16. Johnson et al. is based on the idea of using pharmacological inhibitors of cyclin-dependent kinases (CDKs) to convert normal cells into a radioresistant state by inducing reversible cell cycle arrest at the G1/S transition. The evidence indicates that this approach is likely to be specific for protection of normal cells and may, therefore, have clinical potential as an adjuvant in anticancer therapies.

Importance and challenges of clinical radioprotection

While radiotherapy is a powerful anticancer treatment approach, it is associated with severe side effects that drastically limit its therapeutic capacity. Two of the most radiosensitive tissues — the hematopoietic (HP) system and the gastrointestinal (GI) tract — provide the biggest clinical challenges. The outcome of radiotherapy would be substantially improved if its therapeutic index could be increased by selective reduction of damage to the HP and GI systems, without an increase in the radiosensitivity of tumors. There is evidence indicating that increasing the cumulative radiation dose by as little as 10%–20% might make a difference between incomplete and complete eradication of tumors, as shown, for example, for head and neck cancer (1). In order to allow for increased radiotherapy doses, pharmacological approaches to radioprotection of normal tissues must be developed. These approaches must be strictly selective for healthy tissues and must be safe (i.e., must not promote the development of radiation-induced secondary cancers).

Since the killing of cells by radiation is mediated by the ionization of irradiated matter, the most well-characterized principle of radioprotection involves using antioxidants, which act as scavengers of reactive oxygen species. The only drug currently approved for clinical use to protect against the toxicity of radiotherapy, amifostine, works in this way (2). However, clinical use of amifostine is complicated by its own toxicity and lack of sufficient selectivity in protection of normal versus tumor cells (2).

Targeting apoptotic mechanisms for radioprotection: p53 inhibitors

In the 1990s, it became clear that the massive cell loss that occurs in radiosensitive tissues and embryos after irradiation and leads to lethality is not due to irreversible damage of cells but rather to activation of apoptosis. This apoptosis is largely p53 dependent, and p53-deficient mice are resistant to doses of radiation that kill wild-type mice by inducing lethal HP acute radiation syndrome (3). Importantly, apoptotic mechanisms are frequently inactivated in tumors, as part of their progression toward unconstrained growth (4). These findings presented an attractive opportunity for the development of pharmacological inhibitors of p53 capable of providing reversible radioprotection by temporarily blocking p53-mediated apoptosis. The feasibility of this idea was proven by isolation of small molecule p53 inhibitors, named pifithrins, that demonstrated radioprotective efficacy in mice (5, 6). Since p53-dependent apoptosis is considered a major component of p53 tumor suppressor activity, concerns regarding the safety of pharmacological p53 inhibition have slowed down clinical developments based on this approach.

Radioprotection: smart games with death

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However, it has now been well documented, using both pharmacologic (7) and genetic (8) approaches, that temporary and reversible suppression of p53, resulting in massive rescue of cells in radiosensitive tissues, is not associated with an increase in carcinogenicity. Thus, p53 inhibitors are expected to emerge as p53 inhibitors that mimic a different tumor-specific mechanism of cell survival: activation of the prosurvival NF-kB pathway. Being a major mediator of immune responses, NF-kB is generally inactive in healthy cells under normal conditions, but it is constitutively activated in most tumors, presumably as part of their survival strategy (13). Among other targets, NF-kB drives expression of inflammatory cytokines, which have long been recognized to have radioprotective power (14); however, unacceptable toxicity has prevented their clinical development as radioprotectants. Recently, however, an NF-kB–activating approach to radioprotection has been proven feasible by the demonstration that bacterial flagellin, which is an agonist of TLR5 and a natural NF-kB–activating agent, has outstanding radioprotective properties in mice and primaries (15). Pharmacologic, constitutive engagement of NF-kB activity is highly specific for protection of normal cells in mouse tumor models (15). Like p53 inhibitors, use of NF-kB activators in combination with radiation is safe, in terms of lack of stimulation of radiation-induced carcinogenicity (15).

Both p53 inhibitors and NF-kB activators are currently in clinical development as tissue protectants for a variety of applications (Cleveland BioLabs Inc., www.cbiolabs.com/scientific_founda tion_technol.php and Quark Pharmaceuticals, http://www.quarkpharma.com/qbi-en/products/qpi-1002/). Another radioprotection concept, described in this issue by Johnson et al. (16), may open a new avenue for the development of radio- protectants. This concept is based upon the use of an emerging class of anticancer agents, inhibitors of cyclin-dependent kinases (CDKs), to target the cell cycle control machinery (Figure 1).

**Quiescence as a safe harbor: radioprotection via reversible cell cycle arrest**

The cytotoxicity of DNA-damaging agents, such as ionizing radiation, is cell cycle dependent (17). Cells are generally more sensitive at mitosis, when the chromatin is subject to strong torsional stresses and when DNA breaks have almost no chance of being repaired before chromosome segregation. Cells in early G1 and late S phase are relatively radioresistant, whereas the G1/S transition and G2/M phase are relatively radiosensitive. These observations provided the rationale for Johnson et al. (16) to test cell cycle–targeting agents as potential radioprotectants (Figure 1).

The inhibitor of kinase 4/alternative reading frame (INK4a/ARF) locus encodes proteins that act as negative regulators of the cell cycle by inhibiting particular CDKs to cause cell cycle arrest at specific points of the cell cycle. Analysis of human malignancies has shown that the CDK–cyclin D/INK4/pRb/E2F pathway is abnormal in most cancers. Deregulation of CDK4/6 activity, either through overexpression of D-type cyclins or through loss of INK4 proteins, almost invariably leads to hyperproliferation and eventually to tumor development (18). Given their critical role in cell cycle control, CDKs have been actively considered as targets for anticancer therapy. A number of CDK inhibitors (CDKIs), with different mechanisms of action targeting regulatory pathways implicated in CDK function, have been evaluated in laboratory experiments and in human trials (19).

Johnson et al. (16) tested the effect of previously identified CDKIs that control the transition from G1 to S phase in ionizing radiation–induced cell toxicity. They confirmed that inhibitors specific for CDK4/6, specifically PD0332991 and 2BrIC, caused reversible G1 arrest (pharmacological quiescence) exclusively in Rb-positive and not Rb-deficient human cells. In mice, oral administration of PD0332991 resulted in reversible inhibition of proliferation of different populations of bone marrow cells.
The least differentiated cells (HP stem cells, multipotent progenitor cells, and common myeloid progenitors) appeared to be more dependent on CDK4/6 than did the more differentiated bone marrow elements (granulocyte-monocyte progenitors and megakaryocyte-erythroid progenitors). Consistent with earlier reports (20), cells at the final stages of differentiation were found to be resistant to CDK4/6 inhibition (16).

The PD0332991-induced quiescence of bone marrow progenitor cells observed by Johnson and colleagues was accompanied by radioresistance (16). When administered to mice 4 hours before exposure to a radiation dose sufficient to induce lethal HP syndrome, PD0332991 protected the mice from death. In this scenario, PD0332991 had a beneficial effect on the recovery of all peripheral blood lineages: platelets, erythrocytes, myeloid cells, and peripheral blood lymphocytes. Moreover, no evidence of myeloproliferative disorder or myelodysplasia was found in animals of long-term surviving cohorts. These data indicate that the mechanism of PD0332991-mediated radioprotection is apparently safe and does not exacerbate the late hematological toxicity associated with high-dose total body irradiation.

**Why do CDK4/6 inhibitors protect normal cells but not tumor cells?**

It is expected that CDK4/6 inhibitors should not protect Rb-deficient cells, as they are CDK4/6 independent. Consistent with this, Johnson et al. showed that PD0332991 did not protect CDK4/6-independent tumors against radiotherapy by studying mice that develop autochthonous melanomas, due to melanocyte-specific expression of mutant H-Ras (16). However, this does not mean that radioprotection by CDK4/6 inhibitors should be limited to patients with Rb-deficient tumors. On the contrary, these agents were originally considered as prospective anticancer drugs against tumors “addicted” to the high Rb-suppressive activity of CDK4/6 (Figure 1), a common property of a large proportion of tumors (19). As opposed to normal cells, in which pharmacological inhibition of CDK4/6 results in reversible quiescence, presumably converting them into radiosensitive state, the response of tumor cells to these agents is frequently irreversible and involves the induction of senescence or apoptosis (Figure 2). It has been demonstrated that temporary overexpression of the natural CDK2 inhibitor p21 resulted in establishment of senescence in some human tumor cell lines, while cell cycle arrest remained reversible in normal diploid fibroblasts (21). The choice between senescence and quiescence in response to CDKI treatment is determined in part by p53 activity, specifically, the ability of p53 to serve as an inhibitor of the mTOR pathway (21). Hence, tumor cell radioprotection by PD0332991 or functionally similar compounds is expected to occur only in a minor subset of tumors that retain both Rb and p53 function, including the ability of p53 to negatively regulate mTOR (Figure 2).

**Puzzles and perspectives**

Johnson et al. interpret the radioprotection provided by CDK4/6 inhibitors as a function of cellular quiescence. This is a sound hypothesis but is currently supported only by correlative evidence. Since HP cell death following irradiation occurs primarily through apoptosis, this hypothesis suggests a link between quiescence and resistance to apoptosis, something that has not been previously reported for bone marrow HP precursor cells and that is definitely not true for thymocytes, splenocytes, and other radiosensitive mature HP cells. Hence, the exact mechanisms translating quiescence into radioresistance of HP cells remain to be elucidated.

Johnson et al. found that PD0332991 markedly enhanced the survival of mice, when applied as late as 20 hours after total body irradiation (16); thus, it acted as a radiomitigator. If this rescuing effect is indeed explained by drug-induced quiescence, it would suggest that premature entrance of HP progenitors into the cell cycle following DNA damage could be a lethal event for irradiated HP progenitors and contribute to HP acute radiation syndrome. This is a testable hypothesis that remains to be verified. Other possible explanations for PD0332991-mediated radiomitigation include involvement of direct and indirect effects of the compound on HP progenitors or bone marrow stroma.

Another unknown is the tissue specificity of the reported effects. If PD0332991 is found to be protective/mitigative not only against the HP component of acute radiosensitivity but also against the higher doses of ionizing radiation that induce severe GI damage, the potential clinical impact of CDK4/6 inhibitors would be greatly enhanced, with a broader scope of potential applications as radiotherapy and chemotherapy adjuvants as well as biodefense drugs.

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Autophagy is a tightly regulated catabolic process whereby cells degrade their constituents to dispose of unwanted cytoplasmic elements and recycle nutrients for cellular remodeling. Studies of autophagy in mammals have elicited substantial interest because it is linked to a range of physiologic and pathologic states. In this issue of the JCI, Maríño et al. uncover a role for autophagy in a balance disorder related to inner ear pathologies. Mice lacking the protease autophagy-related 4B (Atg4B, also known as autophagin-1) exhibited a systemic reduction in autophagy and showed defects in the development of otocoria, organic particles that contain calcium carbonate crystals and proteins that are essential for balance perception (equilibriception) in mammals. The intriguing aspect of this work is that an autophagy block impairs the secretion and assembly of otocorial proteins, emphasizing a role for autophagy in functions distinct from macromolecule degradation.

The physiological importance of autophagy
Autophagy, a tightly regulated process by which cells consume unwanted cytoplasmic macromolecular constituents and recycle nutrients for cellular remodeling, is mediated by the coordinated activity of autophagy-specific (ATG) genes. There are several forms of autophagy, but here we focus on the best-characterized form, macroautophagy (referred to herein as "autophagy"). During this evolutionarily conserved process, a double membrane known as the isolation membrane wraps around portions of the cytoplasm to form a double-membrane vesicle, the autophago-some. The engulfed cargo, including organelles, is degraded upon autophagosome fusion with late endosomes or lysosomes.

Autophagy plays a central role in cancer, neurodegeneration, innate immunity, organellar clearance, the organismal response to starvation, and aging (1). Genetic variations in the autophagy-related genes autophagy-related 16–like 1 (ATG16L1) and immunity-related GTPase family M (IRGM) are linked to susceptibility to Crohn disease, a human chronic inflammatory disorder affecting the gastrointestinal tract (2, 3). Mice exhibit basal and induced forms of autophagy, both of which play important physiological roles (4, 5). The former is involved in the homeostasis of cellular constituents, including organelles, and the degradation of long-lived proteins and protein aggregates (4, 5). However, autophagy can also be induced by physiologic and pathologic conditions, such as nutrient starvation and infection by pathogens (4, 5). Consequently, it should not be surprising that mice incapable of autophagy die perinatally, revealing the necessity of autophagy in surviving neonatal starvation (i.e., after the transplacental nutrient supply is stopped), autophagy becomes essential for maintaining the amino acid supply; ref. 6).

Tissue-specific knockouts of autophagy genes have also revealed diverse pathologies. These include severe hepatomegaly and hepatic hypertrophy caused by liver-specific deficiency of autophagy-related 7 (Atg7) (5); behavioral problems, as indicated by abnormal limb claspings, reduced coordinated movement, and neuronal loss, caused by either Atg5 or Atg7 deficiency in the central nervous system (7); the degeneration of islet cells, reduced glucose tolerance, and insulin secretion caused by Atg7 deficiency in pancreatic β cells (8, 9); and the abnormal morphology and function of intestinal Paneth cells caused by deficiency in either Atg16L1 or Atg5 (10). These studies demonstrate that the physiological phenotypes are caused not only by the loss of

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