



Shelterin genes, germ line mutations and chronic lymphocytic leukemia

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Telomeres are distinctive DNA-protein structures that cap the ends of linear chromosomes; they are essential to maintain chromosomal integrity and genome stability. Telomeres are composed by tandem repeats of the non-codificante DNA sequence TTAGGG bound by the shelterin complex. It contains six core proteins: telomeric repeat binding factor 1 (TERF1), TERF2, protection of telomeres 1 (POT1), adrenocortical dysplasia homolog (ACD), telomeric repeat-binding factor 2-interacting protein (TERF2IP) and TERF1 interacting nuclear factor 2 (TINF2), which play fundamental roles in telomere protection, chromosomal stability and regulation of telomere length (TL) (1). In addition, the shelterin complex modulates telomerase activity at chromosome ends, recognizes telomeric DNA and remodels it into a t-loop, which protects the 3' overhang from being recognized as DNA damage. Alterations in the structure and function of any of these proteins may lead to undesirable DNA damage responses that could be associated to a role in tumorigenesis and cancer progression.

Particularly, POT1 has a critical role in protecting the telomere from DNA damage signaling (1). This protein binds specifically to single-stranded telomeric DNA (ssDNA), influencing the maintenance of telomeric DNA by telomerase and protecting the 5' end of the chromosome. In addition, POT1 acts as a regulator of telomerase-dependent TL and it can help telomere to form D-loop structure to stabilize telomere as well (2). POT1 forms a heterodimer with ACD protein contributing to the t-loop formation (3). Experimental

studies support a dual role for ACD acting in telomere protection preventing DNA damage response and telomere maintenance, preserving telomere function (4). This member of the shelterin complex also interacts with ataxia telangiectasia mutated pathway, thus alterations in ACD may stimulate chromosomal instability and large accumulation of mutations leading to cancer development (5). In contrast to the other shelterin components, TERF2IP is not a protective protein, but prevents telomere recombination and fragility. TERF2IP does not bind DNA on its own, but is brought to the telomere by binding to TERF2 (6), and is critical for inhibiting non-homologous end-joining of mammalian telomeres (7).

Chronic lymphocytic leukemia (CLL) is one of the most common types of adult leukemia in Western world. It is also the lymphoid malignancy in which familial aggregation has been described more frequently, showing an eightfold increased risk in first-degree relatives (8). Studies using genome-wide association methodology have contributed to identify several CLL susceptibility loci, providing evidence for a polygenic basis for CLL predisposition (9,10). However, no data of germ-line alleles that might explain this risk had been found. In a recent report, Speedy *et al.* (11) demonstrated for the first time the existence of germ-line mutations associated with familial CLL. The authors identified seven families with loss-of-function mutations in shelterin genes: four of them in POT1, two in TERF2IP and one in ACD genes, supporting the importance of telomere-associated genes in the pathogenesis of this pathology. The

Table 1 Germ line shelterin genes mutations in familial human cancer

Diagnostic	Genes	Reference
Familial melanoma	<i>TRF2IP</i>	(12)
	<i>ACD</i>	(12)
	<i>POT1</i>	(13)
	<i>POT1</i>	(14)
Familial glioma	<i>POT1</i>	(15)
Cardiac angiosarcoma	<i>POT1</i>	(16)
Familial colorectal cancer	<i>POT1</i>	(17)
Familial bone marrow failure	<i>ACD</i>	(18)
Chronic lymphocytic leukemia	<i>POT1, TRF2IP, ACD</i>	(11)

POT1, protection of telomeres 1; *ACD*, adrenocortical dysplasia homolog; *TRF2IP*, telomeric repeat-binding factor 2-interacting protein.

type of mutation was variable: splice acceptor (one family), missense (two pedigrees) and frameshift (one family) for *POT1*, missense in both families that harbored *TRF2IP* mutations and splice acceptor in the only one family with *ACD* mutated gene. It is important to pointing out that germ line mutations in shelterin genes were previously observed in different familial solid tumors (12-17) and in inherited bone marrow failure (18) but, to our knowledge, they had never been found in hematological malignancies (Table 1).

Interestingly, in the report of Speedy *et al.* (11) no differences in TL between *POT1* mutated and unmutated carriers was found. It is different to those observed in cases of familial melanoma with germ line *POT1* mutations (13,14) that showed significantly longer and more fragile telomeres than controls, probably related with the disruption of the interaction between *POT1* and ssDNA. The analysis of an increase number of CLL patients with germ line shelterin gene mutations may clarify these discordant findings.

Nevertheless, somatic shelterin gene mutations were described in different lymphoid malignancies. Particularly, Ramsay *et al.* (19) found somatic *POT1* mutations in CLL patients associated to unmutated IGHV (immunoglobulin heavy chain variable region) status, as well as the presence of telomeric and chromosomal abnormalities in the leukemic

cells. In line with these findings, *POT1* mutations were also observed in cases with cutaneous T-cell lymphomas related to telomere fragility and genome instability (20), suggesting that alterations in this telomere-associated gene could lead to genomic instability and the acquisition of the malignant phenotype. In addition, a somatic *ACD* mutation was observed in a case of childhood pre-B acute lymphoblastic leukemia (21); *in vitro* studies support a role for this genomic abnormality in leukemia cells resistance to apoptosis.

A limited number of studies have also evaluated the expression profile of shelterin genes in CLL patients. Among them, Poncet *et al.* (22) found a global dysregulation of telomere-associated genes, with reduced expression of *TERF1*, *TERF2*, *TERF2IP* and *POT1*, increased mRNA levels of *ACD* and no differences for *TINF2* as compared to controls. On the contrary, Hoxha *et al.* (23) using microarray analysis observed *TERF1*, *POT1* and *TERF2IP* overexpression; these discordant results could be related to the number and clinical characteristics of patients. Furthermore, our group has studied the expression profile of shelterin genes in other lymphoid malignancies. Particularly in mantle cell lymphoma patients, we have observed significant increased expression in all members of the shelterin complex compared to controls (24). In addition, the analysis of cases with plasma cell disorders showed upregulation of shelterin genes in multiple myeloma (MM) patients with respect to cases with monoclonal gammopathy of undetermined significance, with statistical differences for *TERF2* and *POT1*. Particularly, *POT1* overexpression was associated with adverse prognostic factors, poor clinical outcome and short overall survival in MM patients, results confirmed by multivariate analysis (25). These data suggested for the first time the participation of *POT1* in the process of multistage tumorigenesis in plasma cell disorders and permitted to propose it as a molecular marker in these entities.

Concluding, these new findings in familial CLL patients reported by Speedy *et al.* (11) add more complexity to the molecular heterogeneity of this pathology, constituting a possible novel prognostic biomarker for cancer patients, and suggest the importance of germ line mutations in telomere-associated genes on cancer-predisposing conditions.

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