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Telomere Uncapping, Chromosomes, and Carcinomas

Luis F.Z. Batista[1] and Steven E. Artandi[1,*]
[1]Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA
*Correspondence: sartandi@stanford.edu
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Data from mouse models and from human cancers have supported the idea that telomere shortening leads to chromosomal instability and epithelial carcinogenesis. In this issue of *Cancer Cell*, Else et al. demonstrate that telomere uncapping—altering a protein that protects chromosome ends without shortening telomeres—also results in epithelial cancers.

Tumorigenesis is a multistep process that requires the accumulation of multiple genetic changes. In human carcinomas—epithelial cancers—these steps are facilitated by genomic alterations, including aneuploidy, translocations, and chromosomal amplifications and deletions. Although the mechanisms generating these aberrant cancer genomes remain incompletely understood, increasing evidence suggests that carcinoma development can be driven by shortening of telomeres, the nucleoprotein caps that protect chromosome ends.

Telomerase—the enzyme complex that elongates telomeres—is upregulated in the majority of human tumors. However, telomeres are often significantly shorter in cancer cells, compared to neighboring normal cells within a tissue, indicating that telomeres shorten during the tumor development process. When telomere end protection is compromised, a local DNA damage response occurs at the chromosome end. This DNA damage response explains replicative senescence, the growth arrest that occurs in human fibroblasts, which exhibit progressive telomere shortening as these cells divide for many population doublings in the absence of telomerase (Palm and de Lange, 2008). Efforts to model telomere shortening in knockout mice deficient in the telomerase RNA component (TERC) showed that critical telomere shortening led to defects in renewing tissues, due in part to robust activation of the p53 tumor suppressor gene in tissue progenitor cells (Chin et al., 1999). In a setting of an impaired p53 response, telomere shortening in $TERC^{-/-}$ $p53^{+/-}$ mice led to a shift in the tumor spectrum from lymphomas and sarcomas, cancers typically seen in $p53^{+/-}$ mice, to one dominated by carcinomas (Artandi et al., 2000). The chromosomes in these carcinomas were highly rearranged and characterized by nonreciprocal translocations and focal amplifications and deletions (Maser et al., 2007; O'Hagan et al., 2002). These chromosomal aberrations strongly resembled those in human carcinomas, suggesting that telomere shortening may account for many of the widespread genomic changes see in human cancer. Indeed, analyses of human breast cancer by comparative genome hybridization (CGH) revealed that gene copy number aberrations increased substantially at the ductal carcinoma in situ stage and that telomeres shortened during stepwise progression of human breast cancer (Chin et al., 2004). Together with the mouse model data, these findings suggested that dysfunctional telomeres may explain many chromosomal alterations in human carcinomas.

In addition to the gradual loss of telomere sequences that occurs in the absence of telomerase, telomeres can become dysfunctional, or uncapped, through a different mechanism—alterations in telomere-binding proteins. Telomeres are tracts of TTAGGG repeats and terminate in a single-stranded overhang, which folds back into the double-stranded telomere to form a lariat or t-loop structure. Telomeres are protected by the t-loop conformation and by shelterin, a large multisubunit protein complex, which together prevents the chromosome end from being recognized as a DNA break and inhibits inappropriate recombination. For example, loss of TRF2, one of the proteins that bind the double-stranded telomere sequence, causes rapid uncapping and ligation of chromosome ends. The single-stranded overhang is bound by POT1, which in turn is connected to the shelterin complex via interaction with TPP1. Inhibition of either POT1 or TPP1 leads to a potent DNA damage response at telomeres caused by disruption of this complex at the telomere overhang (Guo et al., 2007; Hockemeyer et al., 2007).

Now, in this issue of *Cancer Cell*, Else et al. demonstrate that a mutation in a telomere-binding protein leads to skin carcinoma in mice by inducing telomere dysfunction directly, without telomere shortening (Else et al., 2009). The authors study a mouse strain that harbors a hypomorphic mutation in *TPP1*. In the homozygous state, this mutant—*adrenocortical dysplasia* (*acd*)—exhibits a defect in telomere capping that leads to adrenal gland dysplasia, male germ cell loss, and skin hyperpigmentation. In fact, the telomere defect in $TPP1^{acd/acd}$ mice is sufficiently severe that homozygous mice typically die in utero, and few mice make it to adulthood. These defects are even more severe than those seen in $TERC^{-/-}$ mice, presumably because the prevalence of cells with dysfunctional telomeres is even greater in the *acd* model than in $TERC^{-/-}$ mice. Since p53 is critical in mediating the DNA damage response to uncapped telomeres (Karlseder et al., 1999), as well as to critically short telomeres, Else et al. asked if loss of p53 would rescue the phenotypes of the *acd* mouse. They find a dramatic effect of deleting *p53*, which restores viability, enabling $TPP1^{acd/acd}$ mice to survive in near Mendelian ratios. Furthermore, hyperpigmentation and hair loss are normalized, as is the dysplasia of the adrenal gland in $TPP1^{acd/acd}$ $p53^{-/-}$ mice.

However, the phenotypic rescue by p53 loss comes at a cost. Dysfunctional

telomeres in *acd* mice with an impaired p53 response now render these mice highly cancer prone, even more predisposed to cancer than p53 mutant mice with intact telomere function. *TPP1*$^{acd/acd}$ *p53*$^{-/-}$ mice and *TPP1*$^{acd/acd}$ *p53*$^{+/-}$ mice developed cancers much more rapidly than their *TPP1*$^{+/+}$ *p53*$^{-/-}$ and *TPP1*$^{+/+}$ *p53*$^{+/-}$ counterparts. Strikingly, whereas the *TPP1*$^{+/+}$ *p53*$^{+/-}$ mice developed sarcomas and lymphomas, *TPP1*$^{acd/acd}$ *p53*$^{+/-}$ mice exhibited a shift in the tumor spectrum toward one dominated by carcinomas, including skin carcinoma and adrenocortical carcinoma. This shift toward carcinomas is reminiscent of *TERC*$^{-/-}$ *p53*$^{+/-}$ mice but also in a broad sense resembles the predisposition toward carcinomas seen in aged humans.

Genomic analyses of the carcinomas in *TPP1*$^{acd/acd}$ *p53*$^{+/-}$ mice revealed the presence of focal amplifications and deletions by CGH. While these types of copy number aberrations are common in human cancers, they are rare in mouse tumors. Cytogenetic analysis by spectral karyotyping (SKY) showed that carcinomas from *TPP1*$^{acd/acd}$ *p53*$^{+/-}$ mice harbor complex chromosomal translocations. The genomic instability introduced into these tumors by dysfunctional telomeres likely leads to changes in copy number of critical cancer genes, which provides a selective advantage for tumor initiation and progression. Conventional mouse tumors lack this type of instability and therefore have fairly normal genomic profiles. From this cancer genomics perspective, mouse cancer models that incorporate dysfunctional telomeres more closely resemble human cancers.

The main findings in *TPP1*$^{acd/acd}$ *p53*$^{+/-}$ mice are strikingly similar to those in *TERC*$^{-/-}$ *p53*$^{+/-}$ mice deficient in telomerase. In both models, tumorigenesis was accelerated by dysfunctional telomeres, carcinomas emerged to dominate the tumor spectrum, and these carcinomas exhibited highly aberrant genomes. These common features provide strong evidence to support the hypothesis that dysfunctional telomeres lead to chromosomal instability and carcinoma development. Furthermore, the data provided by Else et al. indicate that dysfunctional telomeres arising through two independent means can similarly promote tumorigenesis (Figure 1). In settings of insufficient telomerase, telomeres shorten gradually, ultimately causing loss of telomere protection at a small subset of telomeres. How the telomere cap is altered at a critically short telomere is still not well understood, but possibilities include reduced binding of shelterin proteins and a conformational change, for example, in the t-loop structure. Alternatively, changes in shelterin proteins themselves can lead to telomere dysfunction in cancer. The hypomorphic mutation in *TPP1*$^{acd/acd}$ mice is one such example, which leads to a marked reduction in TPP1 levels and to decreased loading of POT1 onto telomeres (Guo et al., 2007; Hockemeyer et al., 2007).

In either case—telomere dysfunction induced by critical shortening or telomere dysfunction caused by altered protein binding—a localized DNA damage response at the uncapped telomere can result in ligation of chromosome ends. These fused chromosomes have two centromeres and can therefore break in subsequent mitoses, resulting in cycles of fusion bridge breakage that lead to dramatic reorganization of the cancer genome. Amplification of specific oncogenes and loss of tumor suppressor genes during these cycles of chromosomal instability provide a strong selective advantage for tumor outgrowth within epithelial tissues. These new data showing that telomere uncapping without shortening can drive tumor development provide an important new link to chromosomal instability and suggest experiments to determine if this specific mechanism contributes to human carcinogenesis.

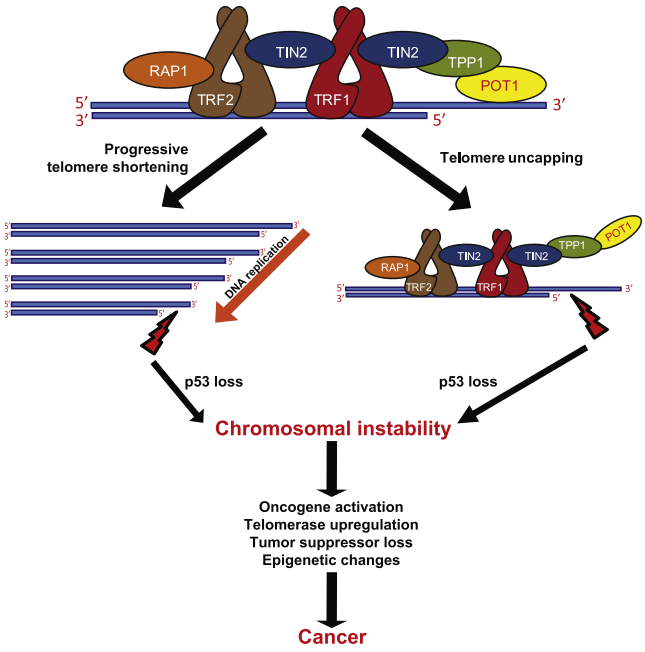

Figure 1. Routes from Dysfunctional Telomeres to Carcinomas
Telomeres protect chromosome ends from being recognized as double-strand breaks and prevent inappropriate recombination. These functions require binding of shelterin, a complex of six proteins that protect the double-stranded telomere and the single-stranded overhang. Dysfunctional telomeres may arise by two independent mechanisms. In settings of insufficient telomerase, telomeres shorten progressively, compromising end protection at a subset of telomeres. Alternatively, interference with telomere-binding proteins can lead to immediate uncapping of telomeres without telomere shortening. For example, low TPP1 levels in the *acd* mouse results in decreased binding of POT1 to the single-stranded overhang. In either case, the chromosome end is recognized as DNA damage, leading to chromosomal ligation, cycles of fusion bridge breakage, and gene amplification and deletion. These stochastic changes in gene copy number can drive carcinoma formation in the absence of p53 function.

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Mammary Tumorigenesis through LPA Receptor Signaling

Jos Jonkers[1] and Wouter H. Moolenaar[2,*]
[1]Division of Molecular Biology
[2]Division of Cell Biology
The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands
*Correspondence: w.moolenaar@nki.nl
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Lysophosphatidic acid (LPA) is a lipid growth factor that is produced by an extracellular phospholipase, termed autotaxin (ATX), and acts via G protein-coupled receptors. In this issue of *Cancer Cell*, Liu et al. show that transgenic overexpression of ATX or LPA receptors leads to invasive and metastatic mammary cancer.

Lysophosphatidic acid (LPA; mono-acyl-*sn*-glycero-3-phosphate) (Figure 1) is a bioactive phospholipid that stimulates the proliferation, migration, and survival of many cell types. Its "lyso" prefix notwithstanding, LPA has no membrane-perturbing effects but acts as a high-affinity ligand for specific G protein-coupled receptors. To date, there are six confirmed LPA receptors (termed LPA_{1-6}), which show a broad tissue distribution and have overlapping signaling properties (van Meeteren and Moolenaar, 2007). LPA signaling has been implicated in a great variety of biological processes, ranging from vascular development and neurite remodeling to inflammation and tumor progression. This multitude of activities may seem confusing but is consistent with the ubiquitous expression of LPA receptors and their coupling to a great diversity of G protein-mediated signaling pathways, including those initiated by Ras and Rho GTPases (van Meeteren and Moolenaar, 2007). The three classic LPA receptors, termed LPA_{1-3}, belong to the so-called EDG family of G protein-coupled receptors. The more recently identified LPA receptors, LPA_{4-6}, are related to the purinergic P2Y receptor family but are far distant from the EDG receptors, implying that LPA receptors have evolved from distinct ancestor genes.

LPA is produced extracellularly from more complex lysophospholipids, particularly lysophosphatidylcholine (LPC, the most abundant phospholipid in plasma), by a secreted (lyso)phospholipase D named autotaxin (ATX; also known as NPP2, nucleotide pyrophosphatase/phosphodiesterase 2), as illustrated in Figure 1 (van Meeteren and Moolenaar, 2007). ATX was originally identified as an "autocrine motility factor" for human melanoma cells, but its mode of action has remained elusive for a decade until it was discovered that ATX is identical to plasma lysophospholipase D, converting LPC to LPA. ATX is widely expressed, with highest mRNA levels detected in brain, lymph nodes, kidney, and testis; it is found overexpressed in various cancers. Gene targeting studies in mice indicate that ATX (encoded by *Enpp2*) is essential for vascular development, an unexpected finding given that none of the previous LPA receptor knockouts has hinted at a role for LPA in vasculogenesis (van Meeteren and Moolenaar, 2007).

Given its growth factor-like activities, it is not surprising that LPA has long been implicated in cancer. The oncogenic potential of the ATX-LPA receptor axis has become evident from studies in nude mice. In xenografted NIH 3T3 cells, overexpressed ATX cooperates with activated Ras to promote tumor aggressiveness and metastasis (Nam et al., 2000). Overexpression of LPA_1 in MDA-MB-231 breast carcinoma cells enhances tumor growth and promotes metastasis to bone (Boucharaba et al., 2004), whereas overexpression of LPA_1, LPA_2, and LPA_4 (but not LPA_3) in embryonic fibroblasts induces cell transformation and tumor formation in conjunction with MYC and TBX2 (Taghavi et al., 2008). Those studies also indicate that distinct oncogenic events must collaborate with ATX-LPA signaling to induce tumor formation.

Of the EDG family LPA receptors, LPA_2 provides the strongest case for a causal link to cancer. Not only is LPA_2 found overexpressed in various common cancers, including ovarian, colon, gastric, and invasive ductal breast carcinoma (Kitayama et al., 2004), but a recent study by Lin et al. (2009) demonstrates that