UCSF
UC San Francisco Previously Published Works

Title
Commonly used mesenchymal stem cell markers and tracking labels: Limitations and challenges.

Permalink
https://escholarship.org/uc/item/7nv519xn

Journal
Cellular and molecular biology, 28(9)

ISSN
0213-3911

Authors
Lin, Ching-Shwun
Xin, Zhong-Cheng
Dai, Jican
et al.

Publication Date
2013-09-01

DOI
10.14670/hh-28.1109

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

NIH Public Access
Author Manuscript
Histol Histopathol. Author manuscript; available in PMC 2013 November 25.

Published in final edited form as:
Histol Histopathol. 2013 September ; 28(9): 1109–1116.

Commonly Used Mesenchymal Stem Cell Markers and Tracking Labels: Limitations and Challenges

Ching-Shwun Lin[1], **Zhong-Cheng Xin**[2], **Jican Dai**[3], and **Tom F Lue**[1]

[1]Knuppe Molecular Urology Laboratory, Department of Urology, School of Medicine, University of California, San Francisco, California 94143, USA

[2]Andrology Center, Peking University First Hospital, Beijing 100009, China

[3]Department of Urology, Affiliated Renji Hospital of Jiaotong University, Shanghai 200001, China

Summary

Early observations that cultured mesenchymal stem cells (MSCs) could be induced to exhibit certain characteristics of osteocytes and chondrocytes led to the proposal that they could be transplanted for tissue repair through cellular differentiation. Therefore, many subsequent preclinical studies with transplanted MSCs have strived to demonstrate that cellular differentiation was the underlying mechanism for the therapeutic effect. These studies generally followed the minimal criteria set by The International Society for Cellular Therapy in assuring MSC identity by using CD70, CD90, and CD105 as positive markers and CD34 as a negative marker. However, the three positive markers are co-expressed in a wide variety of cells, and therefore, even when used in combination, they are certainly incapable of identifying MSCs *in vivo*. Another frequently used MSC marker, Stro-1, has been shown to be an endothelial antigen and whether it can identify MSCs *in vivo* remains unknown. On the other hand, the proposed negative marker CD34 has increasingly been shown to be expressed in native MSCs, such as in the adipose tissue. It has also helped establish that MSCs are likely vascular stem cells (VSCs) that reside in the capillaries and in the adventitia of larger blood vessels. These cells do not express CD31, CD104b, or α-SMA, and therefore are designated as CD34+CD31-CD140b-SMA-. Many preclinical MSC transplantation studies have also attempted to demonstrate cellular differentiation by using labeled MSCs. However, all commonly used labels have shortcomings that often complicate data interpretation. The β-gal (LacZ) gene as a label is problematic because many mammalian tissues have endogenous β-gal activities. The GFP gene is similarly problematic because many mammalian tissues are endogenously fluorescent. The cell membrane label DiI can be adsorbed by host cells, and nuclear stains Hoechst dyes and DAPI can be transferred to host cells. Thymidine analog BrdU is associated with loss of cellular protein antigenicity due to harsh histological conditions. Newer thymidine analog EdU is easier to detect by chemical reaction to azide-conjugated Alexa fluors, but certain bone marrow cells are reactive to these fluors in the absence of EdU. These caveats need to be taken into consideration when designing or interpreting MSC transplantation experiments.

Keywords

mesenchymal stem cell markers; tracking labels; cell therapy; engraftment; differentiation

Corresponding Author: Dr. Ching-Shwun Lin, Department of Urology, University of California, San Francisco, CA 94143-0738, USA Phone: 415-476-3800, Fax: 415-476-3803, clin@urology.ucsf.edu.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Introduction

Stem cells are defined by their ability to self-renew and to differentiate into various cell types. In the adult organism such as the human being, these cells are principally responsible for maintaining the homeostasis of their respective tissues; for example, hematopoietic stem cells (HSCs) for replenishing the blood system. Thus, when isolated and with the option of culture-expansion, they can be transplanted into appropriate patients to treat the underlying tissue defects. Indeed, by using this "regenerative medicine" strategy, HSCs have been successfully employed in the past five decades to treat many types of blood and immune disorders (Appelbaum, 2007).

The bone marrow, which produces HSCs, contains another type of stem cell, namely, mesenchymal stem cells (MSCs), which are defined by their ability to differentiate into mesenchymal tissues such as adipose, bone, and cartilage (tri-lineage differentiation) (Dominici et al., 2006). This definition was subsequently used in many studies to demonstrate the existence of MSCs in extramedullary tissues; in particular, one such study has concluded that MSCs exist in virtually all postnatal tissues (da Silva Meirelles et al., 2006). However, it should be noted that in all of these studies the trilineage differentiation was demonstrated *in vitro*, not *in vivo*. That is, the candidate MSCs were isolated from various tissues, propagated in culture, and then chemically induced to differentiate into the specified lineages. Similarly, the *in vitro* tri-lineage differentiation has been used as a basis for testing these tissue-specific MSCs in numerous preclinical and clinical trials that aimed at treating diseases in virtually all adult tissues, including non-mesenchymal tissues such as the nervous system (Caplan and Correa, 2011; Lalu et al., 2012). Importantly, while some of these studies have attempted to demonstrate the engraftment and/or differentiation of the transplanted cells, none has convincingly shown that cellular differentiation *in vivo* was responsible for these cells' therapeutic effects. This troubling deficit is mainly due to an inability to track MSCs after their transplantation, and this in turn was due to (1) the non-specific nature of commonly used MSC markers, and (2) the unreliability of commonly used cell-tracking molecules and reagents. In this review article we will discuss the pros and cons of these MSC markers and tracking molecules/reagents, with the purpose of urging investigators to consider these reliability issues when designing and interpreting preclinical and clinical trials that concern MSC engraftment and differentiation.

Commonly used MSC markers

It has been recommended that MSCs must fulfill the following cell-surface marker expression criteria: 95% of the population must express CD105, CD73 and CD90, and 2% of the population must not express CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA class II (Dominici et al., 2006). Thus, in the following subsections we will discuss whether CD73, CD90, and CD105 can be used as positive markers for identifying MSCs *in vivo*. In addition, among the recommended negative MSC markers, CD34 has been controversial (Lin et al., 2012a) and therefore will be discussed as well. Furthermore, although not mentioned in the MSC guideline paper, Stro-1 is nonetheless a frequently used positive MSC marker (Kolf et al., 2007); therefore, its validity will also be examined.

CD73

CD73 is an ecto-5'-nucleotidase that converts extracellular adenosine monophosphate to adenosine. It is expressed in a wide variety of cell types including lymphocytes, endothelial cells, smooth muscle cells, epithelial cells, and fibroblasts (Airas et al., 1997; Strohmeier et al., 1997; Hashikawa et al., 2003; Tamajusuku et al., 2006). Although an earlier study (Haynesworth et al., 1992) showed that two monoclonal antibodies, SH3 and SH4, which were later identified as anti-CD73 (Barry et al., 2001), had specificity for MSCs (unreactive

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

with hematopoietic cells and osteoblasts), this would have implied that MSCs express a CD73 molecule that is different from other cell types (i.e., having the unique SH3 and SH4 epitopes). However, although more than 20 years have passed, there has been no evidence that this is the case. In any event, up to this date, there is no verifiable evidence that any anti-CD73 antibody can specifically detect MSCs *in vivo*.

CD90

CD90, also known as Thy1, is a glycosylphosphatidylinositol-linked protein involved in cell-cell and cell-matrix interactions. Although varied between species, CD90 expression has been identified in endothelial cells (both vascular and lymphatic), hematopoietic stem cells, lymphocytes, fibroblasts, and neurons (Craig et al., 1993; Saalbach et al., 1999; Rege and Hagood, 2006; Araki et al., 2007; Jurisic et al., 2010). In addition to this lack of cell type-specificity, another potential limitation for CD90 as an MSC marker is that this molecule appears not to be well conserved evolutionarily, and as a result commonly used anti-CD90 antibodies may not be able to react with MSCs of certain species (Boxall and Jones, 2012). Thus, although recommended as a positive marker, CD90 appears not to be a useful marker for *in vivo* MSC detection.

CD105

CD105, also known as endoglin, is a type I membrane glycoprotein that functions as an accessory receptor for TGF-beta superfamily ligands. As its name suggests, endoglin is highly expressed in vascular endothelial cells. But it is also highly expressed in synctiotrophoblasts (of term placenta) and less abundantly in monocytes, fibroblasts, chondrocytes, and hematopoietic progenitor cells (Nassiri et al., 2011). Interestingly, MSCs from the adipose tissue have been shown to express CD105 at low levels when freshly isolated but become increasingly CD105+ upon culture passages (Yoshimura et al., 2006; Varma et al., 2007). As MSCs from other tissues cannot be isolated in sufficient quantities without culture passages, whether they also express CD105 at low levels when freshly isolated is presently unknown. In any event, the data from adipose MSCs suggest that CD105 may not be a useful marker for *in vivo* MSC detection.

CD34

CD34 is a transmembrane sialomucin whose function remains largely unknown but has been suggested to be adhesive and/or anti-adhesive, depending on the cellular environment (Nielsen and McNagny, 2009). It was recommended as a negative marker for MSCs (Dominici et al., 2006), and numerous research reports and review articles have since adhered to this guideline. However, it should be noted that the recommendation was given without any reason or reference; therefore one can only speculate on why this came about. One possibility is that it could have been born out of a necessity to distinguish MSCs from HSCs, as HSCs are generally believed to express CD34. Another is that most MSC studies have found that culture-expanded MSCs are CD34-. However, it should be pointed out that many cell types that are CD34+ when freshly isolated (e.g., endothelial cells) lose CD34 expression in culture (Fina et al., 1990; Muller et al., 2002; Unger et al., 2002; Ning et al., 2006; Stolzing et al., 2012), and even HSCs can become CD34- (Gangenahalli et al., 2006). Thus, MSC's negativity for CD34 is likely a cell culture-induced phenomenon, not indicative of their actual *in vivo* status. Furthermore, a frequently used MSC marker, Stro-1, was defined by a monoclonal antibody generated by using CD34+ human bone marrow cells as immunogen (see next subsection for detail). Thus, whether CD34 is truly a negative marker for MSCs should be re-evaluated (Lin et al., 2012a).

In contrast to bone marrow-derived MSCs (BMSCs), the adipose tissue-derived MSCs (ADSCs) are generally classified as CD34+, despite the well-known phenomenon of losing

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

CD34 expression in culture (Gimble et al., 2007). Thus, in a dedicated histology study CD34 was used as a defining marker in search of ADSCs in human adipose tissue (Lin et al., 2008). The resulting immunohistochemical and immunofluorescence data indicated the existence of such cells in the capillaries and in the adventitia of larger blood vessels. Subsequently, these findings have been confirmed by all dedicated histological ADSC studies (Zimmerlin et al., 2010; Maumus et al., 2011; Corselli et al., 2012; Lin et al., 2012c; Braun et al., 2013), as summarized in the following.

In larger blood vessels CD34 was localized to the tunica intima and tunica adventitia; thus, the cross-sectional view of these CD34-stained vessels was that of two concentric circles (endothelium and adventitia) sandwiching the smooth muscle layer (which was stained with a different color; for example, red versus green). A similar CD34 staining pattern has previously been identified in the aorta (Anderson et al., 2005; Pasquinelli et al., 2007), femoral artery (Pasquinelli et al., 2007), coronary artery (Torsney et al., 2007), and internal thoracic artery (Zengin et al., 2006). Furthermore, the CD34+ adventitial cells have been shown capable of differentiating into mature hematopoietic cells, endothelial cells, and macrophages (Zengin et al., 2006). Thus, we have proposed the term “adventitial progenitor cells (APCs)” to denote these cells, and we also suggested that APCs are the de facto MSCs that have been identified in most adult tissues (Lin and Lue, 2013).

Immunofluorescence analysis of capillaries in the adipose tissue for CD31 (endothelial marker), α-smooth muscle actin (α-SMA), CD140b (pericyte marker), and CD34 expression revealed a CD34+CD31-SMA-CD140b- population (Lin et al., 2008; Lin and Lue, 2011), which we believe is the equivalent of the CD34+CD31-SMA- APCs (Lin and Lue, 2013). Since capillaries are not layer-structured as larger vessels, and thus lack the tunica adventitia, we proposed the term “vascular stem cells (VSCs)” to denote the CD34+CD31-SMA-CD140b- population in both capillaries and larger vessels (Lin and Lue, 2013).

Stro-1

In a 2007 review article Kolf et al contended that Stro-1 is by far the best-known MSC marker (Kolf et al., 2007). Indeed, since its first report in 1991 (Simmons and Torok-Storb, 1991), the Stro-1 antibody has played a critical role in hundreds of studies that relied on it for the identification and/or the isolation of MSCs from a wide variety of tissues (Lin et al., 2011). While the exact reason for this popularity is not known, a brief history of how this antibody was created may provide a hint.

The antibody was produced by one of several hybridomas that were generated by immunizing mice intrasplenically with human CD34+ bone marrow cells (Simmons and Torok-Storb, 1991). These hybridomas were initially screened against T- and B-cell lines, and then further selected for reactivity with subpopulations of CD34+ cells. Those that fulfilled these negative and positive selection criteria were subsequently cloned, and one hybridoma clone named Stro-1 has since been the source of all brands of Stro-1 antibody currently available. In the data sheet of all of these brands, the Stro-1 antibody is described as having the ability to enrich the MSC population from bone marrow cells as determined by their capacity for tri-lineage differentiation. This MSC enrichment capability thus appears to be the reason why Stro-1 has become one of the most popular antibodies for the identification and/or isolation of MSCs from various tissues.

Stro-1 antibody has been used predominantly for flow cytometric analysis, and, much less commonly, for immunocytochemical staining of candidate MSCs. In both instances, disagreements over whether a particular cell population is Stro-1 positive or negative are not uncommon (Gimble et al., 2007). Also troubling is that, despite rarely performed, immunostaining of tissues with Stro-1 antibody often produced positivity in the vascular

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

endothelium (Lin et al., 2011). Thus, we conducted a dedicated study in which the Stro-1 antibody was compared to the endothelial-specific vWF antibody in immunofluorescence analysis of blood vessel-rich tissues, lung, liver, and kidney. The results showed extensive overlaps between Stro-1 and vWF stains, thus confirming the endothelial identity of Stro-1 (Ning et al., 2011). Furthermore, we also demonstrated by Western blotting analysis that Stro-1 is a 75kd protein expressed in endothelial cells and ADSCs, but not in fibroblasts or smooth muscle cells (Ning et al., 2011). Thus, although expressed in cultured MSCs, Stro-1 as an *in vivo* MSC marker is compromised by its concurrent expression in the endothelium.

Commonly used tracking molecules and reagents

For the purpose of demonstrating post-transplantation engraftment and/or differentiation, MSCs are most commonly labeled with a tracking molecule, followed by transplantation, histology, and microscopic examination of the transplanted tissue. Positive identification is interpreted when the tracking molecule and the differentiation marker of interest (e.g., α-SMA for smooth muscle cells) are seen within the same cells. Thus, it is obvious that the tracking label plays a critical role in the determination of whether the transplanted cells have differentiated or not. In the following subsections we will discuss the reliability issues of some of the commonly used tracking molecules and reagents.

β-galactosidase (β-gal)

β-gal is a hydrolase enzyme encoded by the LacZ gene of *Escherichia coli* and has been utilized in various research settings to convert colorless man-made substrates (most commonly, Blue-gal and X-gal) into a blue reaction product. Specifically, MSCs transfected with LacZ gene or isolated from LacZ-transgenic animals can be tracked histochemically after their transplantation into a suitable host. However, many mammalian cells and tissues exhibit endogenous β-gal activity, which is physiologically important for digesting glycolipids (Weiss et al., 1999). In addition, several β-gal isozymes also exist in mammalian tissues (Weiss et al., 1999). Thus, it has been an ongoing effort for β-gal users to circumvent the host tissue background problem by testing various experimental conditions, including changing the tissue fixative, raising the reaction pH, reducing the time of exposure to substrate, and/or using Blue-gal rather than X-gal. While there are reports of improved detection of bacterial versus mammalian β-gal activity (Weiss et al., 1999; Gioglio et al., 2002), difficulties have persisted (Brazelton and Blau, 2005). In particular, osteoclasts residing in murine bone marrow were specifically and robustly stained blue with X-gal regardless of the staining condition (varying the pH and the time of exposure to substrate) (Kopp et al., 2007). Therefore, it is critically important that proper positive and negative controls are included when assessing the differentiation potential of LacZ-transfected MSCs.

Green Fluorescence Protein (GFP)

GFP is a green fluorescent protein first isolated from the jellyfish *Aequorea victoria*. MSCs transfected with the GFP gene or isolated from GFP-transgenic animals thus can be tracked by fluorescent microscopy after their transplantation in a suitable host. However, mammalian cells' metabolites and structural components are naturally fluorescent (commonly known as "autofluorescence"). Thus, unless the transfected GFP is highly expressed or densely localized, its detection can seem like "seeing the wood through the trees" (Billinton and Knight, 2001). However, GFP expression level in transgenic mice is highly variable among individual animals and even among individual cells within the same animal (Anderson et al., 2005; Brazelton and Blau, 2005; Swenson et al., 2007). In addition, cells that express GFP at a high level before transplantation may become low expressing overtime after transplantation. Furthermore, it has been shown that murine hippocampus persistently contained a prominent population of autofluorescent cells even after the tissue

NIH-PA Author Manuscript

specimens underwent procedures designed to quench autofluorescence (Spitzer et al., 2011). Therefore, the existence of such stubborn autofluorescent cells in other tissues must be taken into consideration when designing or interpreting GFP-based MSC tracking experiments.

Chloromethyl-dialkylcarbocyanine (DiI)

DiI is a fluorescent dye that preferentially binds to cell membrane (Parish, 1999) and has been used frequently to label cells for tracking purposes. It is cytotoxic although the threshold concentration differs from one cell type to another. In situations when DiI sensitivity is not pre-determined, the cell preparation may contain dead cells, and when such a cell preparation is transplanted, the debris of the dead cells' DiI-labeled membrane are adsorbed by host cells, thus leading to misinterpretation of cell differentiation. Even if there are no dead cells in the cell preparation, the non-covalently bound DiI can still dissociate from labeled cells and get picked up by host cells (Onifer et al., 1993; Kruyt et al., 2003; Brulport et al., 2007; Li et al., 2008; Schormann et al., 2008). Thus, when using DiI as a cell-tracking label, it is important to consider the possibility of donor-to-host transfer.

Hoechst dyes

Hoechst dyes are a family of cell membrane permeable bis-benzimides that bind to DNA in the minor groove of AT-rich sequences. They are commonly used to stain the nuclei of fixed (dead) cells in immunofluorescent histological studies, and are much less frequently used for tracking transplanted cells. Because Hoechst dyes' binding to DNA is non-covalent, there have been suspicions that the label could be transferred from transplanted cells to host cells. In one study such transfer was observed within hours of cell transplantation, and the label persisted in the host cells for at least 4 weeks post-transplantation (Iwashita et al., 2000). In another study, co-culture of labeled cells with unlabeled cells resulted in the transfer of label to >50% of the initially unlabeled cells within 6 hours (Mohorko et al., 2005). The authors thus concluded that Hoechst dyes are unsuitable for cell transplantation research.

4',6-diamidino-2-phenylindole (DAPI)

DAPI is often used interchangeably with Hoechst dyes for visualization of cell nuclei in immunofluorescent histological studies. However, it is a poor label for live cells because it does not penetrate intact cell membrane well and is cytotoxic at high concentrations (Kapuscinski, 1995; Zink et al., 2003). More importantly, because of its non-covalent binding to DNA, it can dissociate from the DNA of labeled cells after transplantation and be adsorbed by host cells. Specifically, in one study DAPI-labeled BMSCs were injected into the vitreous cavity of rat eyes with retinal injury, and 8 weeks later, most of the retinal cells became DAPI+ although the transplanted cells remained mostly in the vitreous cavity (Castanheira et al., 2009). This study and the two abovementioned studies (Iwashita et al., 2000; Mohorko et al., 2005) together point out that it is advisable not to use non-covalent DNA-binding dyes when selecting labels for tracking transplanted cells.

5-bromo-2'-deoxyuridine (BrdU)

BrdU is a thymidine analog extensively used for labeling cells through its incorporation into newly synthesized DNA. The labeled cells, when transplanted, can be detected by immunohistochemistry or immunofluorescence with an anti-BrdU antibody. However, because the incorporated BrdU is buried in densely packed chromosomes, its exposure to the antibody requires untangling the chromosomes with strong acids and high temperature, which invariably degrade the cell/tissue structure, resulting in distorted histological images. In addition, the denaturing treatment can cause loss of antigenicity of cellular proteins, making them unsuitable for immunohistochemistry. Thus, when MSCs are labeled with BrdU for tracking, whether they differentiate into a specific cell type cannot be assessed

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

because the cell type-specific protein would have lost antigenicity. Even if the protein survives the denaturing treatment, it is still difficult to identify the BrdU label with confidence because, in immunohistochemically processed tissue samples, its brown color cannot be easily distinguished from the purplish nuclear stain (Rakic, 2002). Furthermore, it has been shown that the donor BrdU label could be transferred to host cells, thereby causing misinterpretation of post-transplantation differentiation (Coyne et al., 2006).

5-ethynyl-2'-deoxyuridine (EdU)

EdU is a newer thymidine analog introduced for the purpose of circumventing the abovementioned technical difficulties that are often associated with BrdU (Salic and Mitchison, 2008). While the introduction paper demonstrated EdU's utility for labeling cultured cells and tissues, we first used it for tracking transplanted cells (Lin et al., 2009) and have since used it in 20 additional studies. In our latest study we further showed that EdU labeling did not interfere with *in vitro* cell proliferation, differentiation, cytokine secretion or migratory response (Ning et al., 2013). For cell tracking, EdU-labeled cells are detected by a rapid, simple chemical reaction using an azide-conjugated fluor (most commonly, the red Alexa594-azide or green Alexa488-azide) that covalently binds to EdU's alkyne moiety. The procedure does not require treating the tissue sample with any harsh chemicals and can be combined with immunofluorescence stain for any cellular protein, thus allowing detection of cellular differentiation. Since the label is covalently bound to DNA, it does not leak as Hoechst dye, DAPI or DiI does from labeled cells. Furthermore, since the fluorescent azide is added at the time of detection, there is no time-associated loss of signal as with GFP. Most importantly, due to the intense red or green fluorescence of Alexa fluors, the scoring of EdU+ cells is unambiguous - unlike BrdU, whose immunohistochemical detection is often guesswork. However, the use of EdU as a tracking label has a surprising shortcoming: certain bone marrow cells of unknown identity are stained positive by Alexa-azide fluors in the absence of an alkyne label (Lin et al., 2012b). This is surprising because an alkyne moiety, which is required for the bonding of azide, is not known to exist in mammalian cells; therefore, how unlabeled bone marrow cells can be stained by Alexa-azide does not seem to have a chemical or biological explanation. In any case, fortunately, this shortcoming can possibly be overcome by DAPI counterstain and viewing the bone marrow tissue sample at a high magnification (>400x) because the "false-positive" stain is in the cytoplasm whereas the true positive stain is in the nucleus (Lin et al., 2012b). Furthermore, the bone marrow appears to be the only tissue that exhibits such false positivity. So, overall, EdU is a highly reliable and easy-to-use tracking label.

Concluding remarks

The concept of using MSCs for therapy was originally based on their potential for multilineage differentiation. In subsequent preclinical studies the demonstration of cellular differentiation has thus become an important indication for MSC's therapeutic effects. To achieve this goal, commonly employed approaches typically consisted of transplantation of MSCs that have been labeled with a "reporter" molecule and visualization of the reporter together with a differentiation marker within the same cells in the transplanted host. Whether these approaches have succeeded in identifying MSC differentiation *in vivo* has been discussed in several excellent review articles, for example, Prockop et al (Prockop et al., 2010) and Caplan and Correa (Caplan and Correa, 2011). In the present review we examined these issues from a different perspective by focusing on commonly used MSC markers and tracking molecules. We pointed out their strengths and weaknesses, in hopes of fostering a better understanding of the challenges we face when employing these tactics for MSC research. In particular, we recommend a re-evaluation of CD34 as a negative marker. We believe that realizing that CD34 is a sometimes-on-sometimes-off molecule will help avoid erroneous interpretations. We also highly recommend the use of EdU as a tracking

NIH-PA Author Manuscript

label because we have shown that it has a greater benefit-versus-risk ratio than others. In conclusion, regardless of which markers or molecules are used, it is important to know their limitations when designing or interpreting a cell tracking experiment.

Acknowledgments

This work was supported in part by the National Institutes of Health (DK64538, DK045370, and DK069655).

References

Airas L, Niemela J, Salmi M, Puurunen T, Smith DJ, Jalkanen S. Differential regulation and function of cd73, a glycosyl-phosphatidylinositol-linked 70-kd adhesion molecule, on lymphocytes and endothelial cells. J. Cell Biol. 1997; 136:421–431. [PubMed: 9015312]

Anderson DA, Wu Y, Jiang S, Zhang X, Streeter PR, Spangrude GJ, Archer DR, Fleming WH. Donor marker infidelity in transgenic hematopoietic stem cells. Stem Cells. 2005; 23:638–643. [PubMed: 15849171]

Appelbaum FR. Hematopoietic-cell transplantation at 50. N. Engl. J. Med. 2007; 357:1472–1475. [PubMed: 17928594]

Araki H, Yoshinaga K, Boccuni P, Zhao Y, Hoffman R, Mahmud N. Chromatin-modifying agents permit human hematopoietic stem cells to undergo multiple cell divisions while retaining their repopulating potential. Blood. 2007; 109:3570–3578. [PubMed: 17185465]

Barry F, Boynton R, Murphy M, Haynesworth S, Zaia J. The sh-3 and sh-4 antibodies recognize distinct epitopes on cd73 from human mesenchymal stem cells. Biochem. Biophys. Res. Commun. 2001; 289:519–524. [PubMed: 11716504]

Billinton N, Knight AW. Seeing the wood through the trees: A review of techniques for distinguishing green fluorescent protein from endogenous autofluorescence. Anal. Biochem. 2001; 291:175–197. [PubMed: 11401292]

Boxall SA, Jones E. Markers for characterization of bone marrow multipotential stromal cells. Stem Cells Int. 2012; 2012:975871. [PubMed: 22666272]

Braun J, Kurtz A, Barutcu N, Bodo J, Thiel A, Dong J. Concerted regulation of cd34 and cd105 accompanies mesenchymal stromal cell derivation from human adventitial stromal cell. Stem Cells Dev. 2013; 22:815–827. [PubMed: 23072708]

Brazelton TR, Blau HM. Optimizing techniques for tracking transplanted stem cells in vivo. Stem Cells. 2005; 23:1251–1265. [PubMed: 16109764]

Brulport M, Schormann W, Bauer A, Hermes M, Elsner C, Hammersen FJ, Beerheide W, Spitkovsky D, Hartig W, Nussler A, Horn LC, Edelmann J, Pelz-Ackermann O, Petersen J, Kamprad M, von Mach M, Lupp A, Zulewski H, Hengstler JG. Fate of extrahepatic human stem and precursor cells after transplantation into mouse livers. Hepatology. 2007; 46:861–870. [PubMed: 17668884]

Caplan AI, Correa D. The msc: An injury drugstore. Cell Stem Cell. 2011; 9:11–15. [PubMed: 21726829]

Castanheira P, Torquetti LT, Magalhas DR, Nehemy MB, Goes AM. Dapi diffusion after intravitreal injection of mesenchymal stem cells in the injured retina of rats. Cell Transplant. 2009; 18:423–431. [PubMed: 19622229]

Corselli M, Chen CW, Sun B, Yap S, Rubin JP, Peault B. The tunica adventitia of human arteries and veins as a source of mesenchymal stem cells. Stem Cells Dev. 2012; 21:1299–1308. [PubMed: 21861688]

Coyne TM, Marcus AJ, Woodbury D, Black IB. Marrow stromal cells transplanted to the adult brain are rejected by an inflammatory response and transfer donor labels to host neurons and glia. Stem Cells. 2006; 24:2483–2492. [PubMed: 16873764]

Craig W, Kay R, Cutler RL, Lansdorp PM. Expression of thy-1 on human hematopoietic progenitor cells. J. Exp. Med. 1993; 177:1331–1342. [PubMed: 7683034]

da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J. Cell Sci. 2006; 119:2204–2213. [PubMed: 16684817]

NIH-PA Author Manuscript

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement. Cytotherapy. 2006; 8:315–317. [PubMed: 16923606]

Fina L, Molgaard HV, Robertson D, Bradley NJ, Monaghan P, Delia D, Sutherland DR, Baker MA, Greaves MF. Expression of the cd34 gene in vascular endothelial cells. Blood. 1990; 75:2417–2426. [PubMed: 1693532]

Gangenahalli GU, Singh VK, Verma YK, Gupta P, Sharma RK, Chandra R, Luthra PM. Hematopoietic stem cell antigen cd34: Role in adhesion or homing. Stem Cells Dev. 2006; 15:305–313. [PubMed: 16846369]

Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. Circ. Res. 2007; 100:1249–1260. [PubMed: 17495232]

Gioglio L, Cusella de A.M. Boratto R, Poggi P. An improved method for beta-galactosidase activity detection on muscle tissue. A light and electron microscopic study. Ann Anat. 2002; 184:153–157. [PubMed: 11936195]

Hashikawa T, Takedachi M, Terakura M, Saho T, Yamada S, Thompson LF, Shimabukuro Y, Murakami S. Involvement of cd73 (ecto-5'-nucleotidase) in adenosine generation by human gingival fibroblasts. J. Dent. Res. 2003; 82:888–892. [PubMed: 14578500]

Haynesworth SE, Baber MA, Caplan AI. Cell surface antigens on human marrow-derived mesenchymal cells are detected by monoclonal antibodies. Bone. 1992; 13:69–80. [PubMed: 1316137]

Iwashita Y, Crang AJ, Blakemore WF. Redistribution of bisbenzimide hoechst 33342 from transplanted cells to host cells. Neuroreport. 2000; 11:1013–1016. [PubMed: 10790874]

Jurisic G, Iolyeva M, Proulx ST, Halin C, Detmar M. Thymus cell antigen 1 (thy1, cd90) is expressed by lymphatic vessels and mediates cell adhesion to lymphatic endothelium. Exp. Cell Res. 2010; 316:2982–2992. [PubMed: 20599951]

Kapuscinski J. Dapi: A DNA-specific fluorescent probe. Biotech. Histochem. 1995; 70:220–233. [PubMed: 8580206]

Kolf CM, Cho E, Tuan RS. Mesenchymal stromal cells. Biology of adult mesenchymal stem cells: Regulation of niche, self-renewal and differentiation. Arthritis Res Ther. 2007; 9:204. [PubMed: 17316462]

Kopp HG, Hooper AT, Shmelkov SV, Rafii S. Beta-galactosidase staining on bone marrow. The osteoclast pitfall. Histol. Histopathol. 2007; 22:971–976.

Kruyt MC, De Bruijn J, Veenhof M, Oner FC, Van Blitterswijk CA, Verbout AJ, Dhert WJ. Application and limitations of chloromethylbenzamidodialkylcarbocyanine for tracing cells used in bone tissue engineering. Tissue Eng. 2003; 9:105–115. [PubMed: 12625959]

Lalu MM, McIntyre L, Pugliese C, Fergusson D, Winston BW, Marshall JC, Granton J, Stewart DJ. Safety of cell therapy with mesenchymal stromal cells (safecell): A systematic review and meta-analysis of clinical trials. PLoS One. 2012; 7:e47559. [PubMed: 23133515]

Li N, Yang H, Lu L, Duan C, Zhao C, Zhao H. Comparison of the labeling efficiency of brdu, dii and fish labeling techniques in bone marrow stromal cells. Brain Res. 2008; 1215:11–19. [PubMed: 18468584]

Lin, CS.; Lue, TF.; Illouz, YG.; Sterodimas, A. Adipose stem cells and regenerative medicine. Springer; New York, NY: 2011. Adipose-derived stem cells: Characterization and application in urology; p. 193-207.

Lin CS, Lue TF. Defining vascular stem cells. Stem Cells Dev. 2013 In press.

Lin CS, Ning H, Lin G, Lue TF. Is cd34 truly a negative marker for mesenchymal stromal cells? Cytotherapy. 2012a; 14:1159–1163. [PubMed: 23066784]

Lin G, Garcia M, Ning H, Banie L, Guo YL, Lue TF, Lin CS. Defining stem and progenitor cells within adipose tissue. Stem Cells Dev. 2008; 17:1053–1063. [PubMed: 18597617]

Lin G, Huang YC, Shindel AW, Banie L, Wang G, Lue TF, Lin CS. Labelling and tracking of mesenchymal stromal cells with edu. Cytotherapy. 2009; 11:864–873. [PubMed: 19903099]

Lin G, Liu G, Banie L, Wang G, Ning H, Lue TF, Lin CS. Tissue distribution of mesenchymal stem cell marker stro-1. Stem Cells Dev. 2011; 20:1747–1752. [PubMed: 21208041]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Lin G, Ning H, Banie L, Qiu X, Zhang H, Lue TF, Lin CS. Bone marrow cells stained by azide-conjugated alexa fluors in the absence of an alkyne label. Stem Cells Dev. 2012b; 21:2552–2559. [PubMed: 22380729]

Lin G, Xin Z, Zhang H, Banie L, Wang G, Qiu X, Ning H, Lue TF, Lin CS. Identification of active and quiescent adipose vascular stromal cells. Cytotherapy. 2012c; 14:240–246. [PubMed: 22070603]

Maumus M, Peyrafitte JA, D'Angelo R, Fournier-Wirth C, Bouloumie A, Casteilla L, Sengenes C, Bourin P. Native human adipose stromal cells: Localization, morphology and phenotype. Int. J. Obes. 2011; 35:1141–1153.

Mohorko N, Kregar-Velikonja N, Repovs G, Gorensek M, Bresjanac M. An in vitro study of hoechst 33342 redistribution and its effects on cell viability. Hum. Exp. Toxicol. 2005; 24:573–580. [PubMed: 16323574]

Muller AM, Hermanns MI, Skrzynski C, Nesslinger M, Muller KM, Kirkpatrick CJ. Expression of the endothelial markers pecam-1, vwf, and cd34 in vivo and in vitro. Exp. Mol. Pathol. 2002; 72:221–229. [PubMed: 12009786]

Nassiri F, Cusimano MD, Scheithauer BW, Rotondo F, Fazio A, Yousef GM, Syro LV, Kovacs K, Lloyd RV. Endoglin (cd105): A review of its role in angiogenesis and tumor diagnosis, progression and therapy. Anticancer Res. 2011; 31:2283–2290. [PubMed: 21737653]

Nielsen JS, McNagny KM. Cd34 is a key regulator of hematopoietic stem cell trafficking to bone marrow and mast cell progenitor trafficking in the periphery. Microcirculation. 2009; 16:487–496. [PubMed: 19479621]

Ning H, Lin G, Lue TF, Lin CS. Neuron-like differentiation of adipose tissue-derived stromal cells and vascular smooth muscle cells. Differentiation. 2006; 74:510–518. [PubMed: 17177848]

Ning H, Lin G, Lue TF, Lin CS. Mesenchymal stem cell marker stro-1 is a 75 kd endothelial antigen. Biochem. Biophys. Res. Commun. 2011; 413:353–357. [PubMed: 21903091]

Ning H, Albersen M, Lin G, Lue TF, Lin CS. Effects of edu labeling on mesenchymal stem cells. Cytotherapy. 2013; 15:57–63. [PubMed: 23260086]

Onifer SM, White LA, Whittemore SR, Holets VR. In vitro labeling strategies for identifying primary neural tissue and a neuronal cell line after transplantation in the cns. Cell Transplant. 1993; 2:131–149. [PubMed: 8143080]

Parish CR. Fluorescent dyes for lymphocyte migration and proliferation studies. Immunol. Cell Biol. 1999; 77:499–508. [PubMed: 10571670]

Pasquinelli G, Tazzari PL, Vaselli C, Foroni L, Buzzi M, Storci G, Alviano F, Ricci F, Bonafe M, Orrico C, Bagnara GP, Stella A, Conte R. Thoracic aortas from multiorgan donors are suitable for obtaining resident angiogenic mesenchymal stromal cells. Stem Cells. 2007; 25:1627–1634. [PubMed: 17446560]

Prockop DJ, Kota DJ, Bazhanov N, Reger RL. Evolving paradigms for repair of tissues by adult stem/progenitor cells (mscs). J Cell Mol Med. 2010; 14:2190–2199. [PubMed: 20716123]

Rakic P. Neurogenesis in adult primate neocortex: An evaluation of the evidence. Nat Rev Neurosci. 2002; 3:65–71. [PubMed: 11823806]

Rege TA, Hagood JS. Thy-1 as a regulator of cell-cell and cell-matrix interactions in axon regeneration, apoptosis, adhesion, migration, cancer, and fibrosis. FASEB J. 2006; 20:1045–1054. [PubMed: 16770003]

Saalbach A, Wetzig T, Haustein UF, Anderegg U. Detection of human soluble thy-1 in serum by elisa. Fibroblasts and activated endothelial cells are a possible source of soluble thy-1 in serum. Cell Tissue Res. 1999; 298:307–315.

Salic A, Mitchison TJ. A chemical method for fast and sensitive detection of DNA synthesis in vivo. Proc. Natl. Acad. Sci. U. S. A. 2008; 105:2415–2420. [PubMed: 18272492]

Schormann W, Hammersen FJ, Brulport M, Hermes M, Bauer A, Rudolph C, Schug M, Lehmann T, Nussler A, Ungefroren H, Hutchinson J, Fandrich F, Petersen J, Wursthorn K, Burda MR, Brustle O, Krishnamurthi K, von Mach M, Hengstler JG. Tracking of human cells in mice. Histochem. Cell Biol. 2008; 130:329–338. [PubMed: 18425526]

Simmons PJ, Torok-Storb B. Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, stro-1. Blood. 1991; 78:55–62. [PubMed: 2070060]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Spitzer N, Sammons GS, Price EM. Autofluorescent cells in rat brain can be convincing impostors in green fluorescent reporter studies. J. Neurosci. Methods. 2011; 197:48–55. [PubMed: 21310182]

Stolzing A, Bauer E, Scutt A. Suspension cultures of bone-marrow-derived mesenchymal stem cells: Effects of donor age and glucose level. Stem Cells Dev. 2012; 21:2718–2723. [PubMed: 22462498]

Strohmeier GR, Lencer WI, Patapoff TW, Thompson LF, Carlson SL, Moe SJ, Carnes DK, Mrsny RJ, Madara JL. Surface expression, polarization, and functional significance of cd73 in human intestinal epithelia. J. Clin. Invest. 1997; 99:2588–2601. [PubMed: 9169488]

Swenson ES, Price JG, Brazelton T, Krause DS. Limitations of green fluorescent protein as a cell lineage marker. Stem Cells. 2007; 25:2593–2600. [PubMed: 17615263]

Tamajusuku AS, Carrillo-Sepulveda MA, Braganhol E, Wink MR, Sarkis JJ, Barreto-Chaves ML, Battastini AM. Activity and expression of ecto-5'-nucleotidase/cd73 are increased by thyroid hormones in vascular smooth muscle cells. Mol. Cell. Biochem. 2006; 289:65–72. [PubMed: 16718378]

Torsney E, Mandal K, Halliday A, Jahangiri M, Xu Q. Characterisation of progenitor cells in human atherosclerotic vessels. Atherosclerosis. 2007; 191:259–264. [PubMed: 16787646]

Unger RE, Krump-Konvalinkova V, Peters K, Kirkpatrick CJ. In vitro expression of the endothelial phenotype: Comparative study of primary isolated cells and cell lines, including the novel cell line hpmec-st1. r. Microvasc. Res. 2002; 64:384–397.

Varma MJ, Breuls RG, Schouten TE, Jurgens WJ, Bontkes HJ, Schuurhuis GJ, van Ham SM, van Milligen FJ. Phenotypical and functional characterization of freshly isolated adipose tissue-derived stem cells. Stem Cells Dev. 2007; 16:91–104. [PubMed: 17348807]

Weiss DJ, Liggitt D, Clark JG. Histochemical discrimination of endogenous mammalian beta-galactosidase activity from that resulting from lac-z gene expression. Histochem. J. 1999; 31:231–236. [PubMed: 10447064]

Yoshimura K, Shigeura T, Matsumoto D, Sato T, Takaki Y, Aiba-Kojima E, Sato K, Inoue K, Nagase T, Koshima I, Gonda K. Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. J. Cell. Physiol. 2006; 208:64–76. [PubMed: 16557516]

Zengin E, Chalajour F, Gehling UM, Ito WD, Treede H, Lauke H, Weil J, Reichenspurner H, Kilic N, Ergun S. Vascular wall resident progenitor cells: A source for postnatal vasculogenesis. Development. 2006; 133:1543–1551. [PubMed: 16524930]

Zimmerlin L, Donnenberg VS, Pfeifer ME, Meyer EM, Peault B, Rubin JP, Donnenberg AD. Stromal vascular progenitors in adult human adipose tissue. Cytometry A. 2010; 77:22–30. [PubMed: 19852056]

Zink D, Sadoni N, Stelzer E. Visualizing chromatin and chromosomes in living cells. Methods. 2003; 29:42–50. [PubMed: 12543070]