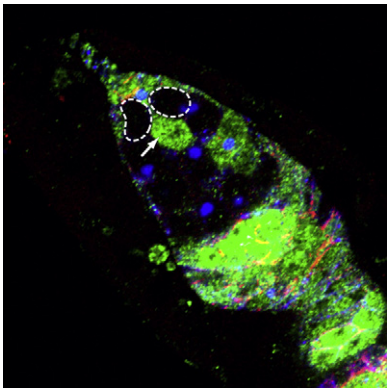
Stem Cell Select

Cell

Stem cell biology covers a wide range of topics, as exemplified by the articles in this special review issue and the papers highlighted in this Stem Cell Select. Understanding the interactions of stem cells with their surroundings—whether they be *Drosophila* germ cells, human bone-forming precursors, or mouse immune modulators—will reveal important target mechanisms that can be exploited therapeutically to promote homing of stem cells to the correct location and to modulate stem cell fate in situ. Developing methods to generate differentiated cells of a desired lineage from stem cells, such as muscle precursors, as well as identifying stem cell populations that give rise to unwanted populations, such as tumor cells, will also be key for moving the field of regenerative medicine forward. To translate stem cell biology into therapeutic action will necessitate that challenges be tackled from a range of angles using diverse animal model systems.

Jostling for Space in the Niche



A germarium tip, in which mutant cells (broken white lines) are in the process of pushing a wild-type GSC (green; white arrow) out of the niche. Image courtesy of T. Xie.

Studies of *Drosophila* germ stem cells (GSCs) have provided an essential framework for understanding how these cells interact with their niche, a model that has been applied to other stem cells in a variety of lineages. In the *Drosophila* ovarium, female GSCs exhibit E-cadherin-dependent binding to the cap cells, which constitute the cellular component of their stem cell niche. Bone morphogenetic proteins (BMPs) produced by the niche inhibit expression of the differentiation-inducing genes *bam* and *bgcn*. Indeed, GSCs unable to express either of these proteins are blocked in a stem cell-like state and have been shown to accumulate beyond points of cap cell contact. In their new study, Ting Xie and colleagues (Jin et al., 2008) have used *bam* and *bgcn* mutant flies to reveal that stem cells are, in fact, capable of competing for physical contact with their niche. By clonally marking a fraction of existing GSCs and calculating the relative loss of marked GSCs over time—which is consistent with the expected turnover of GSCs during differentiation—the authors observed that marked *bgcn*-mutant GSCs were more often retained in the niche relative to control GSCs. More dramatically, niches became increasingly occupied with marked *bam*-deficient GSCs over time, indicating that wild-type GSCs can be displaced entirely. The mechanism behind the increased competitiveness of the *bam* and *bgcn* mutant GSCs appears to involve E-cadherin, as the prolonged maintenance of *bgcn*-mutant GSCs was overcome in compound mutant flies that also lacked E-cadherin expression. Furthermore, using either marked *bam*-deficient flies, or a model of heat-inducible *bam* overexpression, E-cadherin accumulation in the GSC-cap cell junction was inversely proportional to the levels of *bam*. Indeed, overexpression of E-cadherin alone, in the absence of any *bam* mutation, was sufficient to elevate the competitiveness of GSCs for contact with the niche. The authors discuss the possibility that this competitive mechanism may offer a degree of ''quality control'' in maintaining an adequate pool of competent stem cells. In addition, the observation that altered adhesion molecule expression can result in stem cell replacement at an occupied niche offers an attractive model by which mammalian stem cells might be manipulated to promote engraftment during therapeutic transplantation.

Z. Jin et al. (2008). Cell Stem Cell ***2****, 39–49.*

Sweetening the Road Home

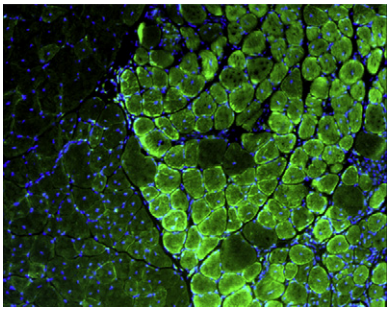
Before transplanted stem cells have an opportunity to compete for access to their niche, they must first migrate, or ''home,'' to the correct location. There is a long history of isolating bone-forming cells from cultures of bone marrow, both from mice as well as from humans. However, the use of these populations for therapeutic bone formation is limited by their inability to home to existing bone surfaces. Now, Sackstein and colleagues (2008) demonstrate that human bone marrow-derived stromal cells (also called ''mesenchymal stem cells,'' or MSCs) can be manipulated ex vivo such that they become competent to home to endosteal surfaces via the marrow and to deposit human osteoid (bone-forming material) when transplanted into murine recipients. Migration of blood cells is a well-studied process that involves the sequential interaction of adhesion molecules and chemokines with their corresponding receptors. One particularly important ligand present on marrow endothelium is E-selectin, which binds to multiple receptors, including a particular sialofucosylated glycoform of CD44 termed HCELL. The authors demonstrate that cultures of MSCs, which do not normally express HCELL, instead express high levels of a sialylated form of CD44. By developing a novel technique to modify surface glycans without impacting cell viability or function, the authors were able to generate human MSCs that expressed HCELL. These modified cells, capable of binding specifically to E-selectin in vitro, were transplanted intravenously into mice and tracked using real-time confocal microscopy of the bone

marrow cavities in the skulls of recipients. The authors demonstrate that the HCELL$^+$ MSCs home to sinusoidal vessels in the marrow and infiltrate the marrow parenchyma. Despite the HCELL modification being stable in culture for only 24 hr, examination of the MSC-injected animals revealed HCELL-dependent migration to endosteal surfaces within 10 days and subsequent bone-forming human osteoid deposits 12 weeks post-transplantation. The results of this study provide not only an important advance in the delivery of functionally relevant bone-forming cells but also open avenues to new methods for the manipulation of the migration patterns of other adoptively transferred cell populations.
R. Sackstein et al. (2008). Nat. Med. ***14****, 181–187*.

T Cells: Moths to an MSC Flame

Adherent stromal cells derived from many tissues, particularly bone marrow, can be expanded in culture and will generate differentiated progeny in response to a variety of in vitro protocols. In addition to forming bone, as in the Sackstein et al. paper, these MSC populations tend to home to sites of inflammation when transplanted. In addition, this migration often, but not always, correlates with improved regeneration/repair at the site of injury. The mechanism of action behind the observed recovery remains largely open to question; however, a growing body of evidence points to MSC-mediated immune suppression being responsible for their therapeutic effect. Recent work from Yufang Shi's laboratory (Ren et al., 2008) outlines at least one mechanism by which MSCs appear to suppress T cell-mediated immune responses. The authors used clonal murine MSC lines and combined a series of in vitro assays to demonstrate that T cell proliferation could be blocked in the presence of MSCs, provided IFN-γ and at least one of three inflammatory cytokines (TNFα, IL-1α, or IL1-β) were also present. These combinations of cytokines induced MSCs to produce nitric oxide synthase, iNOS, and resulted in production of very high levels of NO, which were required for the T cell-suppressive effect. By an independent mechanism, the same inflammatory cocktails induced cultured MSCs to secrete multiple chemokines, and the authors suggest that this effect is likely responsible for the observed migration of activated T cells towards the treated MSCs. Strikingly, using transplanted iNOS$^{-/-}$ MSCs or blocking antibodies against inflammatory cytokines in mouse models of acute, localized, or chronic systemic inflammation, the authors demonstrate that MSC-derived NO may be responsible for the T cell-suppressive effect in vivo. With this study, the authors offer important insight into the pathways responsible for the therapeutic benefit observed in some cases of MSC transplantation. With an improved handle on the mechanisms involved in MSC-mediated effects, therapies can be tailored to a given clinical situation.
G. Ren et al. (2008). Cell Stem Cell ***2****, 141–150.*

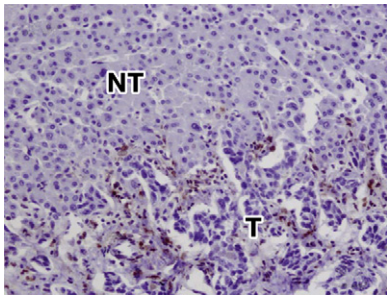
Muscling in on the Therapeutic Action



Pax3-induced ES cell-derived myogenic progenitors (green) contribute to new myofiber formation in dystrophic mice. Image courtesy of R. Darabi.

In order to harness the therapeutic potential of embryonic stem (ES) cells, many hurdles will need to be overcome. Prominent among these challenges are the generation of specific differentiated cells of interest and development of protocols with which to transfer the resulting population such that the cells engraft and contribute to the target tissue in a patient. In a new study, Darabi and colleagues (2008) demonstrate that functional skeletal muscle can be generated after the transfer of differentiated ES cells into two different mouse models of muscle injury. Historically, myogenic cells have been difficult to obtain from ES cell cultures. In this study, the authors engineered an ES cell line to carry an inducible construct controlling expression of Pax3, a transcription factor known to promote the expression of myogenic regulatory genes. By inducing the Pax3 construct with doxycycline, cultured ES cells gave rise to populations with phenotypic and gene expression profiles consistent with myogenic progenitors. However, transfer of unfractionated cultures to cardio-toxin injured muscles in murine hosts resulted in teratoma formation, indicative of residual undifferentiated cells present in the transferred cell population. By preselecting a population of PDGF$^-\alpha$R$^+$Flk-1$^-$ cells, the authors were able to obtain significant in vivo muscle generation and no tumor growth after transplantation. The authors went on to test whether these ES cell-derived myogenic progenitors could contribute functionally to muscle after engraftment. When the Pax3-induced PDGF$^-\alpha$R$^+$Flk-1$^-$ cells were injected locally or systemically into *mdx* mice (a model of human muscular dystrophy), there was a boost in the contractile properties of the dystrophic muscles. The potential to generate cells with myogenic capacity in culture and systemically transfer them to recipients is an important step towards the therapeutic application of ES cell-derived cells. Remaining challenges include improving the level of engraftment so as to achieve further functional recovery and development of a protocol to yield similar populations of human cells.
R. Darabi et al. (2008). Nat. Med. ***14****, 134–143.*

Pinpointing the Cancer Culprit



Distribution of CD90$^+$ cells (brown) in the margin between tumor and nontumor tissues. Image courtesy of Z.F. Yang.

The cancer stem cell hypothesis proposes that bulk tumor populations harbor a subfraction of cells with the potential to generate all other cell types present in the tumor, and that these so-called cancer stem cells (CSCs) are self-replicating such that they retain the capacity to generate additional tumors in a serial fashion. Whereas some studies erroneously designate populations as CSCs based only on surface marker phenotype and/or the capacity to grow in vitro, two recent papers provide ample evidence to support their claims of isolating tumor-initiating populations in human melanoma and liver cancer (Schatton et al., 2008 and Yang et al., 2008, respectively). In the first case, the Frank laboratory demonstrated that the tumor-forming capacity of human melanoma samples was highly enriched in the subpopulation expressing a protein involved in chemoresistance, ABCB5, the frequency of which in a given sample correlated with escalating clinical progression. Transplantation of purified cells led to dose-dependent tumor formation in immune-compromised mice. Serially passaged repurified primary tumor ABCB5$^+$ cells harbored tumor-initiating activity at a frequency of 1 in 120,000 cells. Dramatically, systemic injection of an ABCB5-targeted antibody into murine hosts was sufficient to significantly reduce the tumor burden of established tumors derived from primary patient samples. Meanwhile, in a related but independent study, Yang et al. (2008) performed similarly rigorous analyses of human liver cancer cell lines and primary tumor samples from patients. They discovered that the CD90$^+$ fraction contained tumor-forming potential when injected into murine hosts at limiting dilutions and could be maintained for three serial passages in vivo. In addition, the CD90$^+$ population could be further fractionated according to CD44 expression, and systemic injection of a CD44-specific antibody was sufficient to prevent the growth of both primary and metastatic liver tumors induced by purified CD90$^+$ populations. The authors also detected CD90$^+$ tumor-forming cells in patient blood samples, the frequency of which correlated with tumor burden, suggesting that screening blood samples from cancer patients might prove useful for monitoring disease progression. These two studies add to the growing body of literature suggesting that identification of tumor-initiating populations may advance the development of new and effective cancer therapies.

T. Schatton et al. (2008). Nature ***451****, 345–349.*
Z.F. Yang et al. (2008). Cancer Cell ***13****, 153–166.*

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