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Cancer chromosomes going to POT1

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Abstract

Alterations in the single-stranded telomere-binding protein POT1 have recently been identified in chronic lymphocytic leukemia. This discovery provides novel insights into how genomic instability induced by dysfunctional telomeres contributes to tumorigenesis.

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in Western countries, with an estimated incidence of 3.9 cases per 100,000 people in the United States and a median age of 72 years at the time of diagnosis¹. CLL is clinically heterogeneous, with variable presentations and patient outcomes. Whereas it is indolent in many patients, in others it is highly aggressive, resulting in rapid mortality. This clinical variability is likely due to the fact that CLL is genetically heterogeneous. Recent next-generation whole-genome and exome sequencing approaches have shown that CLLs possess strikingly complex genomic profiles, highlighted by multiple aberrations in chromosome structure and pathogenic mutations in at least seven distinct genetic pathways^{2–4}. On page 526 of this issue, Carlos López-Otín and colleagues implicate telomere end protection as yet another genetic pathway perturbed in CLL⁵. They found that somatic mutations in the *POT1* gene (encoding protection of telomere 1) are present in 3.5% of all CLL cases, occurring exclusively in the clinically aggressive CLL subtype with wild-type *IGHV*. When they ranked CLL mutations by corrected frequency scores, which take into account gene size, the number of mutations found in the gene and codon composition, *POT1* emerged as the second most frequently mutated gene. Clonal and subclonal analyses showed that *POT1* mutations arise early, suggesting that they likely represent driver mutations in CLL⁴. POT1 is the first component of the telomere shelterin complex found to be mutated in human cancer, and this discovery further supports the hypothesis that telomere dysfunction results in the genomic rearrangements that are permissive for cancer initiation and progression⁶.

POT1 mutations induce chromosomal aberrations

Telomeres are specialized DNA-protein structures that cap the ends of all eukaryotic chromosomes and have important roles in genome stability. They are maintained by the enzyme telomerase and the shelterin complex, composed of the duplex telomere-binding proteins TRF1 and TRF2 and the single-stranded telomere-binding protein POT1 (ref. 7).

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POT1 forms a heterodimer with TPP1, and TPP1 in turn tethers POT1 to TRF1 and TRF2-RAP1 through TIN2 (Fig. 1a). POT1 contains two highly conserved oligonucleotide/oligosaccharide-binding (OB) folds through which it binds to the 3' terminus of the single-stranded G overhang. Remarkably, 19 of the 25 POT1 alterations in CLL clustered within both OB folds, and most affected phylogenetically conserved residues. In accord with a previous report⁸, POT1 OB-fold mutants could not bind telomeric single-stranded DNA (ssDNA) *in vitro* but were able to localize to telomeres, likely through recruitment by TPP1. CLL samples harboring *POT1* mutations and the telomerase-positive HT1080 cell line expressing mutant POT1 all had chromosomal breaks and structural aberrations, indicative of loss of telomere capping function (Fig. 1b). Importantly, these lesions were not observed when wild-type POT1 was overexpressed or in cells heterozygous for the mouse ortholog *Pot1a*^{8,9}, suggesting that these chromosomal aberrations arose as a consequence of mutation acquisition. Because all *POT1* mutations in CLL are heterozygous, they likely have a dominant negative effect on the wild-type protein at telomeres. Analysis of the types of chromosomal aberrations shed light on the repair pathways disrupted by *POT1* mutations in CLL. For example, the fusion of sister telomeres suggests the failure of the mutant POT1 to protect newly replicated telomere ends¹⁰, and the presence of fragile telomeres suggests that POT1 might have an unrecognized role in telomere replication¹¹. These cytogenetic aberrations, coupled with chromosomal fusions generating dicentric chromosomes, are postulated to promote the genomic instability observed in CLL.

***POT1* mutations promote CLL survival**

Despite the cytogenetic abnormalities observed in CLL cells and HT1080 cells expressing POT1 mutants, telomeres did not show evidence of a DNA damage response (DDR), which is a prominent characteristic of *Pot1a*-null cells^{8,9}. This unexpected result is likely due to the loading of wild-type POT1 onto telomeric ssDNA, thus preventing RPA binding and the initiation of CHK1-dependent DDR, which would be deleterious for cell survival¹². In addition, POT1 mutants induced significant telomere elongation in HT1080 cells over many population doublings, with telomerase activity itself not upregulated. Insight into this unique mechanism of telomere length extension comes from observations that the POT1-TPP1 heterodimer functions as both a positive and negative regulator of telomerase activity at telomeres^{13,14}. POT1 sequesters the 3' single-strand overhang, preventing telomerase access to telomeric ssDNA. Translocation of POT1 to an internal site, coupled with TPP1-mediated recruitment of telomerase, promotes telomere extension. Because POT1 OB-fold mutants cannot bind to telomeric ssDNA to repress telomerase access, telomerase is able to greatly lengthen telomeres (Fig. 1b). As telomerase is limiting in CLL, *POT1* mutations likely confer a survival advantage to B cells with wild-type *IGHV@*, maintaining their replicative potential and ability to generate additional subclones. This mechanism may also explain why *POT1* mutations in CLL preferentially disrupt the OB fold. In contrast with these observations, critical telomere shortening resulting in end-to-end chromosome fusions has previously been documented in CLL, with very short telomere length being a negative predictor for disease survival¹⁵. Together, these results suggest the possibility of two distinct subtypes of CLL—one bearing *POT1* mutations and elongated telomeres and the other with critically shortened telomeres with wild-type POT1 function.

Clinical directions

The discovery of *POT1* mutations in CLL provides a compelling link between telomere dysfunction, genomic instability and human cancer, a connection previously documented in mouse models of telomere dysfunction and cancer⁶. Genomic instability due to *POT1* mutations could contribute to the stepwise accumulation of mutational changes in favor of tumor initiation and progression, initially in a genetically heterogeneous cell population, then by clonal selection of more aggressive traits, including the ability to evade the impact of chemotherapeutic agents⁴. CLL disease progression is currently predicted by monitoring the mutation status of *IGHV@*, *ZAP70* expression and the presence of recurrent cytogenetic aberrations¹. Given the prevalence of *POT1* mutations in the most aggressive form of this disease and the likelihood that these mutations confer survival advantages, monitoring a patient's *POT1* mutational status is warranted. This information will be a first step toward the development of future personalized therapy to target CLL harboring *POT1* mutations.

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a Wild-type B cell **b** *POT1*-mutant B cell without *IGHV@* mutation **Figure 1.**

POT1 mutations in CLL promote chromosomal aberrations and telomere extension. **(a)** In wild-type B cells, the shelterin complex, composed of TRF1, TRF2-RAP1, TIN2 and POT1-TPP1, protects telomeres and maintains chromosomal stability. The POT1-TPP1 complex functions to protect telomeres and to modulate telomerase access to telomeric 3' ssDNA. TPP1 recruits telomerase to telomeres, promoting telomere extension, and POT1 competes with telomerase for access to the 3' single-stranded telomeric overhang via its OB fold (pink box), preventing telomere extension. Red spheres on chromosomes represent telomeric DNA. **(b)** In B cells with wild-type *IGHV@*, mutations affecting the POT1 OB fold (denoted by the black dot in the pink box) abolish binding to telomeric 3' ssDNA. Telomerase recruitment to telomeres is unrestrained, resulting in progressive telomere lengthening ①. In the presence of mutant POT1, chromosomal aberrations, including sister telomere fusions ②, fragile telomeres ③, chromosomal breaks ④ and chromosomal fusions with telomere signals at fusion sites ⑤, are prominent. This dysfunctional telomere–

induced genomic instability, coupled with the acquisition of additional driver mutations, promotes progression to CLL.