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REVIEW

It takes four to tango: Long noncoding RNA PANDA, SAF-A, polycomb repressive complexes and NF-Y in senescence regulation

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Cellular senescence is a stable cell cycle arrest that inhibits the outgrowth of pre-cancerous cells but is also implicated in wound healing, embryonic development, aging and age-related pathologies. Our knowledge on gene regulatory circuits that establish and maintain the senescence phenotype is highly fragmentary. Here, we provide several lines of evidence supporting a critical and novel function of scaffolding-attachment-factor A SAF-A and long, noncoding RNA PANDA in the establishment and maintenance of the senescence phenotype. First, we demonstrate that SAF-A and PANDA are differentially expressed in presenescent compared to senescent cells. Second, we show that both SAF-A and PANDA actively contribute to senescence induction and maintenance. Finally, we establish that SAF-A and PANDA physically and functionally interact to directly repress senescence- and proliferation-promoting genes by regulating access of polycomb repressive complexes PRC1 and PRC2 as well as transcription factor NF-YA to their cognate target genes. Together, our data identify DNA-RNA-binding protein SAF-A and long, noncoding RNA PANDA as key actors in senescence cell fate decision and highlight the importance of cell fate dependent target gene changes of transcription factors and noncoding RNAs.

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Cellular senescence not only plays important roles during embryonic development and wound healing, but is also considered a major regulator of a number of age-related diseases, including cancer [1, 2]. The senescence arrest is essentially permanent and accompanied by widespread changes in metabolism, cytomorphology, chromatin structure, gene expression including a senescence-associated secretory phenotype (SASP) - the expression and secretion of inflammatory cytokines, growth factors, proteases and other molecules that can alter tissue microenvironments. Among the most prominent stimuli that induce a senescence response are genomic and epigenomic perturbations caused by hyper-activated oncogenes, DNA damage or telomere dysfunction [1].

The senescence response appears to be conserved, at least among vertebrate species, and is a doubled-edged sword. On the positive side, it protects young organisms from cancer, promotes tissue repair, and fine-tunes embryonic morphogenesis. On the negative side, it is thought to drive late life pathology. Senescent cells accumulate with age, are prominent at sites of many age-related diseases -- both

degenerative and hyperplastic -- and likely fuel age-related pathology by virtue of the SASP [3].

One hallmark of senescence is the stable repression of proliferation-promoting genes regulated by the E2F-family of transcription factors (TFs) [4] and the activation/de-repression of senescence-promoting genes that are, at least in part, regulated by polycomb-repressive complexes 1 and 2 (PRC1 and 2) [5]. PRC1 comes in different flavors and three main sub-complexes can be distinguished based on the presence either of BMI1, MEL18 or NSCP1. The enzymatic center of all PRC1 complexes is comprised of the RING1A/B ubiquitin ligase that catalyzes the mono-ubiquitination of lysine 119 on histone H2A (H2AK119ub) [6]. The core component of PRC2 complex is the histone methylase EZH2 or 1 that catalyzes the di- and tri-methylation of lysine 27 on histone H3 (H3K27me3) [7]. Both, H2AK119ub and H3K27me3, are associated with transcriptionally inactive chromatin. An unresolved question, at least in mammalian cells, is the recruitment mechanism of PRCs to their target genes because the latter lack a DNA-binding domain and thus need to piggy-back on DNA binding factors including TFs and noncoding RNAs.

SAF-A (*alias* hnRNPU) is a member of ubiquitinously expressed heterogeneous nuclear ribonucleoproteins (hnRNPs) that also includes the likes of hnRNPA, B and K among others [8]. SAF-A was first shown to play a crucial role in organization of higher order chromatin structure in the interphase nucleus by tethering chromatin loops to nuclear matrix via matrix attachment regions (MARs) [9], but it has now also been implicated in a diverse array of RNA metabolic events including gene regulation and splicing [10]. Biochemical studies revealed that SAF-A contains three conserved protein domains: a SAF-Box (DNA-binding domain), a SPRY-box of unknown function, and an RGG-box (RNA binding domain) [11]. Protein-protein interaction studies established that SAF-A functions both in gene activation and repression. While the gene repressive function of SAF-A stems, at least in part, from its interaction with HP1α [12] the gene activating function is likely linked to its interaction with RNA polymerase II [13, 14], coactivator p300 acetyl transferase [15] and BRG1 [16], a member of the the ATP-dependent SWI/SNF chromatin-remodeling complex. Genome-wide analysis using CLIP-seq revealed that SAF-A virtually binds to all classes of regulatory noncoding RNAs, including small nuclear RNAs (snRNAs) required for splicing [10]. Given SAF-A's DNA and RNA binding domains this makes it a perfect protein to act as an adaptor between the DNA and RNA world to direct chromatin and gene expression changes. A case in point, SAF-A plays a pivotal role for inactivation of the X-chromosome (XCI) by facilitating the deposition of inactive chromatin marks histone macroH2A, H3K27me3 and Xist long, noncoding RNA (lncRNA) [17].

LncRNAs have emerged as key molecules in the control of a wide variety of biological pathways and cellular processes [18]. The emerging paradigm is that lncRNAs are principal and instructive components of a highly dynamic chromatin scaffold that can respond to internal and external cues [19]. According to this view lncRNAs are to impact both global as well as specific gene expression programs thereby shaping cellular diversity and phentoypes. Many lncRNAs are deregulated in human cancers including breast, liver, colorectal, prostate and have been found to function as oncogenes and tumor suppressors [20-23]. Others and we have shown that lncRNAs are also differentially regulated in senescence [5,24-26]. The precise function(s) of only a few lncRNAs have been defined in these processes. For example, lncRNA MALAT1, also known as non-coding nuclear enriched abundant transcript 2 (NEAT2), modulates the speckle association of splicing factors and is overexpressed in many cancer tissues [27, 28]. In line with this, MALAT1 expression is negatively correlated with senescence [29]. Similarly, lncRNAs ANRIL is overexpressed in a subset of prostate cancers [30]. *ANRIL is* expressed from the INK4a tumor suppressor gene locus where it facilitates recruitment of PRCs and repression of this locus in *cis* [31,32]. Accordingly, *ANRIL* expression was found to decrease in senescent cells concomitant with the derepression of the INK4a locus and senescence onset. LncRNA *MEG3* is repressed in many human cancer cell lines. Forced expression of *MEG3* inhibited the growth of human cancer cell lines suggesting that *MEG3* may act as a tumor suppressor [33,34]. *7SL* lncRNA is highly expressed in cancer cells. *7SL* silencing was found to promote cell cycle arrest and senescence in cancer cells. These effects were mediated, at least in part, through binding to and suppressing *TP53* mRNA translation by competing with the RNA-binding protein HuR [35]. LncRNA MIR31HG was recently shown to control senescence by regulating the expression of senescence-promoting gene CDKN2A and a negative correlation between lncRNA MIR31HG and CDKN2A expression in melanomas exists [25]. Expression correlation analyses revealed that lncRNA HOTAIR, encoded in the HOXC cluster, is as a strong predictor of breast cancer metastasis. The fact that enforced expression of HOTAIR was sufficient to drive breast cancer metastasis, involving epithelial-to-mesenchymal-ransitions (EMT), support the idea that lncRNAs might be involved in cell fate transitions in development and in cellular reprogramming [36]. Our own studies have established a significant role of PANDA in cellular senescence and provided a potential mechanistic link

for PANDA downregulation in hepatocellular carcinoma (HCC) [5].

The past few years have seen a growing number of examples for lncRNAs controlling the access to or dissociation of regulatory proteins from chromatin. Several studies showed lncRNAs to recruit chromatin modifiers/remodellers to regulate transcription [37]. For example: the human lncRNA HOTAIR physically associates with PRC2 and modulates the localization of PRC2 and H3K27me3 at hundreds of sites genome-wide [38]. HOTAIR and Xist interface with PRC2 via the catalytic methyltransferase subunit EZH2 [39]. Two p53-regulated lncRNAs, lncRNA-p21 and PANDA, were recently shown to interact with hnRNPK and TF NF-YA, leading to transcriptional repression at specific gene loci [40,41]. Although many studies initially focused on lncRNAs associated with repressive chromatin-modifying complexes, it has become evident that active chromatin states are also associated with lncRNAs. Recently, novel technologies including ChIRP-Seq and CHART-Seq have paved the way to decipher genome wide interactions between RNA and chromatin with unprecedented precision to reveal genomic maps of lincRNA occupancy and general principles of RNA-chromatin interactions [42, 43]. Collectively, these studies demonstrate the critical importance of lncRNAs in chromatin biology to control gene expression.

Based on the role SAF-A and lncRNAs play in gene regulation and tumorigenesis, we recently probed into the involvement of these factors in senescence emphasizing on their role in transcriptional regulation of senescence-promoting and inhibiting genes [5]. We demonstrated that both factors, indeed, play a critical role for the repression of senescence promoting genes in proliferating cells by recruiting BMI1-PRC1 and EZH2-PRC2 to the respective gene promoters. In cells undergoing senescence, SAF-A:PRC:lncRNA complexes decommission from respective targets leading to the de-repression of them. Congruent with this, acute depletion of SAF-A impaires the association of PRCs with the respective targets and leads to de-repression of these genes and induction of senescence. Given the RNA and DNA-binding domain of SAF-A we speculated that an RNA component could be important for the interaction with PRCs. Indeed, we demonstrate that specifically the interaction between BMI1:PRC1 is RNA-dependent whereas the interaction with EZH2:PRC2 was not. Subsequently, we identified lncRNA PANDA (p21 Associated ncRNA DNA damage Activated) [40] as the RNA component that mediates SAF-A:BMI1 complex formation and recruitment to gene targets. In line with this, PANDA depletion, akin to SAF-A depletion, in proliferating cells renders them senescent. In addition, we identified cyclin-dependent kinase inhibitor CDKN1A (alias p21CIP, WAF1) as a crucial target gene for senescence induction and congruent with this PANDA-induced senescence could be bypassed in CDKN1A-knock-out cells.

Another important finding of our study was that PANDA differentially interacts with protein components in a cell-fate dependent manner. PANDA, along with a bevy of other lncRNAs (our unpublished data), is strongly up-regulated in senescent cells compared to proliferating cells and its interaction with SAF-A: PRC1 complex is lost in senescence. In senescent cells, however, PANDA takes on another function by sequestering transcriptional activator NF-YA from proliferation-promoting genes, thus, giving way to their repression and enforcing senescence arrest. Hence, silencing of PANDA in proliferating and senescent cells has opposite outcomes. While in proliferating cells PANDA depletion leads to senescence entry by destabilizing PRC function, in senescent cells it leads to senescence exit by relieving the decoy function on NF-YA. These unique properties suggest that PANDA acts as a locking device that confines cells in a given proliferative state or cell fate.

Finally, we unearthed an unexpected role for TF NFYA in the regulation of senescence. The histone-like TF NF-Y consists of three subunits: NF-YA, NF-YB and NF-YC, which are all necessary for DNA binding of CCAAT boxes [44]. Loss of function studies highlighted the importance of NF-Y in cell cycle progression and NF-Y trimeric complex controls the expression of many cell cycle genes in collaboration with the E2F family of TFs [45]. As mentioned above, in cells undergoing senescence PANDA decoys NF-YA from its target genes. We reasoned that ectopic expression of NF-YA in senescent cells might override this decoy effect and reactivate expression of cell cycle genes to force cells back into the cell cycle and proliferation. Indeed, this occurred; hence, we identified NF-YA inhibition by PANDA as a major senescence-maintaining mechanism. Surprisingly, NFYA depletion in senescent cells resulted in cell death, thus, bringing forth a novel and unexpected function for NFYA in survival of senescent cells. Indeed, NF-YA depletion leads to the activation of many pro-apoptotic (*e.g.* TNFα, TNFR1) and repression of many anti-apoptotic genes, notably BCL2A1 (our unpublished results). This is a very important finding against the backdrop of inherent cell death resistance of senescent cells and could provide an inroad for future therapy to specifically eliminate senescent cells, given that an accumulation of senescent cells could have detrimental organismal effects [46].

In essence, our study provides compelling evidence that long, noncoding RNAs and multimodular DNA: RNA-binding proteins, such as SAF-A, mediate widespread

PRC and TF recruitment to target genes and are important factors for senescence establishment and maintenance. Additionally, we are yet to fully appreciate the significance of ubiquitously expressed TFs, like the NF-Y complex, in many settings, and this argues for a comprehensive reassessment of the roles other housekeeping TFs may play in cell fate specification.

Conclusion

Cellular senescence is increasingly recognized as a major contributor to health and disease. As such, research on therapeutic strategies exploiting senescence targeting for healthspan improvement has gained traction. Importantly, elimination of senescent cells in a premature mouse aging model was shown to improve overall tissue fitness and healthspan [46]. Thus, a detailed understanding of the causes/stresses, signalling networks and mechanisms underlying cellular senescence is critical to provide inroads for new therapeutic strategies to improve health and to target hyper/neoplastic and degenerative age-related pathologies with the final goal to improve overall healthspan [3].

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References

1. Campisi J. Aging, Cellular Senescence, and Cancer. Annu. Rev. Physiol. 2013; 75: 17.1-17.21.
2. Muñoz-Espín D, Serrano M. Cellular senescence : from physiology to pathology. Nat Rev Mol Cell Biol. 2014; 15:482-96.
3. Tchkonia T, Zhu Y, van Deursen J, Campisi J, Kirkland JL. Cellular senescence and the senescent secretory phenotype : therapeutic opportunities. J Clin Invest. 2013;123:966-72.
4. Narita M, Nũnez S, Heard E, Narita M, Lin AW, Hearn SA, Spector DL *et al*. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell 2003;113:703-16.
5. Puvvula PK, Desetty RD, Pineau P, Marchio A, Moon A, Dejean A *et al*. Long noncoding RNA PANDA and scaffold-attachment-factor SAFA control senescence entry and exit. Nat Commun. 2014; 5:5323.
6. Gil J, O'Loghlen A. PRC1 complex diversity: where is it taking us? Trends Cell Biol. 2014; 24:632-41.
7. Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. Nature. 2011; 469:343-9.
8. Han SP, Tang YH, Smith R. Functional diversity of the hnRNPs: past, present and perspectives. Biochem J. 2010; 430:379-92.
9. Romig H, Fackelmayer FO, Renz A, Ramsperger U, Richter A. Characterization of SAF-A, a novel nuclear DNA binding protein from HeLa cells with high affinity for nuclear matrix/scaffold attachment DNA elements. EMBO J. 1992; 11:3431-40.
10. Xiao R, Tang P, Yang B, Huang J, Zhou Y, Shao C *et al*. Nuclear matrix factor hnRNP U/SAF-A exerts a global control of alternative splicing by regulating U2 snRNP maturation. Mol. Cell 2012; 45:656-68.
11. Kiledjian, M, Dreyfuss, G. Primary structure and binding activity of the hnRNP U protein: binding RNA through RGG box. EMBO J. 1992; 11:2655-64.
12. Ameyar-Zazoua M, Souidi M, Fritsch L, Robin P, Thomas A, Hamiche A *et al*. Physical and functional interaction between heterochromatin protein 1alpha and the RNA-binding protein heterogeneous nuclear ribonucleoprotein U. J. Biol. Chem. 2009; 284:27974-9.
13. Kukalev A, Nord Y, Palmberg C, Bergman T, Percipalle P. Actin and hnRNP U cooperate for productive transcription by RNA polymerase II. Nat. Struct. Mol. Biol. 2005; 12:238-44.
14. Obrdlik A, Kukalev A, Louvet E, Farrants AK, Caputo L, Percipalle P. The histone acetyltransferase PCAF associates with actin and hnRNP U for RNA polymerase II transcription. Mol. Cell. Biol. 2008; 28:6342-57.
15. Martens JH, Verlaan M, Kalkhoven E, Dorsman JC, Zantema A. Scaffold/matrix attachment region elements interact with a p300-scaffold attachment factor A complex and are bound by acetylated nucleosomes. Mol. Cell. Biol. 2002; 22: 2598-606.
16. Vizlin-Hodzic D, Runnberg R, Ryme J, Simonsson S, Simonsson T. SAF-A forms a complex with BRG1 and both components are required for RNA polymerase II mediated transcription. PLoS One 2011; 6:e28049.
17. Hasegawa Y, Brockdorff N, Kawano S, Tsutui K, Tsutui K, Nakagawa S. The matrix protein hnRNP U is required for chromosomal localization of Xist RNA. Dev. Cell 2010; 19:469-76.
18. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. Annu. Rev. Biochem. 2012; 81:145-66.
19. Kanduri, C. Long noncoding RNA and epigenomics. Adv Exp Med Biol 2011; 722:174-195.
20. Martin L, Chang HY. Uncovering the role of genomic 'dark matter' in human disease. J. Clin. Invest. 2012; 122:1589-95.
21. Shi X, Sun M, Liu H, Yao Y, Song Y. Long non-coding RNAs: a new frontier in the study of human diseases. Cancer Lett. 2013; 339:159-66.
22. Gutschner T, Diederichs S. The Hallmarks of Cancer: A long non-coding RNA point of view. RNA Biol. 2012; 9:703-19.
23. Tano K, Akimitsu N. Long non-coding RNAs in cancer progression. Front. Genet. 2012; 3:219.
24. Grammatikakis I, Panda AC, Abdelmohsen K, Gorospe M. Long noncoding RNAs (lncRNAs) and the molecular hallmarks of aging. Aging (Albany NY) 2014; 6:992-1009.
25. Montes M, Nielsen MM, Maglieri G, Jacobsen A, Højfeldt J, Agrawal-Singh S *et al*. The lncRNA MIR31HG regulates p16(INK4A) expression to modulate senescence. Nat. Commun. 2015; 6, 6967.
26. Bischof O, Martínez-Zamudio RI. MicroRNAs and lncRNAs in

senescence: A re-view. IUBMB Life 2015; 67:255-67.

27. West JA, Davis CP, Sunwoo H, Simon MD, Sadreyev RI, Wang PI *et al*. The Long Noncoding RNAs NEAT1 and MALAT1 Bind Active Chromatin Sites. Mol. Cell 2014; 55:791-802.
28. Lai MC, Yang Z, Zhou L, Zhu QQ, Xie HY, Zhang F *et al*.. Long non-coding RNA MALAT-1 overexpression predicts tumor recurrence of hepatocellular carcinoma after liver transplantation. Med. Oncol. 2011; 29:1810-6.
29. Tripathi V, Shen Z, Chakraborty A, Giri S, Freier SM, Wu X *et al*.. Long noncoding RNA MALAT1 controls cell cycle progression by regulating the expression of oncogenic transcription factor B-MYB. PLoS Genet. 2013; 9:e1003368.
30. Pasmant E, Sabbagh A, Vidaud M, Bieche I. ANRIL, a long, noncoding RNA, is an unexpected major hotspot in GWAS. FASEB J 2011; 25:444-448.
31. Yap KL, Li S, Munoz-Cabello AM, Raguz S, Zeng L, Mujtaba S *et al*..Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a. Mol. Cell 2010; 38:662-74.
32. Kotake Y, Nakagawa T, Kitagawa K, Suzuki S, Liu N, Kitagawa M *et al*.. Long non-coding RNA ANRIL is required for the PRC2 recruitment to and silencing of p15(INK4B) tumor suppressor gene. Oncogene 2011; 30:1956-62.
33. Wang P, Ren Z, Sun P. Overexpression of the long non-coding RNA MEG3 impairs in vitro glioma cell proliferation. J Cell Biochem 2012; 113:1868-1874.
34. Zhou Y, Zhang, X, Klibanski A. MEG3 noncoding RNA: a tumor suppressor. J. Mol. Endocrinol. 2012; 48:R45-53.
35. Abdelmohsen K, Panda A, Kang MJ, Xu J, Selimyan R, Yoon JH *et al*. 7SL RNA represses p53 translation by competing with HuR. Nucleic Acids Res. 2014; 42, 10099-111.
36. Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ *et al*. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 2010; 464:1071-1076.
37. Khalil AM, Guttman M, Huarte M, Garbera M, Rajd A, Morales DR *et al*. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proc Natl Acad Sci USA 2009; 106:11667-11672.
38. Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA *et al*. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell 2007; 129:1311-23.
39. Zhao J, Ohsumi TK, Kung JT, Ogawa Y, Grau DJ, Sarma K *et al*. Genome-wide identification of polycomb-associated RNAs by RIP-seq. Mol. Cell 2010; 40:939-53.
40. Hung T, Wang Y, Lin MF, Koegel AK, Kotake Y, Grant GD *et al*. Extensive and coordinated transcription of noncoding RNAs within cell-cycle promoters. Nat. Genet. 2011; 43:621-9.
41. Huarte M, Guttman M, Feldser D, Garber M, Koziol MJ, Kenzelmann-Broz D *et al*. A large intergenic noncoding RNA induced by p53 mediates global gene repression in the p53 response. Cell 2010; 142:409-19.
42. Chu C, Qu K, Zhong FL, Artandi SE, Chang H. Y. Genomic maps of long noncoding RNA occupancy reveal principles of RNA-chromatin interactions. Mol. Cell 2011; 44:667-78.
43. Simon MD, Wang CI, Kharchenko PV, West JA, Chapman BA, Alekseyenko AA *et al*. The genomic binding sites of a noncoding RNA. Proc. Natl. Acad. Sci. U. S. A. 2011; 108:20497-502.
44. Dolfini D, Gatta R, Mantovani, R. NF-Y and the transcriptional activation of CCAAT promoters. Crit. Rev. Biochem. Mol. Biol. 2012; 47:29-49.
45. Benatti P, Dolfini D, Viganò A, Ravo M, Weisz A, Imbriano C *et al*. Specific inhibition of NF-Y subunits triggers different cell proliferation defects. Nucleic Acids Res. 2011; 39:5356-68.
46. Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van den Sluis B *et al*. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature 2011; 479:232-6.