Radiation induced suppression of p16 levels in human mammary epithelial cells

Rituparna Mukhopadhyay, Alexey Bazarov, Mary Helen Barcellos-Hoff and Paul Yaswen
Life Sciences division, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720

Background:
We are using cultured human mammary epithelial cells (HMEC) to evaluate the potential of a prototypical environmental stressor, ionizing radiation (IR), to cause pre-malignancy-associated changes. In serum-free growth medium, HMEC from histologically normal breast tissue growth arrest after 5-20 population doublings, exhibiting senescent morphologies and elevated expression of the cyclin-dependent kinase inhibitor $p16^{INK4a}$. This p16-dependent "stasis" involves activation of the RB tumor suppressor. Variable p16 expression is also observed in epithelial cells *in vivo*, suggesting that the conditions that induce it may have physiologic relevance. In HMEC cultures from normal tissues, a variant p16(-) cell population that is capable of long-term growth (50-100 PD total) arises spontaneously with frequencies of $1x10^{-5}$ and $1x10^{-8}$, depending on specimen and culture conditions. Epigenetic silencing of p16 in these cells is associated with methylation of the p16 promoter by unknown mechanism(s). Such silencing is also frequently observed in many human cancers, including breast.
Objective:
Quantify the effects of low doses of radiation on growth potential, p16 expression, and p16 promoter methylation in HMEC.
Methods:
Using standard conditions for the culture of adherent cells, assay the long-term growth potential of control and irradiated cells, and determine whether a correlation exists with p16 expression and p16 promoter methylation, as determined by immunoblotting and methylation-specific PCR. Use senescence-associated beta-galactosidase (SA-beta gal) activity as an indication of stasis.
Results:
HMEC irradiated with 2 Gy showed accelerated proliferation and outgrowth of SA-beta gal (-) cells, compared to unirradiated controls. The irradiated cells emerged from stasis faster than control cells, and expressed low or undetectable levels of p16.
Conclusions:
Our results indicate that radiation increases the frequency with which HMEC escape stasis. We are currently examining the hypothesis that radiation-induced p53-dependent de-repression of a DNA methyltransferase causes heritable changes in the methylation state of the p16 gene, and possibly other critical growth regulatory genes. If this hypothesis is correct, it may provide a mechanistic explanation for radiation-induced persistent phenotypic changes that are not associated with specific mutational events. The studies will lead to the development of a better scientific basis for understanding exposures and risks to humans from low doses of ionizing radiation and other environmental carcinogens.