Elucidating the Secretion Proteome of Human Embryonic Stem Cell-derived Mesenchymal Stem Cells

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Transplantation of mesenchymal stem cells (MSCs) has been used to treat a wide range of diseases, and the mechanism of action is postulated to be mediated by either differentiation into functional reparative cells that replace injured tissues or secretion of paracrine factors that promote tissue repair. To complement earlier studies that identified some of the paracrine factors, we profiled the paracrine proteome to better assess the relevance of MSC paracrine factors to the wide spectrum of MSC-mediated therapeutic effects. To evaluate the therapeutic potential of the MSC paracrine proteome, a chemically defined serum-free culture medium was conditioned by MSCs derived from human embryonic stem cells using a clinically compliant protocol. The conditioned medium was analyzed by multidimensional protein identification technology and cytokine antibody array analysis and revealed the presence of 201 unique gene products. 86–88% of these gene products had detectable transcript levels by microarray or quantitative RT-PCR assays. Computational analysis predicted that these gene products will significantly drive three major groups of biological processes: metabolism, defense response, and tissue differentiation including vascularization, hematopoiesis, and skeletal development. It also predicted that the 201 gene products activate important signaling pathways in cardiovascular biology, bone development, and hematopoiesis such as Jak-STAT, MAPK, Toll-like receptor, transforming growth factor-β, and mTOR (mammalian target of rapamycin) signaling pathways. This study identified a large number of MSC secretory products that have the potential to act as paracrine modulators of tissue repair and replacement in diseases of the cardiovascular, hematopoietic, and skeletal tissues. Moreover our results suggest that human embryonic stem cell-derived MSC-conditioned medium has the potency to treat a variety of diseases in humans without cell transplantation. Molecular & Cellular Proteomics 6:1680–1689, 2007.

Mesenchymal stem cells (MSCs) are multipotent stem cells that have been used in clinical and preclinical applications to treat a wide range of diseases (1, 2) including musculoskeletal tissue bioengineering (3, 4) and heart disease (5, 6). They are routinely isolated from adult tissues such as bone marrow and adipose tissues and expanded ex vivo. Ex vivo expanded MSCs have lineage-restricted differentiation potential and can be induced to differentiate into mesenchymal lineages such as osteoblasts, chondrocytes, adipocytes, myocytes, tendon-ligament fibroblasts, and cardiomyocytes. Transplantation of these MSCs has been shown to enhance repair of musculoskeletal injuries, reduce tissue damage and improve cardiac function in ischemic heart disease, and ameliorate severity of graft versus host disease (1). Unlike embryonic stem cells, these lineage-restricted stem cells have negligible risk of teratoma formation (7).

The therapeutic capacity of MSCs to treat a wide spectrum of diseases has been attributed to their potential to differentiate into many different reparative cell types. However, the efficiency of transplanted MSCs to differentiate into functional reparative cells in the injured tissues or organs and in therapeutically relevant numbers has never been adequately documented or demonstrated. Recent reports have suggested that some of these reparative effects are not mediated by 1 The abbreviations used are: MSC, mesenchymal stem cell; hESC, human embryonic stem cell; hESC-MSC, hESC-derived MSC; ITS, insulin, transferrin, and selenoprotein; CM, conditioned medium; NCM, non-conditioned medium; SCX, strong cation exchange; qRT, quantitative RT; mTOR, mammalian target of rapamycin; MAPK, mitogen-activated protein kinase; STAT, signal transducers and activators of transcription; Jak, Janus kinase; TGF, transforming growth factor; DMEM, Dulbecco’s modified Eagle’s medium; PDGF, platelet-derived growth factor; FA, formic acid; GO, gene ontology; ECM, extracellular matrix; LOD, limit of detection.
tissue-derived MSCs, MSC and share the basic distinctive characteristics of adult (hESC-MSC) lines fulfilled the minimal criteria of a multipotent clinically compliant protocol (21). These hESC-derived MSCs derived from a human embryonic stem cell (hESC) line using HuES9.E1 MSC line, one of three lines that we have previously chemically defined culture medium conditioned by the technology (20) and cytokine antibody array analysis on a MSCs by performing multidimensional protein identification effects.

This paracrine hypothesis will introduce a radically different dimension to the use of MSCs in regenerative medicine. Instead of using cells, repair of injured tissues will be mediated by enhancing endogenous tissue repair using biologics secreted by MSCs. This will bypass the present confounding issues associated with cell-based therapy, i.e. immune compatibility, tumorigenicity, xenozootic infections, costs, and waiting time for ex vivo expansion of autologous cell preparations. Such an approach will have a greater potential for the development of “off-the-shelf” MSC-based therapeutics at affordable costs and with better quality control and consistency.

Numerous reports have invoked the secretion of paracrine factors as a mechanism for the reparative effects of MSCs on injured tissues (8, 19). However, there has been no systematic or comprehensive profiling of the paracrine proteome that will enable an adequate assessment of the general validity of the paracrine hypothesis and the relevance of this paracrine hypothesis to the wide spectrum of MSC-mediated therapeutic effects.

In the present study we assessed the secretion proteome of MSCs by performing multidimensional protein identification technology (20) and cytokine antibody array analysis on a chemically defined culture medium conditioned by the HuES9.E1 MSC line, one of three lines that we have previously derived from a human embryonic stem cell (hESC) line using a clinically compliant protocol (21). These hESC-derived MSC (hESC-MSC) lines fulfilled the minimal criteria of a multipotent MSC and share the basic distinctive characteristics of adult tissue-derived MSCs, i.e. these cell lines are plastic-adherent when maintained in standard culture conditions; express CD105, CD73, and CD90 and lack expression of CD45 and CD34; and can differentiate into osteoblasts, adipocytes, and chondroblasts (21, 22). Although hESC-derived MSCs resemble adult bone marrow-derived MSCs, there are differences. For example, genes that were preferentially expressed in the HuES9.E1 MSC line are associated with embryonic processes such as proliferation and early developmental processes of embryogenesis and segmentation, whereas those in bone marrow-derived MSCs were over-represented in biological processes associated with more mature cell types such as metabolic processes, cell structure, and late developmental processes of skeletal development and muscle development (21). This phenomenon is consistent with the observation that the biology of MSCs isolated from fetal and adult tissues is different and is characteristic of the developmental stage of their tissue of origin (24–26). In general, MSCs from younger donors or developmentally less mature tissues are more proliferative and have a more robust differentiation potential and greater therapeutic efficacy (24, 25). Therefore by extrapolation, MSCs derived from embryonic hESC lines will be more proliferative and have a more robust differentiation potential and greater therapeutic efficacy than those derived from adult tissues.

The use of these hESC-MSCs to characterize the secretory proteome of MSCs offers several advantages over the use of MSCs derived from adult tissues. 1) hESC-MSCs can be stably propagated in culture for at least 80 population doubling time as monitored by genome-wide gene expression profile and karyotyping analysis (21), thereby ensuring a stable and renewable source of cells for repeated verifications during proteomics profiling. This advantage is further bolstered by the reproducible generation of highly similar MSC cultures from either the same hESC line or different hESC lines (21). 2) The use of hESCs in contrast to the use of adult tissues, e.g. bone marrow as the tissue source of MSCs, virtually ensures an infinitely renewable and consistent tissue source and enhances the reproducible and consistent batch to batch preparation of MSCs and therefore secretory products. 3) We have also demonstrated that the hESC-MSC lines derived using our protocol are highly similar to single cell-derived hESC-MSC cultures and are therefore homogenous (21).

Analysis of the secretory proteome of hESC-MSCs revealed a total of 201 unique gene products. 29 of these gene products have been previously reported to be secreted by adult tissue-derived MSCs. Four other proteins that were reported to be secreted by adult tissue-derived MSCs, namely IGFBP3, MIP3α, Oncostatin M, and TGFβ3, were not present in our list of 201 gene products. The spectrum of secreted gene products is consistent with the reported paracrine effects of MSCs on different diverse cellular systems and diseases (9–18, 27) and provides a molecular basis for the use of hESC-MSC-conditioned medium in local or systemic treatment of diseases including repair of the heart after myocardial infarction.

EXPERIMENTAL PROCEDURES

Preparation of Conditioned Medium—HuES9.E1 cells were cultured as described previously (21). 80% confluent HuES9.E1 cell cultures were washed three times with PBS and cultured overnight in a chemically defined medium consisting of DMEM without phenol red (catalog number 31053, Invitrogen) and supplemented with insulin, transferrin, and selenoprotein (ITS) (Invitrogen), 5 ng/ml FGF2 (Invitrogen), 5 ng/ml PDGF AB (Peprotech, Rocky Hill, NJ), glutamine-penicillin-streptomycin, and β-mercaptoethanol. The cultures were then rinsed three times with PBS, and then fresh defined medium was added. After 3 days, the medium was collected and centrifuged at 500 × g, and the supernatant was filtered using a 0.2-μm filter. For LC-MS/MS analysis, the conditioned medium was placed in dialysis cassettes with molecular weight cutoff of 3500 (Pierce), dialyzed against three changes of 10 volumes of 0.9% NaCl, then concentrated 20 times using Slide-A-Lyzer concentrating solution, and then dialyzed against 10 changes of 100 volumes of 0.9% NaCl before filtering with a 0.2-μm filter. The same volume of non-conditioned
medium was dialyzed and concentrated in parallel with the conditioned medium.

**Cytokine Antibody Blot Assays**—1 ml of conditioned or non-conditioned medium (CM) or non-conditioned medium (NCM) was reduced, alkylated, and digested with trypsin as described previously (20). The samples were then desalted by passing the digest mixture through a conditioned Sep–Pak C₁₈ solid phase extraction cartridge (Waters, Milford, MA), washed twice with a 3% ACN (J. T. Baker Inc.) and 0.1% formic acid (FA) buffer, and eluted with a 70% ACN and 0.1% FA buffer. The eluted samples were then dried to about 10% of their initial volumes by removing organic solvent in a SpeedVac. The samples were kept at 4 °C prior to LC-MS/MS analysis. The desalted peptide mixture was analyzed by multidimensional protein identification technology with an LC-MS/MS system (LTQ, ThermoFinnigan, San Jose, CA). The sample was loaded into a strong cation exchange (SCX) column (Biobasic SCX, 5 μm, Thermo Electron, San Jose, CA) and fractionated by six salt steps with 50 μl of buffers (0.2, 5, 10, 100, and 1000 mM ammonium chloride in 5% ACN and 0.1% FA) in the first dimension. The peptides eluted from the SCX column were concentrated and desalted in a Zorbax peptide trap (Agilent Technologies, Foster City, CA). The cDNA was diluted with distilled water to a volume of 100 μl. 1 μl was used for each primer set in pathway-specific RT² Profiler PCR Arrays (SuperArray, Frederick, MD) according to the manufacturer’s protocol. The plates used for the analysis were Chemokines and Receptors PCR Array (catalog number APH-022), NFκB Signaling Pathway PCR Array (catalog number APH-025), Inflammatory Cytokines and Receptors PCR Array (catalog number APH-011), Common Cytokine PCR Array (catalog number APH-021), and JAK/STAT Signaling Pathway PCR Array (catalog number APH-039).

**RESULTS**

**Preparation of CM and NCM**—To ensure that there was minimal contamination of conditioned medium by medium supplements such as serum replacement medium, HuES9.E1 MSCs were grown to about 80% confluence, washed three times with PBS, and incubated overnight in a chemically defined medium consisting of DMEM supplemented with ITS, 5 ng/ml FGF2, 5 ng/ml PDGF AB, glucose-penicillin-streptomycin, and β-mercaptoethanol. HuES9.E1 MSCs can be propagated in this minimal medium for at least a week. The next day, the cell culture was again washed three times with PBS and incubated with the fresh defined medium. The medium was collected after 3 days of conditioning. The CM was always analyzed or processed in parallel with an equivalent volume of NCM. For LC-MS/MS analysis, the medium was concentrated ~10× before dialyzing extensively against 0.9% saline as described under “Experimental Procedures.” The average protein concentrations of concentrated CM and NCM were 98.0 ± 17.9 and 41.6 ± 1.2 μg/ml (n = 3), respectively. The conditioning of medium by MSCs was monitored by running aliquots of the medium on protein gels. Protein composition of the medium increased in complexity with time (Fig. 1). The CM had a more complex protein composition than NCM.

**Analysis of MSC-conditioned Medium by LC-MS/MS and Antibody Array**—LC-MS/MS analysis identified 247 proteins that were present in two independently prepared batches of CM but not in a similarly processed NCM (Supplemental Table 1). Together these 247 proteins are encoded by 132 unique known genes (Supplemental Table 2a). There were 29 unknown proteins (Supplemental Table 2b).

MSCs have been shown to secrete a broad spectrum of cytokines and growth factors that affect cells in their vicinity (8). Many of these factors are small molecules that are not easily detectable during shotgun LC-MS/MS analysis. Therefore, the CM and NCM were also analyzed by hybridization to five different antibody arrays that together carried antibodies.
against 101 cytokines/growth factors (Fig. 2 and Supplemental Table 3a). 72 of the cytokines/growth factors were found to be reproducibly secreted by HuES9.E1 MSCs in at least three of four independently prepared batches of CM but not in NCM (Supplemental Table 3, b and c). However, only three gene products, namely IGFBP2, TIMP1, and TIMP2, were also detected by LC-MS/MS analysis possibly because many of cytokines and growth factors are small molecules and are not detectable during conventional LC-MS/MS analysis.

Because the lists of 132 and 72 gene products identified by LC-MS/MS and antibody array analysis, respectively, have three common genes, namely IGFBP2, TIMP1, and TIMP2, the final tally was 201 unique gene products (Table I). 29 of these gene products, namely ENA78 (CXCL5), FGF4, FGF7, FGF9, GCP2 (CXCL6), granulocyte colony-stimulating factor, granulocyte/macrophage colony-stimulating factor, GRO-α, NCC4 (CCL16), hepatocyte growth factor, IGFBP1, IGFBP2, IGFBP4, IL1β, IL6, IL8, IP10 (CXCL10), leukocyte migration inhibitory factor, MCP1 (CCL2), macrophage colony-stimulating factor, macrophage migration inhibitory factor, Osteoprotegerin, pulmonary and activation-regulated chemokine, placenta growth factor, stem cell factor, TGFβ2, TIMP1, TIMP2, and vascular endothelial growth factor, have been previously reported to be secreted by adult tissue-derived MSCs (7, 13, 16, 19, 28). Four other proteins that were reported to be secreted by adult tissue-derived MSCs, namely IGFBP3, MIP3α, Oncostatin M, and TGFβ3 (Supplemental Table 3b), were not present in our list of 201 gene products.

Verification by Genome-wide Gene Expression Analysis—Comparison of the 201 gene products to a genome-wide gene expression profile of the hESC-MSCs generated by hybridizing total RNA to an Illumina BeadArray revealed that 134 or 67% of the gene products had gene transcript levels that were present at above the limit of detection (LOD) with a 99% confidence (Table I). Although 115 or 88% of the 132 gene products identified by LC-MS/MS had detectable transcript levels (Table I), only 27 or 38% of the 72 gene products identified by antibody array had detectable transcript levels, and 45 or 62% had no detectable transcript level (Table I). Probes for two of the gene products, ENO1B and SVEP1, were not present on the Illumina BeadArray. It is possible that transcript levels for most of the 72 gene may be too low in abundance for detection by Illumina BeadArray because...
mRNAs encoding for cytokines/chemokines are known to contain AU-rich elements that caused rapid degradation of the mRNA during translation (29, 30). More sensitive qRT-PCR assays were therefore performed. 42 of the 72 gene products were randomly selected and tested. 36 or 86% of the 42 gene products had detectable transcript levels defined as having a normalized Ct value of <35 (Table II). This frequency was similar to the 88% frequency observed for gene products identified by LC-MS/MS and antibody array.

**Biological Processes That Are Modulated by the Secreted Proteins**—To investigate whether the secreted products have the potential to repair the injured tissues or organs, gene products were first classified according to their biological processes and pathways according to GO. The frequency of unique genes in the secreted MSC proteome associated with each process or pathway was then compared with the gene frequency for the respective pathway or process in a database collated from Unigene, Entrez, and GenBank using GeneSpring GX7.3 Expression Analysis software. Significantly higher frequencies of genes (p < 0.05) were associated with 58 biological processes and 30 pathways (Supplemental Table 4). The 58 biological processes could be approximated into the three major groups of metabolism, defense response, and tissue differentiation, whereas the 30 pathways could be broadly categorized into receptor binding, signal transduction, cell-cell interaction, cell migration, immune response, and metabolism (Figs. 3 and 4). The postulated biological processes and pathways both suggest that the secreted proteins have a major impact on the cellular metabolism that will

### Table I

**Alphabetical list of 201 unique gene products identified by LC-MS/MS and antibody array**

The proteins identified by LC-MS/MS and antibody array as listed in Supplemental Tables 2a and 3b were combined and are represented by their gene symbol. The transcript level for each gene was assessed using a high throughput Illumina BeadArray.

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| Symbol | Illumina BeadArray | Normalized Ct |
|--------|--------------------|---------------|
| BDNF  | >LOD               | 13.38         |
| CCL2  | >LOD               | 11.28         |
| CCL7  | >LOD               | 17.35         |
| CCL8  | >LOD               | 30.28         |
| CXCL1 | >LOD               | 14.09         |
| CXCL12| >LOD               | 16.83         |
| CXCL5 | >LOD               | 19.93         |
| IL1A  | >LOD               | 16.6          |
| IL1B  | >LOD               | 11.44         |
| IL6   | >LOD               | 18.92         |
| IL8   | >LOD               | 8.53          |
| MIF   | >LOD               | 16.23         |
| MMP3  | >LOD               | 15.96         |
| TGFβ2 | >LOD               | 15.16         |
| TNFRSF11B | >LOD | 12.28         |
| CCL1  | <LOD               | 34.67         |
| CCL11 | <LOD               | 26.13         |
| CCL23 | <LOD               | 37.33         |
| CCL24 | <LOD               | 10.82         |
| CCL26 | <LOD               | 30.38         |
| CCL5  | <LOD               | 27.97         |
| CSF1  | <LOD               | 14.35         |
| CSF2  | <LOD               | 23.92         |
| CSF3  | <LOD               | 32.47         |
| CXCL1 | <LOD               | 25.04         |
| CXCL11| <LOD               | 22.38         |
| CXCR3 | <LOD               | 28.41         |
| IL10  | <LOD               | 27.4          |
| IL12B | <LOD               | 22.17         |
| IL13  | <LOD               | 14.96         |
| IL16  | <LOD               | 32.98         |
| IL2   | <LOD               | 31.87         |
| IL3   | <LOD               | 32.24         |
| IL7   | <LOD               | 22.3          |
| TGFβ1 | <LOD               | 10.63         |
| TNF   | <LOD               | 31.72         |
| CCL15 | <LOD               | >35           |
| CCL16 | <LOD               | >35           |
| CCL13 | <LOD               | >35           |
| IFNG  | <LOD               | >35           |
| VEGF  | <LOD               | >35           |
| XCL1  | <LOD               | >35           |
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modulate energy production and the breakdown, biosynthesis, and secretion of macromolecules, processes essential for the removal of damaged tissues and regeneration of new tissues (Figs. 3 and 4). Consistent with the predominant presence of cytokines and chemokines in the MSC-conditioned medium, the analysis also predicted that the secreted factors could elicit many cellular responses that are dependent on external stimuli, e.g., chemotaxis, taxis, and many immune responses (Fig. 3). Notably, the conditioned medium could also induce biological processes that are important in tissue differentiation, particularly processes that promote vascularization, hematopoiesis, and bone development (Fig. 3). In those pathways predicted to be modulated by the secreted proteome, receptor-mediated binding of cytokine and ECM pathways were consistent with the predominance of cytokines and ECM components in the secreted proteome (Fig. 4).

The main signal transduction pathways that could be activated by the secreted proteome include Jak-STAT signaling pathway, MAPK signaling pathway, Toll-like receptor signaling pathway, TGFβ signaling pathway, mTOR signaling pathway, FcεRI signaling pathway, and epithelial cell signaling in Helicobacter pylori infection. The computational analysis of the secreted proteome also suggested that MSC secretion could enhance cell-cell interaction, migration, and immune responses.

**DISCUSSION**

MSCs have been used in preclinical and clinical trials to treat a myriad of diseases (3–5, 31–34). However, the underlying mechanism remains unclear. Although MSCs have the potential to differentiate into numerous cell type, e.g., endothelial cells, cardiomyocytes, and chondrocytes, that can potentially repair or regenerate damaged tissues, differentiation of MSCs into reparative cell types is generally too inefficient to
effectively replace damaged tissue or restore tissue function. There is increasing evidence that some of the therapeutic effects of MSCs may be mediated by paracrine factors secreted by MSCs (8). Therefore, elucidating the molecular composition of the secretion from MSCs will enhance our understanding of the paracrine effects of MSCs. The significant similarity between hESC-derived MSCs and adult tissue-derived MSCs (21) suggests that conditioned medium of either MSC culture is likely to have similar biological activities. hESC-derived MSCs, however, have several advantages over adult tissue-derived MSCs. It has been observed that the age of the donor and the developmental stage of the tissue from which MSCs are derived have an impact on the proliferation, differentiation potential, and therapeutic efficacy of the MSCs. In general, younger donors and developmentally less mature tissues generate MSCs that are more proliferative and have a more robust differentiation potential and greater therapeutic efficacy (24–26). The use of hESC cell lines as a tissue source of MSC constitutes an infinitely renewable and expandable tissue source and enhances the reproducible and consistent batch to batch preparation of MSCs. We have shown previously that our protocol for generating MSCs from hESCs is not only clinically compliant but also generates MSC cultures that are homogenous and highly similar (21). The use of hESC-MSC lines also enhances the scalability of preparing CM and the potential of developing low cost off-the-shelf therapeutics. In addition, the development of a serum-free chemically defined medium for the preparation of hESC-derived MSCs (CM) reduces confounding and variable contaminants associated with complex medium supplements such as serum or serum replacement medium.

Here we describe the composition of the secreted proteome of hESC-MSCs through a combination of two techniques, LC-MS/MS and antibody arrays. Although shotgun proteomics analysis by LC-MS/MS is a sensitive technique and has high throughput capability, it is difficult to detect small proteins/peptides that include most of the cytokines, chemokines, and growth factors. This was partially mitigated by the use of antibody arrays. The qualitative proteomic profile of the MSC secretion using the two techniques was highly reproducible. Proteins identified by LC-MS/MS were present in two independently prepared batches of CM, whereas those identified by antibody array were present in at least three of four independently prepared batches of CM. The resulting proteomic profile of secretion by hESC-MSCs included almost all the factors that were previously reportedly secreted by adult tissue-derived MSCs (7, 13, 16, 19, 28) including twice as many factors that have not been described. The robustness of the proteomics profiling was further substantiated by the detection of transcripts for 86–88% of gene products in the proteomic profile using a high throughput genome-wide gene expression assay and quantitative RT-PCR assays. Although this proteomic profile was developed using a population of cells rather than single cells, it is nevertheless a robust representation of hESC-derived MSC secretion for several reasons. First, the cell population used in the elucidation of the secreted proteome can be reproducibly generated from either the same parental hESC line or a different hESC line with a high degree of similarity between sorted single cell-derived lines and unsorted population-derived lines (21). Second, the cell population maintained a stable gene expression profile and karyotype for at least 60 population doublings (21). Third, >99% of cells in this cell population differentiate to form adipocytes when exposed to an adipocyte differentiation medium, and >90% of the cells produce proteoglycans when exposed to chondrocyte differentiation medium, suggesting that a large majority of cells are least bipotential (21). Therefore, the elucidated proteome is largely reflective of an hESC-derived MSC population. However, it remains to be determined whether this secreted proteome is unique to hESC-derived MSCs and not to other hESC-derived cell populations or differentiated MSCs. A major hindrance in resolving this issue is that, unlike hESC-derived MSCs, we have not been able to maintain any hESC-derived cell population or differentiate MSCs using a chemically defined medium without serum or serum replacement medium. The high protein content in serum and serum replacement medium precludes any meaningful proteomics analysis of secretion by cells into the medium.

To evaluate and assess the potential functions of the MSC secretion on a global scale, we utilized the more readily available computational tools for gene expression analysis. Consistent with the predominance of cytokines and chemokines in the secretion, computational analysis not unexpectedly predicted many processes and pathways that are generally associated with the functions of cytokines and chemokines such as chemotaxis, taxis, cellular response to external stimuli, breakdown, biosynthesis, and secretion of macromolecules, cytokine-cytokine receptor interactions, cell-cell communication, and basal metabolism, e.g. glucose and amino acid metabolism. Although these processes and pathways are not specific to the process of injury, repair, and regeneration in any particular cell or tissue type, their facilitation of immune cell migration to the site of injury, ECM remodeling, and an increase in the cellular metabolism will have reparative effects on most injured or diseased tissues.

Aside from these generic pathways associated with cytokines and chemokines, computational analysis also predicted...
that the secreted proteins regulate many processes involved in vascularization, hematopoiesis, and skeletal development. Coincidentally most reported MSC-mediated tissue repair and regeneration are associated with cardiovascular, hematopoietic, and musculoskeletal biology. For example, Jak-STAT signaling is associated with cardioproteinosis (35), hematopoiesis (36, 37), and skeletal repair and remodeling (38, 39); MAPK signaling plays a crucial role in many aspects of cardiovascular responses (40, 41), skeletal repair and remodeling (39, 42), and hematopoiesis (43); Toll-like receptor signaling has been implicated in the initiation and progression of cardiovascular pathologies (44) and modulation of innate and adaptive immunity (45); TGFβ signaling is critical in correct heart development cardiac remodeling, progression to heart failure, and vascularization (46–48), hematopoiesis (49), formation and remodeling of bone and cartilage (34, 50), and general wound healing (51); and mTOR as an important regulator of cell growth and proliferation plays a nonspecific yet critical role in both normal physiology and diseases (23, 52, 53).

In conclusion, our analysis of the secreted proteome in hES-derived MSCs not only identified many of the cytokines that were previously reportedly to be secreted by adult tissue-derived MSCs but also more than 150 proteins that were not known to be secreted by MSCs. Collectively the secreted proteome could potentially exert modulating effects on tissue repair and regeneration particularly in the cardiovascular, hematopoietic, and musculoskeletal tissues and therefore provide molecular support for an MSC-mediated paracrine effect on tissue repair and regeneration in MSC transplantation studies. Although the composition of secretion by MSCs in vitro is consistent with the hypothesis that paracrine factors could potentially mediate the tissue repair and regeneration observed in MSC transplantation, validation of the hypothesis will require demonstration that transplanted MSCs in vivo also secrete a similar spectrum of proteins or a spectrum of proteins that could support tissue repair and regeneration. Our present elucidation of the in vitro MSC secretion proteome will not only facilitate verification of this hypothesis by providing candidates for in vivo verification, it also unveiled many highly testable hypotheses for the molecular mechanisms in MSC-mediated tissue repair and also potential “druggable” targets to modulate tissue repair and regeneration. Therefore, our elucidation of the CM is very relevant to the translation of MSC-based biologics to clinical applications.

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