

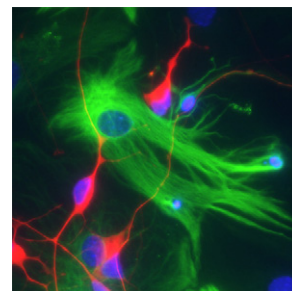
The stem cell field is gradually moving toward translational applications as exemplified by the papers highlighted in this Stem Cell Select. In vitro human embryonic stem cell (hESC) models of disease may prove useful for drug screening. Resolving whether cancer stem cells are important for the pathogenesis of all or only some human cancers should accelerate development of new stem cell-specific therapeutic strategies. Finally, visualizing how hematopoietic stem cell grafts colonize bone marrow in real time will provide insights enabling optimization of transplant protocols.

Mutant Astrocytes Stress Out Motor Neurons

The pathogenesis of the neurodegenerative disease amyotrophic lateral sclerosis (ALS) is complex. However, genetic evidence implicates mutations in the gene encoding superoxide dismutase (SOD1)—an enzyme that breaks down toxic free radicals—as contributing to the selective death of motor neurons. Animal models of ALS have shown that once the motor neurons are damaged and the disease process triggered, then disease progression depends on nonneuronal cells such as astrocytes, which are thought to be crucial mediators of mutant SOD1 toxicity. Now, the Gage and Eggan groups (Marchetto et al., 2008; Di Giorgio et al., 2008) present in vitro models of human ALS comprising motor neurons derived from hESCs cultured with astrocytes expressing either mutant or wild-type SOD1. Marchetto et al. (2008) grew hESC-derived motor neurons together with primary human astrocytes expressing either wild-type or mutant SOD1 (SOD1^{G37R}) and labeled the motor neurons using a lentivirus-Hb9::GFP construct. In their assay, Di Giorgio and colleagues generated an Hb9::GFP transgenic hESC line, which they induced to form motor neurons that were then cocultured with primary astrocytes derived from either SOD1^{G93A} transgenic or control mice. Both groups found that when cocultured with astrocytes expressing mutant SOD1 (but not normal SOD1), the number of motor neurons decreased by ~50%; coculture with fibroblasts expressing mutant SOD1 did not result in motor neuron death. Marchetto et al. then found that a greater number of mutant astrocytes were activated compared with control astrocytes. Activated astrocytes are part of the inflammatory response, and SOD1^{G37R} mutant astrocytes were found to boost production of reactive oxygen species (ROS), iNOS, NOX2, and CHGA, a neurosecretory protein that interacts specifically with mutant SOD1. These authors then screened antioxidant compounds in the coculture system and found that the NOX2 inhibitor apocynin and the antioxidant α -lipoic acid decreased ROS generation. They further demonstrated that apocynin could abrogate death of motor neurons cocultured with SOD1^{G37R} astrocytes. Di Giorgio and colleagues performed gene expression profiling to identify genes that are uniquely transcribed in astrocytes overexpressing the SOD1^{G93A} mutation. The authors found increased expression of 53 genes but assayed a subset of genes implicated in inflammation and immunity for motor neuron survival in the coculture system. Of the agents tested, prostaglandin D2 dramatically decreased motor neuron survival, and blocking the prostaglandin D2 receptor could partly rescue motor neuron loss caused by astrocytes expressing mutant SOD1. Both studies reveal that the inflammatory component of ALS pathogenesis can be specifically recapitulated in cultured motor neurons derived from hESCs and that in vitro hESC-based ALS models may be valuable for screening compounds that specifically affect motor neuron survival.

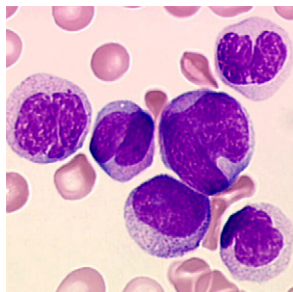
F.P. Di Giorgio et al. (2008). *Cell Stem Cell* 3, 637–648.

M.C.N. Marchetto et al. (2008). *Cell Stem Cell* 3, 649–657.



Motor neurons (red) derived from hESCs, cocultured with mouse astrocytes (green). Image courtesy of F.P. Di Giorgio.

Cancer Be Gone



Differentiation of APL cells induced by arsenic trioxide. Image courtesy of M.T. Daniel.

Cancer relapse is believed to be caused by a small number of drug-resistant cancer cells that linger behind after surgery and chemotherapy. According to the cancer stem cell (CSC) hypothesis, these cells are exclusively able to self-renew and can, in phoenix-like fashion, recreate the original tumor. There is much interest in exploring whether specific targeting of these CSCs could be exploited to treat cancer. Nasr et al. (2008) now demonstrate that a combination of retinoic acid (RA) and arsenic trioxide targets leukemia initiating cells (LICs), which are the root cause of acute promyelocytic leukemia (APL). The synergistic effect of these compounds leads to rapid remission in most APL patients, but the molecular basis of this therapeutic benefit is a matter of debate. RA activates the oncoprotein PML-RARA (the fusion protein PML-retinoic acid receptor- α), which induces terminal differentiation of leukemia cells. However, the ability of arsenic trioxide to induce differentiation of leukemia cells is limited. Meanwhile, both agents are known to trigger degradation of PML-RARA, suggesting that mechanisms other than differentiation may underlie the synergistic effects mediated by these drugs. In their study, Nasr and colleagues report that bone marrow from mice injected with

murine APL cells, and treated with RA, showed a marked reduction in the number of LICs in secondary recipients. Furthermore, RA and arsenic trioxide acted synergistically to reduce the number of LICs but did not seem to affect the differentiation of APL cells, leading the authors to conclude that LIC eradication and not differentiation is in fact the primary basis for APL remission. They also found that LIC loss was dependent on proteolysis of PML-RARA and was regulated by c-AMP-mediated phosphorylation, which could explain the synergy in vivo between RA, arsenic trioxide and phosphodiesterase inhibitors. The

authors discuss that controlling production of PML-RARA *in vivo* is crucial to prevent LIC self-renewal and hypothesize that oncoprotein degradation, if linked to CSC self-renewal in other forms of cancer, could be a new anticancer therapeutic strategy.

R. Nasr *et al.* (2008). *Nat. Med.* **14**, 1333–1342.

Cancer Stem Cells—A Numbers Game

Not all researchers agree that cancer stem cells (CSCs) are the sole drivers of tumor progression, but most agree that these tumor-forming cells are a small, distinct subpopulation of the tumor. This assumption comes from limiting dilution transplant assays where the frequency of a range of human cancer cells that form tumors after transplantation into NOD/SCID mice is exceedingly low. As a result, many believe that only rare human cancer cells have the potential to proliferate extensively and that most human cancer cells have little capacity to proliferate or to contribute to disease. Quintana *et al.* (2008) now reveal that tumor-forming cells in human melanomas in fact are neither rare nor distinct. Melanoma-initiating cells appeared to be rare when assayed under conventional conditions in NOD/SCID mice. However, when the authors altered several key assay parameters, they could boost the frequency of melanoma-forming cells by several orders of magnitude. These alterations included using as recipients a NOD/SCID $\text{Il2r}\gamma^{-/-}$ mouse strain, which is more immunocompromised than the NOD/SCID strain, enabling more efficient engraftment of human cells. Other alterations included injecting the NOD/SCID $\text{Il2r}\gamma^{-/-}$ recipient mice with melanoma cells mixed with matrigel and monitoring tumor formation in the transplanted mice for longer time periods. Using these modified conditions, the authors injected single unselected melanoma cells and reported that tumors arose in a striking 27% of the NOD/SCID $\text{Il2r}\gamma^{-/-}$ recipient mice. They then assessed whether tumor- and non-tumor-forming cells could be distinguished on the basis of differential expression of cell surface markers. Of the 50 markers surveyed, including known markers for other CSC populations, the authors found that all melanoma subpopulations injected, irrespective of their marker profiles, seemed equally capable of regrowing the parental melanoma. They concluded that cells with tumor-forming potential are common within the tumor and suggest that the frequency and heterogeneity of tumor-forming cell populations in other types of cancer may be greater than previously thought. Identifying all cancer cells that are able to propagate the disease remains an important goal, whether they are true stem cells, rare, or neither, but the new findings highlight the challenge in pinpointing which properties define those cells that actually have the potential to contribute to cancer progression.

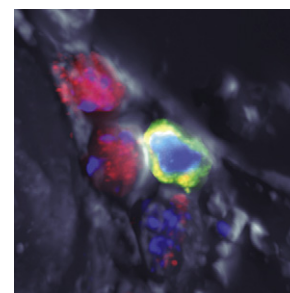
E. Quintana *et al.* (2008). *Nature* **456**, 593–598.

Blood and Bone Intertwined

Hematopoiesis is regulated by both the vascular cells and bone cells of bone marrow, but the complex interface between these cells has made it difficult to decipher the distinct roles played by each component. Two recent studies visualize the bone marrow in real time and conclude that the bone and vascular niches, rather than being distinct, are intimately associated at the endosteum (the inner surface of bone). Combining confocal microscopy and two-photon video imaging, Lo Celso *et al.* (2008) devised a way to follow the movement of hematopoietic stem and progenitor cells (HSPCs) in real time. Using the Col2.3-GFP mouse, in which osteoblasts express green fluorescent protein (GFP), the authors labeled blood vessels with quantum dots and followed the appearance of DiD-labeled HSPCs in live animals. Meanwhile, Xie *et al.* (2008) took an *ex vivo* approach, following GFP-expressing HSPCs in isolated bone sections and using immunodetection of CD31 and N-cadherin to identify blood vessels and osteoblasts, respectively. Both groups report that the endosteal surface is highly vascularized, with osteoblasts and blood vessels in intimate proximity. To study engraftment, both groups injected HSPCs into irradiated mice. Lo Celso and colleagues reported that HSPCs were found closer to the endosteal surface in irradiated compared to control mice and observed that the HSPCs divided over time on the inner bone surface. Irradiation damages blood vessels, which Lo Celso *et al.* visualized by monitoring leakage of dye. In *c-Kit*-receptor-deficient mice, which allow HSPC engraftment in the absence of irradiation, and hence with undamaged vessels, these authors showed that HSPCs still settled closer to the endosteal surface. Xie and colleagues further revealed that because the trabecular region of the bone expressed higher levels of the chemotactic factor *Sdf1* in response to irradiation, more HSPCs homed to this region rather than to compact bone. These researchers next discovered that HSPCs tended to directly contact preosteoblasts expressing N-cadherin. Lo Celso and coworkers also demonstrated that the more differentiated the HSPCs, the further away from the endosteum they were located. They comment that their imaging system cannot decipher whether HSPC engraftment and proliferation requires direct contact with osteoblasts, and Xie *et al.* note that not all HSPCs are associated with osteoblasts expressing N-cadherin. Taken together, these studies show that the endosteum is the preferential site to which HSPCs home and that HSPCs may be regulated by gradients of factors derived from osteoblasts, blood vessels, and the extracellular matrix.

C. Lo Celso *et al.* (2008). *Nature*. Published online December 3, 2008. 10.1038/nature07434.

Y. Xie *et al.* (2008). *Nature*. Published online December 3, 2008. 10.1038/nature07639.



A hematopoietic stem cell (green) attached to N-cadherin-expressing preosteoblasts (red). Image courtesy of Y. Xie.