CANCER

Suppressing Cancer: The Importance of Being Senescent

Judith Campisi

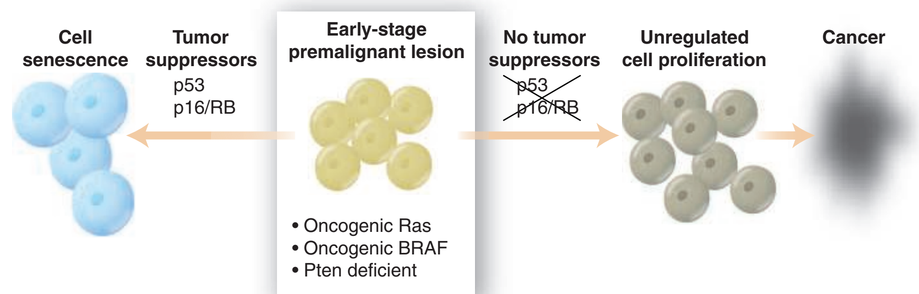
Cancer is a potentially lethal disease in mammals and other complex organisms with renewable tissues. Tumors originate from cells that are actively dividing. Such cells are at much greater risk than postmitotic (nondividing) cells for acquiring mutations, a major driving force for cancer development. Cell division is extensive during development and continues during maturation and adulthood. Yet cancer is typically an age-related disease, developing primarily in older adults. Why, then, don't mammals develop cancer earlier and more frequently? The answer lies in the tumor suppressor mechanisms that evolved to protect complex organisms from malignant tumors (*1*). Some of these mechanisms protect the genome from damage or mutation. Others eliminate or arrest the proliferation of potential cancer cells by processes called apoptosis or cellular senescence. There is ample evidence that apoptosis, or cellular suicide, suppresses tumorigenesis in vivo. However, evidence that cellular senescence, the permanent arrest of cell division, suppresses cancer has been largely circumstantial. Four recent papers dispel doubts that cellular senescence is an important anticancer defense in vivo (*2*–*5*). Furthermore, they show that activated oncogenes—mutant genes that have the potential to transform normal cells into a cancerous state—induce cellular senescence in vivo (see the figure), a phenomenon that previously had been seen only in cell culture. The findings support the idea that the senescence response is a failsafe mechanism that prevents the proliferation of cells at risk for neoplastic transformation.

Cellular senescence was first identified as a process that limits the ability of normal human cells to proliferate in culture. We now know that this limit is caused by at least two intertwined mechanisms (*1*). First, the erosion of telomeres, regions at the ends of chromosomes that stabilize DNA, elicits a DNA damage response that causes the cell division cycle to arrest. This response requires the activity of a signaling pathway that includes the tumor suppressor protein p53. Second, cumulative stress of an unknown nature induces expression of the p16 tumor suppressor. This activates a signaling pathway that involves another tumor suppressor protein, pRB, which in turn halts cell cycle progression. We also now know that many stimuli induce a senescence response. These include nontelomeric DNA damage that engages the p53 pathway and certain oncogenes that trigger the p16-pRB pathway. How relevant is the senescence response, particularly the response to oncogenes, in vivo?

Collado *et al*. (*2*) used a mouse model in which inducible expression of an oncogenic allele of the Ras gene (*K-rasV12*) causes multiple lung adenomas, a few of which progress to malignant adenocarcinomas. Ras proteins transduce growth factor signals and oncogenic Ras forms deliver unregulated signals. Collado *et al*. show that the adenomas, but not adenocarcinomas, express several markers characteristic of senescent cells in culture. Likewise, they observed premalignant lesions in the pancreas and skin that expressed senescence markers, whereas malignant tumors did not. Thus, activated oncogenes induce a senescence response in vivo, at least in mice. Because malignant tumors that eventually developed were largely devoid of senescent cells, it is likely that rare variants overcome senescence and then progress to full-blown malignancy.

Using an entirely different mouse model in which the Pten tumor suppressor is inactivated in the prostate at puberty, Chen *et al*. (*3*) similarly found that premalignant or nonlethal cancers expressed senescence markers, whereas malignant tumors did not. Pten dampens growth-promoting signals. Its absence in mice resulted in lethal invasive prostate cancer only when p53 was inactivated. In culture, Pten-deficient cells from these animals entered a senescent state that was overcome by loss of p53 function. Thus, p53 restricts the growth and malignant progression of Pten-deficient cells—both in culture and in vivo—by inducing cellular senescence. This is very likely relevant to human prostate cancer because cells expressing a senescence marker were found in early-stage human prostate cancers, but not in frankly malignant tumors.

Michaloglou *et al*. (*4*) explored the role of cellular senescence in human cancer progression by examining benign melanocytic tumors (nevi), many of which express an oncogenic form of BRAF, a downstream mediator of RAS. When expressed in cultured human melanocytes and fibroblasts, mutant BRAF caused a transient burst of proliferation, followed by senescence and increased p16 expression. This arrest was overcome by expressing the viral oncogene SV40 T antigen, which inactivates both p53 and pRB. In human skin samples, the melanocytes present in nevi expressed markers of senescence. It may be that these lesions are benign because of senescent melanocytes that harbor BRAF mutations. Interestingly, p16 expression in nevi was heterogeneous and its elimination had no



Cell senescence as an anticancer defense. Mutations that activate the oncogenes Ras or BRAF or inactivate the tumor suppressor Pten produce premalignant (early-stage) lesions. If there is additional loss of p53 or p16/pRB function, premalignant cells cannot undergo cellular senescence and progress instead into malignant tumors.

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effect on senescence. Moreover, telomere erosion was not apparent, suggesting that some cells senesce by telomere- and p16-independent mechanisms. One possible mechanism is DNA damage caused by the reactive oxygen species that mediate RAS-dependent mitogenic signals (*6*).

Finally, Braig *et al*. (*5*) used a mouse model in which oncogenic Ras (Eμ-*N-Ras*) is constitutively expressed in hematopoietic cells. The study shows that a deficiency in Suv39h1, a histone methyltransferase, markedly accelerates the development of lethal tumors. Suv39h1 is thought to promote the heterochromatic silencing of growth-promoting genes in senescent cells. This silencing causes the senescence response of lymphocytes to oncogenic Ras. Lymphomas that develop in Eμ-*N-Ras* mice undergo senescence in response to chemotherapy, but this did not occur in Suv39h1-deficient tumors. Rather, Suv39h1-deficient tumor cells underwent apoptosis. Thus, cell senescence suppressed lymphomagenesis in these mice.

Together, these papers support the idea that cellular senescence, like apoptosis, plays an important role in suppressing tumorigenesis in mice and humans in vivo. Needless to say, many questions remain. What are the mechanisms that determine whether cells undergo senescence or apoptosis when challenged by potentially oncogenic insults? Are there pathways other than the p53 and p16-pRB pathways that cause the senescence response? And, is senescence as effective as apoptosis at preventing cancer? The latter question is especially important because senescent cells secrete factors that can stimulate the proliferation and malignant progression of neighboring cells (*7*, *8*). And thus, a potential irony lurks: Prolonged presence of senescent cells may eventually facilitate the development of malignant cancers from benign lesions.

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APPLIED PHYSICS

Droplet Control for Microfluidics

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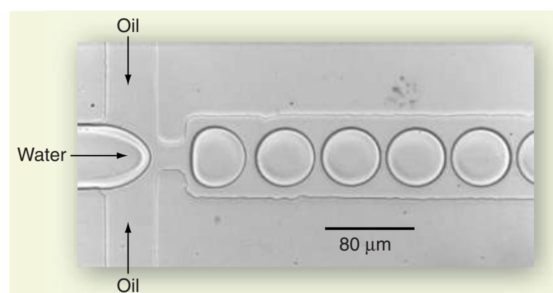
Nanoliter droplets of uniform size spontaneously form in microchannels when two immiscible fluid streams merge (*1*) (see the figure). This nonlinear process involves basic physics (*2*), with the local geometry and surface chemistry of the microchannel strongly affecting the competition between viscous forces tending to draw the fluids along the channel and capillary forces tending to form droplets so as to minimize the total interface between the two fluids. This results in droplets of sizes comparable to the channel diameter (*3*), and these sizes can be tuned by adjusting the flow rates of the various streams (*4*, *5*). The determination of which liquid is inside the droplet and the extension of the regime where droplets are emitted periodically are controlled by the wetting competition (which fluid preferentially interacts with the channel surfaces) and by added surfactants (*6*). This new route for easy and steady production of calibrated emulsions opens a stimulating field for applications of microfluidic devices [for a recent review, see (*7*)].

Typical microfluidic channel sizes (height and width) are in the range of 10 to 100 μm, and flow rates are between 10 and 1000 nl/s. This leads to nanoliter-size droplets, produced at frequencies of 10 Hz to 10 kHz, moving at speeds from micrometers per second to centimeters per second. As a result, these tiny droplets are almost ideal chemical reactors because they create homogeneous controlled conditions (*8*). In the first place, the very high surface-to-volume ratio (owing to the small size of the droplets) grants very fast thermal transfer. In addition, each droplet moves as an independent nanoliter batch reactor, with no hydrodynamic dispersion. For a steady flow, each location along the channel directly corresponds to a unique residence time after droplet formation. And internal recirculation within the droplet permits fast and efficient mixing, especially if wiggly channels are used (*8*). Such ideal reactors allow one to follow reactions in time. An interesting tool for such studies is confocal Raman microspectroscopy, which can be used to determine the chemical composition anywhere along the channel, providing a mapping that is a direct measurement of the whole kinetics at once. For short distances and high flow rates, reaction times as short as a few milliseconds can be measured (*8*). By varying the initial composition of the droplets, one can assess its effect on yield and kinetics, providing a useful operational research tool for laboratories. Control of residence time also enables synthesis of quite monodisperse small particles within the droplets.



Tunable droplets. Water droplets form at a rate of 1000 per second in an asymmetric microchannel containing hexadecane. The channel is 30 μm high.

There is another area in which these devices can be valuable tools for materials engineering, namely the transformation of each droplet into a single colloidal object. For example, one can dissolve the desired molecules or polymers into an organic phase and flow the latter into an aqueous stream to generate droplets. To dry the resulting emulsion, the organic solvent is either exchanged with the aqueous phase or slowly evaporated through it. A last step of ultraviolet-induced cross-linking or polymerization can then be used to solidify the colloids. The polydispersity of the particles can be as low as a few percent, far better than what is achievable with classical means of generating emulsions. Colloids of various shapes (disks, cylinders, and so forth) can be obtained by solidifying confined droplets (*9*). Monodisperse droplets of liquid crystals can also be obtained (*10*).

As suggested earlier, the use of hydrophobic channels results in formation of water droplet in a stream of hydrophobic liquid (oil), whereas hydrophilic channels favor creation of water-in-oil droplets. Now imagine a two-step process whereby a hydrophobic channel, in which a water-in-oil emulsion is generated, connects to a hydrophilic channel in which water flows. This can result in a multiple emulsion of

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