# *PICS*-ure This: Prosenescence Therapy?

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**Senescence is increasingly recognized as a critical feature of mammalian cells to suppress tumorigenesis, acting together with cell death programs. Whether senescence, like programmed cell death, can be exploited therapeutically has been unclear. Pandolfi and coworkers now propose that *P*TEN-loss-*i*nduced *c*ellular *s*enescence (PICS) may be triggered in vivo for therapy.**

Originally, cellular senescence was described as a state of cell-cycle arrest occurring in response to prolonged culturing of untransformed human cells in vitro. In addition to the progressive shortening of telomeres, other stress signals, owing to inadequate culturing conditions, can elicit this premature form of arrest. Senescence can be triggered also by other alterations, like the expression of an oncogene that is activated by mutation. The associated cessation of cell proliferation is largely irreversible: although cells can be senescent for long periods of time without loss of metabolic activity, they can re-enter the cell cycle only upon disruption of specific signaling cascades (Campisi, 2005). Senescence is often associated with the secretion of dozens of factors that mediate communication between senescent cells and their microenvironment (Kuilman and Peeper, 2009). The execution of the senescence program relies on the activation of several tumor suppressor routes, most if not all of which are frequently altered in human cancers.

Since 2005, evidence demonstrating that cellular senescence corresponds to a common phenomenon bearing strong physiological relevance has accumulated rapidly. Senescence biomarkers have been identified in a number of human lesions, including melanocytic nevi (moles), neurofibromas, and prostate intraepithelial neoplasia (PIN) (Collado and Serrano, 2010). These observations are corroborated in an increasing series of mouse models. For example, expression of the cancer-derived $BRAF^{V600E}$ protein kinase in the melanocytic compartment triggers lesions closely resembling human nevi. Only in the presence of a second mutation they massively progress to malignancy and form metastasizing melanomas (Dankort et al., 2009). Other models have highlighted that not only oncogene activation but also loss of tumor-suppressor genes can activate a senescence program in vivo.

The activation of senescence by loss of tumor-suppressor genes can be exemplified by *PTEN*, which is among the most commonly mutated tumor suppressors in human cancer. *PTEN* encodes a phosphatase catalyzing the conversion of the membrane lipid PIP3 to the PI3K substrate PIP2, fueling downstream signaling cascades, including the AKT pathway. *PTEN* is often mutated in prostate cancer, the most frequently diagnosed cancer in men. Peculiarly, whereas loss of a single *PTEN* allele acts mitogenically, loss of both alleles instead sets in motion a senescence program, in a p53-dependent fashion (Chen et al., 2005). It thus suppresses prostate cancer, with PTEN dosage inversely correlating with disease progression (Trotman et al., 2003).

The powerful tumor-suppressing role of senescence in vivo prompts the question as to whether its (re-)activation in tumors would be a realistic option for cancer therapy. Similarly, induction of apoptosis in tumor cells, whether by chemotherapeutics or signaling molecules, can be successfully utilized clinically. Studying *P*TEN-loss-*i*nduced *c*ellular *s*enescence (PICS) in detail, Pandolfi and coworkers now propose that prosenescence therapy, too, may be feasible for blocking tumor progression, in particular prostate cancers driven by *PTEN* loss (Alimonti et al., 2010). Given that most prostate tumors have lost one allele of *PTEN*, the authors argue that its haploinsufficient nature renders prostate tumors amenable to temporary pharmacological inhibition of the protein that is expressed from the remaining allele.

The investigators compared PICS to OIS (*o*ncogene-*i*nduced *s*enescence) in vitro. Typically, the cell-cycle arrest of OIS is preceded by a brief period of hyperproliferation. At least for some activated oncogenes, this is accompanied by hyperreplication stress and a DNA damage response (DDR). Upon loss of *Pten*, mouse embryo fibroblasts (MEFs) activate p53 and its downstream effectors p21 and PAI-1. The authors noted that, in contrast to OIS, PICS kicked in soon after the loss of PTEN, giving rise to increased SA-β-Galactosidase activity (as a measure of senescence) and cessation of proliferation. Features of the senescence response were seen also when *Pten* was deleted in aphidicolin-arrested cells, indicating that PICS does not rely on DNA replication. In contrast to OIS, MEFs undergoing PICS exited the cell cycle without overt signs of a DDR. Correspondingly, PIN lesions in mice displayed increased SA-β-galactosidase activity yet lacked γ-H2AX foci, a marker for DNA breaks.

The uncoupling of PICS from DDR activation prompted the authors to consider the possibility of activating PICS in early tumors, in an effort to prevent them from progressing to full malignancy. The concept of activating senescence in early tumors may sound generally appealing (particularly for those tumors that are highly resistant to induction of death). However, it is questionable whether this should be a therapeutic aim for lesions that undergo OIS, given that this may be accompanied by DNA damage and subsequent outgrowth of mutated cells. For PICS, Alimonti et al. reasoned, this is different because in contrast to OIS, it does not involve proliferation nor mounting a DDR.

When *Pten*-heterozygous, but not WT, MEFs were exposed to VO-OHpic, an

antidiabetes drug that inhibits PTEN, a considerable fraction increased SA-β-galactosidase activity and slowed down proliferation. Homozygous *Pten*-deficient cells, of which many are already senescent, failed to show this response, indicating that the impact of the drug occurs as a function of PTEN levels. Also the tumorigenic capacity of mouse xenografts from a human prostate carcinoma cell line (MDA PCa-2b), expressing low quantities of PTEN, was decreased upon VO-OHpic treatment. Similar to the in vitro settings, the proliferative activity of the cancer cells dropped substantially, which was accompanied by an increase in the number of SA-β-galactosidase-positive cells.

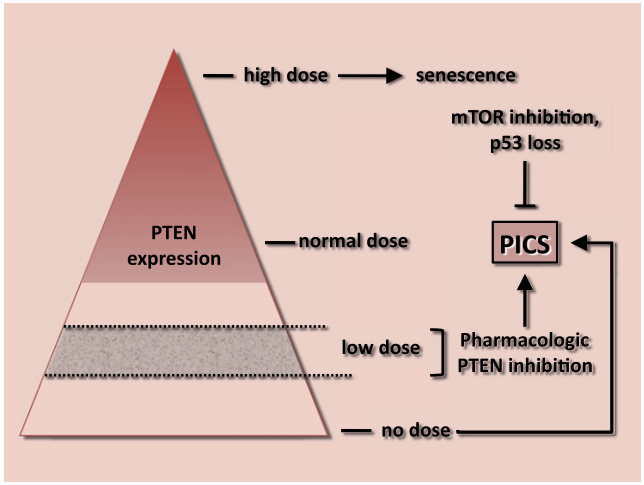


**Figure 1. Pharmacologic Induction of PICS**
Loss of both gene copies ("no dose") of PTEN sets in motion a senescence program, "PICS," in a p53- and mTOR-dependent fashion. It occurs in the absence of a DNA damage response and can be established also in already arrested cells. Also overexpression of PTEN ("high dose"), or inactivation of PI3K, can cause senescence. Cells with a "low dose" (30%–50% of the normal dose in WT cells) can be forced to enter senescence upon pharmacologic inhibition of PTEN.

Although we do not yet know how commonly, senescence can be triggered in tumors upon chemotherapy. Similarly, reactivation of p53 can elicit not only apoptosis but also senescence in vivo (Sharpless and DePinho, 2007). This observation is consistent with the prevailing model that cancer proliferation and survival rely on a set of "driver" genes: activated oncogenes and inactivated tumor-suppressor genes. Instead, the paper by Alimonti et al. argues that, at least within the context of partial PTEN deficiency, (further) tumor-suppressor inactivation can suppress, rather than drive, tumorigenesis. Because it was shown previously that ectopic expression of PTEN or suppression of PI3K also causes senescence (Courtois-Cox et al., 2006), the current observations suggest that the PI3K/PTEN pathway requires delicate fine-tuning in order to be compatible with cell proliferation: too little or too much may act cytostatically.

Is clinical extrapolation of the current findings, "prosenescence therapy" as the authors put forward, now within reach? For this to be feasible, several issues need to be investigated further. For example, what is the effect of PTEN inhibition in different genetic contexts? It is shown that inactivation of p53 or mTOR abrogates PICS; it is conceivable that other (epi)genetic alterations also affect the outcome of PTEN inhibition. In addition, it appears that targeting PTEN would be beneficial only within a narrow window of its expression level (Figure 1), which may fluctuate resulting from tumor heterogeneity. And although two-thirds of prostate tumors suffer from heterozygous *PTEN* allelic loss, other mechanisms contribute to loss of PTEN expression in advanced prostate cancer (Whang et al., 1998). Before prosenescence therapy becomes a realistic goal, we need to find the answers to these and other clinically oriented questions. This notwithstanding, the findings by Alimonti et al. are food for thought because they highlight one out of several vulnerabilities of cancer cells, representing a feature that cancer researchers may wish to further explore for future clinical application.

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