SLUGging away at cell death

Programmed cell death (PCD) plays an important role in normal and malignant hematopoiesis. In this issue of *Cancer Cell*, Inoue et al. (2002) demonstrate that the CED-1 homolog, Slug, is a key regulator of apoptosis in the response of early hematopoietic progenitors to γ radiation.

Blood cells are generated in the bone marrow through a process called hematopoiesis by which the host continuously maintains adequate numbers of terminally differentiated cells of different lineages. Hematopoiesis is arranged as a hierarchy whereby the mature elements of the hematopoietic system are derived in a clonal fashion from hematopoietic stem cells (HSC). The stem cell compartment consists of long-term reconstituting HSCs (LT-HSC), which alone possess the capacity for self-renewal as well as the capacity to form all elements of the mature hematopoietic system for the life of the animal, and short-term reconstituting HSCs (ST-HSC), which can only briefly form blood cells (Figure 1). The bone marrow has the ability to markedly increase the production of blood cells to compensate for hematological stresses such as blood loss and infection. The need to continuously vary production of different types of lympho-hematopoietic cells necessitates strict control over relevant committed progenitor cell numbers, and programmed cell death (PCD) plays a key role in this homeostasis (Wickremasinghe and Hoffbrand, 1999). However, the dose-limiting toxicity of chemotherapy or γ radiation is the induction of PCD in hematopoietic progenitor and/or stem cells leading to anemia, bleeding, and infections.

PCD is executed by the activation of a cascade of proteases called caspases that irreversibly cause the cell to undergo apoptosis. There are two major apoptosis pathways, the death receptor (caspase 8- or 10-dependent) pathway and the intrinsic death (mitochondrial, caspase 9-dependent) pathway. After activation of one of the cell death pathways, the effector caspases (caspases 3, 6, and 7) then execute the programmed death of the cell. The former pathway is triggered by activation of death receptors of the tumor necrosis receptor (TNF-R) family such as Fas, while the latter is triggered by cell stresses such as γ radiation. The mitochondrial death machinery can be mediated by proapoptotic members of the *bcl-2* family such as *bax* and can be inhibited by antiapoptotic members of the *bcl-2* family or by members of the IAP family (Hengartner, 2000). Thus, the decision to live or die after an insult is often determined by the balance of pro- and antiapoptotic proteins in a cell.

PCD also protects cells from malignant transformation. In the event of severe DNA damage, apoptosis prevents the survival of cells that have accumulated potentially cancer-inducing mutations. Furthermore, many of the events that lead the deregulation of cellular proliferation also trigger apoptosis. Not surprisingly, many of the mutations that lead to malignant transformation occur in genes that regulate PCD. The fusion protein, E2A-HLF, found in a rare

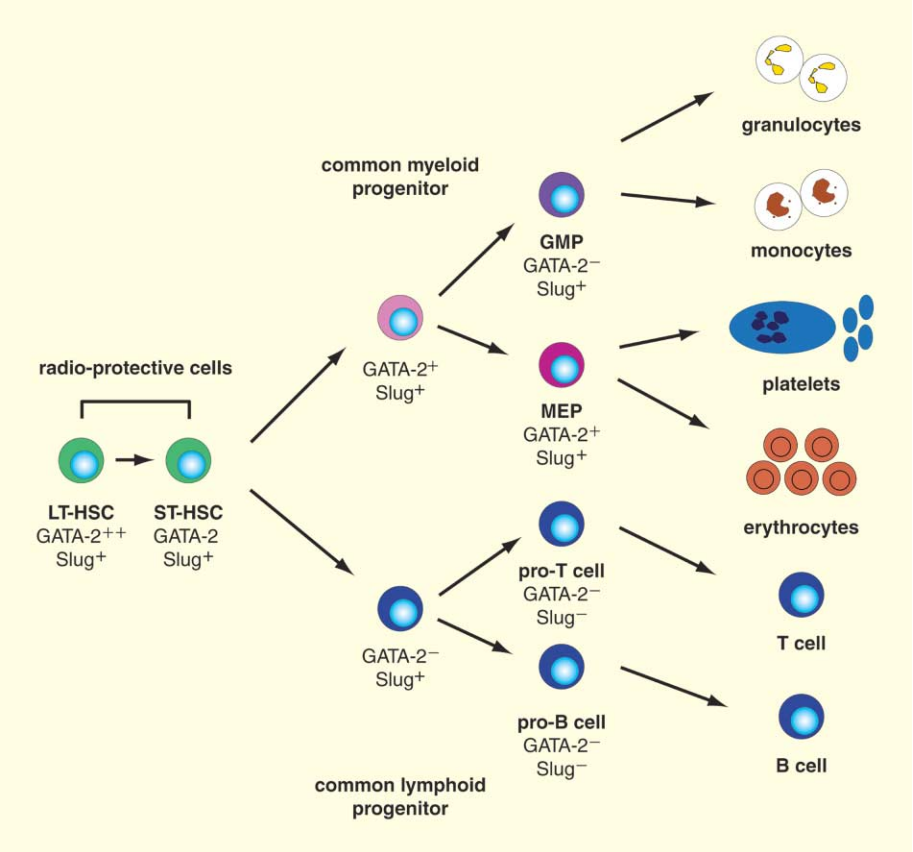

Figure 1. Proposed model for normal hematopoietic maturation in the adult mouse

In this model, the population of normal hematopoietic cells capable of multilineage reconstitution of a lethally irradiated host is strictly contained within two identifiable and separate population of cells: the long-term hematopoietic stem cell and the short-term hematopoietic stem cell. Using flow cytometry, Inoue et al. isolated enriched populations of LT-HSCs and ST-HSCs as well as populations of committed progenitors. They then examined the expression of *Slug*, as well as GATA-2, in 2000 cell aliquots using RT-PCR with primers specific for *Slug* and GATA-2. As shown here, they demonstrated the expression of *Slug* in both LT-HSC and ST-HSC as well as the committed progenitors of the myeloid series. *Slug* was not detected in beyond the earliest identifiable lymphoid progenitor, CLP, or in terminally differentiated peripheral blood elements.

form of chemotherapy-resistant B cell ALL, contains the N-terminal domains of E2A fused with the C-terminal basic region and leucine zipper domain of HLF. E2A-HLF retains both the DNA binding capacity of HLF and transcriptional activation domains of E2A. In *C. elegans,* CES-2 has a DNA binding domain homologous to that of HLF and has been shown to be involved in the regulation of neuronal apoptosis.

In 1999, Inukai et al. identified *Slug* as a downstream target of the E2A-HLF (Inukai et al., 1999). By amino acid analysis, SLUG is a homolog of CES-1, the downstream target of CES-2 in *C. elegans*. Since CES-1 and CES-2 regulate apoptosis in the worm, the capacity of *Slug* to inhibit apoptosis following growth factor withdrawal was tested. IL-3-dependent BAF3 cells were infected with a retrovirus that expressed the murine *Slug*. Following withdrawal of IL-3, BAF-3 cells expressing *Slug* were shown to have a prolonged survival with the antiapoptotic activity of *Slug* approaching that of Bcl-2 and Bcl-x_L.

In the current paper, Inoue et al. examine the effects of *Slug* in normal hematopoiesis. *Slug*$^{-/-}$ mice were viable and demonstrated only mild growth retardation as well as eyelid deformities. Examination of the peripheral blood compartment demonstrated equivalent numbers of mature lympho-hematopoietic cells, but there was a 2-fold increase in the number of hematopoietic colony-forming progenitors compared to wild-type litter mates. Since colony scoring was performed relatively early post culture, days 3 and 7, there may have been a bias toward later progenitors. The authors hypothesize that the increased colony formation in *Slug*$^{-/-}$ is likely due to expansion of the progenitor pools to maintain normal levels of mature blood cells in the peripheral blood. Analysis of the numbers of specific progenitor cells by flow cytometry will be needed for confirmation (Morrison et al., 1995; Akashi et al., 2000a, 2000b).

In *C. elegans*, CES-2 and CES-1, the orthologs of HLF and Slug, are involved in the developmentally regulated cell death of two serotonergic neurons. In this system, CES-1 represses the expression of downstream genes required for cell death signaling and is regulated by CES-2. To examine the role of *Slug* in vertebrate cellular responses to DNA damage, the authors then studied the effects of γ radiation on the hematopoietic system of *Slug*$^{-/-}$ mice. Wild-type mice exposed to 8 Gy of total body irradiation demonstrated a survival rate of 50%; in contrast, all *Slug*$^{-/-}$ mice died following this dose of radiation. Studies of the peripheral blood of moribund mice as well as pathologic studies of the *Slug*$^{-/-}$ mice post radiation suggested bone marrow aplasia as the likely cause of mortality. This conclusion was strengthened by the observation that a single dose of the hematopoietic growth factor Peg-rmMGDF protected *Slug*$^{-/-}$ mice against γ radiation-induced cell death.

In thymocytes, γ radiation-induced cell death has been shown to be p53 dependent. The authors therefore analyzed the radiosensitivity of bone marrow cells and thymocytes of *Slug*$^{-/-}$ mice. In contrast to the bone marrow cells, the thymocytes were equally sensitive to γ irradiation as wild-type littermates. Furthermore, there was no difference in the expression of p53 in either cell type in *Slug*$^{-/-}$ and wild-type mice after irradiation. These studies suggest that a key function of *Slug* is the protection of hematopoietic progenitors against cell death following DNA damage via a mechanism other than p53.

To begin to understand which cells are protected by *Slug*, TUNEL assays were done using Lineage$^+$ as well as Lineage-depleted bone marrow cells from *Slug*$^{-/-}$ and wild-type mice following γ irradiation. Compared to wild-type littermates, *Slug*$^{-/-}$ mice had increased levels of TUNEL$^+$ cells (56.8% $\pm$ 9.7% versus 30.3% $\pm$ 12.5%). By TUNEL assay, lineage-positive cells from both sets of animals were equally resistant to γ irradiation. In addition, the expression of *Slug* was increased in the Lineage-negative fraction of wild-type mice following γ irradiation, suggesting that *Slug* is directly induced by DNA-damaging effects of γ irradiation in normal animals. Consistent with this observation, *Slug* was found to be expressed at higher levels in the stem and early progenitor cells than in more mature hematopoietic cells.

These studies strongly implicate *Slug* as an important inhibitor of apoptosis in the hematopoietic system following DNA damage via a p53-independent pathway. The rapid decline in peripheral cell counts and early deaths seen in the *Slug*$^{-/-}$ mice are consistent with a profound loss of committed progenitors by the irradiated mice (Na Nakorn et al., 2002). This is supported by the TUNEL assays. Since stem cells comprise only $\sim$1/1000 of Lineage cells, most of the large number TUNEL$^+$ cells seen in the authors' study must have been progenitor cells. While they determined that *Slug* is expressed in the HSC, its role in inhibition of HSC apoptosis following γ irradiation was not addressed in this study. Future studies will hopefully address the role of *Slug* in inhibiting apoptosis in the HSC.

The data presented are intriguing and offer insight into the mechanisms protecting hematopoietic progenitor cells from γ irradiation-induced apoptosis. Potentially as interesting would be the role of *Slug* in mediating apoptosis following other agents capable of inducing similar levels of DNA damage, i.e., chemotherapy. *Slug* was initially identified following representational difference analysis with an E2A-HLF-positive human leukemic cell line. An important clinical feature of this cytogenetic abnormality is its association with leukemias highly refractory to chemotherapy. This suggests that *Slug* may play a role in inhibiting apoptosis following exposure to other cytotoxic agents. In their initial study, Inukai et al. (1999) examined primary samples from patients with acute leukemia and determined that *Slug* expression was found only in patients possessing the E2A-HLF abnormality. This suggests that *Slug* is not a common means of developing resistance to chemotherapy in acute leukemia. More interesting, if *Slug* is important in inhibiting apoptosis following cytotoxic therapies in normal but not malignant hematopoiesis, perhaps it represents a target for therapies designed at protecting patients from the morbidities associated with their treatment.

Michael W. Becker and
Michael F. Clarke[1]

Division of Hematology and Oncology
Department of Internal Medicine
University of Michigan Medical Center
Ann Arbor, Michigan 48109
[1]E-mail: mclarke@umich.edu

Selected reading

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MMP9 potentiates pulmonary metastasis formation

Tumor cell dissemination to distant organ sites is a complex process involving multiple cell types, soluble growth factors, adhesion receptors, and tissue remodeling. A new study in this issue of *Cancer Cell* shows that MMP9-expressing tumor-associated macrophages play a key role in prepping premetastatic sites for eventual malignant cell growth in a manner dependent upon vascular endothelial growth factor receptor-1 (VEGFR-1).

Metastasis is the primary cause of morbidity and mortality for patients with cancer. While it has been recognized for many years that movement of neoplastic cells is not a random process, the molecular and cellular mechanisms governing their movement, survival through foreign tissue environments, and parameters for choosing and taking up residence at a final destination have remained uncertain. Several contrasting theories have emerged to explain metastatic specificity. The "homing" theory suggests that organs distal to sites of primary malignancy actively attract and/or arrest ("trap") malignant cells via expression of adhesion receptors, e.g., selectins, or by secretion of soluble chemotactic factors, e.g., chemokines. Indeed, there is good experimental evidence implicating chemokine receptors and their ligands for the chemoattraction aspect of the homing theory (Muller et al., 2001; Wilson and Balkwill, 2002). In addition, identification of "molecular addresses" or adhesion receptors on endothelial cells in vascular beds of distal organs that specifically trap circulating malignant cells supports the active "arrest" view of homing (Borsig et al., 2002; Laakkonen et al., 2002). In contrast, the "fertile soil" theory proposes that different organ environments provide optimal growth conditions for specific circulating cell types. Since underlying mechanisms supporting these theories are not mutually exclusive, it seems likely that distinct mechanisms/molecules might govern a malignant cells journey to an ectopic tissue, separate from those regulating its growth and/or survival once its destination has been achieved. An exciting observation by Shibuyu and colleagues (Hiratsuka et al., 2002 [this issue of *Cancer Cell*]) provides some clues into the later aspects of the metastatic cascade implicating an extracellular metalloproteinase, e.g., matrix metalloproteinase-9 (MMP9), made by tumor-associated macrophages (TAMs) and alveolar endothelial cells, in microenvironmental remodeling necessary for metastatic cell survival in the lung.

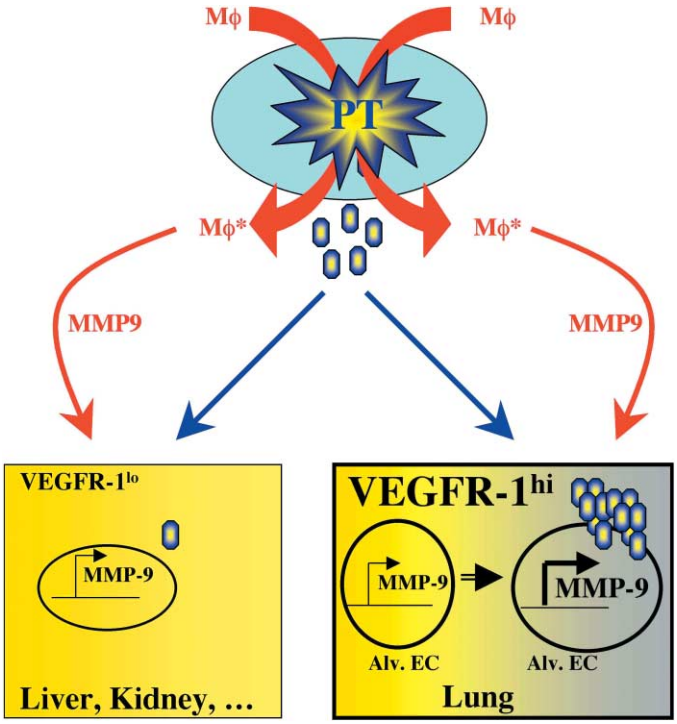

Figure 1. A model for the role of tumor-associated macrophages and MMP9 in priming premetastatic tissue

Macrophages (Mϕ) are recruited to sites of malignant growth. Upon activation by primary tumor (PT) cells, activated macrophages (Mϕ*) expressing MMP9 then reenter the circulation and induce MMP9 expression in (alveolar) endothelial cells (Alv. EC) in a VEGFR-1-dependent manner, thereby prepping premetastatic tissue for malignant cell colonization. Distal organs harboring low-level VEGFR-1 expression are not suitable environments for metastatic growth and fail to induce MMP9 expression in response to the presence of activated macrophages.

Tumor cells produce various cytokines and chemokines that attract leukocytes (macrophages, neutrophils, dendritic cells, eosinophils, and mast cells) that are variably loaded with molecules affecting primary neoplastic growth, e.g., cytokines, cytotoxic mediators, serine-, cysteine-, and metallo-proteases, membrane-perforating agents, and soluble mediators of cell killing, such as tumor necrosis factor-α (TNF-α), interleukins (IL), and interferons (IFNs) (Wilson and Balkwill, 2002). TAMs play a dual role in neoplasms. Whereas TAMs