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POSITION PAPER

Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement

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The considerable therapeutic potential of human multipotent mesenchymal stromal cells (MSC) has generated markedly increasing interest in a wide variety of biomedical disciplines. However, investigators report studies of MSC using different methods of isolation and expansion, and different approaches to characterizing the cells. Thus it is increasingly difficult to compare and contrast study outcomes, which hinders progress in the field. To begin to address this issue, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposes minimal criteria to define human MSC. First, MSC must be plastic-adherent when maintained in standard culture conditions. Second, MSC must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR surface molecules. Third, MSC must differentiate to osteoblasts, adipocytes and chondroblasts in vitro. *While these criteria will probably require modification as new knowledge unfolds, we believe this minimal set of standard criteria will foster a more uniform characterization of MSC and facilitate the exchange of data among investigators.*

Keywords

MSC, stem cells, adherent cells, immunophenotype, differentiation.

Biologic and clinical interest in MSC has risen dramatically over the last two decades, as shown by the ever-increasing number of research teams studying these cells. Not only are established laboratories focusing on MSC but new investigators are rapidly being attracted to the field, which will undoubtedly accelerate scientific discovery and the development of novel cellular therapies. However, this soaring interest has also generated many ambiguities and inconsistencies in the field.

To begin to address these issues, a recent report from the International Society for Cellular Therapy (ISCT) stated that 'multipotent mesenchymal stromal cells' (MSC) is the currently recommended designation [1] for the plastic-adherent cells isolated from BM and other tissues that have often been labeled mesenchymal stem cells [2].

The defining characteristics of MSC are inconsistent among investigators. Many laboratories have developed methods to isolate and expand MSC, which invariably have subtle, and occasionally quite significant, differences. Furthermore, investigators have isolated MSC from a variety of tissues with ostensibly similar properties [3]. These varied tissue sources and methodologies of cell

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preparation beg the question of whether the resulting cells are sufficiently similar to allow for a direct comparison of reported biologic properties and experimental outcomes, especially in the context of cell therapy. This question of cell equivalence is, in part, because of the lack of universally accepted criteria to define MSC. Most importantly, the inability to compare and contrast studies from different groups is likely to hinder progress in the field.

To address this problem, the Mesenchymal and Tissue Stem Cell Committee of the ISCT proposes a set of standards to define human MSC for both laboratory-based scientific investigations and for pre-clinical studies. These identifying criteria should not be confused with release specifications for clinical studies, as the current proposal is intended solely as identifying criteria for research purposes. The aim of this position statement is to provide the scientific community with a standard set of criteria, based on the best currently available data, to define the identity of MSC, recognizing that future research will probably mandate a revision of the criteria as new data emerge.

We propose three criteria to define MSC:

- adherence to plastic
- specific surface antigen (Ag) expression
- multipotent differentiation potential (Table 1).

First, MSC must be plastic-adherent when maintained in standard culture conditions using tissue culture flasks. Second, $\geq 95\%$ of the MSC population must express CD105, CD73 and CD90, as measured by flow cytometry. Additionally, these cells must lack expression ($\leq 2\%$ positive) of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA class II. Third, the cells must be able to differentiate to osteoblasts, adipocytes and chondroblasts under standard *in vitro* differentiating conditions.

Table 1. Summary of criteria to identify MSC

1	Adherence to plastic in standard culture conditions		
2	Phenotype	Positive ($\geq 95\%+$)	Negative ($\leq 2\%+$)
		CD105	CD45
		CD73	CD34
		CD90	CD14 or CD11b
			CD79α or CD19
			HLA-DR
3	*In vitro* differentiation: osteoblasts, adipocytes, chondroblasts (demonstrated by staining of *in vitro* cell culture)		

Plastic adherence is a well-described property of MSC, and even unique subsets of MSC that have been described maintain this property [4,5]. While MSC may be maintained, and possibly expanded, without adherence [6], these protocols typically require very specific culture conditions, and these cells, if maintained under more standard conditions, would be expected to demonstrate adherence if the cells are to be considered a population of MSC.

Surface Ag expression, which allows for a rapid identification of a cell population, has been used extensively in immunology and hematology. To identify MSC, we propose that cells should express CD105 (known as endoglin and originally recognized by the MAb SH2), CD73 (known as ecto 5′ nucleotidase and originally recognized by the MAb SH3 and SH4) and CD90 (also known as Thy-1). Novel surface markers that may be identified in the future could lead to modifications of these criteria. To assure that studies of heterogeneous populations of MSC are not confounded by other cells, we recommend that lack of expression of hematopoietic Ag be used as additional criteria for MSC as they are not known to express these Ag. For this purpose, we recommend that a panel of Ag be used to exclude the cells most likely to be found in MSC cultures. CD45 is a pan-leukocyte marker; CD34 marks primitive hematopoietic progenitors and endothelial cells; CD14 and CD11b are prominently expressed on monocytes and macrophages, the most likely hematopoietic cells to be found in an MSC culture; CD79α and CD19 are markers of B cells that may also adhere to MSC in culture and remain vital through stromal interactions; and HLA-DR molecules are not expressed on MSC unless stimulated, e.g. by IFN-γ. Only one of the two macrophage and B-cell markers needs to be tested. Each group of investigators should select the marker(s) that is (are) most reliable in their laboratory.

Finally, the biologic property that most uniquely identifies MSC is their capacity for trilineage mesenchymal differentiation. Thus, cells must be shown to differentiate to osteoblasts, adipocytes and chondroblasts using standard *in vitro* tissue culture-differentiating conditions. Differentiation to osteoblasts can be demonstrated by staining with Alizarin Red or von Kossa staining. Adipocyte differentiation is most readily demonstrated by staining with Oil Red O. Chondroblast differentiation is demonstrated by staining with Alcian blue or immunohis-

tochemical staining for collagen type II. Most published protocols for such differentiations are similar, and kits for such assays are now commercially available; thus, we believe that demonstrating differentiation should be feasible for all investigators.

Several of the criteria merit further comment. First, we encourage investigators to test for as many surface markers (both positive and negative) as they deem important, especially as it relates to their own research. The optimum flow cytometric assay would utilize multicolor analyzes (i.e. double staining, triple staining, etc.) to demonstrate that individual cells co-express MSC markers and lack hematopoietic Ag. Our proposal represents the minimum requirements, but additional evidence is always useful.

We also recognize that the proposed panel of Ag does not uniquely identify MSC compared with some other cells [7]. However, the surface phenotype, in conjunction with the other functional criteria, best identifies MSC with the current state of knowledge.

Second, MSC express HLA-DR surface molecules in the presence of IFN-γ but not in an unstimulated state. Thus, if HLA-DR expression is found, and in fact such expression may be desirable for some applications, the cells may still be termed MSC, assuming the other criteria are met, but should be qualified with adjectives, such as 'stimulated MSC', or other nomenclature to indicate that the cells are not in the baseline state.

Third, the level of MSC purity we suggest ($\geq 95\%$ expression of CD105, CD73, CD90; $\leq 2\%$ expression of hematopoietic Ag) should be considered as minimal guidelines. Greater levels of demonstrated purity may be required for some experimental systems.

Finally, MSC have great propensity for *ex vivo* expansion. Investigators who utilize extensively passaged cells may be well served by verifying a normal karyotype to reduce the probability of chromosomal abnormalities, including potentially transforming events. Such events could potentially lead to the establishment of a novel cell line, and the resulting cells should no longer be considered MSC. However, karyotype analysis is not being recommended for routine identification of MSC.

These criteria apply only to human MSC. While adherence and trilineage differentiation are characteristics of cells from other species, for example murine MSC, surface Ag expression is not universally well characterized [8] and the Ag recommended may not apply to non-human systems. Moreover, these criteria should be employed in a control study fashion. Investigators should demonstrate that the cells prepared in their laboratory for a given project meet the stated criteria; however, each cell preparation does not need to be re-evaluated.

Our goal is to encourage MSC investigators to adopt these minimal criteria in an effort to standardize the cell preparations and allow for a comparison of scientific studies among laboratories. These criteria should become the basis for additional characterization of these cells. By this approach, we anticipate more rapid advances in the use of these cells in pre-clinical studies and in the subsequent development of clinical therapies.

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