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AUTHOR'S VIEWS

Stimulation of epidermal hyperplasia and tumorigenesis by resident p16^{INK4a}-expressing cells

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ABSTRACT

p16^{INK4a} (CDKN2A) is a central tumor-suppressor and activator of senescence. We recently found that prolonged expression of p16^{INK4a} in epidermal cells induces hyperplasia and dysplasia through Wnt-mediated stimulation of neighboring keratinocytes. The study suggests a pro-tumorigenic function of p16^{INK4a} in early epidermal lesions, which could potentially be targeted by senolytic therapy.

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Gatekeeper tumor suppressor proteins often activate coordinated cell-intrinsic programs, such as apoptosis and senescence, in cells suffering damage or oncogene activation. These programs prevent the proliferation of defective cells and propagation of active oncogenes, and are important barriers for tumorigenesis. The functions of cellular senescence are particularly intriguing, since senescent cells remain viable and can be retained in tissues, and therefore may have secondary long-term effects.[1]

p16^{INK4a} (CDKN2A, p16 hereafter) is a central activator of senescence and is the most consistent marker of this program. The numbers of cells expressing p16 increase with age in multiple tissues,[2,3] indicating that resident senescent cells accumulate during aging. Cumulative cellular damage, combined with reduced removal of senescent cells by the immune system, are likely to contribute to this increase,[4] yet its direct causes are poorly characterized. p16 can be activated by persistent DNA damage, oncogene activity, and exposure to external stresses such as UV light and cigarette smoke.[5] Tissues that are continuously exposed to such agents may accumulate higher numbers of p16-expressing (p16^{+}) cells.

It is therefore of importance to understand the biological traits of p16^{+} cells and their effects on tissue physiology, aging and cancer. Recent studies in mouse models revealed that resident p16^{+} senescent cells accelerate normal aging and mortality, including cancer-caused deaths.[3] The targeted elimination of these cells through genetic manipulation or by treatment with senolytic drugs (that preferentially kill senescent cells), had positive effects on a broad range of disease models.[6] This raises the possibility that senolytics represent a new therapeutic mode with broad applications.

Currently there is only limited understanding of the mechanisms by which p16^{+} cells exert physiologic effects. The dramatic changes in cell structure and metabolism upon senescence, and the occupation of important niches by senescent cells, could influence the tissue microenvironment in ways that are largely unknown. The senescence-associated secretory phenotype (SASP), which involves secretion of cytokines, growth factors and ECM remodelers, is the most likely mechanism by which senescent cells could influence tumorigenesis and other diseases positively or negatively.[7] SASP components can directly stimulate cancer cells and modify the immune environment to favor cancer growth, for example through low-level inflammation; conversely, senescent cells can suppress tumorigenesis by enhancing immune killing of cancer cells or by inducing cell cycle arrest in neighboring cells.[7] Further elucidation of the ways these cells influence cancer propensity in different contexts is therefore necessary.

The contribution of p16 itself to specific phenotypic components of the senescence program also requires further elucidation. For example, p16 does not activate the SASP,[8] which is regulated by NFκB and other factors and is dependent on DNA damage. Whether every cell expressing p16 enters senescence, fully or partially, is also unclear.

In our recent work,[9] we set out to study these questions by generating mice with an inducible p16 transgene. We found that expression of p16 in the basal epidermis of young adult mice led to a transient proliferative arrest, followed by a return to normal proliferation rates within several days. Since the transgene was expressed in 20–40% of keratinocytes, this likely represents compensatory proliferation by neighboring cells. The epidermis retained its overall structure, albeit with increased keratinocyte size, whereas proliferation of hair-follicle stem cells was inhibited, resulting in hair loss. These short-term effects are all consistent with the known cell-cycle inhibitory functions of p16.

We then proceeded to ask what would be the consequences of prolonged p16 expression. This led to more pronounced alterations: the epidermis of mice expressing p16 for six months displayed a dramatic increase in keratinocyte

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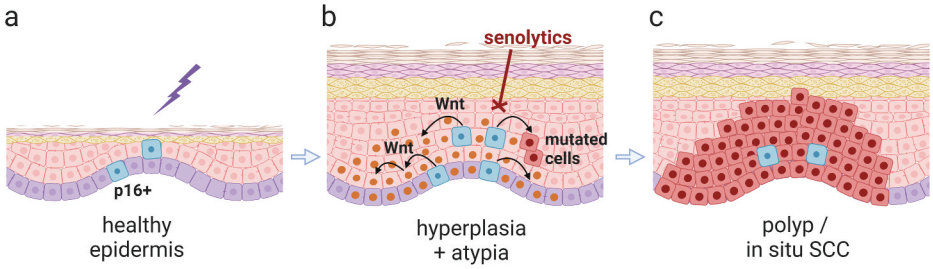

Figure 1. Potential actions of p16$^+$ cells during early epidermal tumorigenesis. (a) Epidermis exposed to damage accumulates p16$^+$ cells (blue). (b) Long-term resident p16$^+$ cells (blue) secrete Wnt ligands, stimulating further Wnt secretion by neighboring cells (pink and purple), inducing hyperplasia and atypia. Neighboring cells carrying oncogenic driver mutations (red) are stimulated to proliferate. p16$^+$ cells can be targeted by senolytic therapy, such as the Bcl2-family inhibitor ABT-737. (c) Stimulated mutated cells (red) form early benign lesions – polyps, or *in situ* squamous cell carcinoma (SCC). Such lesions may harbor p16$^+$ cells (blue), whose presence and activity impede lesion growth and progression, generating selective pressure for p16 loss-of-function mutations.

proliferation rates, manifested as epidermal hyperplasia, with regions of atypia (dysplasia). Proliferation rates of basal keratinocytes not expressing the transgene exceeded 90%. Chronic p16 expression was thus sufficient to induce a premalignant state in the tissue. The dysplastic regions closely resembled human actinic keratosis, a common UV-induced premalignant lesion that is a precursor to squamous cell carcinoma. We found that in such patient lesions p16$^+$ cells are indeed often intermixed with more proliferative p16 $^-$ cells.

When we applied the DMBA/TPA skin carcinogenesis model to mice, and activated p16 subsequent to treatment with the carcinogen DMBA, p16-expressing mice developed twice the number of skin papillomas than controls. This indicated that p16$^+$ cells promote the ability of neighboring cells harboring a driver mutation to initiate a growing tumor. The cell-autonomous tumor suppressive action of p16 thus appears to be coupled, over time, with a paracrine pro-tumorigenic activity. Interestingly, while papilloma numbers were doubled, their growth rate was slowed, with p16 restricting proliferation and enhancing cell differentiation, further illustrating its double-edged sword function.

To elucidate the mode of interaction between p16$^+$ cells and surrounding keratinocytes, we isolated these cells and studied their transcriptome. This revealed an upregulation of Wnt ligand and target genes, which was reflected in increased nuclear localized β-catenin (Ctnnb1) protein in the tissue. We overexpressed p16 in cultured primary keratinocytes and found that this indeed led to elevated Wnt ligand secretion. Conditioned medium from p16$^+$ cells stimulated the proliferation of naïve keratinocytes. Interestingly, the treated cells also increased their Wnt secretion levels, generating a positive feedback loop, and a similar process was found in the p16-induced mouse skins. Blocking the Wnt pathway in p16-induced mice, either pharmacologically (using the tankyrase inhibitor XAV-939), or genetically (by co-expression of Tcf7l1, also known as Tcf3), reversed the observed hyperplasia. Wnt activation is therefore a newly identified means by which resident p16$^+$ cells can stimulate their environment.

p16 expression induced only partial features of senescence, and, as previously observed, did not induce a full SASP. The mouse model thus outlines the contribution of p16 to downstream senescence-like phenotypes in the absence of cellular damage that can co-activate additional pathways. The findings illustrate that p16-expressing, partially senescent cells, can have substantial effects in the absence of a full SASP, in this case acting through Wnt. Importantly, we found that despite their partial phenotype, the p16$^+$ cells were susceptible to senolytic elimination: ABT-737, an inhibitor of the Bcl2 family of anti-apoptotic proteins,[10] eliminated many of the p16$^+$ cells, and proportionally reversed hyperplasia and Wnt activation, illustrating the dependence of these effects on the continued presence of p16$^+$ cells.

The study introduces a new aspect of the actions of p16, one of the most important tumor suppressors. The chronic activation of p16 in a subset of cells, which can occur in damaged tissues or during aging, may elicit pro-proliferative and pro-tumorigenic paracrine effects (Figure 1). Wnt activation as a downstream effector of p16 is added to the known senescence-associated paracrine mediators. The findings provide insight into the mechanisms by which accumulation of resident p16$^+$ cells could enhance cancer initiation rates, and senolytic therapy could be considered as a means to eliminate these cells from premalignant settings (Figure 1).

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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