

A novel pathway links telomeres to NK-cell activity

Implications for immunotherapy

Julien Cherfils-Vicini¹, Pasquale Zizza², Eric Gilson^{1,3}, and Annamaria Biroccio^{2,*}

¹Institut for Research on Cancer and Aging; Nice (IRCAN); CNRS UMR7284/INSERM U1081; Faculty of Medicine; Nice University; Nice, France;

²Regina Elena National Cancer Institute; Rome, Italy; ³Department of Medical Genetics; Archet 2 Hospital; CHU of Nice; Nice, France

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Abbreviations: DAMP, damaged-associated molecular pattern; DDR, DNA damage response; HSPG, heparan-sulfate proteoglycan; IFN γ , interferon γ ; ITS, interstitial telomeric sequence; NK, natural killer; SASP, senescence-associated secretory profile; TERC2, telomeric repeat-binding factor 2

Here, we describe a model in which telomeric repeat-binding factor 2 (TERF2) can control tumorigenesis not only via cancer cell-intrinsic mechanisms but also via non-cancer cell autonomous pathways. Indeed, we have recently shown that TERC2 regulates tissue homeostasis as it promotes the elimination of aged, damaged, and neoplastic cells by the immune system, opening the way to new therapeutic options against cancer.

Telomeres, the most distal structures of chromosomes, play an important role in genome stability and have recently emerged as integrators of various stress signals.¹ Thus, structural changes in telomeres profoundly affect the ability of cells to proliferate and to adapt to the changing microenvironment. Cells respond to telomere dysfunction by initiating the DNA damage response (DDR) through the ataxia telangiectasia mutated (ATM) or ataxia telangiectasia and Rad3 related (ATR) protein kinase, which leads to apoptosis or senescence. This mechanism represents an intrinsic barrier to carcinogenesis. The DDR also promotes the acquisition of the so-called senescence-associated secretory profile (SASP), resulting in the establishment of a pro-inflammatory microenvironment and hence in the activation of innate immune responses (which generally remove senescent cells from the organism).^{2,3} Therefore, genome integrity is kept under strict control by both cell-intrinsic and cell-extrinsic mechanisms.

Telomeres are protected from the inappropriate activation of the DDR by the multiprotein complex shelterin,

including telomeric repeat-binding factor 2 (TERF2), which is at the heart of the molecular events that maintain telomere integrity.⁴ In line with this notion, the downregulation of TERC2 promotes, depending of cell type, apoptosis or senescence. In collaboration with the laboratory of Eric Vivier, we have recently unveiled a cell-extrinsic function of TERC2, which is capable of regulating the activity of natural killer (NK) cells⁵ independently of its role in telomere protection⁶ (Fig. 1). This conclusion was based on the following results: (1) TERC2-deficient cancer cells rapidly stop dividing upon injection into mice, are unable to promote angiogenesis and recruit NK cells; (2) the recruitment of NK cells is repressed or promoted by TERC2-overexpressing or TERC2-deficient cells, respectively; and (3) the depletion of NK cells restores the capacity of TERC2-deficient cells to form vascularized tumors in mice. The fact that the NK-cell population can be controlled by changes in the levels of telomere-regulating factors suggest a sophisticated mechanism whereby telomere integrity and the immune system have co-evolved to

preserve tissue homeostasis. Now it will be interesting to see whether such role of TERC2 is part of a general mechanism by which cells bearing telomere damage are eliminated by the innate immune system.

Until recently, the oncogenic properties of TERC2 had been attributed to its cell-intrinsic role in the control of telomere stability and proliferation. The fact that the expression of *RAS*^{V12} protects telomeres against a partial TERC2 dysfunction offered us the unique opportunity to separate the cell-extrinsic antineoplastic effects of the loss of TERC2 from the cell-intrinsic inhibition of proliferation.⁶ The ability of an oncogenic form of *RAS* to render cancer cells resistant to the loss of TERC2 appears to be a general phenomenon, since cells derived from a breast cancer expressing a mutant form of *KRAS* did not display defective growth or DDR activation in response to TERC2 dysfunctions. This raises the interesting possibility that oncogenic transformation reinforces telomere capping functions and allows cells to extend their replicative lifespan.

Interferon γ (IFN γ) is a candidate mediator of the antiangiogenic activity

*Correspondence to: Annamaria Biroccio; Email: biroccio@ifo.it.

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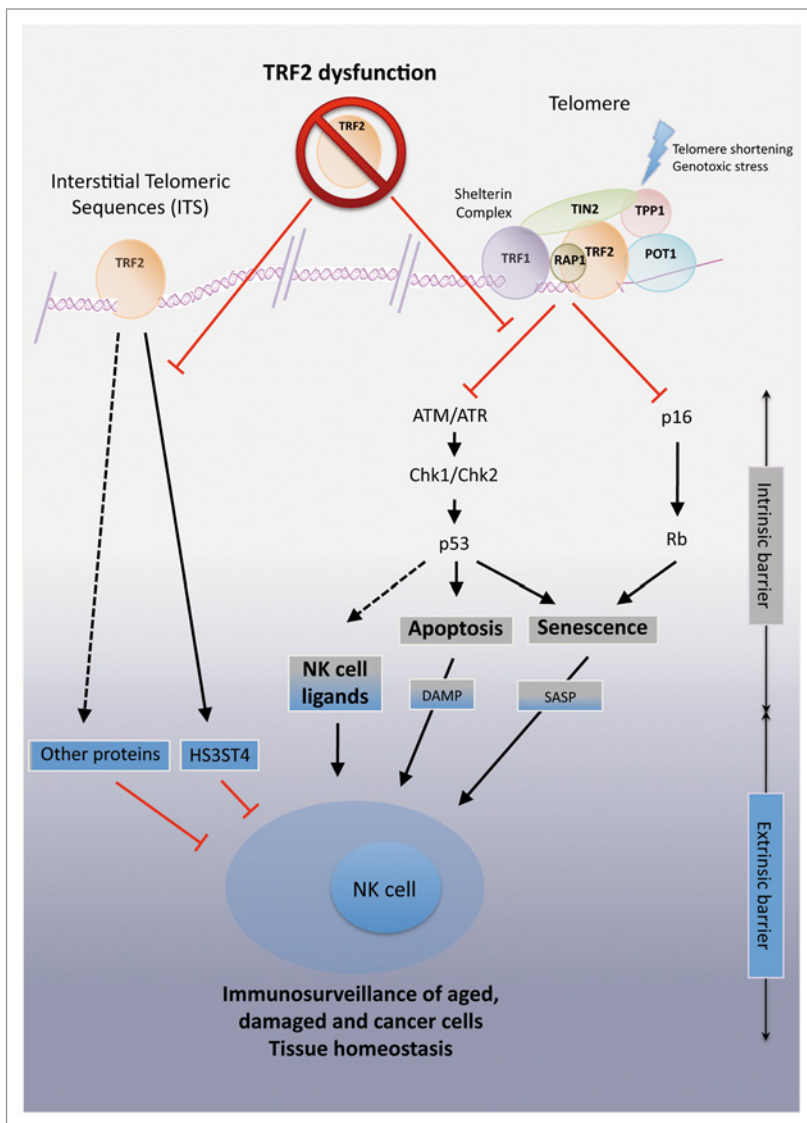


Figure 1. TERF2 controls innate immunosurveillance through cell-intrinsic and cell-extrinsic pathways. Two pathways link telomeres to the activation of natural killer (NK) cells. On the one hand, telomere dysfunction triggers the DNA damage response (DDR), which promotes apoptosis or senescence, constituting a cell-intrinsic barrier against oncogenesis. This can lead to the recruitment of NK cells through a p53-dependent signaling pathway, the release of damaged-associated molecular patterns (DAMPs) or the activation of the senescence-associated secretory program (SASP). On the other hand, the binding of telomeric repeat-binding factor 2 (TERF2) to DNA regions other than telomeres results in the transactivation of heparan sulfate (glucosamine) 3-O-sulfotransferase 4 (HS3ST4). HS3ST4 actually inhibits the recruitment of NK cells. The dysfunction of TERF2, compromising both cell-intrinsic and -extrinsic barriers to carcinogenesis, exerts a positive effect on (NK cell-dependent) cancer immunosurveillance.

of NK cells in our model for the following reasons: (1) $\text{IFN}\gamma$ is known as a potent antiangiogenic factor;⁷ (2) $\text{IFN}\gamma$ -elicited angiostatic molecules as well as their receptors are all expressed by the lymphoid cells that infiltrate TERF2-deficient microtumors; (3) $\text{IFN}\gamma$ is required for the antitumor activity of TERF2; and (4) $\text{IFN}\gamma$ is produced in

large quantities upon the inoculation of TERF2-deficient cells in mice. Taken together, these data delineated an extracellular model for the oncosuppressive effects of TERF2 inhibition according to which the secretion of large amounts of $\text{IFN}\gamma$ by NK cells inhibits the proliferation of cancer cells and their ability to promote angiogenesis.

By screening for extratelomeric genomic targets of TERF2,⁸ we found that TERF2 binds to an interstitial telomeric sequence (ITS) present within the intron of heparan sulfate (glucosamine) 3-O-sulfotransferase 4 (*HS3ST4*). We demonstrated that *HS3ST4* is positively regulated by TERF2 and inhibits the recruitment of NK cells by cooperating with TERF2 in an epistatic manner. In a context in which the role of syndecan 2 (SDC2, also known as heparan-sulfate proteoglycan, HSPG) in NK-cell recognition has been questioned for a long time, our findings raise the hypothesis that the sulfation of HSPG can regulate NK-cell recruitment. This notion is in agreement with recent data from an independent group.⁹ The clinical relevance of our results is suggested by the fact that during the early stages of colorectal carcinogenesis, the progressive upregulation of TERF2 correlates with a decrease in the density of tumor-infiltrating NK cells. Globally, the overexpression of TERF2 appears to be a critical step for developing tumors to bypass innate immunosurveillance. This mechanism might be particularly effective at the early stages of oncogenesis, a time frame in which TERF2 has been found to be upregulated in hepatic, pulmonary, and colon neoplasms. The fact that TERF2 plays a critical role in tumorigenesis might explain why *TERF2* loss-of-function mutations have not yet been found in human cancers despite the fact that telomere dysfunction promotes disease initiation in various mouse tumor models and possibly in human cancers.

In conclusion, our findings have profound implications for basic and applied biomedical research as well as for the clinical management of cancer patients. They reveal a new pathway depending on TERF2 that links telomeres to the recruitment of NK cells, opening new avenues for innovative combination of immunotherapy,¹⁰ especially for the treatment of tumors characterized by high TERF2 levels. Our findings also highlight TERF2 as a pertinent 2-hit therapeutic target, acting on both cell-intrinsic and cell-extrinsic oncosuppressive mechanisms. In this scenario, molecules targeting TERF2 could represent valuable multimodal drugs that combine different therapeutic activities

in one single component, with obvious advantages in term of simplicity of treatment and selectivity for cancer cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Lin J, Epel E, Blackburn E. Telomeres and life-style factors: roles in cellular aging. *Mutat Res* 2012; 730:85-9; PMID:21878343; <http://dx.doi.org/10.1016/j.mrfmmm.2011.08.003>
2. Coppé JP, Patil CK, Rodier F, Sun Y, Muñoz DP, Goldstein J, Nelson PS, Desprez PY, Campisi J. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol* 2008; 6:2853-68; PMID:19053174; <http://dx.doi.org/10.1371/journal.pbio.0060301>
3. Xue W, Zender L, Miething C, Dickins RA, Hernando E, Krizhanovsky V, Cordon-Cardo C, Lowe SW. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* 2007; 445:656-60; PMID:17251933; <http://dx.doi.org/10.1038/nature05529>

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4. Giraud-Panis MJ, Pisano S, Benarroch-Popivker D, Pei B, Le Du MH, Gilson E. One identity or more for telomeres? *Front Oncol* 2013; 3:48; PMID:23509004; <http://dx.doi.org/10.3389/fonc.2013.00048>
5. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. *Nat Immunol* 2008; 9:503-10; PMID:18425107; <http://dx.doi.org/10.1038/ni1582>
6. Biroccio A, Cherfils-Vicini J, Augereau A, Pinte S, Bauwens S, Ye J, Simonet T, Horard B, Jamet K, Cervera L, et al. TRF2 inhibits a cell-extrinsic pathway through which natural killer cells eliminate cancer cells. *Nat Cell Biol* 2013; 15:818-28; PMID:23792691; <http://dx.doi.org/10.1038/ncb2774>
7. Takai S, Tokuda H, Matsushima-Nishiwaki R, Saio M, Takami T, Kozawa O. TGF-beta superfamily enhances the antigen-induced IFN-gamma production by effector/memory CD8+ T cells. *Int J Mol Med* 2010; 25:105-11; PMID:19956908
8. Simonet T, Zaragosi LE, Philippe C, Lebrigand K, Schouteden C, Augereau A, Bauwens S, Ye J, Santagostino M, Giulotto E, et al. The human TTAGGG repeat factors 1 and 2 bind to a subset of interstitial telomeric sequences and satellite repeats. *Cell Res* 2011; 21:1028-38; PMID:21423270; <http://dx.doi.org/10.1038/cr.2011.40>
9. Brusilovsky M, Cordoba M, Rosental B, Hershkovitz O, Andrade MD, Pecherskaya A, Einarson MB, Zhou Y, Braiman A, Campbell KS, et al. Genome-Wide siRNA Screen Reveals a New Cellular Partner of NK Cell Receptor KIR2DL4: Heparan Sulfate Directly Modulates KIR2DL4-Mediated Responses. *J Immunol* 2013; 191:5256-67; PMID:24127555; <http://dx.doi.org/10.4049/jimmunol.1302079>
10. Zitvogel L, Kepp O, Kroemer G. Immune parameters affecting the efficacy of chemotherapeutic regimens. *Nat Rev Clin Oncol* 2011; 8:151-60; PMID:21364688; <http://dx.doi.org/10.1038/nrclinonc.2010.223>