Local tumor control following irradiation, as evidenced by clonogenic survival, is achieved through the elimination of tumor stem cells. Logically, this beneficial effect could be deleterious when normal tissue stem cells are depleted. Few studies, however, document the importance of radiation-induced premature senescence in non-hematopoietic progenitor cells. The Citrin et al. article in this issue of the Journal (1) is among the first to reveal the biological significance of this process in epithelial precursor cells. Using molecular array and histochemical techniques, Citrin et al. found that alveolar epithelial type II (AECII) cells from irradiated C57BL/6NCr mice underwent dose-dependent senescence. Apoptosis was not dependent on dose or correlated with toxicity. The senescence increase was seen even when the number of AECII cells recovered. They propose that oxidative stress induces senescence and that depopulation of AECII progenitor cells leads to pulmonary inflammation and fibrosis.

Pathologists, as summarized by Fajardo et al., describe radiation injury as accelerated aging with persistent fibrosing inflammation and depopulation of epithelial cells (2). Chronic oxidative stress, such as proliferation and premature maturation of fibroblasts, is commonly assumed to be the stimulus for inflammation and a cause of this injury. Radiation-induced cellular damage has been demonized as the primary mechanism causing pulmonary injury, including endothelial cell apoptosis and fibroblast proliferation. Ultimately, the accumulation of mature fibroblasts produces excess collagen and causes downstream tissue damage and inflammation. Pathological studies regarding activated transforming growth factor beta (TGF-β), interleukin-1 alpha (IL-1α), and interleukin 6 (IL-6) support the notion of an inflammatory damage mechanism (3,4). In the present investigation, gene expression studies were performed in vitro, which remains the gold standard for identifying in vivo specimens (8). The clonogenic assays, however, were performed with whole tissue extracts; therefore, they do not distinguish the mature pneumocytes from AECII cells, endothelial cells, fibroblasts, or AECII cells. However, the histological studies show that the fates of fibrosis and AECII cells are reciprocal, and the co-culture studies show that irradiation of AECII cells results in a lasting fibrogenic phenotype. These studies contribute to the discussion regarding the interaction between oxidative stress, inflammation, cytokine cascades, and now senescence.

Citrin et al. are the first to illustrate that gene expression for senescence increases rapidly and remains high long after irradiation and in concert with fibrosis. Supporting the authors’ observation, the anti-inflammatory and antifibrotic role of exogenous stem cells is increasingly being recognized as having clinical applications for the mitigation of autoimmune and possibly radiation-related fibrotic disease. Ra et al. show that ex vivo–expanded autologous mesenchymal stem cells acquired from patients with autoimmune tissue damage can be injected to reduce inflammation and autoimmune injury (5). The role of exogenous AECII delivery in radiation-induced pulmonary fibrosis (RIPF) requires further evaluation. One might expect that transplants using maturing AECII cells acquired from irradiated lungs would not neutralize the effects of radiation, whereas normal AECII cells might mitigate radiation-induced damage.

The C57Bl/6 murine strain is known for its naturally high propensity for late radiation-induced fibrosis, whereas strains with reduced TH1 cytokine profiles (eg, C3H/HeJ) experience pneumonitis and lung dysfunction without fibrosis (6). Indeed, transgenic mice overexpressing IL-1α/β or TGF-β develop pulmonary fibrosis as they age, even without irradiation. It would be interesting to know if the inverse correlation of AECII populations and RIPF corresponds with fibrosis between murine strains that are prone or resistant to fibrosis.

The mechanism of the AECII effect in co-culture warrants further study. These irradiated AECII cells appear to have been altered phenotypically and are either producing a factor that promotes oxidative injury and fibrosis or, perhaps more likely, are failing to produce factors that suppress fibrosis. Determining the protein expression levels of secreted inflammatory mediators would help illuminate the impact these cells have on tissue fibrosis and redox state.

The discovery that chronic NOX inhibition mitigates radiation-induced fibrosis is important for many reasons. Most notably, this intervention was associated with improved survival of AECII cells and with reduced RIPF. Causality, however, between AECII survival after NOX inhibition with AECII senescence and pulmonary damage has not yet been shown. AECI and AECII cells provide a physical barrier, with AECI cells covering 95% of the alveolar surface. AECII cells play an important role in pulmonary homeostasis by undergoing proliferation and differentiation into AECI cells. One of the hallmarks of the type II cell is that it undergoes hyperplastic response with most pulmonary injuries, including radiation-induced damage. Perhaps the time-dependent and dose-dependent changes in AECII senescence and mRNA production after fibrogenic irradiation are an incidental observation and do not directly contribute to fibrosis. This might occur if there was selective depopulation or select proliferation of AECII or AECI cells in response to the actual cause of fibrosis.

A question naturally remains regarding the reproductive integrity of the apparently normal but irradiated AECII cells in vivo and the validity of the genetic expression patterns and senescence-associated β-galactosidase assay for identifying cells that have become senescent (7). Unfortunately, as there are no fully satisfactory assays for identifying reproductively intact stem or progenitor cells after irradiation, marker studies of irradiated tumor stem cells have led to misleading conclusions, particularly when used with in vivo specimens (8). The clonogenic assays, however, were performed in vitro, which remains the gold standard for identifying reproductively intact cells. Ultimately, we need better methods for...
identifying reproductively intact cells in tissues to fully document the maturation of AECII to senescence.

This manuscript represents the first gene expression analysis demonstrating changes in AECII senescence after irradiation and adds a whole new set of markers that can and should be used in evaluating interventions for the mitigation of radiation-induced pulmonary injury.

References

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Novel Facts About FAK: New Connections to Drug Resistance?
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Resistance to taxane chemotherapy presents a major hurdle in the treatment of recurrent ovarian cancer. The majority of epithelial ovarian cancers are sensitive to paclitaxel or docetaxel with initial treatment, but resistance ultimately develops in the majority of women. Development of a successful method to maintain susceptibility to taxane and platinum chemotherapy is an unmet clinical need. The article by Kang and colleagues in the current issue of the Journal describes a novel mechanism of taxane resistance, through FAK-dependent upregulation of YB-1, a transcription factor that in turn upregulates CD44 (1). Intriguing findings in their work include the importance of nuclear-activated p397Tyr-FAK for this resistance process, and the demonstration that nuclear colocalization of activated FAK and p102Ser-YB1 dichotomized overall survival of patients with ovarian cancer.

How might this relate to taxane resistance in ovarian cancer? The mechanism of anticancer activity of taxanes relies on their ability to bind and stabilize polymerized tubulin. Stabilization of tubulin filaments prevents spindle disassembly during mitosis, leading to catastrophic cell death. Lack of tubulin depolymerization also prevents transport of proteins within nondividing cells, a likely cause for neuropathy associated with taxane therapy. Additionally, the decrease in free tubulin leads to mitochondrial hyperpolarization and release of cytochrome c, triggering proapoptotic cascades (2,3).

Classically, resistance to taxanes has been attributed to alterations in tubulin structure preventing the binding of taxane to its target. Other potential mechanisms include imbalance in the cellular apoptotic machinery (4), increased expression of the multidrug-resistance drug efflux pumps (5,6), and increased activation of MAPK (7,8).

Class III β-tubulin overexpression in clear cell carcinoma of the ovary was shown to discriminate poor prognosis; in the same study, p-glycoprotein did not correlate with clinical outcome (9). However, β-tubulin changes have not been borne out as common mechanisms of taxane resistance in ovarian cancer; nor has MDR-1 been demonstrated as a major mechanism of chemotherapy resistance in ovarian cancer (10). When analyzed together, however, colocalization of urokinase plasminogen activator, CD44, and MDR1 together did prognosticate a poor outcome for ovarian cancer (11).

FAK is a cytoplasmic tyrosine kinase that mediates cell adhesion, migration, and survival by coupling integrins with cytoskeletal signaling cascades (12). In addition to autophosphorylation (p397Tyr), it is phosphorylated and activated by Src and Src family kinases, downstream of receptor tyrosine kinases, including c-MET/HGFR, EGFR, VEGFR, and other receptor tyrosine kinases active in ovarian cancer and the ovarian cancer microenvironment. Activation of FAK can trigger prosurvival and antiapoptotic/antianoikis cascades via its activation of the ERK (13) and the phosphatidylinositol-3’ kinase (PI3K)/AKT pathways. One of the earliest prosurvival functions of FAK was shown to be abrogation of anoikis, programmed cell death occurring in the absence of cell attachment (14), a concept key to ovarian cancer, which relies on malignant effusions as a primary mechanism of early dissemination. Relevant to the current topic, increased MET activity, which leads to FAK phosphorylation, can induce taxane resistance in ovarian cancer (15). FAK activation stimulates the ERK/MEK pathway. Similarly, transient activation of ERK downstream of paclitaxel mediates resistance, and inhibition of ERK in ovarian cancer cells abrogates resistance (7,8).


Notes
The authors have no conflicts of interest to declare.

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