

p21 and p27 a shared senescence history

Juana María Flores^{1,*}, Juan Martín-Caballero², and Rosa Ana García-Fernández¹

¹Department of Animal Medicine and Surgery; Veterinary School; Complutense University; Madrid, Spain; ²Barcelona Biomedical Research Park; Barcelona, Spain

Senescence is an irreversible loss of cellular proliferative capacity and, together with apoptosis, is considered a major pathway to control cell proliferation. Senescent cells remain metabolically active but are permanently in growth arrest.¹ Two types of senescence can be distinguished: replicative and stress-induced. Replicative senescence (RE) occurs by continuous telomere shortening after each cell division, which causes the exhaustion of their proliferative potential, leading to activation of ataxia telangiectasia mutated (ATM) kinase, which, in turn, initiates cell cycle arrest. Stress-induced premature senescence (SIPS) is triggered by multiple mechanisms of cellular stress, such as persistent DNA damage, oxidative stress, or oncogene activity, and occurs independently of telomere length.¹ The first evidence of premature senescence induced by tumor suppressors involved Pten in prostate and Nras in hematopoietic cells. Later, an association between tumor suppressors and induced senescence was observed in multiple mouse models of cancer.^{1,2} Although it was originally thought that RE and SIPS followed different pathways, it is currently admitted that a combination of mechanisms could participate in senescence, and the relative contribution of different pathways depends on the cell type and their environmental conditions. The upregulation of tumor suppressors is linked to SIPS in human and mice tumors by multiple evidences.¹ In this regard, classical pathways such as p53, which activates p21^{cip1/waf1}, and p16^{INK4a} prevent phosphorylation of the retinoblastoma protein (Rb). Another well known senescent pathway is PTEN-p27^{Kip1}. In fact, p27 protein (with inhibitory activity on CDK2-cyclin E) is frequently downregulated in many mice

and human cancers and correlates with a worse prognosis.^{1,3}

In a previous study, we showed that a combined deficiency in p21 and p27 proteins in mice is linked to more aggressive spontaneous tumorigenesis, resulting in a decreased lifespan as compared with p21-KO mice or p27-null mice.⁴ Histopathological analysis of the neoplasias revealed a broad tumor spectrum, although most of the proliferative lesions developed in p21–p27 double-KO mice had an endocrine origin (83.4%), arising from pituitary, adrenal gland, and thyroid gland.⁴ To analyze if SIPS could play a role in these proliferative glandular lesions we recently developed a study combining different senescent biomarkers such as γ -H2AX (a molecule involved in DNA damage response and applicable to standard formalin-fixed paraffin-embedded material), p53, p16, PTEN, and a cell proliferation index marker such as Ki-67.⁵ Nowadays, the combination of senescent biomarkers with cell proliferation detection improves confidence in the estimation of senescent cells in tissue sections.¹

Our work shows that glandular hyperplasias developed in p21–p27 double-KO mice displayed statistically significant lower values of γ H2AX positive-cells when compared with similar lesions in mice lacking either p21 or p27. These data, together with the low cell proliferation confirmed by the Ki-67 index values, suggest that p21 and p27 proteins enhance cellular senescence in these pre-tumoral lesions.⁵ Glandular hyperplasias arising from p27-KO mice showed a decrease in γ H2AX-positive cells when compared with p21-KO mice, suggesting that the loss of p27 could play a relevant role in the reduced SIPS observed in glandular pre-neoplastic lesions that

developed in the double-null mice, as was previously described in other mice glandular tumors.^{1,3} When we assessed SIPS in adenomas arising from thyroid, pituitary, and adrenal glands, the lowest number of γ H2AX-positive cells was observed in double-KO mice, which suggests a cooperative role of both CKIs in triggering the phosphorylation of H2AX. As expected, the malignant tumors developed in this study, pheochromocytomas and thyroid carcinomas, did not show γ H2AX immunopositivity but displayed high levels of Ki-67 cell proliferation index.

In our work, only glandular hyperplasias developed in p21-KO mice showed significantly lower p53 expression when compared with other mice groups, whereas in adenomas similar values of p53 were found among groups. These results suggest that the Arf/p53 pathway seems to play a minor role in SIPS observed in p21–p27 double-KO glandular lesions. The role of p53 in the induction of a senescent phenotype has been debated in recent studies which have shown that p53 can either activate or suppress senescence, suggesting that a moderate activation of p53, unable to inhibit mTOR signaling, will induce senescence, while strong p53 activation results in quiescence.^{6,7} Highest positivity of p16 expression was noted in hyperplasias, but no differences were detected among groups, suggesting that this tumor suppressor does not have a pivotal effect on SIPS in this cancer model. PTEN expression was not observed in lesions of double-KO mice, and no differences among groups were noted. Taken together these data point out that the deletion of p21 and p27 prevented premature senescence in pre-malignant lesions, and that p53, p16, and PTEN expression does not seem to trigger the senescence

*Correspondence to: Juana María Flores; Email: jflores@ucm.es

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observed in proliferative benign glandular lesions that develop in this double-KO mice. In this regard, p27, p21, and ARF induction triggers senescence in adrenal and prostatic gland tumors in other cancer mouse model.⁸

In summary, an intrinsic cooperation between p21 and p27 CKIs was observed in SIPS of spontaneous proliferative glandular lesions developed in double-KO mice, suggesting that premature senescence, which would explain the

low malignancy observed in those lesions, could prevent tumor cell proliferation.

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