REFERENCES

Bordería, A.V., Isakov, O., Moratorio, G., Henningsson, R., Agüera-González, S., Organtini, L., Gnädig, N.F., Blanc, H., Alcover, A., Hafenstein, S., et al. (2015). PLoS Pathog. *11*, e1004838.

Diehl, W.E., Lin, A.E., Grubaugh, N.D., Carvalho, L.M., Kim, K., Kyawe, P.P., McCauley, S.M., Donnard, E., Kucukural, A., McDonel, P., et al. (2016). Cell *167*, 1088–1098.e6.

Herfst, S., Schrauwen, E.J.A., Linster, M., Chutinimitkul, S., de Wit, E., Munster, V.J., Sorrell, E.M., Bestebroer, T.M., Burke, D.F., Smith, D.J., et al. (2012). Science *336*, 1534–1541.

Kew, O.M., Sutter, R.W., de Gourville, E.M., Dowdle, W.R., and Pallansch, M.A. (2005). Annu. Rev. Microbiol. *59*, 587–635.

Schuffenecker, I., Iteman, I., Michault, A., Murri, S., Frangeul, L., Vaney, M.-C., Lavenir, R., Pardigon, N., Reynes, J.-M., Pettinelli, F., et al. (2006). PLoS Med. *3*, e263.

Stapleford, K.A., Coffey, L.L., Lay, S., Bordería, A.V., Duong, V., Isakov, O., Rozen-Gagnon, K., Arias-Goeta, C., Blanc, H., Beaucourt, S., et al. (2014). Cell Host Microbe *15*, 706–716.

Stern, A., Yeh, M.T., Zinger, T., Smith, M., Wright, C., Ling, G., Nielsen, R., Macadam, A., and Andino, R. (2017). Cell *169*, this issue, 35–46.

Tsetsarkin, K.A., Vanlandingham, D.L., McGee, C.E., and Higgs, S. (2007). PLoS Pathog. *3*, e201.

Tsetsarkin, K.A., Chen, R., Yun, R., Rossi, S.L., Plante, K.S., Guerbois, M., Forrester, N., Perng, G.C., Sreekumar, E., Leal, G., et al. (2014). Nat. Commun. *5*, 4084.

Urbanowicz, R.A., McClure, C.P., Sakuntabhai, A., Sall, A.A., Kobinger, G., Müller, M.A., Holmes, E.C., Rey, F.A., Simon-Loriere, E., and Ball, J.K. (2016). Cell *167*, 1079–1087.e5.

Rejuvenation by Therapeutic Elimination of Senescent Cells

Paul Krimpenfort[1] and Anton Berns[1,*]

[1]Division of Molecular Genetics, the Netherlands Cancer Institute, Amsterdam, the Netherlands

*Correspondence: a.berns@nki.nl

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In this issue of *Cell*, Baar et al. show how FOXO4 protects senescent cell viability by keeping p53 sequestered in nuclear bodies, preventing it from inducing apoptosis. Disrupting this interaction with an all-D amino acid peptide (FOXO4-DRI) restores p53's apoptotic role and ameliorates the consequences of senescence-associated loss of tissue homeostasis.

Senescence arises as the consequence of cellular stress such as telomere erosion, unresolved DNA damage, or oncogenic signaling. For a cell, it embodies a twilight zone between regaining its normal function and death, and it serves as one of the mechanisms to stop incipient cancer cells in their tracks. Senescence is characterized by a persistent proliferative arrest in which cells display a distinct pro-inflammatory senescent-associated secretory phenotype (SASP) characterized by secretion of factors such as IL6 and IL8 (Kuilman et al., 2008). Whereas SASP exerts a supportive paracrine function during early development and wound healing (Demaria et al., 2014), the continuous secretion of these SASP factors has detrimental effects on normal tissue homeostasis and is considered to significantly contribute to aging (DiLoreto and Murphy, 2015). In this issue of *Cell*, Baar and colleagues have uncovered the molecular mechanisms underlying maintenance of senescent cells and their effect on tissue homeostasis (Figure 1).

In 2016, Van Deursen and coworkers showed that prophylactic ablation of cells that express the $p16^{Ink4a}$ senescent marker mitigates tissue degeneration and extends the healthspan of mice (Baker et al., 2016). Although the Baker et al. study established the causative role of senescent cells in tissue degeneration, it did not reveal the underlying mechanisms responsible for their generation. This is now addressed in the article by Baar et al. (2017). The authors compared the expression profiles of normal human IMR90 cells with their radiation-induced senescent counterparts. Whereas they expected that the senescent cells would show reduced levels of pro-apoptotic and/or an increase in anti-apoptotic proteins, they observed the opposite, suggesting lack of an upstream trigger to activate the death program. Therefore, they searched for changes in the expression levels of transcription regulators that could underlie these changes. One immediate candidate was FOXO4, a member of a transcription factor family previously implicated in aging and longevity (Martins et al., 2016), which appeared upregulated upon senescence induction. Therefore, FOXO4 was further scrutinized for its potential role in arresting cells in a senescent state. Indeed, inhibition of FOXO4 expression using a lentiviral shRNA made cells undergo apoptosis rather than become senescent upon irradiation. More importantly, FOXO4 inhibition in already senescent cells reduced their viability. This established that FOXO4 plays a critical role in consolidating the senescent state and that its inactivation causes senescent cells to undergo apoptosis.

To gain insight into the underlying mechanism, the authors went on to determine the subnuclear localization of

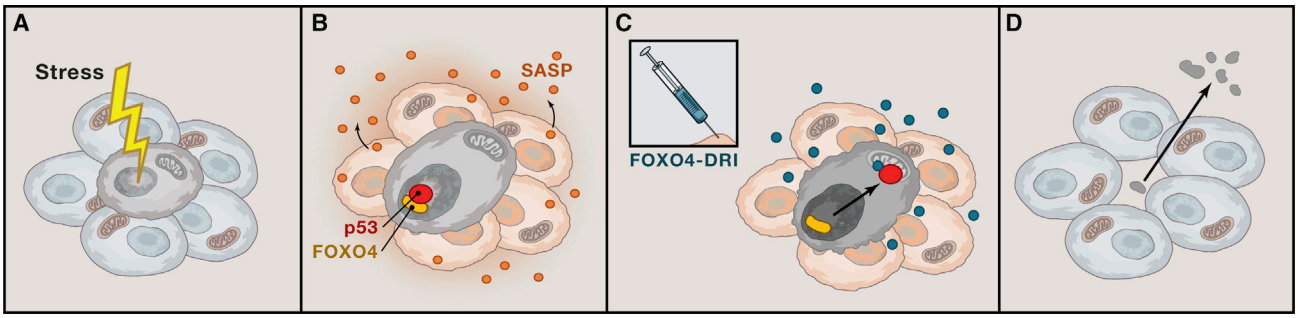

Figure 1. Tissue Rejuvenation by FOXO-DRI Treatment
(A) Environmental stress (yellow lightning bolt) causes damage to individual cells within healthy tissue.
(B) Nuclear sequestration of p53 (red) by FOXO4 (yellow) leads to senescence of damaged cells, which secrete SASP factors (orange speckles) that damage healthy surrounding cells.
(C) FOXO4-DRI (teal) treatment releases p53 from FOXO4, enabling its translocation to mitochondria and activation of cytochrome-*c*-mediated apoptosis in the damaged cell.
(D) Apoptotic cells are removed from the system and the affected tissue can rejuvenate.

FOXO4 in senescent cells. They showed that, upon radiation, FOXO4 becomes associated with nuclear bodies directly neighboring 53BP1-containing DNA-SCARS (DNA segments with chromatin alterations reinforcing senescence) (Rodier et al., 2011). Since p53 was found to co-localize with these structures and FOXOs are known to bind p53, Baar et al. subsequently tested whether FOXO4 could directly sequester p53 in these bodies. In order to disrupt the suspected interaction between FOXO4 and p53, they synthesized a D-retro inverso peptide (DRI-isoform) fully composed of D-amino acids (Guichard et al., 1994) and corresponding to the reverse sequence of the FOXO4 domain that interacts with p53. D-retro-inversion can endow peptides with new chemical properties with augmented potency in vitro and in vivo (Borsello et al., 2003). To facilitate cellular uptake, the inverso peptide was fused to a HIV-TAT peptide. The resulting FOXO4-DRI was found to bind p53 with even higher affinity than the corresponding forkhead region of FOXO4 and caused dissociation of the FOXO4-p53 complex. Similar to what was observed with FOXO4 shRNAs, treatment of senescent cells with FOXO4-DRI resulted in nuclear exclusion of p53, permitting it to interact with mitochondria and catalyze cytochrome *c* release and apoptosis of the cells. In contrast, FOXO4-DRI treatment of normal cells did not show any detrimental effects.

Next, the authors assessed whether FOXO4-DRI could also ablate senescent cell in vivo and restore tissue homeostasis in damaged tissues. They tested this in both fast-aging $Xpd^{TTD/TTD}$ and naturally aged mice, as well as in chemotherapy-treated animals. All three models are characterized by an abundance of senescent cells. In all instances, they noted significant functional improvements as based on a set of established parameters for tissue homeostasis. These included standard tissue function tests (for liver and kidney), fur density, exploratory behavior, and voluntary running wheel activity. Importantly, administration of FOXO-DRI did not lead to any of the negative side effects reported for other "senolytics," such as ABT compounds that target BCL2/W/XL anti-apoptotic proteins.

Therefore, FOXO4-DRI or compounds with similar specificity appear to be very promising therapeutic agents for further development. In fact, given the mechanism of action, administration of such compounds for a short period could be sufficient to clear out senescent cells that have accumulated upon an insult, such as chemotherapy treatment, as a result of environmental genotoxic exposures or as the consequence of heritable aging syndromes or even natural aging. In this way, the negative effects of senescent cells could be eliminated without affecting the capacity of a tissue to produce senescent cells de novo if these were needed, e.g., in the case of wound healing.

Does this imply that we can expect that a periodic "senescent cell clean-out" with compounds such as FOXO4-DRI will lead to a substantial healthspan extension? In the long run this may become a reality, and the work by Baar et al. (2017) certainly has taken an important hurdle toward such a future. However, we will first need to get better insight into the potential short- and long-term side effects. In view of the strong affinity of FOXO4-DRI to p53, one would like to know whether FOXO4-DRI binding to p53 modulates its functions in normal cells, causing unwanted side effects that only become apparent in the longer term or under specific conditions. Could it be that treatment with FOXO4-DRI causes rare senescent cells carrying oncogenic lesions, such as those found in nevi, to re-enter the cell cycle rather than undergo apoptosis, thereby increasing the risk of tumor development? One would also like to know more about the pharmacokinetic properties of FOXO4-DRI and whether it is immunogenic, as the latter would limit its repeated use. Otherwise it might be wiser to focus on the development of small-molecule drugs with similar activity and specificity. Further tests in animal models should provide answers to at least some of these questions.

Clearly, potential side effects will have to be weighted toward the objective benefits of such interventions. Patients suffering from severe heritable degenerative diseases or cancer patients experiencing tissue damage from chemotherapy would likely be the first to benefit. If these benefits are evident and no or only minor side effects are seen, this intervention could also enter the picture for

healthy individuals who want to keep running around beyond their natural expiration date.

REFERENCES

Baar, M.P., Brandt, R.M.C., Putavet, D.A., Klein, J.D.D., Derks, K.W.J., Bourgois, B.R.M., Stryeck, S., Rijksen, Y., van Willigenburg, H., Feijtel, D.A., et al. (2017). Cell *169*, this issue, 132–147.

Baker, D.J., Childs, B.G., Durik, M., Wijers, M.E., Sieben, C.J., Zhong, J., Saltness, R.A., Jeganathan, K.B., Verzosa, G.C., Pezeshki, A., et al. (2016). Nature *530*, 184–189.

Borsello, T., Clarke, P.G., Hirt, L., Vercelli, A., Repici, M., Schorderet, D.F., Bogousslavsky, J., and Bonny, C. (2003). Nat. Med. *9*, 1180–1186.

Demaria, M., Ohtani, N., Youssef, S.A., Rodier, F., Toussaint, W., Mitchell, J.R., Laberge, R.-M., Vijg, J., Van Steeg, H., Dollé, M.E.T., et al. (2014). Dev. Cell *31*, 722–733.

DiLoreto, R., and Murphy, C.T. (2015). Mol. Biol. Cell *26*, 4524–4531.

Guichard, G., Benkirane, N., Zeder-Lutz, G., van Regenmortel, M.H., Briand, J.P., and Muller, S. (1994). Proc. Natl. Acad. Sci. USA *91*, 9765–9769.

Kuilman, T., Michaloglou, C., Vredeveld, L.C.W., Douma, S., van Doorn, R., Desmet, C.J., Aarden, L.A., Mooi, W.J., and Peeper, D.S. (2008). Cell *133*, 1019–1031.

Martins, R., Lithgow, G.J., and Link, W. (2016). Aging Cell *15*, 196–207.

Rodier, F., Muñoz, D.P., Teachenor, R., Chu, V., Le, O., Bhaumik, D., Coppé, J.P., Campeau, E., Beauséjour, C.M., Kim, S.H., et al. (2011). J. Cell Sci. *124*, 68–81.