EDITORIAL

Potential for new medical countermeasures for radiation injury by targeting the Hedgehog signaling pathway

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Ionizing radiation (IR) induces apoptosis in many cell types in a dose-dependent fashion. Of all cells of the body, the Sertoli cells of the testes are the most sensitive to IR-induced apoptosis and necrosis. Hematopoietic elements are also highly impacted by radiation effects. For example, the rate of reduction in the absolute lymphocyte count following exposure to IR correlates so well with cumulative radiation dose and dose rate that this decline is commonly used as a clinical surrogate marker for whole-body radiation dose.^{1,2} Hematopoietic progenitor cells are also reduced after exposure to IR with a Do (the dose at which survival is reduced to e^{-1} or 0.37 of its previous value on the exponential portion of the survival curve) of 1.18 ± 0.24 Gy for human circulating progenitors at a dose rate of 0.8 Gy/min, and 1.02 ± 0.05 Gy for human BM-derived colony-forming unit (CFU)-GM at a dose rate of 2.0 Gy/min.^{3,4} This pro-apoptotic effect leads to impaired myeloid differentiation and resultant neutropenia. Together with radiation-induced lymphopenia, impaired lymphokine gene expression and bacterial translocation across disrupted cutaneous and gastrointestinal mucosal barriers, neutropenia predisposes to fatal infections in irradiated humans.^{1,2,5}

Hematopoietic cytokines have been used to mitigate the effect of radiation on hematopoietic progenitor/stem cell populations and attendant cytopenias. A review of the quality of evidence supporting this practice for management of the hematopoietic syndrome (defined as the development of one or more cytopenias in the setting of whole-body or significant partial-body acute radiation exposure) was undertaken by a panel of experts assembled in Geneva by the World Health Organization.⁶ Using the Grading of Recommendations Assessment Development and Evaluation (GRADE) system, data were abstracted from English-language articles identified in MEDLINE and PubMed. Although no randomized controlled trials of cytokines have been (or will likely be) conducted in irradiated humans, the quality of the evidence was found to be consistent with a beneficial effect on overall survival with a particular benefit in patients with exposure doses of <5 Gy, and the only likely critical organ failure is the hematopoietic system. On the basis of this analysis, a strong recommendation was made for the use of G-CSF or GM-CSF when the ANC is $<500 \times 10^9$ cells/L.⁶

In May 2013, this recommendation, together with newly published results showing efficacy of G-CSF in irradiated macaques,⁷ was considered at a joint meeting of two subcommittees of the US Food and Drug Administration (FDA). The Medical Imaging Drugs Advisory Committee and the Oncologic Drugs Advisory Committee recommended that the FDA add radiation injury to the list of approved indications for this class of myeloid cytokines. Nevertheless, the Geneva consultancy recognized that new medical countermeasures are needed to prevent or enhance the recovery from radiation-induced myelosuppression, reduce infection rate and improve survival.⁵

Hedgehog (hh) was first reported in 1980 as a gene that regulates the pattern of the embryonic cuticle of *Drosophila*.⁸ The sonic hh (Shh), Indian hh and desert hh genes were subsequently discovered in vertebrates. As recently reviewed by Jiang and Hui, humans were found to have a hh counterpart that is important for regulating tissue pattern formation and cell growth control.⁹ Hh signaling has been implicated as a requisite for stem cell maintenance as well as for tissue repair and regeneration in adults.¹⁰ The hh/GLI signaling pathway is involved in the regulation of stem cell activation and self-renewal,¹¹ whereas errant activation of this pathway has been reported to contribute to the development of hematological malignancies (reviewed in Aberger *et al.*¹²) In addition to the Wnt and Notch signaling pathways, the Shh pathway has been implicated in the regulation of hematopoiesis, exerting an effect on the hematopoietic microenvironment through regulation of bone morphogenetic protein.^{13,14}

In the February 2014 issue of *BMT*, Drouet *et al.*¹⁵ exploit the impact of Shh on the hematopoietic microenvironment to identify a new potential category of medical countermeasures for IR-induced myelosuppression. When irradiated nonhuman primates were given intraosseous porcine adipocyte-derived stem cells (ASCs) that underwent Shh gene transfer, the duration of absolute neutropenia, anemia and thrombocytopenia was significantly reduced, relative to that observed in irradiated nonhuman primates receiving mock-transfected ASCs.

Although the results are significant, the number of rhesus monkeys in test ($n = 4$), sham treated ($n = 4$, two of which expired from radiation toxicity and were not evaluable for the hematological end points of this study) and control (administered no ASCs, $n = 2$, 1 of which expired) groups is small, raising the need for additional studies. Unfortunately, the nonhuman primate facility at the Institut de Recherche Biomedicale des Armees is slated for closure, leaving only a few research facilities wherein such studies can be performed in the future. Nevertheless, the intriguing possibility remains that chemicals or agents that alter or regulate sites in the hh/GLI signaling pathway of accessory cells may ultimately be identified as potential medical countermeasures for mitigation of radiation injury to the hematopoietic system.

Precisely how accessory cells in the microenvironment modulate the survival and growth of hematopoietic stem cells (HSCs) is unknown. The restrictive tissue microenvironment in which dormant HSCs reside is referred to as a niche.¹⁶ Two separate but not necessarily exclusive hypotheses suggest a role for osteoblasts located at the interface of marrow and bone or the endosteum (that is, the osteoblastic niche) and endothelial cells located at venous sinusoids where cells transit from the marrow to the circulation (that is, the vascular niche). Direct physical contact or soluble factors may mediate signaling from accessory cells to HSCs.^{17–22} In addition, signals may be trafficked both within the local microenvironment and throughout the circulation via plasma membrane-derived vesicles (more recently referred to as ‘microvesicles’ or ‘exosomes’) that are exfoliated in a directed, energy-dependent process from structurally distinct regions of the surface of accessory cells.^{23–27} We have shown that shed microvesicles carry cytokines, pro-apoptotic molecules (that

are upregulated by exposure to IR) and MHC Ags on their surface, and that these regulatory molecules are enriched, compared with the activity expressed on parent membranes from which they arise.^{28–31} Recently, microvesicles have been shown to contain intravesicular microRNA whose biological significance is under investigation.^{32,33} As Shh has been found to upregulate angiogenic growth factors,³⁴ it is tempting to speculate that its mechanism of action in hematopoiesis involves these intercellular signaling pathways within the vascular niche.

Hh is also involved in regulating the output from human pluripotent stem cells (iPS) to produce hematopoietic cells. Using human embryonic stem cell lines, McIntyre *et al.*³⁵ showed that transient GLI3-mediated inhibition of hh with chemical- and gene-targeting-mediated inactivation of hh signaling resulted in initiation of the transition from embryonic to adult hematopoiesis. Although hh inhibition increased the total number of hematoendothelial progenitors, the potential of individual CFU-derived progeny from hematopoietic lineage progenitors was unchanged, consistent with an effect that is primarily directed at early-lineage fate decisions. Using induced human iPS, Amabile *et al.*³⁶ generated teratomas in immunodeficient mice from which transplantable human hematopoietic cells develop, indicating for the first time that hematopoietic stem cells can be derived from human iPS cells. When iPS cells were co-transplanted with stromal cells, the number of CD34 + CD45 + cells was significantly increased in teratomas, suggesting that it is possible to modulate hematopoietic differentiation with accessory cells that are known to have a supportive role in hematopoiesis.

In theory, the promise of a personalized approach to medical care using patient-derived stem cells may be extended to individuals who are exposed to IR.³⁷ For example, autologous iPS cells may be collected from somatic cells of individuals exposed to a high radiation dose, treated with a chemical that alters hh in favor of hematopoietic differentiation and returned to the exposed individual. Substantial hurdles must be overcome first, ranging from the consequences of random integration of viruses used to transduce somatic cells, including virus reactivation and stochastic activation of endogenous genes that predispose to human disease such as cancer, to malignant transformation of the transplanted cells, retention of genetic 'memory' from the somatic cell of origin and immune rejection.^{37,38} However, it may be feasible one day to manipulate hh in human iPS cells. Application of this technology to a large animal model may uncover yet additional methods to mitigate radiation injury to the hematopoietic system via hh with the ultimate goal of application to humans.

Translating promising molecular observations to clinical care requires assessment in large animals. To this end, governments should support nonhuman primate facilities that may be used to conduct mechanistic and efficacy studies of potential medical countermeasures in the setting of radiation exposure. Although the provocative findings reported by Drouet *et al.* require additional study, they raise the possibility that manipulation of hh in accessory cells of the marrow microenvironment, stem cells and/or hematopoietic lineage progenitor cells may one day yield a new class of medical countermeasures for the prevention and/or treatment of the hematopoietic syndrome resulting from radiation exposure.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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