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COMMENTARY

Telomerase Reverse Transcriptase and Wnt Signaling[▽]

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More than 25 years ago, Carol Greider, then a graduate student in Elizabeth Blackburn's laboratory, detected activity of an unusual polymerase capable of synthesizing telomeric repeats *in vitro* (4). This enzyme, called telomerase, is a reverse transcriptase that uses an intrinsic RNA template to extend the 3′ ends of chromosomes. In the absence of the catalytic reverse transcriptase (TERT) and/or the telomerase RNA component (TR), telomeres gradually shorten through the inability of the DNA replication machinery to completely duplicate chromosome ends. While it was initially thought that telomerase is specialized for telomere addition, the enzyme has been reported to play roles in mitochondrial function, growth signaling, apoptosis, and DNA damage response under conditions in which changes in steady-state telomere length are not observed (reviewed in reference 5). However, because catalytic activity is required for many of these functions, it has been difficult to rule out a role at telomeres. As a result, there was great interest when J. Choi and colleagues reported that TERT (in a catalytically dead form or in the absence of the telomerase RNA) stimulates Wnt pathway activation (3). This observation suggested that phenotypes previously ascribed to defects in telomere maintenance might have other origins, a possibility with important implications for human diseases associated with telomerase deficiency.

In this issue, Strong and colleagues take a close look at the consequences of TERT deficiency in mice (10a). The authors reason that any functionally relevant roles of murine TERT (mTERT) that are independent of its action with the telomerase RNA at telomeres will cause phenotypes different from those previously observed upon loss of murine TR (mTR). Despite extensive analysis of telomere maintenance, viability, and physiology, Strong and colleagues do not find any significant differences between mice lacking mTERT and those lacking mTR. The authors conclude that phenotypes arising in the mouse model can be explained on the sole basis of telomere shortening. What do these results mean for a potential role of TERT in the Wnt pathway? This commentary examines the evidence of a physiological role for TERT in Wnt signaling and considers the implications of these results for human disease in the context of telomerase dysfunction.

Evidence for a role of TERT in Wnt signaling. In 2005, Sarin et al. reported that forced overexpression of mTERT in mouse skin triggered hair follicles to enter or remain in the anagen, or active phase (9). The remarkable result was furry mice in which the normal regulation of hair growth was disrupted. Even more remarkable was the observation that this effect of mTERT overexpression occurred in mice lacking mTR (9) and was supported by a catalytically inactive protein ($mTERT^{ci}$) (3), ruling out the possibility that mTERT's role in telomere replication was responsible. To identify genomic targets that might explain this effect, gene expression changes following rapid downregulation of $mTERT^{ci}$ in skin were monitored. Affected genes strongly correlated with those regulated by the Myc and Wnt pathways (3), and Park et al. went on to show that an endogenously tagged version of mTERT expressed in mouse embryonic stem (ES) cells associated with BRG1, an ATP-dependent chromatin-remodeling factor implicated in the Wnt pathway (6). Consistent with this interaction, a T-cell factor (TCF)-binding site reporter construct (TOPFLASH) was upregulated by overexpression of either mTERT or $mTERT^{ci}$ in a BRG1-dependent, but mTR-independent, manner (6).

While provoking, these results carried the caveat that overexpression might create gain-of-function phenotypes. Several results build a strong case that TERT has an endogenous function in Wnt signaling. First, the authors detected specific binding of an epitope-tagged version of mTERT at Wnt-regulated promoters under conditions in which the protein was not overexpressed (6). Second, to demonstrate a role for TERT in Wnt pathway activation, Park et al. examined the consequences of TERT loss in three different contexts. Conditional *mTERT* knockout ES cells were created and shown to exhibit muted basal and induced expression of the Wnt target gene encoding Axin2 upon *mTERT* excision (6), demonstrating that acute loss of mTERT function impairs Wnt signaling. Because Wnt signaling is important during *Xenopus laevis* development, the impact of *Xenopus TERT* (*xTERT*) knockdown was examined. The injection of two different morpholinos directed against *xTERT* into frog embryos caused striking defects in anterior-posterior axis formation. These defects were rescued by coinjection with morpholino-resistant *xTERT* or $xTERT^{ci}$ mRNAs, strongly supporting the conclusion that effects are specific and due to a noncatalytic role of TERT (6). These results caused the authors to reexamine the phenotype of mTERT-deficient mice. First-generation mTERT-deficient mice (which still have long and functional telomeres) are superficially normal, but detailed examination revealed a partially penetrant (~50% of animals) homeotic transformation of the vertebrae, observed as loss of the 13th rib on one or both sides, suggesting a role for TERT in Wnt signaling during development (6).

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Evidence that mTERT-specific functions do not contribute to phenotype. If the loss of TERT function contributes to stem cell or developmental defects in a manner independent of its role in telomere maintenance, then mice lacking mTERT are expected to display phenotypes different from those generated by loss of mTR. To address this issue, Vidal-Cardenas and Greider previously undertook an analysis of gene expression patterns in the livers of first-generation mTERT- or mTR-deficient mice within the C57BL/6 background (11). Overall gene expression profiles were extremely similar to those observed in the wild type (WT), with no genes showing greater than 2-fold change. Importantly, no significant changes were observed in levels of RNA for genes specifically implicated in the DNA damage response, apoptosis, cell cycle, and cell growth pathways (Wnt, epidermal growth factor [EGF], and mitogen-activated protein kinase [MAPK] genes). To examine the possibility that the modulation of compensatory pathways might mask the effect of mTERT deficiency, mouse embryonic fibroblasts (MEFs) derived from the WT and homozygous mutant progeny (embryonic days 12.5 [E12.5] to 14.5) of heterozygous parents were analyzed for changes in gene expression (11). The absence of significant differences suggests that any compensatory mechanisms involve either subtle changes in gene expression or posttranscriptional mechanisms.

In this issue, Strong and colleagues take the analysis further by introducing the mTERT deficiency into a short-telomere CAST/EiJ background (10a). Telomere shortening in both heterozygous and homozygous progeny was indistinguishable from that observed upon *mTR* deletion. Importantly, mice lacking mTERT were generated at the expected Mendelian ratio, ruling out the possibility that developmental defects are masked by intrauterine lethality. To examine the effect of mTERT loss on Wnt signaling, MEFs were generated from homozygous WT or mutant embryos resulting from the cross of two heterozygous parents. The extents to which the Wnt3a ligand activated a TOP-FLASH reporter in WT and mTERT-deficient MEFs were indistinguishable. The authors also did not observe any evidence of homeotic transformations of the vertebrae (10a), in contrast to the report from Park et al. (6). Strong and colleagues conclude that the lack of any recognizable differences between mice lacking mTERT and mice lacking mTR implies that independent roles of TERT are not manifested in the context of inherited gene deletion and that phenotypes observed result from gradual telomere attrition in the absence of telomerase activity.

Are these observations truly at odds? In the case of the homeotic transformations in mTERT-deficient mice, the observations by Park et al. (6) are incompatible with those by Strong et al. However, differences in strain background or in the laboratory environment (8) may give rise to this discrepancy and could be addressed by independent analyses of phenotypes or by sharing of strains between the two groups. The conclusion that mTERT contributes to development would be strengthened by showing that mTR-deficient mice lack the phenotype under similar strain and environmental conditions. Furthermore, the combination of mTERT deficiency with mutations that partially compromise Wnt signaling might reveal synthetic phenotypes.

Beyond the skeletal phenotype, comparison is difficult because the two groups have not done the same experiments. Strong and colleagues detect no evidence of Wnt dysfunction in MEFs generated from embryos lacking mTERT (10a). Although derived from heterozygous parents, these embryos have undergone the early stages of development in the absence of TERT function. In contrast, Park et al. examine the effect of exogenously overexpressing mTERT in $TERT^{-/-}$ MEFs but do not test basal or induced Wnt signaling in that context. Instead, muted Wnt signaling is demonstrated in ES cells 4 days after acute TERT loss through recombination (6). The different conclusions reached suggest that cellular context influences the effect of mTERT on Wnt signaling and/or that compensatory mechanisms occur early in development and are maintained during generation of MEFs.

Should the role of TERT on Wnt signaling be dismissed because obvious phenotypes are not manifested in $mTERT^{-/-}$ mice under all conditions? I would argue not. *xTERT* morpholinos cause clear developmental defects in *Xenopus* embryos (6). This compelling result, not discussed by Strong et al., strongly argues for a physiologically relevant role of TERT in Wnt signaling, at least in some organisms. Why then are phenotypes of Wnt dysfunction not more evident in mice lacking TERT? As mentioned above, genetic or epigenetic factors may compensate for the loss of mTERT during early development in mice. Epigenetic changes are the more likely explanation because compensation must manifest early in development with high penetrance. Strong and colleagues make a significant contribution by showing convincingly that telomere shortening is the cause of disease symptoms in *mTERT*-deficient mice, but their work does not rule out the possibility that TERT has modulatory roles in the wild-type animal or that the impact of TERT on Wnt signaling may become relevant when TERT expression is upregulated (a possibility mentioned by Strong et al. [10a]).

What are the implications of these findings for human disease? Telomere shortening was recognized as a cause of human disease through studies of dyskeratosis congenita (DC), a genetically heterogeneous disorder characterized by mucocutaneous features, including oral leukoplakia, skin hyperpigmentation, and nail dystrophy (reviewed in reference 1). Mutations in human TR (hTR) and human TERT (hTERT) cause autosomal dominant DC through haploinsufficiency (1, 2, 12). A high incidence of aplastic anemia (AA) and pulmonary fibrosis among DC patients led to the realization that mutations in hTERT and hTR underlie a fraction of cases of familial and sporadic AA and idiopathic pulmonary fibrosis (IPF) (reviewed in reference 1). While the phenotypic variability observed in these diseases raises the possibility that nontelomere functions of TERT or TR might influence the outcome of disease, several observations argue against this possibility. First, both IPF and AA can occur within the same individual or family, suggesting that variability in presentation is likely the result of differing environmental or genetic factors (7). Second, mutations in TINF2, a component of the telomere-binding shelterin complex, cause severe forms of dominant DC accompanied by dramatic telomere shortening (10). The fact that both hTERT and hTR are still present in patients with TINF2 mutations argues that DC phenotypes can manifest even when hTERT function is retained. These observations are consistent with the results of Strong et al. in mice (10a). However, time will tell whether the role of TERT in Wnt signaling has con-

sequences in mammals for phenotypes that have not been (or cannot easily be) tested in the laboratory.

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