (16). In our series, we also detected shorter survival in MM patients in GIII (with high levels of hTERT and very short TL) compared with GI, supporting the connection between dysfunctional telomeres and tumor progression. This observation is consistent with those reported by Wu et al. (18), who found that the combination of both short telomeres and high telomerase activity defined a subgroup of patients with poor prognosis.

In reference to MGUS, the molecular basis of its evolution to a malignant monoclonal gammopathy remains poorly understood (3). Different predictors of risk progression (size of M protein, type of immunoglobulin, BMPCI, serum free light chain ratio) have emerged, and there is growing evidence that the stromal component of the BM microenvironment may also play an important role in disease evolution (3,30). In our series of MGUS cases, no association with clinical characteristics was found, but the presence of a group of patients with both short TL and high expression of telomere-associated genes permits us to suggest that these parameters are probable prognostic factors that will likely be useful in allowing more accurate risk stratification for individual patients. The short time of transition from MGUS to MM in one of these patients supports this hypothesis. Nevertheless, more studies will be necessary to confirm these findings.

Telomere dysfunction is considered one of the molecular causes of genetic instability (31). Telomere shortening has been demonstrated to contribute directly to the presence of chromosomal abnormalities usually found in diverse types of cancer. This telomere reduction may trigger the formation of telomeric fusions between chromosome arms, which may result in novel karyotypic rearrangements (32,33). Different reports showed similar genetic alterations in MGUS and MM, which suggests a model for MGUS evolution based on genomic instability manifested by aneuploidy as the permissive event for the occurrence of chromosome alterations (34,35). Furthermore, gene expression profiling studies allowed the identification of a clear oncogenic pathway deregulation from normal plasma cells to relapsed MM characterized by increased chromosome instability and overexpression of MYC and E2F genes (36). The results of this study and a previously reported study by our group (17) in both MM and MGUS patients support the presence of genomic instability manifested by telomere dysfunction and the probable participation of these factors in the development and/or progression of the disease.

Results of a recently reported study (13) showed that short telomeres in nontransformed leukocytes are a risk factor for lymphomagenesis. In our study, we were able to find short telomeres and changes in the expression of telomere maintenance genes as well as a strong correlation among genes in mononuclear cells of patients with MGUS and MM, providing the first evidence of a modification in the expression of telomere-associated genes in
these pathologies. This finding is important, considering the new therapeutic approaches with diverse telomere-targeting inhibitors (37) and suggests that a number of MM patients may be particularly sensitive to telomere-damaging drugs.

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DISCLOSURE
The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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