Editorial

Telomeric zinc-finger associated protein (TZAP): a new player in telomere diseases?

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In humans, telomere length homeostasis regulates the balance between cellular immortalization and senescence. In particular, aberrant activation of telomere lengthening allows evasion of cellular senescence leading to uncontrolled replication, both considered major hallmarks of cancer (1). Conversely the regenerative potential of an organ depends on the replicative capacity of its stem cell compartment that is limited by telomere shortening. Indeed, excessive telomere shortening is a condition typical of chronic diseases involving lung and liver (such as fibrosis), blood (aplastic anemia) and skin (2). Due to its fundamental role in maintaining the physiological life-cycle of a cell, telomere homeostasis is tightly regulated by a wide intertwinement of molecular mechanisms. The main process controlling telomere length in somatic cells is "telomere attrition", that induces the progressive shortening of telomeres at each mitosis, thereby acting as a "mitotic clock" to sense cell aging. When telomeres become critically short (so-called "Hayflick limit"), a DNA-damage program is activated to protect chromosomes' integrity during cell division, leading to cell senescence or apoptosis. On the contrary, the constitutive expression of telomerase (a ribonuclear enzymatic complex responsible for telomere elongation) allows immortal cells, such as stem or cancer cells, to maintain long telomeres (3). The shelterin proteins complex also participates to this mechanism contributing to telomere protection by binding specifically to telomeres. The main actors of this complex are the telomeric repeat-binding factors 1 and 2 (TRF1-TRF2), that bind double strand DNA conferring telomere stability (2). Furthermore, other mechanisms, such as alternative lengthening of telomeres (ALT) based on homologous recombination (1), are known to participate to telomere elongation independently from telomerase.

Telomeres play an essential role in the maintenance of genomic stability. Their important function is strictly connected to their structure. They are composed by thousands copies of a short DNA strand repeated in tandem (TTAGGG) located at the end of chromosomes and terminating with a single stranded G-rich overhang, which forms a structure named telomere loop (T-loop) (3-5). The T-loop, a stabilized homologous recombination intermediate (6), protects chromosomes tips from end-to-end fusion, abnormal rearrangement and translocation (7,8).

On the resolution of the T-loop structure it is based the telomere trimming, the most recently described process involved in telomere regulation (9), that reckons on the release of extrachromosomal circular telomeric molecules (t-circles) by Homologous Recombination (9-11). This mechanism limits telomere elongation beyond a threshold length, most likely sensed by shelterin proteins, but in contrast to telomere attrition is compatible with

cell growth (1,9) and it does not induce nor DNA damage response neither loss of telomere capping function (8). This phenomenon is exacerbated in the condition of increased telomerase activity and/or extended telomere lengthening. Consistently, t-circles generation is probably necessary to counteract the elevated levels of HR that mediates telomere elongation in telomerase negative ALT cells (6,11,12) as well as telomere lengthening induced by telomerase (9-11). The relevance of telomere trimming is reflected in its conservation across many species, including yeast (13), plants (14) and mammals (10), suggesting that this mechanism is crucial in telomere biology, however the proteins that functionally mediate this process were still not characterized.

In this context, the work by Li *et al.* (15) adds new information to the molecular mechanisms controlling telomere trimming. Authors described telomeric zinc-finger associated protein (TZAP) (previously known as kruppel-like zinc finger protein, ZBTB48) as a new specific telomere binding protein responsible for the initiation of trimming process. Using a ChiP-seq approach, they showed that TZAP binds exclusively to telomeres, and proved that the interaction of this DNA-binding protein with the TTAGGG telomere repeats is direct. Indeed, by a domain swap approach by fusing the catalytic domain of TRF2 with the zinc-finger DNA-binding domain of TZAP, they demonstrated that the resulting chimeric protein could correctly localized to telomere, maintaining the correct TRF2 binding and consequently avoiding telomere end-to-end fusion. Next, by an array of truncation mutants, the three terminal zinc finger domains (9-11) were identified as the portion of TZAP required and necessary for telomere binding.

These observations are particularly intriguing since up to this work the only proteins known to specifically associate with the TTAGGG telomere repeats were the shelterin proteins. Thus, TZAP may compete with TRFs for the binding to telomere ends and therefore interfering with their function. However, while TRF2 overexpression could displace TZAP binding from telomere ends, TZAP overexpression did not result in a significant inhibition of TRF2 binding. This suggest that the binding of TZAP to telomeres was disadvantaged by high concentration of shelterin proteins. It is known that shelterin amount is not affected by telomere length and consequently that cells with long telomere have low-density of TRFs proteins on their telomere (16). Consistently, in HeLa cell lines that differed in telomere length, TZAP binding was largely favored at long telomeres with low concentration of shelterins.

By relying on their observations, the Authors investigated the possibility that TZAP, beside binding telomeres, may take part to the regulation of their homeostasis. Indeed, TZAP overexpression in telomerase-negative cells was correlated with progressive reduction of telomere length and accumulation of extrachromosomal DNA (t-circles), suggesting that TZAP plays a pivotal role in telomere trimming. Finally, silencing of TZAP by CRISPR-CAS9 in mouse embryonic stem cells (mES), which are known to regulate telomere length by trimming (10), led to telomere re-elongation and a decreased amount of extrachromosomal DNA. Overall, these data provide a model in which TZAP prevents excessive telomere elongation, binding to long telomeres and inducing the activation of trimming process.

Consistently with these data, TZAP has recently been described in a study examining several vertebrate species, in which a phylo-interactomics map of telomeres has been outlined in order to find out new highly conserved telomere regulating proteins. This approach combines interaction proteomics with phylogenomics that investigates evolutionary relationship based on high-throughput DNA sequencing data. The zinc-finger family of protein including TZAP has been identified at the TTAGGG telomeric sequence in at least five species (17). However, this last study suggests that even the other zinc finger proteins found enriched at telomere, such as ZBTB7A and ZBTB10, may be equally involved in telomere regulation by trimming, and therefore they need to be further investigated for a complete comprehension of this mechanism. In parallel, also Jahn *et al.* described TZAP relationship with telomere trimming tightening to zinc finger domain 11 the sufficient region for telomere binding. Moreover, Authors showed that TZAP exerted its function even in presence of short telomeres, suggesting a more complex and intriguingly regulation for trimming. Furthermore, TZAP also acted as transcription factor on the promoter of selected genes, among which mitochondrial proteins, while Li *et al.* supported the hypothesis that TZAP was specifically enriched at telomere (18). Thus, even if the two papers agree and enforce each other on the role of TZAP in a regulated and physiologic deletion of telomere extension, more work needs to be done in order to clarify which are the stimuli that activate this protein and how it exerts its function.

The study by Li *et al.* highlighted a key role of TZAP in telomere homeostasis, paving the way for the study of a new protein involved in telomere regulation that may represent a new therapeutic target for cancer, and degenerative and

age-dependent diseases. For example, the role of telomere regulation in cancer has been studied in deep in the years and it is currently of large interest thanks to next generation sequencing approaches that are extending the spectrum of proteins involved in telomere regulation and related disease phenotypes. Since telomere length is a strong heritable tract, mutations in the genes responsible for their control may be investigated in order to identify predisposing causes to the development of telomere diseases. In particular, the identification of genetic factors may be important for the risk stratification of patients in term of disease susceptibility. For instance, we recently demonstrated that short telomeres, a hallmark of cirrhosis, predispose to hepatocellular carcinoma (HCC) development in patients with nonalcoholic fatty liver disease (NAFLD), the most common liver disease, in which telomere attrition has been involved in disease progression (2,19). Furthermore, we found that germline mutations in telomerase reverse transcriptase (TERT) are enriched in NAFLD-HCC patients (20,21). Interestingly, an enrichment in TERT mutations has also been found in patients with HCC due to other liver diseases (22). However, telomere length was shortened irrespective of the presence of telomerase mutations (20). All in all, our data, as well as other evidence in this field, suggests that beyond TERT other proteins among the hundreds included in the telomere proteome may be considered for the study of genetic factors influencing cancer pathogenesis. Mutations in TZAP, able to impair its ability to bind dsDNA or its likely catalytic activity, may favor the mechanisms of telomere lengthening promoting tumor growth. Indeed, according to The Human Protein Atlas (www.proteinatlas.org), TZAP is expressed in at least 20 different type of cancers. In this context, we speculate a role for TZAP as a tumor-suppressor that may directly influence cancer susceptibility. Moreover, the identification of somatic mutations in tumor tissues might result in the individuation of biomarkers useful for the classification of diseases and the adoption of personalized therapy. In particular, TZAP might be modulated for limiting telomere lengthening and undefined capacity of cells replication, both in telomerase positive and ALT positive cancer cells. On the other hand, targeting of TZAP may be exploited in disease characterized by telomere shortening and exhaustion of tissue staminal compartment, a process accentuated in chronic degenerative conditions associated with high cell replication rate and genomic instability, that may once again indirectly promote cancer onset. Finally, ample evidence links telomeres to metabolic disorder (such as diabetes and obesity) through regulation of mitochondrial metabolism. It has been noticed that telomerase late generation knockout mice showed repression of peroxisome proliferator-activated receptor gamma coactivator-1 alpha and beta (Pgc-1α and Pgc-1β), the master regulators of mitochondrial physiology and metabolism, resulting in altered mitochondrial biogenesis and function and increased reactive oxygen (23). Consistently it has been very recently demonstrated that TERT deficient mice models, upon dietary stress, failed to engage genes involved in the metabolic response to fatty diet and presented functionally impaired mitochondria in liver cells (19). These observations represent another evidence for the involvement of liver dysfunctions in telomere diseases, and in particular a new indication that telomerase abnormalities may trigger liver disease progression from NAFLD onset to cirrhosis and cancer development. Interestingly there is evidence also describing TZAP participation in mitochondrial regulation. In particular, it binds the promoter of the mitochondrial fission protein (MTFP1), consistently cells in which TZAP is lacking show a complete loss of MFTP1 and are characterized by abnormal mitochondrial biogenesis (24). This evidence acquire relevance since it is known the importance of mitochondrial functionality for cancer cells sustenance and growth (25).

In conclusion, this study identifies a new important dowel in the complex regulation of telomere homeostasis. Studying in deep the mechanisms linking TZAP to telomere related diseases may provide new perspectives for the individuation of treatment for degenerative conditions, such as progressive liver disease, as well as targets towards which address cancer therapy.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Pickett HA, Reddel RR. The role of telomere trimming in normal telomere length dynamics. Cell Cycle 2012;11:1309-15.

2. Donati B, Valenti L. Telomeres, NAFLD and chronic liver disease. Int J Mol Sci 2016;17:383.
3. Blackburn EH. Switching and signaling at the telomere. Cell 2001;106:661-73.
4. Wright WE, Tesmer VM, Huffman KE, et al. Normal human chromosomes have long G-rich telomeric overhangs at one end. Genes Dev 1997;11:2801-9.
5. Blackburn EH. Structure and function of telomeres. Nature 1991;350:569-73.
6. Cesare AJ, Griffith JD. Telomeric DNA in ALT cells is characterized by free telomeric circles and heterogeneous t-loops. Mol Cell Biol 2004;24:9948-57.
7. de Lange T. Protection of mammalian telomeres. Oncogene 2002;21:532-40.
8. D'Souza Y, Lauzon C, Chu TW, et al. Regulation of telomere length and homeostasis by telomerase enzyme processivity. J Cell Sci 2013;126:676-87.
9. Pickett HA, Cesare AJ, Johnston RL, et al. Control of telomere length by a trimming mechanism that involves generation of t-circles. EMBO J 2009;28:799-809.
10. Pickett HA, Henson JD, Au AY, et al. Normal mammalian cells negatively regulate telomere length by telomere trimming. Hum Mol Genet 2011;20:4684-92.
11. Wang RC, Smogorzewska A, de Lange T. Homologous recombination generates T-loop-sized deletions at human telomeres. Cell 2004;119:355-68.
12. Nabetani A, Ishikawa F. Unusual telomeric DNAs in human telomerase-negative immortalized cells. Mol Cell Biol 2009;29:703-13.
13. Raices M, Verdun RE, Compton SA, et al. C. elegans telomeres contain G-strand and C-strand overhangs that are bound by distinct proteins. Cell 2008;132:745-57.
14. Zellinger B, Riha K. Composition of plant telomeres. Biochim Biophys Acta 2007;1769:399-409.
15. Li JS, Miralles Fusté J, Simavorian T, et al. TZAP: a telomere-associated protein involved in telomere length control. Science 2017;355:638-41.
16. Takai KK, Hooper S, Blackwood S, et al. In vivo stoichiometry of shelterin components. J Biol Chem 2010;285:1457-67.
17. Kappei D, Scheibe M, Paszkowski-Rogacz M, et al. Phylointeractomics reconstructs functional evolution of protein binding. Nat Commun 2017;8:14334.
18. Jahn A, Rane G. ZBTB48 is both a vertebrate telomere-binding protein and a transcriptional activator. EMBO Rep 2017;18:929-46.
19. Alves-Paiva RM, Kajigaya S, Feng X, et al. Telomerase enzyme deficiency promotes metabolic dysfunction in murine hepatocytes upon dietary stress. Liver Int 2017. [Epub ahead of print].
20. Donati B, Pietrelli A, Pingitore P, et al. Telomerase reverse transcriptase germline mutations and hepatocellular carcinoma in patients with nonalcoholic fatty liver disease. Cancer Med 2017;6:1930-40.
21. Valenti L, Dongiovanni P, Maggioni M, et al. Liver transplantation for hepatocellular carcinoma in a patient with a novel telomerase mutation and steatosis. J Hepatol 2013;58:399-401.
22. Donaires FS, Scatena NF, Alves-Paiva RM, et al. Telomere biology and telomerase mutations in cirrhotic patients with hepatocellular carcinoma. PLoS One 2017;12:e0183287.
23. Sahin E, DePinho RA. Axis of ageing: telomeres, p53 and mitochondria. Nat Rev Mol Cell Biol 2012;13:397-404.
24. Garcia-Exposito L, O'Sullivan RJ. TZAP-ing telomeres down to size. EMBO Rep 2017;18:861-3.
25. Wallace DC. Mitochondria and cancer. Nat Rev Cancer 2012;12:685-98.

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