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Stem Cells: A Minireview

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Abstract The identification of adult-derived stem cells which maintain plasticity throughout the course of a lifetime, has transformed the field of stem cell biology. Bone marrow derived hematopoietic stem cells (HSC) are the most well-characterized population of these multipotential cells. First identified for their ability to reconstitute blood lineages and rescue lethally irradiated hosts, these cells have also been shown to differentiate and integrate into skeletal muscle, cardiac myocytes, vascular endothelium, liver, and brain tissue. Various populations of HSC are being studied, exploiting cell surface marker expression, such as Sca-1, c-kit, CD34, and lin^-; as well as the ability to efflux the vital dye Hoecsht 33342. Detection of engrafted donor derived cells into various tissue types in vivo is a laborious process and may involve detection of β-galactosidase via colorimetric reaction or antibody labeling or green fluorescent protein (GFP) via fluorescence microscopy, as well as in situ hybridization to detect the Y-chromosome. Using these techniques, the search has begun for tissue specific stem cells capable of host tissue regeneration, self renewal, and trans-differentiation. Caution is urged when interpreting these types of experiments because although they are stimulating, limitations of the technologies may provide misleading results. J. Cell. Biochem. Suppl. 38: 1–6, 2002.
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In recent years, there has been an increased interest in the biology of adult-derived stem cells. This interest has been sparked in part by the controversy surrounding the potential use of embryonic stem (ES) cells in the treatment of human diseases. Over the last few years, a substantial amount of data has accumulated, which suggests that adult-derived stem cells may have a broader differentiation potential than originally thought. If this is the case and holds true in humans, these cells could be used as a source of tissue for clinical use. In addition, adult-derived stem cells provide the potential for autologous therapies, thereby eliminating the risk of graft rejection.

Stem cells exist in most adult organs, but have only been well characterized in a few systems including bone marrow and gut. They are defined as cells that undergo asymmetric division resulting in self-renewal of the parent stem cell as well as a daughter cell capable of differentiating down specific lineages. Another long-held belief regarding stem cells is that they only differentiate into cell types associated with the tissue from which they were isolated. This tenet of stem cell biology has been called into question in recent years with the mounting body of evidence suggesting that adult stem cells exhibit a type of plasticity not previously thought to occur.

Most work on stem cell plasticity has centered on the bone marrow. Following bone marrow transplantation in mice, bone marrow-derived cells have been shown to differentiate into such diverse tissues as skeletal muscle, cardiac muscle, endothelial cells, liver, and brain

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[Ferrari et al., 1998; Shi et al., 1998; Bittner et al., 1999; Gussoni et al., 1999; Petersen et al., 1999; Brazelton et al., 2000; Mezey et al., 2000; Jackson et al., 2001]. In addition, it has been shown that following bone marrow transplantation in humans, bone marrow-derived cells can be found in liver, demonstrating that the same plasticity observed in mice may also occur in humans [Alison et al., 2000]. Plasticity has also been suggested to occur in cells isolated from other tissues including skeletal muscle and brain [Bjornson et al., 1999; Gussoni et al., 1999; Jackson et al., 1999; Pang, 2000].

PLASTICITY OF BONE MARROW-DERIVED CELLS

The first significant paper addressing stem cell plasticity was published by Ferrari et al. [1998], and demonstrated that bone marrow cells could differentiate into skeletal muscle tissue. Bone marrow was isolated from transgenic mice expressing the *lacZ* gene under a myosin light chain promoter and was transplanted into irradiated scid/bg recipient mice. Following detection of hematopoietic engraftment, the tibialis anterior muscle was injured via cardiotoxin injection. Two to three weeks post injury, β-gal positive muscle fibers were detected in the regenerating skeletal muscle. β-gal positive muscle fibers were also detected following direct injection of bone marrow into injured muscle.

Although the experiments described above clearly indicated that bone marrow-derived cells are capable of differentiating into skeletal muscle, they do not indicate which cell types are responsible for the plasticity observed. At least two stem cell populations reside within the bone marrow: hematopoietic stem cells (HSC) and mesenchymal stem cells (MSC). HSC give rise to all of the differentiated blood lineages, whereas MSC give rise to stromal cells of the bone marrow including osteogenic, chondrogenic, and adipogenic lineages. MSC have also been shown to differentiate into myoblasts, which are skeletal muscle precursors [Wakitani et al., 1995].

Gussoni et al. [1999] were the first to demonstrate the multipotential nature of HSC. Highly purified male HSC from normal donors were transplanted into lethally irradiated female *mdx* mice, a mouse model for muscular dystrophy. Four to six weeks following transplantation, muscle fibers from the hind limb of recipients were examined for dystrophin expression in conjunction with the presence of Y-chromosome. Between 1% and 10% of fibers expressed dystrophin, and a portion of these was also Y-chromosome positive. This was the first significant evidence that a hematopoietic stem cell population was able to differentiate into tissues not confined to those of the hematopoietic system.

In our laboratory, our work provides further evidence to the multipotential nature of HSC by documenting their ability to contribute to cardiomyocytes and endothelial cells in a cardiac ischemia/reperfusion injury model [Jackson et al., 2001]. In this study, HSC were isolated from Rosa26 donors, which express the *lacZ* gene in most cells, and transplanted into lethally irradiated normal recipients. Coronary artery ligation and reperfusion injury was performed following stable engraftment of the hematopoietic system. Two and four weeks after injury, cardiomyocytes and endothelial cells of donor origin were found in the peri-infarct region of the ischemic heart. Approximately 0.02% of cardiomyocytes and 3% of endothelial cells in microvessels were of donor origin.

Lagasse et al. [2000] published the most definitive study thus far on the plasticity of HSC. They compared the ability of different cell populations purified from mouse bone marrow to regenerate liver following bone marrow transplantation in a model of fatal hereditary tyrosinemia, the FAH knockout mouse. The only population of bone marrow cells capable of contributing to liver regeneration was those with a hematopoietic stem cell phenotype. These researchers demonstrated that transplantation with as few as 50 HSC rescued mice from an otherwise lethal liver defect.

In all studies described above, a population of cells, whether whole bone marrow or purified HSC, was introduced. With the possibility of contamination within a purified population of cells, it is difficult to say that these data definitively prove that HSC are the bone marrow cells which contribute to the tissue plasticity described above. Recent work by Krause et al. [2001] addressed this issue by transplanting single cells in a murine hematopoietic transplantation assay. Eleven months after transplantation, engraftment in epithelial tissues was found in all tissues examined with up to nearly 20% of lung pneumocytes of one animal being of donor origin. In contrast to most other

studies in which severe tissue injury or genetic deficiency were a requirement for non-hematopoietic engraftment, Krause et al. [2001] observed engraftment with total body irradiation alone. The long interval between transplantation and examination may have contributed to the high levels of engraftment in this situation. Indeed, they noted that the tissues exhibiting high engraftment were also those that are targets of graft-versus-host disease. Although this work suggests that single HSC are able to differentiate into multiple tissues, further studies are necessary to corroborate this finding.

PLASTICITY OF NON-BONE MARROW DERIVED STEM CELLS

The first reputed case of plasticity of a cell type outside of bone marrow was the story of turning "brain to blood". Bjornson et al. [1999] used cultured neural stem cells and clonal neural stem cell lines from Rosa26 mice as donor cells in a bone marrow transplant into sublethally irradiated recipients. Over a period of months, the neural stem cells appeared to contribute to peripheral blood lineages. This was the first demonstration that a single cell could give rise to two tissues of distinct embryonic origins. Although this data was very stimulating, corroborating evidence is still lacking.

Another intriguing case is the story of turning "muscle to blood". Three groups have reported that cell populations isolated from murine skeletal muscle could generate all cells of the peripheral blood after transplantation into lethally irradiated mice [Gussoni et al., 1999; Jackson et al., 1999; Pang, 2000]. We have shown that crude cultures of skeletal muscle cells introduced into lethally irradiated mice were able to engraft and give rise to all peripheral blood cell lineages [Jackson et al., 1999]. These cells were able to engraft recipients in secondary transplants, although to a lesser extent, demonstrating their true stem cell characteristics. Gussoni et al. [1999] found that when given in a bone marrow transplantation setting, the side population (SP) of muscle provided radioprotection from lethal irradiation. In addition, donor bone marrow cells were detected in recipients by FISH analysis. Although this data was very intriguing, it has not yet been determined whether this population of muscle-derived HSC is the endogenous muscle stem cell population or a bone marrow derived cell type that resides in the muscle. Therefore, this data does not in itself prove plasticity of muscle-derived stem cells.

STEM CELLS

Thus far, there is a considerable amount of evidence documenting the existence of adult tissue-specific stem cells. In contrast, a shortage of consistent data exists regarding the protein expression patterns in many stem cell populations, making identification and purification of these cells variable and difficult. Table I outlines the known markers of several tissue-specific stem cell populations and demonstrates the lack of information in this area.

The best-characterized stem cell population with regard to protein expression is HSC. Mouse HSC were routinely isolated based on their Sca-1 and c-Kit expression and lack of expression of lineage markers. Recently, Phillips et al. [2000] used subtractive hybridization to compile a list of mRNAs expressed on HSC isolated from fetal murine livers. This technique can be applied to other stem cell populations as well, but requires the ability to purify or at least enrich for the stem cells of interest.

Numerous questions arise when addressing protein expression in stem cells:

1. Is there a marker common to all tissue-specific stem cells?
2. Are there markers specific to stem cells not expressed on more differentiated cells?
3. Do multipotent stem cells express markers that distinguish them from non-multipotent stem cells?

Answers to these questions will provide important insights into the biology of stem cells as well as information regarding what defines a stem cell. In addition, identification of markers that are similar between characterized stem cell populations might aid in the identification of tissue-specific stem cells.

METHODS FOR THE ISOLATION OF STEM CELLS

In order to study stem cells at any level, one must be able to purify or at least enrich for stem cells from the tissue of interest. Based on this, it is not surprising that most of the data supporting plasticity has been obtained using HSC, since the hematopoietic system is one of the best

TABLE I. Markers of Tissue-Specific Stem Cells

Organ	Stem cell type	Markers
Bone marrow	HSC	Mouse: Sca-1, c-Kit, CD34; Human: KDR, CD34 [Ziegler et al., 1999]
	MSC	Human: $SH2^+$, $SH3^+$, $CD34^-$, $CD45^-$ [Pittenger et al., 1999]
Brain	Neural stem cells/ependymal cells	Nestin, Notch-1 [Lendahl et al., 1990; Weinmaster et al., 1992]
Cornea	Corneal epithelial stem cells	No known specific markers
Gut	Intestinal stem cells	No known specific markers
Heart	No known stem cell	
Liver	Oval cells	Rat: OV6, OC2, OC3, Thy-1, c-Kit, CD34 [Alison and Saarf, 1998]
Lung	Likely to exist [Emura, 1997]	Unknown
Mammary gland	Mammary epithelial stem cells	Human: CALLA, MUC1 [Pechoux et al., 1999; Stingl et al., 1998]
Pancreas	Pancreatic stem cells	Mouse: Nestin, Neurogenin-3 [Hunziker and Stein, 2000; Jensen et al., 2000]
Retina	Retinal stem cells	Mouse and chicken: Nestin, CHX-10 [Ahmad et al., 2000; Fischer and Reh, 2000]
Skin	Epidermal stem cells	Mouse: α_6^{bri}, $CD71^{dim}$ [Tani et al., 2000]
Testes	Spermatogonial stem cells	α6- and β1- integrin [Shinohara et al., 1999]

characterized system with regard to the isolation of the stem cell population. This section will outline various methods used to isolate stem cells as well as potential pitfalls that may be encountered.

One method used to isolate stem cells is long term culture. This method is commonly used for the purification of MSC and neural stem cells. Briefly, whole cell populations are isolated from the tissue of interest and grown in culture conditions that are conducive to proliferation of stem cells and maintenance of stem cell characteristics [Bjornson et al., 1999; Pittenger et al., 1999]. This technique is not useful for the isolation of stem cells in which proper culture conditions are not known.

Other increasingly popular methods of enriching for particular cell types are fluorescence activated cell sorting (FACS) or magnetic activated cell sorting (MACS). Both of these techniques rely on the ability of antibodies to bind to proteins expressed on the cell surface. This method has commonly been used to isolate HSC based on their expression of Sca-1 and c-Kit and their lack of expression of lineage markers. One drawback of these methods is that one must already know or have an idea regarding the cell surface antigens expressed on the stem cells of interest. Also, the tissues outside of the hematopoietic system may be digested with collagenases in order to get cells into a single cell suspension. When using collagenases, one must determine whether the digestion removes antigens of interest.

In our laboratory, HSC are isolated based on their ability to efflux the vital dye Hoescht 33342. Goodell et al. [1996] first described this method of isolating HSC, when they demonstrated that bone marrow stained with Hoescht 33342 and analyzed by FACS resulted in a small population of cells, which they referred to as the SP, coming off the main population. Upon bone marrow transplantation into lethally irradiated mice, all of the hematopoietic activity resided in the SP population making this a useful tool for the isolation of HSC. Since this time, a SP has been found in skeletal muscle [Gussoni et al., 1999; Jackson et al., 1999]. Although SP populations may exist in other tissues, more experiments must be performed to determine if the SP populations from these tissues are true stem cells.

TECHNIQUES FOR DONOR CELL DETECTION

In transdifferentiation studies, both naturally occuring and artificial systems have been employed to detect donor cell engraftment. Sex-mismatch is the most commonly used method and relies on detection of the Y-chromosome. This technique involves in situ hybridization with probes against nucleotide sequences on the Y-chromosome [van den Berg et al., 1991]. One complication with this detection method is that non-specific binding of the probe may occur so stringent controls must be performed.

Marker systems making use of allelic differences have fostered numerous elegant transplantation studies in the mouse. CD45 (Ly5) has been referred to as common leukocyte antigen because it is found on all cells of a hematopoietic origin. A polymorphism in this gene was first described by Boyse and colleagues, who found an antigenic difference between leukocytes of different mouse strains [Shen et al., 1986]. This polymorphism was crossed into the C57Bl/6 strain allowing for the detection of chimerism of

all nucleated hematopoietic cells in a syngeneic setting. However, in transdifferentiation studies, the Ly5 system has limitations. Although this system allows for facile detection of chimerism in the blood after non-hematopoietic cell transplantation, it is not applicable to following chimerism outside of the hematopoietic system since CD45 will not be expressed [Jackson et al., 1999].

Several artificial marker systems have also been employed in the detection of transdifferentiation events. The *lacZ* gene is a useful tool in these experiments due to the ease of detection by enzymatic color reaction with Xgal or antibodies against the bacterial enzyme [Friedrich and Soriano, 1991]. A transgenic animal has been generated, the ROSA26 mouse, in which the *lacZ* gene was found to be expressed in the cytoplasm of most cell types during development [Zambrowicz et al., 1997]. Although this method of detection is appealing due to its ease of use, complications can arise due to endogenous lysosomal β-galactosidase activity present in some cell types. Lysosomal β-galactosidase is active at a lower pH, so background can be limited by performing the staining at a higher pH [Shimohama et al., 1989].

The lacZ marker system is more effective in transdifferentiation studies when given a nuclear localization signal and placed under the control of a tissue specific promoter. For example, in the work of Ferrari et al. [1998], the detection of reporter activity not only revealed the origin of the engrafted cells, but also simultaneously indicated its transcriptional activity towards myogenic differentiation. In addition, nuclear localization of the transgene allowed for quantitation of chimerism in the skeletal muscle tissue.

A second transgenic animal used in transdifferentiation studies ubiquitously expresses green fluorescent protein (GFP) which is detectable by direct fluorescence [Okabe et al., 1997]. This may be the simplest of all marker systems, but may be problematic in cells which exhibit high autofluorescence with a similar emission profile to that of GFP so this marker system must also be used with rigorous controls.

CONCLUDING REMARKS

In summary, adult stem cells isolated from many tissues may provide a source for autologous therapies ranging from graft creation to gene therapy and transplantation. Various populations of stem cells are being isolated and characterized. Primarily, the differentiation potential is typically assessed, followed by the mechanisms of differentiation to specific cell types. Single cell analyses, although more difficult to perform are a necessity, and will provide an enriched basis to obtain an understanding of the 'true' multipotential nature of adult stem cells. The scrutiny of the field, generated by current legislation and popular interest has led to a surge in publications. The continuous spotlight and evolution of the stem cell field holds the promise of clinical therapies for many different diseases.

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