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Global proteomic signature of undifferentiated human bone marrow stromal cells: Evidence for donor-to-donor proteome heterogeneity

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Abstract The clinical application of human bone marrow stromal cells (hBMSCs) largely depends on their capacity to expand *in vitro*. We have conducted a comprehensive comparative proteomic analysis of culture-expanded hBMSCs obtained from different human donors. The data reveal extensive donor-to-donor proteomic heterogeneity. Processing and database-searching of the tandem MS data resulted in a most comprehensive to date proteomic dataset for hBMSC. A total of 7753 proteins including 712 transcription and translation regulators, 384 kinases, 248 receptor proteins, and 29 cytokines were confidently identified. The proteins identified are mainly nuclear (43.2%) and the share of proteins assigned to more than one subcellular location constitutes 10% of the identified proteome. Bioinformatics tools (IPA, DAVID, and PANTHER) were used to annotate proteins with respect to cellular locations, functions, and other physicochemical characteristics. We also compared the proteomic profile of hBMSCs to recently compiled datasets for human and mouse pluripotent stem cells. The result shows the extent of similarity between the three cell populations and also identified 253 proteins expressed uniformly by all lines of hBMSCs but not reported in the proteomic datasets of the two pluripotent stem cells. Overall, the proteomic database reported in this paper can serve as a reference map for extensive evaluation of hBMSC to explain their biology as well as identify possible marker candidates for further evaluation.
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Introduction

Mesenchymal stromal cells were originally described as stromal cells from bone marrow in the hematopoietic microenvironment that formed adherent colonies when cultured *ex vivo* and demonstrate osteogenic potential (Friedenstein et al., 1968, 1970; Sensebe et al., 2010). Since their first description, cells with similar characteristics have been derived from numerous tissues including cord blood, adipose tissue, cartilage, dental pulp, and muscle (Kuhn and Tuan, 2010). The cells obtained from bone marrow were named mesenchymal stem cells in 1991 by Caplan (Caplan, 1991). In 2005, the International Society for Cellular Therapy (ISCT) recommended the term multipotent

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mesenchymal stromal cells to be used to refer to fibroblast-like cells with a set of properties including plastic-adherence, *in vitro* trilineage differentiation capacity, and expression of a defined set of cell-surface antigens (Dominici et al., 2006; Horwitz et al., 2005). The ISCT's definition has been widely adopted although recent evidence has shown that the characteristics of stromal cells vary depending on their tissue sources. Moreover, the true multipotency and self-renewing capacity of stromal cells from various tissues have not been confirmed with rigorous bioassays (Bianco et al., 2013). A particular challenge to the field has been the absence of the unique set of markers that can be used to enrich MSCs from other connective tissue cell populations and define them functionally. There is much discussion of the functional definition, nomenclature, and experimental handling of multipotent stem cells as can be observed in recent reviews (Bianco et al., 2010, 2013; Keating, 2012). In this paper the term bone marrow stromal cells (BMSCs) is used to refer to plastic adhering bone marrow-derived colonies of stromal progenitors that express a set of cell-surface phenotypes defined by ISCT. Such cells have been referred to by various names in the literature including mesenchymal stem/stromal cells (MSCs).

While stem cell-based therapies hold great potential for the treatment of a wide array of medical conditions they are so novel that product characterization is particularly challenging. Despite considerable progress, the molecular regulatory mechanisms of self-renewal and lineage specification in these cell types are largely unexplored. In recent years a number of "omics" technologies were applied to investigate MSCs (Jansen et al., 2010; Kulterer et al., 2007; Ng et al., 2008; Ren et al., 2011). The majority of earlier proteomic studies were performed by a combination of two dimensional electrophoresis (2DE) and matrix assisted laser desorption ionization mass spectrometry (MALDI MS). The first proteomic investigation reported by Colter et al. resulted in the identification of 40 differentially regulated proteins between rapidly self-renewing and mature human BMSCs (hBMSCs) (Colter et al., 2001). Similar techniques were used to study the effect of transforming growth factor beta (TGF-β) (Wang et al., 2004), shear stress (Yi et al., 2010), and mechanical strain and TGF-β (Kurpinski et al., 2009), or disease conditions such as rheumatoid arthritis (Kastrinaki et al., 2008), osteoarthritis (Rollin et al., 2008), and idiopathic scoliosis (Zhuang et al., 2011) on hBMSCs. Other studies used combinations of 2DE and MALDI MS to compare the proteomic variability between MSCs isolated from various sources such as amniotic fluid, bone marrow, umbilical cord, placenta, adipose tissue, and synovial membrane (Roche et al., 2009).

However, the well-documented poor performance of 2DE with regard to membrane, basic, and low abundance proteins limited the exploration of such complex biological samples as MSCs (Chevalier, 2008). Recent trends show that on-line multi-dimensional liquid chromatography (LC) coupled with MS significantly improves proteomic coverage. This approach dramatically increased the number of proteins identified (~900) including hundreds of membrane proteins from hBMSCs (Niehage et al., 2011). On the other hand, off-line 2D-LC fractionation followed by MALDI MS was also applied successfully to study the proteomic architecture of distinct populations of hBMSC (Mareddy et al., 2009).

The number of proteins identified to date in hBMSC (<1000) clearly indicates that we have only scratched the surface of the proteome and detected mainly abundantly and moderately expressed proteins. A deeper molecular analysis of the proteome, transcriptome, and protein interactome of hBMSCs would lead to a better understanding of these cells. An additional difficulty is the absence of a unified analytical approach which makes the comparison of data obtained in different laboratories challenging, particularly in combination with the well-documented heterogeneity of hBMSCs.

In this study we applied a combined proteomic approach that included pressure cycling-based protein harvesting, 3D fractionation, and complementary MS strategies (electrospray ionization (ESI) and MALDI) to improve proteomic characterization of culture-expanded hBMSCs obtained from different human donors. We created the largest proteomic database for hBMSCs reported to date. The results obtained highlight the surprisingly large degree of proteomic variability in hBMSC cell lines obtained from six human donors. Furthermore, results of extensive bioinformatic analyses of relevant molecular events, biological processes, signaling pathways, and protein–protein interaction networks (interactome) that are operating in these cell populations are also presented and discussed.

Materials and methods

Cell cultures

HBMSC lines from six human donors (four females and two males, Table 1) were obtained from commercial sources. Cell lines PCBM1641, PCBM1632, and PCBM1662 were obtained at passage 1 (P1) from All Cells. Lines 167696, 110877, and 8F3560 were purchased from Lonza at P1. All donors fulfilled institutional requirements at the time of cell collection. According to the manufacturers, following informed consent bone marrow aspirates were taken and plastic adherent hBMSCs were harvested. The time of culture prior to harvest was 15, 15, and 14 days for cell lines 167696 (at 90% confluence), 110877 (at 45% confluence), and 8F3560 (at 35% confluence). At this stage cells were designated as P0 by the supplier. Cells were further cultured to P1 for 6, 6, and 7 days, respectively, collected at 95% confluence, and frozen. Cell lines PCBM1641, PCBM1632, and PCBM1662 were

Table 1 HBMSC lines and donor characteristics. Cells from two commercial sources were obtained at P1 and a proteomic comparison was performed at P3, P5, and P7.

Donor #	Cell line	Sex	Age	Cell source	Race
1	PCBM1641	F	23	All Cells	Hispanic
2	167696	F	22	Lonza	Hispanic
3	PCBM1632	M	24	All Cells	Black
4	110877	M	22	Lonza	Black
5	8F3560	F	24	Lonza	Hispanic
6	PCBM1662	F	31	All Cells	Caucasian