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Telomere protein complexes and their role in lymphoid malignancies

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1. ABSTRACT

Telomeres are highly regulated and dynamic complexes that protect the genomic DNA and prevent the end of linear chromosomes from being misrecognized as a broken DNA. Due to the end replication problem, telomeres of somatic cells shorten with each cell division, inducing cell senescence. Telomerase is a reverse transcriptase capable of compensating telomere attrition by adding telomere repeats to the ends of chromosomes. Human telomeres are associated with the shelterin complex which consists of six telomere-associated proteins that specifically bind to telomeric DNA. Alterations or removal of individual shelterin components would lead to telomere uncapping and telomere dysfunction, resulting in cellular senescence and transformation to a malignant state. Another complex of multifunctional proteins, named non-shelterin complex, is thought to prevent telomere degradation and facilitate telomerase-based telomere elongation. As telomerase is highly expressed in most human tumor cells, it is considered an attractive target for new therapeutic strategies. In this review, we will summarize the characteristics of telomeres and telomerase in lymphoid malignancies and discuss the role of telomere-associated proteins in these entities.

2. INTRODUCTION

2.1. Telomeres and telomerase

Telomeres are highly regulated and dynamic complexes at chromosome ends, consisting in human cells of tandem repeats of the sequence TTAGGG and associated protective proteins (1). The main role of telomeres is to protect the genomic DNA and prevent the end of linear chromosomes from being misrecognized as a broken DNA. As known, due to the end-replication problem, the DNA replication machinery cannot completely copy the DNA of linear chromosomes, leading to telomeres progressively shorter with repeated cell division.

In eukaryotes, this deficiency can be resolved by the cellular ribonucleoprotein enzyme telomerase, which can add telomeric repeat sequences to the ends of chromosomes, thus elongating them to compensate for their attrition (2). The core of the telomerase holoenzyme complex consists of the catalytic reverse transcriptase (TERT) subunit, the RNA template (TERC) and dyskerin (DKC1) (3). Most normal human cells lack sufficient levels of telomerase to maintain telomere length (TL), hence telomeres shorten over time and result in replicative senescence (4). By contrast, in most human tumor cells telomerase is highly expressed, and TL is maintained (5). Approximately 10% to 15% of human cancers lack detectable telomerase activity and the mechanism for maintaining the lengths of telomeres is referred to as alternative lengthening of telomere (ALT). Defects in the protection of telomeres have been implicated in cancer and aging (6).

Despite their heterochromatic state, telomeres are transcribed giving rise to long non-coding RNAs (lncRNA) called TERRA (telomeric repeat-containing RNA). TERRA molecules play critical roles in telomere biology, including regulation of telomerase activity, heterochromatin formation at chromosome ends and capping of telomeres. Nevertheless, the mechanisms of

action of telomeric non-coding RNAs remain largely to be elucidated (7).

2.2. Shelterin and non-shelterin complexes

Human telomeres are associated with the shelterin complex, which contains six proteins: TRF1, TRF2, POT1, TIN2, TPP1 and RAP1. Among them, TRF1 and TRF2 (*Telomeric Repeat Binding Factor 1 and 2*) are homodimeric proteins that bind to the double-stranded telomeric DNA. Several *in vitro* studies have suggested a DNA remodeling role for TRF1 and TRF2 (8, 9). POT1 (*Protection Of Telomeres 1*) binds specifically to single-stranded telomeric DNA and forms a heterodimer with TPP1 (*ACD* gene: *Adrenocortical Dysplasia Homolog*) protein (10). TIN2 (*TRF1-Interacting Protein 2*) is a hub that interacts with TRF1, TRF2, and POT1/TPP1 (10, 11), mediating the assembly of the entire complex. RAP1 (*Repressor/Activator Protein 1*) is recruited through its interaction with TRF2 (9). Alterations or removal of individual shelterin subunits leads to severe telomere uncapping, which triggers specific DNA damage response (DDR) pathways as they are recognized as double-strand breaks. For instance, TRF1 was shown to prevent the activation of both ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3 related (ATR) pathways (12). In addition, TRF2, RAP1, and POT1/TPP1 were shown to inhibit the activation of ATM (13), homology-directed recombination (14), and ATR (15) pathways, respectively.

A current model for how telomere shortening activates a DDR suggests that critically short telomeres would fail to recruit the shelterin amount required for repressing the DNA repair pathways (16). On the contrary, long telomeres recruit more TRF1 and TRF2, facilitating the t-loop formation. In the t-loop state, telomerase would no longer be able to elongate the chromosome ends, leading to loss of sequences with successive cell divisions. In agreement with this model, Takai *et al* (17) have shown that short telomeres serve as a better substrate for telomerase than long telomeres, creating a feedback mechanism to maintain the TL. As telomeres gradually shorten, they switch to an open state due to a lower number of shelterin subunits. At this stage, the telomere elongation would again take place, preventing complete loss of the telomeric DNA and resulting in the stabilization of the telomeres at a short length.

Another complex of multifunctional proteins, named the non-shelterin complex, comprise a set of multifunctional factors such as DNA repair proteins MRE11/NBS/RAD50 (MNR complex) and Replication Protein A1 (RPA1) that prevent telomere degradation and facilitate telomerase-based telomere elongation (18). Maintenance of the telomere architecture involves a highly regulated network of protein-protein, protein-DNA and protein-RNA interactions; thus its impairment can result in telomere dysfunction, cellular senescence and transformation to a malignant state (14, 19).

In addition, there is another complex, named ribonucleoprotein (RNP) complex, composed of four evolutionarily conserved proteins, DKC1, NHP2 (*NHP2 ribonucleoprotein*), NOP10 (*NP10 ribonucleoprotein*), and GAR1 (*GAR1 ribonucleoprotein*), and a function-specifying, noncoding H/ACA RNAs (20, 21). DKC1, NHP2 and NOP10 form a trimer that bound directly to H/ACA small nucleolar (sno)/small Cajal body-specific (sca) RNAs (sno/scaRNA) and the 3´ domain of TERC (22, 23). H/ACA RNPs contribute to telomerase assembly and stabilization, and posttranscriptional processing of nascent ribosomal RNA and spliceosomal RNA (24-26). DKC1, NHP2 and NOP10 are interdependent of each other for stability (27); the loss of function of any of these proteins reduces TERC stability and decreases telomerase activity. On the contrary, GAR1 binds only to DKC1 and is needed for proper functioning of the H/ACA RNPs (28). In a recent report, von Stedingk *et al* (29) suggests that at early tumor stage cells with low *DKC1, NHP2* and *GAR1* expression levels may undergo genetic alterations and instability associated to telomere dysfunction. However, at advanced stage, over-expression of H/ACA RNP components, associated to increased telomerase activity, would favor tumor progression.

Several studies using genetically modified mice for different components of the telomere complexes suggest a role for these proteins in cancer susceptibility and age-related diseases even in the presence of normal telomerase activity and normal TL (30-32). Telomere dynamics have been extensively studied in hematologic malignancies. In this review we will consider the current evidence for the role of telomere-associated proteins in lymphoid neoplasm.

3. TELOMERE HOMEOSTASIS IN LYMPHOID MALIGNANCIES

3.1. Chronic lymphocytic leukemia (CLL)

CLL is the most common type of adult leukemia in the Western world, representing about 30% of all leukemias; the disease mainly affects individuals >60 years of age. It is characterized by the accumulation of small B lymphocytes with a mature appearance in blood, bone marrow, lymph nodes and other lymphoid tissues (33). It is a heterogeneous disorder with a highly variable clinical course, with time to progression ranging from months to decades. In the last years several prognostic biomarkers, including genomic alterations and mutational status of *IGHV* (immunoglobulin heavy chain variable) region, have been identified, allowing the subdivision of CLL into clinical relevant subgroups (34-37). More recently, advances in molecular and genetic profiling have led to the ability to identify

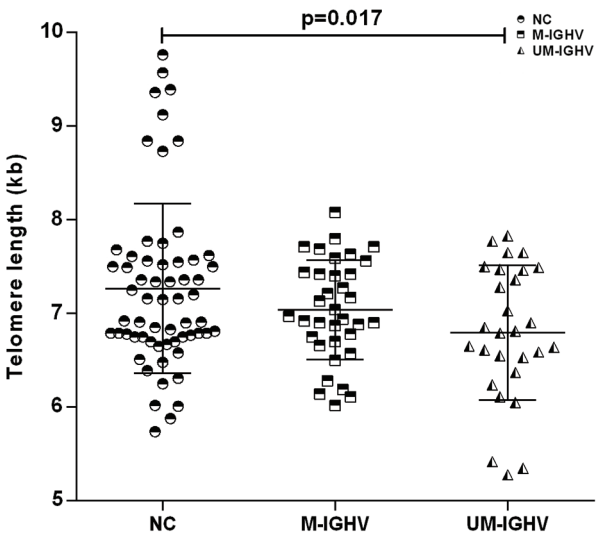

Figure 1. Telomere length in mutated (M)-IGHV and unmutated (UM)-IGHV chronic lymphocytic leukemia patients and normal controls (NC).

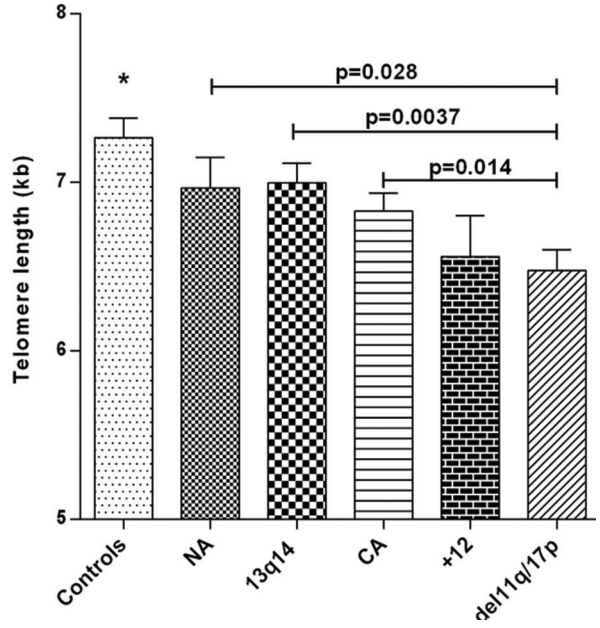

Figure 2. Telomere length in genetic risk groups of chronic lymphocytic leukemia patients and controls. *Significant differences with respect to del11q/17p, +12 and chromosome abnormalities groups (p<0.0.001). NA: No alterations.

sub-groups of patients with CLL whose disease may respond to selected therapy (38).

In CLL, average TL has been reported as an emerging prognostic factor. In general, the TLs of B-CLL cells are significantly shorter than those from B cells of age-matched normal controls (39-43). Short telomeres have been associated with genetic complexity and a high risk of genomic aberrations (40, 42, 44), and ultimately implicated in disease outcome (42, 44, 45). Besides, an association between short TL and a large of copy number aberrations detected by high resolution SNP-arrays was described (45).

Furthermore, the TLs of unmutated (UM) CLL cases, associated to poor outcome, are much shorter than those of age-matched normal donors, and they are even shorter than those of the mutated (M) cases, related to a better prognosis (39-42, 44-46). Studies of our group support these findings, showing short TL in UM-CLL patients compared to both M-CLL cases and normal controls, with significant differences with respect to the last group (Figure 1). Besides shortened TL, telomere fusion and genomic instability was also observed in patients with CLL (47). We have recently found that CLL patients with two or more chromosome aberrations displayed a significant TL reduction (Figure 2) and more importantly shorter treatment free survival when compared to cases with one or no anomalies (43). These findings indicate the importance of telomere dysfunction in driving genomic instability. In this regard, Lin *et al* (47) showed that a subset of early-stage patients exhibited extensive telomere erosion and fusion, indicating that telomere shortening and dysfunction may be critical in the progression of the disease.

Increased telomerase expression and activity were also observed in CLL patients (Table 1). Some studies proved that patients expressing *TERT* have significantly shorter survival than *TERT*-negative cases, regardless of disease stage (42, 48-50). In addition, the levels of *TERT* expression were significantly increased in advanced CLL stages and, within all stages, in the clinically aggressive UM-CLL cases. However, this relationship was not seen by Damle *et al* (51) in blood-derived CLL cells grouped as a whole, although high telomerase activity was found in the poor outcome, UM-CLL subgroup. In addition, a recent report (52) did not find significant association between *TERT* expression and the most important prognostic factors of CLL as well as clinical outcome. Thus, studies in larger series of CLL patients will be necessary to clarify the prognostic significance of *TERT* expression in this pathology.

Altered expression of telomere-associated proteins has also been reported in this entity (Table 1). Poncet *et al* (53) have carried out transcriptomic analysis of telomerase components, shelterin proteins and a set of non-shelterin proteins. They found that the mRNA levels are lower in CLL cells for *TRF1* and *RAP1*, slightly reduced for *TRF2*, and almost unchanged for *TIN2*. A decrease in mRNA levels of *KU80*, *MRE11*, and *RAD50* and an increase in *RPA1* were also observed. These findings suggest that the capping complex might be disrupted, facilitating telomere reduction independently of telomerase levels. In agreement with this hypothesis, Augereau *et al* (54) have shown that shelterin

Table 1. Expression profiles of telomere-associated genes in lymphoid malignancies

Lymphoid malignancy	Expression profile when compared to controls							
	Shelterin			Non-shelterin			Telomerase complex	
	Upregulated	Downregulated	Unchanged	Upregulated	Downregulated	Unchanged	Upregulated	Downregulated
CLL	TRF1[52]	TRF1[53]	TRF2[52]	MRE11[52]	RAD50[53]	NBS[52]	TERT[48,52]	TERT[53]
	POT1[52]	TRF2[53]	TPP1[52]	RAD50[52]	MRE11[53]			DKC1[53]
	RAP1[52]	RAP1[53]	TIN2[52,53]	RPA1[52,53]	Ku80[53]			
BL	TRF2[77]		TRF1[77]	PIF[77]		Tankyrase[77]	TERT[77]	
FL			TRF1[77]			Tankyrase[77]		TERT[77]
			TRF2[77]			PIF[77]		
DLBCL			TRF1[77]			Tankyrase[77]		TERT[77]
			TRF2[77]			PIF[77]		
MCL	TRF1[80]		TRF1[77]			Tankyrase[77]	TERT[80]	TERT[77]
	TRF2[80]		TRF2[77]			PIF[77]	DKC1[80]	
	POT1[80]							
	TIN2[80]							
	TPP1[80]							
	RAP1[80]							
Plasma cell disorders	TRF2[94,96]	TRF1[94]		MRE11[97]			TERT[94,96,97]	
	POT1[80]			RAD50[97]			DKC1[97]	
	TIN2[80]			NBS[97]				
	TPP1[80]			RPA1[97]				
	RAP1[80]			Tankyrase[94]				

CLL: Chronic lymphocytic leukemia; BL: Burkitt's lymphoma; FL: Follicular lymphoma; DLBCL: Diffuse large B cell lymphoma; MCL: Mantle cell lymphoma

deregulation correlates with the presence of telomere damage-induced foci (*TIF*) in early stages of CLL. Since the presence of *TIF* is a hallmark of senescent cells (55), Augereau *et al* (54) propose that early stage CLL is associated to accelerated B lymphocyte senescence. This might result from the accumulation of various telomere dysfunctions in the B cell lineage, including telomerase and shelterin down-regulation (53, 54). On the contrary, Hohxa *et al* (52) studying untreated CLL patients at early clinical stage by microarrays, found a significant increased expression for *POT1*, *TRF1* and *RAP1*, meanwhile no differences were observed for *TRF2*, *TPP1* and *TIN2*. The analysis of non-shelterin genes also showed a significant up-regulation for *MRE11A*, *RAD50* and *RPA1* as compared with controls, but no statistically difference was observed for *NBS*. Although these results need to be confirmed at the protein level, they suggest that modulation of telomere-associated genes, together with telomere shortening, represent an early event in CLL. Simultaneously, Véronèse *et al* (56) performed an unsupervised hierarchical clustering analysis based on the combination of cytogenetics and telomeric characteristics. This study allowed the subdivision of patients in three different clusters, in which cluster I -associated to good prognosis parameters- showed long telomeres and high expression of *TRF1, TRF2* and *POT1* genes. On the contrary, patients from clusters II and III, related to poor prognostic features, had a striking decrease in TL and gene-expression levels, suggesting a relationship between high risk cytogenetic alterations and severe telomere and chromosome instability. Discrepancies among data of the literature may be related with the particular characteristics of series studied and/or the number of patients analyzed. Interestingly, Ramsay *et al* (57) found somatic mutations in *POT1* gene in 3.5% of all CLL cases, occurring exclusively in clinically aggressive CLL patients with UM-*IGHV* status. *POT1*-mutated CLL cells showed high frequency of telomeric and chromosomal abnormalities, suggesting mutations of this gene favor the acquisition of malignant features of leukemic cells and, that they likely represent driver mutations in this pathology. Thus, *POT1* appear as the first component of the telomere shelterin complex found to be mutated in human cancer. In addition, within the

shelterin complex, ACD is required for POT1 to perform its role in protecting telomeres from being recognized as DNA damage (58, 59); this subunit is also necessary for the recruitment of telomerase to telomeres (60, 61). Recent studies found ACD mutations in patients with childhood pre-B acute lymphoblastic leukemia (62) and inherited bone marrow failure (63). These findings indicate the importance of mutations in telomere-associated genes as a disease-causing in humans and support the hypothesis that telomere dysfunction results in genomic rearrangements that may prone cancer initiation and progression (64).

Finally, the H/ACA RNP complex was scarcely studied in lymphoid malignancies. In CLL, Ronchetti *et al* (65) explored the expression profile of snoRNAs and scaRNAs associated to the H/ACA RNP complex in a series of Binet stage A CLL patients. This study could define two subgroups of patients, one with low expression of SNORA74A and SNORD116-18 associated to better prognosis and the other characterized by the high expression of at least one of the two snoRNAs, related to a high risk disease. These findings support a possible role of non-coding RNA deregulation in the prognosis of CLL as well as its potential useful to predict the clinical outcome of early stage patients. In addition, as previously referred, there are four proteins associated to H/ACA RNP complex (DKC1, NOP10, NHP2 and GAR1). To our knowledge, there is only one report in the literature that analyzed *DKC1* expression in CLL (53), detecting a significant reduced transcription level of this gene. Preliminary results of our group showed a global deregulation of *DKC1, NOP10, NHP2* and *GAR1* genes compared to normal controls (data not shown). Its association with a high number of genetic alterations and UM-*IGHV* mutational status suggests a role for these telomere-associated genes in genomic instability and telomere dysfunction in CLL.

3.2. B-cell malignant lymphomas

Non-Hodgkin lymphomas (NHL) comprises a group of closely related heterogeneous diseases derived from malignant transformation of lymphoid cells, characterized by distinctive morphologic, immunophenotypic, genetic and clinical features. They are clonal tumours of mature and immature B, T or natural killer cells at various stages of differentiation, that show variable clinical behavior ranging from highly aggressive to an indolent course. Among them, B-cell neoplasms include numerous histological subtypes that correspond approximately to 90% of all NHL cases (66).

Telomere reduction was vastly studied in B-cell lymphomas. In general, mantle cell lymphoma (MCL) and CLL display the shortest TLs, whereas follicular lymphoma (FL) and diffuse large B cell lymphoma (DLBCL) show the longest TL (67, 68). Studies performed by our group in MCL, DLBCL and FL (69, 70) showed comparable results as those reported by Walsh *et al* (68). However, the shortest telomeres were observed in DLBCL secondary to FL, a very aggressive subtype not studied in other series, supporting the participation of telomere shortening as other genetic change involved in the transformation process (69). In reference to MCL, our study and data of the literature (70, 71) showed that TL reduction in this pathology is independent of the clinical characteristics, morphology and karyotype and, as opposed to CLL, did not reveal any prognostic relevance.

Several publications were reported on telomerase activity in B-cell malignant lymphomas. In these studies, increased telomerase activity was found in patients with Hodgkin disease compared to reactive lymph nodes (72, 73). Furthermore, a positive correlation of telomerase activity with the rate of proliferation in different subtypes of B-cell NHLs as well as lymphoid cell lines was observed (74-76). In contrast to these studies, Klapper *et al* (77) found similar telomerase activity and *TERT* expression in patients with MCL, FL and DLBCL, when compared to normal lymph nodes (Table 1). In addition, Burkitt's lymphoma was the only subtype that showed significantly higher telomerase activity and *TERT* expression, which expressed approximately 17 times the activity found in the other entities. A more recent report, found that B-cell malignancies with translocations affecting the locus 5p13.3.3, in which *TERT* gene is located, showed higher transcriptional expression of this gene as well as increased telomerase activity. These findings suggest a role of these chromosomal abnormalities in *TERT* deregulation and its possible contribution to lymphomagenesis (78). As for MCL, contradictory results have been observed for telomerase activity and expression (Table 1). Trentin *et al* (79) detected increased activity levels in five leukemic MCL cases, whereas Klapper *et al* (77) found low levels of telomerase activity in association with low *TERT* expression in patients compared to normal lymph nodes. In a recent study of our group (80), we identified somatic mutations in the *TERT* promoter (*TERT*p) region, upstream of the ATG start site, that were associated with higher *TERT* mRNA expression in MCL cells. Somatic mutations in the *TERT*p region were detected in many solid tumors (81-83). Different authors (84, 85) had previously demonstrated that these mutations generate *de novo* consensus binding motifs for E-twenty-six (ETS) transcription factors, and in *in vitro* reporter assays, the mutations increased transcriptional activity from the *TERT* promoter by two- to fourfold. In our study**,** the upregulation of *TERT* caused by *TERT*p mutations appeared to influence molecular, cellular, and clinical behavior of MCL. This is explained by the observation that most of *TERT*p-mutant MCL showed UM or minimally M *IGHV* status, overexpressed the transcription factor *SOX11* (SRY (Sex Determining Region Y)-box 11) -associated with adverse prognosis- and displayed altered expression of telomere-associated genes. Thus, our findings suggest

an important role of these mutations as a driver event in MCL development and maintenance.

In addition, the expression of shelterin and non-shelterin subunits was scarcely evaluated in NHLs (Table 1). *TRF1* as well as *Tankyrase*, an inhibitor of *TRF1*, did not show significant differences in their expression levels among all tissues examined, including Burkitt's lymphoma. However, transcript levels of *TRF2* and the helicase *PIF1 (*5'-to-3' DNA helicase) were the highest in Burkitt's lymphoma and showed only minor differences among benign lymph nodes, MCL, FL and DLBCL. The level of *TRF2* as well as *PIF1* correlated positively with telomerase activity (77). These findings highlight a differential expression of *TRF2* and *PIF1* in NHL with an upregulation in Burkitt's lymphoma. A study of our group found upregulation of genes that encode for different telomere associated proteins (TRF1, TRF2, POT1, TIN2, TPP1, RAP1 and DKC1) in MCL samples when compared to controls (80). Studies in larger cohorts would be desirable in order to investigate the clinical significance of telomere dysfunction in this entity.

3.3. Plasma cell disorders

Plasma cell disorders are characterized by the proliferation of a single clone of plasma cells in the bone marrow and by the detection of a monoclonal protein in blood and/or serum. These disorders may range from a phenotypically benign entity, monoclonal gammopathy of undetermined significance (MGUS), to symptomatic multiple myeloma (MM) with lytic bone lesions, bone marrow failure, and renal damage. Approximately 1% of individuals with MGUS evolve to MM per year (86). Recent advances in molecular cytogenetic, genomic, and proteomic studies of tumor cells and their normal counterparts have allowed for increased understanding of the pathogenesis of MM. They have also provided the basis for molecular prognostic classification, identified potential therapeutic targets, and improve patient outcome (87).

As known, MM is associated with considerable cytogenetic instability, involving translocations and additions or deletions of whole chromosomes. When Cottliar *et al* (88) studied telomere length measured by Terminal Restriction Fragments in patients with MM and MGUS, they observed a reduction in TL in MM patients, in agreement with other studies (89), as well as a significant increase in the occurrence of chromosome instability, a critical factor in the initiation and progression of human cancers (90). Simultaneously, Wu *et al* (89) have observed a very strong correlation between cytogenetic abnormalities and the presence of high telomerase activity levels and/or short TL. For instance, deletion of chromosome 13, a marker of poor prognosis, and duplication of chromosome 3, in which *TERC* gene is located, were strongly associated with both high telomerase activity and short TL. Given that MM patients with abnormal karyotypes have a worse prognosis than those with a normal pattern (91, 92) the association of abnormal karyotypes with telomere reduction supports the importance of this mechanism in the development and/or progression of the disease.

Xu *et al* (93) reported elevated telomerase activity in 78% of MM patients and all cases with plasma cell leukemia. However, telomerase levels were not elevated in MGUS. As shown in Table 1, studies of our group have found increased expression levels of *TERT,* in MM as well as in MGUS, providing the first evidence of a modification in the expression of telomerase gene in these entities (94). Interestingly, in both pathologies a similar pattern of *TERT* expression was observed, in which patients displaying the highest telomerase transcription levels had the shortest TLs. More recently, Diaz de la Guardia *et al* (95) using gene expression arrays have identified that *TERT* along with other 16 genes are directly involved in TL maintenance in MM cells. The expression levels of these genes were even higher than those in human embryonic stem cells and induced pluripotent stem cells, which have unlimited proliferation capacity.

The expression profile of shelterin genes in plasma cell disorders have been extensively studied by our group (Table 1) (94, 96, 97). Our findings showed increased expression of shelterin components in MM compared to MGUS. Among the six shelterin subunits, *POT1* showed particular significance for being strongly associated with clinical features such as advanced clinical stages, high calcium and β2-microglobulin levels and the presence of bone lesions (96). Moreover, in multivariate analysis, *POT1* expression was a significant independent prognostic factor for overall survival as well as the International Staging System. Thus, our findings suggest this gene as a useful prognostic factor in MM as well as a possible molecular target for new therapeutic approaches. Our group has also investigated a set of non-shelterin genes involved in essential processes such as replication (*RPA1*), DNA damage repair pathways (*MRE11-RAD50-NBS*) and stabilization of telomerase complex (*DKC1*). We observed, for the first time, a significant increase in the expression of all these genes along with an upregulation of *TERT* and reduced TL in MM compared with MGUS (97), providing new insights into the intricate mechanisms by which telomere-associated proteins collaborate in the maintenance of plasma cells immortalization and suggesting a role for the upregulation of these genes in the progression of the disease. Nevertheless, further studies at the protein level are needed to confirm all these transcriptional changes.

As it was referred for CLL, recent studies have also evidenced altered expression of sno/scaRNAs, associated to the H/ACA RNP complex, in plasma cell disorders. For instance, Lopez-Corral *et al* (98) found overexpression

of SNORD25, SNORD27, SNORD30, and SNORD31 in smoldering MM patients correlated with shorter time to progression to symptomatic disease. Furthermore, two different studies (99, 100) reported the upregulation of ACA11 (SCARNA22) in MM patients harboring the recurrent translocation t(4;14)(p16;q32) (101), associated to short survival in this pathology (87, 91). This scaRNA, located within intron 18-19 of the WHSC1 (Wolf-Hirschhorn syndrome candidate 1) gene (also known as MMSET: multiple myeloma SET Domain Containing Protein), can suppress oxidative stress both in vitro and in vivo, facilitate cell proliferation and protect cells from the effects of chemotherapy, suggesting its importance in tumor development. In addition, Ronchetti *et al* (100) found a general pattern of down regulation of sno/scaRNAs expression in MM and secondary plasma cell leukemia patients compared with a non-neoplastic counterpart, as well as a specific pattern of sno/scaRNAs associated to distinct molecular subtypes of MM. Particularly, upregulation of SNORD36C, SNORD63, SNORD95 and SNORA40 was observed in hyperdiploid MM cases, and a signature overexpressing members of SNORD115 and SNORD116 families, in patients showing low-to-moderate levels of the *CCND1* (*Cyclin D1*) gene in the absence of any primary IGH (immunoglobulin heavy chain) translocation and hyperdiploid status. Overall, these findings add more complexity to the molecular heterogeneity of plasma cell disorders, constituting possible new prognostic biomarkers in these pathologies.

4. PERSPECTIVES

Telomerase has emerged as an attractive target for future cancer treatments since telomerase is the mechanisms employed by a vast majority of cancer cells to enable unlimited proliferation. Several strategies have been developed considering two main aspects. First, telomerase must be the main mechanism of telomere maintenance; second, normal somatic cells, with very low or no telomerase activity, would be unaffected as they have longer telomeres compared to telomerase-positive cancer cells. In this context, increasing knowledge on shelterin components and telomere-associated genes has brought insight into their specific role in the regulation of telomere structure and function. As seen in this review, different forms of cancer show unique expression profiles of telomere-associated genes. It would be interesting to investigate whether this heterogeneity defines a subgroup of patients that may be particularly sensitive to telomerase-targeted therapy.

5. ACKNOWLEDGEMENTS

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Abreviations: ACD: Adrenocortical Dysplasia Homolog; ATM: ataxia telangiectasia mutated; ATR: ataxia telangiectasia and Rad3 related; CCND1: Cyclin D1; CLL: chronic lymphocytic leukemia; DDR: DNA damage response; DKC1: dyskerin; DLBCL: diffuse large B cell lymphoma; ETS: E-twenty-six; FL: follicular lymphoma; GAR1: GAR1 ribonucleoprotein; IGH: immunoglobulin heavy chain; IGHV: immunoglobulin heavy chain variable region; M: mutated; MCL: mantle cell lymphoma; MGUS: monoclonal gammopathy of undetermined significance; MM: multiple myeloma; MMSET: multiple myeloma SET domain containing protein; MRE11: homolog A, double strand break repair nuclease; MRN: MRE11, RAD50 and NBS; NBS: nibrin; NHL: non-Hodgkin lymphomas; NHP2: NHP2 ribonucleoprotein; NOP10: NP10 ribonucleoprotein; PIF1: 5'-to-3' DNA helicase; POT1: protection of telomeres 1; RAD50: RAD50 homolog, double strand break repair protein; RAP1 repressor/activator protein 1; RNP: ribonucleoprotein; RPA1: replication protein A1; scaRNAs: small cajal body-specific RNAs; snoRNAs: small nucleolar RNAs; SOX11: SRY (Sex Determining Region Y)-box 11; TERC: telomerase RNA component; TERT: telomerase reverse transcriptase; TERTp: telomerase reverse transcriptase promoter**;** TIF: telomere damage-induced foci; TIN2: TRF1-interacting protein 2; TL: telomere length; TPP1: adrenocortical dysplasia homolog; TRF1: telomeric repeat binding factor 1; TRF2: telomeric repeat binding factor 2; UM: unmutated; WHSC1: Wolf-Hirschhorn syndrome candidate 1.

Key Words: Telomere Length, Telomerase, Shelterin, Telomere-associated Proteins, Lymphoid Malignancies, Review.

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