

Alternative end joining, clonal evolution, and escape from a telomere-driven crisis

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Abbreviations: A-NHEJ, alternative non-homologous end-joining; BRCT, BRCA1 C-terminal; C-NHEJ, classical non-homologous end-joining; LIG3, Ligase III; LIG4, Ligase IV.

Telomere dysfunction and fusion play key roles in driving genomic instability and clonal evolution in many tumor types. We have recently described a role for DNA ligase III (LIG3) in facilitating the escape of cells from crisis induced by telomere dysfunction. Our data indicate that LIG3-mediated telomere fusion is important in facilitating clonal evolution.

In the absence of telomerase, telomere shortening limits the replicative lifespan of human cells thus providing a stringent tumor suppressive mechanism. However if DNA damage checkpoints are compromised, cells can continue to divide until the telomeres lose their end-capping function and are subjected to DNA double-strand break repair activity that results in telomere-telomere fusion events. The resulting dicentric chromosomes can initiate cycles of anaphase-bridging, breakage, and fusion that generate the large-scale genomic rearrangements common in human cancer.¹ Telomere-driven mutation is therefore a mechanism that generates variation in tumor cell populations, upon which clonal selection can operate and facilitate progression.

We have developed high-resolution, single-molecule technologies to study telomere length and fusion in detail; these methods have provided a level of clarity that was hitherto impossible to achieve in human cells.² In colorectal cancer we showed that telomere erosion, dysfunction, and fusion not only precede the adenoma/carcinoma transition, but may also be pre-existent in the normal cells in which the initial mutation occurs.³ In

chronic lymphocytic leukemia (CLL) we observed extreme telomere erosion and fusion, consistent with CLL B-cells undergoing a telomere-driven crisis.^{4,5} Importantly, this was also detected in a subset of patients with early-stage disease prior to clinical progression.⁴ Our recent large-scale analysis of telomere length and fusion in early-stage CLL has allowed definition of the telomere length threshold below which telomere fusion is detected and revealed the prognostic value of stratifying patients according to this parameter. Patients with telomeres below the telomere fusion threshold had a significantly shorter overall survival that was even more prognostic in early-stage disease patients ($P < 0.0001$, HR = 19.3), and telomere dysfunction was the dominant variable in a multivariate analysis.⁶ Based on these observations we hypothesize that short dysfunctional telomeres provide a mutator mechanism capable of driving genomic instability and disease progression. It is therefore important to understand the molecular basis of telomere fusion and how this drives mutation and clonal evolution.

We have also investigated the process of fusion between short dysfunctional

telomeres in human cells by the direct isolation and characterization of the DNA sequence of telomere fusion events.⁴ Irrespective of the tissue or cell model analyzed, we observed a consistent mutational profile with deletion into the subtelomeric DNA and microhomology at the fusion junction;⁷ this mutational profile is consistent with alternative non-homologous end-joining (A-NHEJ) processes. A-NHEJ involves the coordinated interaction of multiple proteins with nucleating, scaffolding, and resection activity, as well as those ultimately executing DNA ligation. We therefore examined the contribution of DNA ligase III (LIG3)-dependent A-NHEJ and DNA ligase IV (LIG4)-dependent classical-NHEJ (C-NHEJ) pathways in mediating fusion between short dysfunctional telomeres.⁸ Using a dominant negative telomerase (DN-hTERT) we induced telomere erosion, fusion, and the onset of a telomere crisis in HCT116 cells in which the LIG3 or LIG4 genes had been inactivated using recombinant adeno-associated virus-mediated gene targeting.^{9,10} Both wild-type and LIG4^{-/-} clones displayed large-scale genomic rearrangements and telomere fusions, but readily escaped crisis

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following the re-establishment of telomerase activity. Fusions were also detected in $LIG3^{-/-}$ cells but strikingly no clones escaped crisis; after 2 to 3 months no cells remained in these cultures. All $LIG3^{-/-}$ clones escaped crisis following complementation with a wild-type $LIG3$ cDNA, but none escaped following complementation with cDNAs containing either a deletion in the $LIG3$ BRCA1 C-terminal domain or a A874D point mutation, both of which are required for the interaction of $LIG3$ with X-ray repair cross-complementing protein 1 (XRCC1). These data demonstrate an absolute requirement for $LIG3$ in mediating the escape from a telomere-driven crisis.⁸

In order to gain some insight into the underlying mechanisms by which $LIG3$ facilitated the escape from crisis, we undertook a detailed molecular characterization of telomere fusion events mediated by $LIG3$ or $LIG4$. Sister chromatid fusion events were detected in both $LIG3^{-/-}$ and $LIG4^{-/-}$ cells, however there was a marked reduction in interchromosomal events in $LIG4^{-/-}$ cells. Sequencing of interchromosomal fusions from $LIG3^{-/-}$ cells revealed a higher incidence of breakpoints within telomere repeats and a reduction in microhomology at the fusion junction. Our data demonstrated the involvement of both $LIG3$ and $LIG4$ in the fusion of short dysfunctional telomeres, but also indicated that fusions involving $LIG3$ provide a selective advantage to cells undergoing a telomere-driven crisis that facilitates clonal evolution and escape from crisis.

The mechanism by which $LIG3$ facilitates the escape from crisis is not clear; our hypothesis is that interchromosomal fusions, which predominate in cells that cannot escape crisis, are more mutagenic and detrimental to the cells in which they occur; whereas fusion between sister chromatids—resulting in

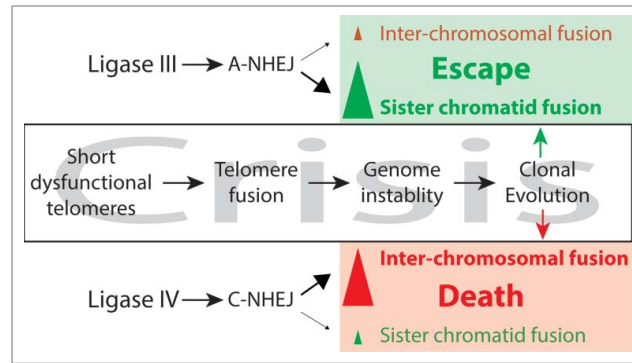


Figure 1. Schematic illustrating our hypothesis for how $LIG3$ may affect the ability of cells to escape a telomere-driven crisis. Cells with short dysfunctional telomeres undergo crisis, during which telomere fusion results in the creation of dicentric chromosomes and the initiation of anaphase-bridging, breakage, and fusion cycles; the ensuing genomic instability leads to large-scale genomic rearrangements. Clonal evolution results in cells that escape crisis; these cells exhibit fewer interchromosomal fusion events compared to fusions between sister chromatids, whereas interchromosomal fusions predominate in cells that cannot escape crisis. We consider that this is governed by the relative activities of Ligase III-dependent alternative non-homologous end-joining (A-NHEJ) compared to Ligase IV-dependent classic non-homologous end-joining (C-NHEJ) pathways.

localized amplification and deletion events—may confer a selective advantage. We consider that the relative balance between these events dictates the ability of cells to escape crisis and that this is modulated by the activities of A-NHEJ and C-NHEJ at short dysfunctional telomeres (Fig. 1). Further work should elucidate the contributions of other components of the A-NHEJ pathway in telomere fusion and the escape from crisis, in addition to examining the mutational impact that these pathways have in the context of the evolving cancer genome.

Telomere fusion during crisis is ultimately a cellular survival mechanism that provides short-term relief from telomere erosion, but importantly also facilitates genome instability that allows for the generation of the genomic rearrangements that can facilitate progression. Our work identifies the A-NHEJ pathway as essential for the ability of cells to clonally evolve and escape a telomere crisis. This indicates that intervention(s) in this pathway may sensitize cells with short dysfunctional

telomeres and thus provide a potential therapeutic target in the subsets of tumors in which short dysfunctional telomeres confer such a poor prognosis. Moreover, given that telomere dysfunction occurs early in tumorigenesis this may open up the possibility of therapeutic intervention prior to clinical progression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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