

Research Article

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Genomic Analysis of Invasive Human Bone Marrow Derived Mesenchymal Stem Cells

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Abstract

Background: Human bone marrow derived mesenchymal stem cells (hMSCs) are capable of differentiation into multiple cell lineages and demonstrate a wide variety of use in various therapeutic applications. Only recently has research begun to understand the gene expression profiles of hMSCs and their differentiated counterparts *in vivo* and *ex vivo*.

Purpose: The research presented here aimed at gaining a better understanding of gene expression patterns present during hMSC invasion through a basement membrane.

Methods: Changes in gene expression were evaluated between invasive and non-invasive cells using Agilent's gene expression arrays and Matrigel invasion chambers. The cells were specifically attracted to a defined stem cell media called SCM.

Results: A total 435 genes were up-regulated by 2- fold or more in the invasive population of cells and classified into developmental programs and immunological/inflammatory signaling pathways determined by Ingenuity Pathway Analysis (IPA). This list included a variety of regulators of growth and differentiation including NANOG, STAT3 and STAT5A and members of the polycomb repressive complex-2 (PCRC2) EZH2 and SUZ12. The known regulator of inflammation and hypoxia HIF-1 α was also increased suggesting that regulation of the microenvironment is important during this process. Finally, the invasion process could be reversed using the STAT3 inhibitor Static.

Conclusions: Overall these data will increase the understanding of the genetic pathways functioning during hMSC invasion and aid in the development of their therapeutic applications.

Keywords: hMSCs; Invasion; Gene expression; STAT3

Abbreviations: hMSCs: Human Bone Marrow Derived Mesenchymal Stem Cells; SCM: Stem Cell Media; qRT-PCR: Quantitative Real Time Polymerase Chain Reaction; IPA: Ingenuity Pathway Analysis; STAT3: Signal Transducer And Activator Of Transcription 3; STAT5A: Signal Transducer And Activator Of Transcription 5A; PCRC2: Polycomb Repressive Complex-2; EZH2: Enhancer of Zeste-2; SUZ12: Suppressor of Zeste-12; HIF-1 α : Hypoxia-Inducible Factor-1 Alpha; EGF: Epidermal Growth Factor; FGF: Fibroblast Growth Factor; ER β : Estrogen Receptor Beta; NCI: National Cancer Institute; AR: Androgen Receptor; IL13RA1: Interleukin-13 Receptor Alpha-1; TLR2/4: Toll-Like Receptor-2/4; FGF12/7: Fibroblast Growth Factor-12/716; IGF2: Insulin-Like Growth Factor-2; KRT14: Cytokeratin-14; ITGB1/4: Integrin Beta-1/4; RAC3: Ras Related C3 Botulinum Toxin Substrate-3; JAG1: Jagged-1; TREM1: Triggering Receptor Expressed On Myeloid Cells-1; IL: Interleukin; NF κ B: Nuclear Factor Kappa B; ES: Embryonic Stem; AKT: Protein Kinase B; MAPK: Mitogen Activated Protein Kinase; INF1 β : Interferon 1 Beta; CXCR4/10: C-X-C Chemokine Receptor Type-4/10; OCT4: Octamer-Binding Transcription Factor 4; BMPs: Bone Morphogenic Proteins; SHH: Sonic Hedgehog; WNT: Wingless-Type MMTV Integration Site Family; EPCs: Endothelial Precursor Cells; VCAM-1: Vascular Cellular Adhesion Molecule-1; MEF: Mouse Embryonic Fibroblast

Introduction

Adult human bone marrow derived mesenchymal stromal cells (hMSCs), also called mesenchymal stem cells, were first identified as highly proliferative/radiation resistant cells isolated from human bone marrow transplants [1]. These cells are capable of undergoing self-renewal and are multipotent, meaning that under the appropriate

stimuli are capable of differentiating into multiple cell lineages including osteoblasts, chondrocytes, adipocytes and myoblasts [2-4]. With their diverse potential, these cells demonstrate a wide variety of use in many therapeutic applications such as osteogenesis imperfecta [5], myocardial infarction [6], muscular dystrophy [7], spinal cord injury [8,9], graft versus host disease [10,11] and have even recently been examined for their role in the treatment of cancer [12-14]. Previous research has characterized that injured or wounded tissues produce inflammatory cytokines which seem to attract hMSCs expressing CD29, CD44, CD51, CD73, CD105, CD166 and Stro-1 (bone morphogenic protein-3: BMP3) [15,16]. The expression of certain markers, however, does seem to be specific to the microenvironment. Additionally, the process of hypoxia, which is a reduction in the amount of available oxygen, is exhibited by a variety of tumors.

This process is regulated by hypoxia-inducible factor-1 α (HIF1 α) [17] and injured tissues or tumors which are present in a hypoxic microenvironment produce these same angiogenic and inflammatory cytokines, further perpetuating the homing of hMSCs. Stem cell niches

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are often located in anatomical regions characterized by hypoxic conditions as they require low levels of oxygen to minimize damage caused by DNA oxidation. The relationship between oxygen and MSCs is under intense investigation as MSCs reside in locations close to the vascular structures, yet the tissues where MSCs are found exhibit low oxygen levels [18]. The exact mechanism(s) by which oxygen regulates MSCs is unknown, but it is clear that oxygen is a critical regulator of MSC fate.

The research presented here is aimed at gaining a better understanding of what these additional pathways regulating hMSCs might be. An in depth investigation of gene expression patterns present during hMSC invasion through a basement membrane was performed using a 44,000 probe-based gene expression array. These data have provided a unique gene expression signature of invasion toward a stem cell niche.

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Methods

Cell lines and reagents

Early passage (P3) human bone marrow derived mesenchymal stem cells (hMSCs) were obtained from Lonza (Gaithersburg, MD) and maintained using their recommended conditions with addition of 500 U/mL penicillin/ 500 µg/mL streptomycin and 0.250 µg/mL amphotericin-B all from Gibco (Invitrogen, Carlsbad, CA). The cultures were maintained in 5% CO₂ air at 37°C. The STAT3 inhibitor Stattic (Calbiochem, Gibbstown, NJ) was dissolved in molecular grade ethanol.

Matrigel invasion assay

Matrigel-coated 24-well inserts (8 µm pore size) and non-coated control inserts purchased from BD Biosciences Clontech (Palo Alto, CA) were used according to manufacturer's instructions. Between 60,000 to 100,000 cells were seeded for the invasion in serum-free RPMI and migrated toward media specific for stem cells (SCM) containing DMEM/F12 with human supplementation of 10 ng/mL bFGF, 20 ng/mL EGF and 5 µg/mL insulin along with 0.4% BSA (each from Sigma, St. Louis, MO). Routine invasion assays were performed for 24 hours and then stained with the Diffi-Quick Staining kit (Dade Behring, Deerfield, IL). The direct STAT-3 inhibitor Stattic was used at 1 µM. Three to five microscopic fields (20X) were photographed and counted for each sample. The experiment was repeated three independent times.

Microarray Analysis

RNA was isolated and labelled as previously described from invasive and non-invasive cells [19], with the following modifications: Reverse transcriptase was heat inactivated at 65°C for 10 minutes and again 100 ng of RNA was amplified using the Message Amp a RNA Amplification Kit. A total of 2 µg of Universal Reference RNA (Stratagene, La Jolla, CA) was labelled with Cy3-dUTP and experimental samples were labelled with Cy5-dUTP. Samples were hybridized to an Agilent whole genome gene expression array following manufacturer's directions. Arrays were scanned using a GenePix 4000B scanner and extracted

using the mAdb portal from the National Cancer Institute. The data was normalized using the Loess method and bad/or not found spots were excluded for extraction. The array data was further analyzed using Cluster and Tree view offered by Michael B. Eisen as freeware (<http://rana.lbl.gov/EisenSoftware.htm>) to produce the heat map. The complete list of genes whose expression changed ≥ 2 - or ≤ 2 - fold is available in Supplementary Table S1.

Proliferation Assays

Cells were seeded overnight in a 96 well plate in 100 µL of regular media at a density of 2000 cells per well. Cells were treated with either DMSO or 1 µM Stattic. Cell proliferation was measured using the CellTiter-Glo assay from Promega (Madison, WI, USA) after 24 hours using 100 µL of reagent and an incubation time of 20 minutes. The relative luciferase units (RLU) were quantified using a Tecan Infinite 200 plate reader. Samples were performed using an N of 8.

Quantitative Real Time Polymerase Chain Reaction (QRT-PCR)

Total RNA was isolated using TRIzol (Invitrogen Corporation, Carlsbad, CA). RNA from 'top' cells was isolated using a cell pellet acquired from trypsinizing cells from one membrane after bottom cell were removed with a cotton swab. Conversely, RNA from the bottom cells was isolated by combining three membranes where the top cells were removed using a cotton swab. The membranes were pooled and placed in TRIzol for 10 minutes at room temperature, and the conventional procedure for isolation of RNA was then followed. To increase the yield of RNA, 5 µg of linear acrylamide (Ambion, Austin, TX) was added prior to precipitation of RNA with isopropanol. Additional to increase overall yield, 100 ng of RNA was amplified using the Message Amp aRNA Amplification Kit (Ambion, Austin, TX). cDNA was prepared using the SuperScript[®]III First-Strand Synthesis System (Invitrogen Corporation, Carlsbad, CA). Quantitative real time polymerase chain reaction (QRT-PCR) analysis was performed using a StepOne Real-time PCR machine (Applied Biosystems, Foster City, CA) with TaqMan Gene Expression Assay reagents and probes (Applied Biosystems). A total of 4 µL of cDNA was used in a 20 µL reaction resulting in a 1:5 dilution. The following SYBR based human probes were used: ER-β (Hs01112040_m1), EZH2 (Hs00544830_m1), HIF1α (Hs00153153_m1), NANOG (Hs02387400_g1), STAT3 (Hs01047580_m1), STAT5A (Hs00559643_m1), SUZ12 (Hs00248742_m1) and 18S rRNA (Hs99999901_s1). Relative fold induction of mRNA was compared between top and bottom cells using the Delta-Delta CT method of quantitation, and 18S rRNA was used as a loading control.

Ingenuity and Genomatrix Software

Agilent databases (excel 2003 format) were uploaded for analysis using software available at <http://www.ingenuity.com/> or <http://www.genomatix.de/>

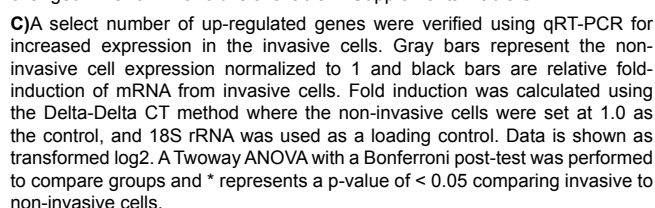
Statistical Analysis

Using Graph Pad Prism (Version 4) a Two-way ANOVA with a Bonferroni post test was performed to compare groups and * represents a p-value of <0.05 comparing invasive to non-invasive cells.

Results

Microarray analysis of invasive hMSCs

Initially it was determined that a small percentage (~20%) of hMSC were able to invade though the Matrigel toward a highly defined media



Recent data suggests a number of these pathways that are increasing with invasion of hMSCs intersect using the STAT3 pathway [22-25]. Using this same invasion assay, our lab has recently demonstrated that the invasion of prostate cancer cells toward SCM can be blocked using

Agilent ID	hMSC Non- Invasive	hMSC_Invasive	Fold Change	UNIQID	Gene Name
A_24_P919916	-1.7055	5.8604	7.5659	WID:6488014	PTF1A
A_23_P42065	-2.8123	0.9734	3.7857	WID:6484664	TNFRSF21
A_23_P30736	-2.4033	0.8032	3.2065	WID:6496619	HLA-DOB
A_23_P60306	-1.43	1.7307	3.1607	WID:6502707	TLR4
A_23_P113111	-1.7102	1.2511	2.9613	WID:6488464	AR
A_23_P77223	-1.0517	1.9013	2.953	WID:6513234	MESP1
A_23_P210763	-2.7955	0.1168	2.9123	WID:6486716	JAG1
A_24_P38387	-3.0463	-0.1468	2.8995	WID:6504089	NDRG1
A_32_P122579	0.6126	3.5082	2.8956	WID:6480399	EZH2
A_23_P207367	-2.2092	0.5955	2.8047	WID:6481655	STAT5A
A_23_P84320	-1.8188	0.8944	2.7132	WID:6495148	HMX1
A_24_P265346	-2.8338	-0.1382	2.6956	WID:6504873	KRT14
A_23_P77440	-2.7784	-0.1771	2.6013	WID:6502102	NFATC3
A_32_P160537	2.0805	4.6637	2.5832	WID:6492904	FGF12
A_23_P206441	-2.1401	0.3806	2.5207	WID:6586963	FANCA
A_24_P598836	1.5009	3.9523	2.4514	WID:6517296	ITGB1
A_23_P214011	-1.9233	0.4855	2.4088	WID:6494488	CDH6
A_23_P23947	-1.2674	1.096	2.3634	WID:6489062	MAP3K8
A_23_P156953	-1.7175	0.6025	2.32	WID:6517216	IGF2R
A_23_P125001	-0.4863	1.8332	2.3195	WID:6482994	RAC3
A_23_P204640	-4.0766	-1.7784	2.2982	WID:6487751	NANOG
A_23_P92499	0.3371	2.5755	2.2384	WID:6486692	TLR2
A_24_P889103	-2.3142	0.6211	2.9353	WID:6516766	SUZ12P
A_23_P54100	-1.5936	0.4465	2.0401	WID:6506930	ESR2
A_23_P137196	-1.3207	0.6947	2.0154	WID:6488748	IL13RA1
A_23_P46964	0.0039	1.6847	1.6808	WID:6478939	HIF1AN
A_23_P100795	-0.1833	1.42	1.6083	WID:6480615	STAT3

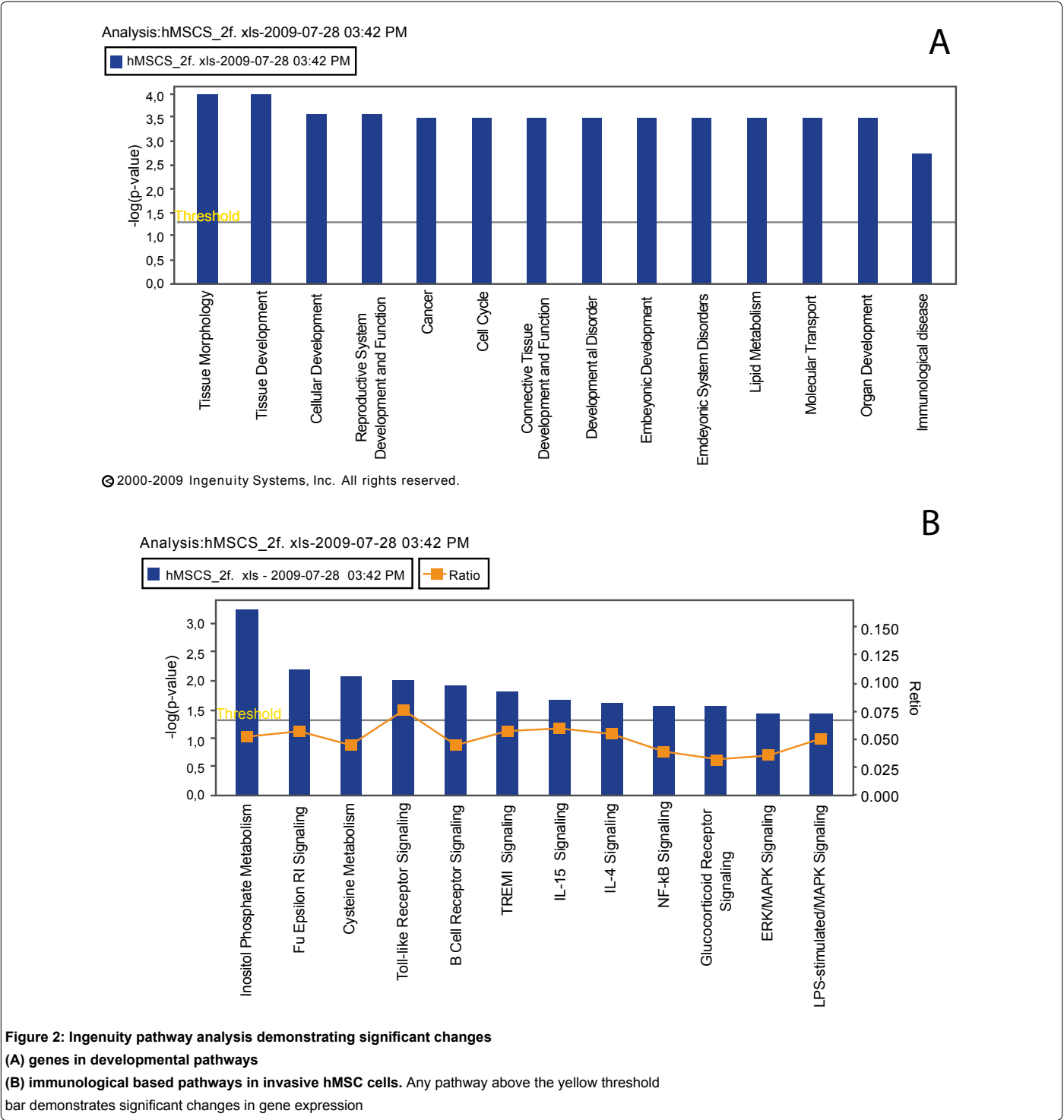
Table 1: Genes up-regulated in invasive hMSC cells determined by microarray analysis. Significant changes in selected genes demonstrating a 1.6-fold or higher increase. The Agilent ID corresponds to the probe ID from the array, values for non-invasive and invasive hMSCs as well as their fold change (Invasive-Non-Invasive) are provided.

the STAT3 inhibitor Stattic [26]. This prompted us to test if Stattic could also block invasion of hMSCs toward SCM. In the presence of Stattic, invasion of the hMSCs was completely abolished without affecting normal cell proliferation, suggesting a strong role for the STAT proteins in mediating this process (Figure 3A and 3B).

Discussion

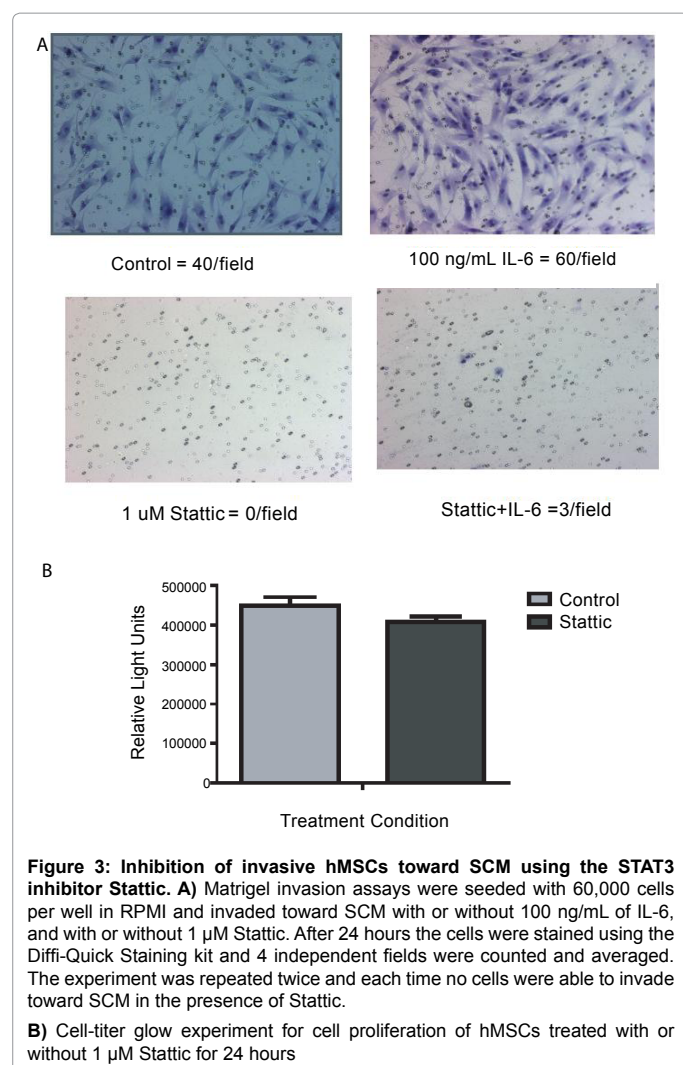
Human bone marrow derived mesenchymal stem cells (hMSCs) have the potential to revolutionize medicine with their donor specific treatment of diseases. hMSCs represent an attractive model for a number of reasons, including the lack of ethical controversy regarding their isolation since the cells themselves can be isolated from the patient's own blood/ tissues [1], as well as their lack of significant immunogenicity allowing for allogeneic transplantation without the use of potentially harmful immunosuppressive drugs [27]. Albeit as provocative as this system may seem there is still much unknown about the behaviour of these patient derived hMSCs. In order to begin to determine which genes might be differentially regulated during the process of hMSCs invasion we analyzed changes in gene expression patterns of the cells invading through a basement membrane of Matrigel. Using the defined media SCM we were able to identify a specific population of invasive hMSCs moving across the Matrigel membrane. This demonstrates that only a portion of the MSCs have the ability to be attracted by the SCM. Clearly, it can be assumed that the invasive cells express receptors for the components of the SCM, yet there is a significant amount of cross-talk and activation that occurs in response to signalling by each EGF, bFGF and insulin independently and synergistically when used

in combination. From the microarray, we found that 435 genes were up-regulated by 2-fold or more after normalization and background subtraction. An increase in a number of classical chemokine receptors such as IL13RA1 and TLR2 and TLR4 was observed, in addition to an increase in AR and ER- β . Traditionally these sex hormone receptors were not thought to be expressed on stem cells, but in a recent review by Ray et al., the authors summarize their roles in the differentiation of hMSCs and embryonic stem (ES) cells [28]. For example, in four independent mouse ES cell line there is functional expression of AR which can interact with transfected androgen response elements [29]. In addition, treatment of hMSCs with 17 β -estradiol is able to help induce osteogenic differentiation [30]. Furthermore, AR expression has been validated on CD34+ cells [31] and with regards to more tissue specific stem cells, AR expression has been seen in putative stem cell population from prostate tumor lines [32]. The increased expression of these sex steroid hormone receptors within the invasive population of hMSCs could be utilized for further differentiation of the cells depending on the environment and/or niche to which they are homed to. The increased expression of TLRs are very interesting in the context of this study. Toll-like receptors (TLRs) involved in mediating stress responses of hMSCs occur through the activation of many pathways, including NF κ B, AKT and MAPK [33]. Additionally, activation of TLRs with their various ligands, including lipopolysaccharide (LPS)-induced expression of a variety of chemokines and cytokines including C-X-C motif chemokine 10 (CXCL10), interferon-1 β (INF1 β) and interleukin-6 (IL-6) resulted in an enhanced migratory ability [33]. Furthermore, the importance and role of TLRs, as well as other growth hormones, chemokine/cytokines receptors and adhesion molecules



expressed by hMSCs is summarized nicely by Spaeth et al. [16] in a review regarding 'the migratory itinerary of mesenchymal stem cells.' Observing an increased expression of TLR2 and TLR4 in the invasive cells was not surprising based on this evidence. Evolutionarily conserved co-expression of transcription factors in human ES cells have been investigated for a number of years, and of interest to our research is the overlap in expression of the such genes as NANOG and STATs [34]. Our data demonstrates an increase in each of these gene families within the invasive population of hMSCs. As previously mentioned, we

observed a significant increase in STAT5A within the invasive cells, and a slight increase in STAT3, both of which were validated by qRT-PCR. The role of STATs in the regulation of stem cells has been investigated for a number of years, but only recently has their role in the regulation of hMSCs, tissue specific stem cells and invasion surfaced within the literature [24,25,35-41]. To further determine the importance of STATs/IL-6 since this has been a longstanding interest in our lab, we placed the STAT3 specific inhibitor Stattic in the chemoattractive media and we observed a complete block in invasion (Figure 3). Although



addition of exogenous IL-6 did slightly increase invasion, the presence of this inhibitor was again able to block this invasion (Figure 3). The observation that inflammatory regulator HIF-1 α was also increased in the invasive cells was of interest and in direct relation to the STAT data. Recently, it has been shown that HIF-1 α regulates IL-6 in low oxygen environments, and IL-6 increases when HIF-1 α is active [42]. A recent review relates the relationships of HIFs with such pathways governing stem cell regulation such as octamer binding transcription factor 4 (OCT4), bone morphogenic proteins (BMPs), NOTCH, sonic hedgehog (SHH) and β -catenin/WNT [43]. Thus, taken together with our data, we believe that IL-6/STAT plays a key role in hMSC invasion and homing and should be taken into consideration when developing therapeutic uses of hMSCs. Additionally, selective withdrawal of IL-6 and induction with tissue specific factors could ensure proper timing and specificity of hMSCs differentiation. Additional changes we observed that we believe are important in the regulation of hMSC homing and invasion based on previous research by our lab and others include increases in gene members of the Glinsky Signature (SUZ12 and EZH2), which determine likelihood of death from metastatic disease [44-46]. EZH2 is involved in histone methylation and deacetylation in stem cells and a recent paper shows that retroviral overexpression of EZH2 in mouse embryonic fibroblasts (MEFs) resulted in bypassing of the senescence program [47]. With regards to normal HSCs, which were

rapidly exhausted after serial transplantations, overexpression of EZH2 completely conserved long-term repopulating potential [47]. Finally, recent data suggests that EZH2 regulated by STAT3 is correlated to the pathological stage and progression of prostate cancer [48].

Conclusion

Overall, we have determined that a number of genes involved in immunological maintenance, developmental processes and the regulation of 'stemness' are increased in invasive hMSCs. By understanding the pathways functioning during hMSC invasion we believe this will aid in the development of their therapeutic applications.

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References

- Golde DW, Hocking WG, Quan SG, Sparkes RS, Gale RP (1980) Origin of human bone marrow fibroblasts. *Br J Haematol* 44: 183-187.
- Friedenstein AJ (1980) Stromal mechanisms of bone marrow: cloning in vitro and retransplantation in vivo. *Haematol Blood Transfus* 25: 19-29.
- Owen M (1985) Lineage of osteogenic cells and their relationship to the stromal system. *Journal of Bone and Mineral Research* 1-25.
- Dexter TM (1982) Stromal cell associated haemopoiesis. *J Cell Physiol*: 87-94.
- Caplan AI (1995) Osteogenesis imperfecta, rehabilitation medicine, fundamental research and mesenchymal stem cells. *Connect Tissue Res* 31: S9-14.
- Pittenger MF, Martin BJ (2004) Mesenchymal stem cells and their potential as cardiac therapeutics. *Circ Res* 95: 9-20.
- Gonçalves MA, de Vries AA, Holkers M, van de Watering MJ, van der Velde I, et al. (2006) Human mesenchymal stem cells ectopically expressing full-length dystrophin can complement Duchenne muscular dystrophy myotubes by cell fusion. *Hum Mol Genet* 15: 213-221.
- Zhao L.R (2000) Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats. *Exp Neurol* 174: 11-20.
- Sanchez-Ramos J, Song S, Cardozo-Pelaez F, Hazzi C, Stedeford T, et al. (2000) Adult bone marrow stromal cells differentiate into neural cells in vitro. *Exp Neurol* 164: 247-256.
- Ikehara S (2003) A novel strategy for allogeneic stem cell transplantation: perfusion method plus intra-bone marrow injection of stem cells. *Exp Hematol* 31: 1142-1146.
- van Laar JM, Tyndall A (2006) Adult stem cells in the treatment of autoimmune diseases. *Rheumatology (Oxford)* 45: 1187-1193.
- Nakamizo A, Marini F, Amano T, Khan A, Studeny M, et al. (2005) Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. *Cancer Res* 65: 3307-3318.
- Stoff-Khalili MA, Rivera AA, Mathis JM, Banerjee NS, Moon AS, et al. (2007) Mesenchymal stem cells as a vehicle for targeted delivery of CRAds to lung metastases of breast carcinoma. *Breast Cancer Res Treat* 105: 157-167.
- Qiao L, Xu ZL, Zhao TJ, Ye LH, Zhang XD (2008) Dkk-1 secreted by mesenchymal stem cells inhibits growth of breast cancer cells via depression of Wnt signalling. *Cancer Lett* 269: 67-77.
- Ries C, Egea V, Karow M, Kolb H, Jochum M, et al. (2007) MMP-2, MT1-MMP,

- and TIMP-2 are essential for the invasive capacity of human mesenchymal stem cells: differential regulation by inflammatory cytokines. *Blood* 109: 4055-4063.
16. Spaeth E, Klopp A, Dembinski J, Andreeff M, Marini F (2008) Inflammation and tumor microenvironments: defining the migratory itinerary of mesenchymal stem cells. *Gene Ther* 15: 730-738.
 17. Dvorak HF (1986) Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med* 315: 1650-1659.
 18. Mohyeldin A, Garzón-Muvdi T, Quiñones-Hinojosa A (2010) Oxygen in stem cell biology: a critical component of the stem cell niche. *Cell Stem Cell* 7: 150-161.
 19. Hurt EM, Kawasaki BT, Klarmann GJ, Thomas SB, Farrar WL (2008) CD44+ CD24(-) prostate cells are early cancer progenitor/stem cells that provide a model for patients with poor prognosis. *Br J Cancer* 98: 756-765.
 20. Mathews LA, Hurt EM, Zhang X, Farrar WL (2010) Epigenetic regulation of CpG promoter methylation in invasive prostate cancer cells. *Mol Cancer* 9: 267.
 21. Klarmann GJ, Hurt EM, Mathews LA, Zhang X, Duhagon MA, et al. (2009) Invasive prostate cancer cells are tumor initiating cells that have a stem cell-like genomic signature. *Clin Exp Metastasis* 26: 433-446.
 22. Kim HL, Cassone M, Otvos L Jr, Vogiatzi P (2008) HIF-1 α and STAT3 client proteins interacting with the cancer chaperone Hsp90: therapeutic considerations. *Cancer Biol Ther* 7: 10-14.
 23. Xu Q, Briggs J, Park S, Niu G, Kortylewski M, et al. (2005) Targeting Stat3 blocks both HIF-1 and VEGF expression induced by multiple oncogenic growth signaling pathways. *Oncogene* 24: 5552-5560.
 24. Rattigan Y, Hsu JM, Mishra PJ, Glod J, Banerjee D (2010) Interleukin 6 mediated recruitment of mesenchymal stem cells to the hypoxic tumor milieu. *Exp Cell Res* 316: 3417-3424.
 25. Nilsson CL, Dillon R, Devakumar A, Shi SD, Greig M, et al. (2010) Quantitative phosphoproteomic analysis of the STAT3/IL-6/HIF1 α signaling network: an initial study in GSC11 glioblastoma stem cells. *J Proteome Res* 9: 430-443.
 26. Mathews LA, Hurt EM, Zhang X, Farrar WL (2010) Epigenetic regulation of CpG promoter methylation in invasive prostate cancer cells. *Mol Cancer* 9: 267.
 27. Pansky A, Roitzheim B, Tobiasch E (2007) Differentiation potential of adult human mesenchymal stem cells. *Clin Lab* 53: 81-84.
 28. Ray R, Novotny NM, Crisostomo PR, Lahm T, Abarbanell A, et al. (2008) Sex steroids and stem cell function. *Mol Med* 14: 493-501.
 29. Goldman-Johnson DR, de Kretser DM, Morrison JR (2008) Evidence that androgens regulate early developmental events, prior to sexual differentiation. *Endocrinology* 149: 5-14.
 30. Wang Q, Yu JH, Zhai HH, Zhao QT, Chen JW, et al. (2006) Temporal expression of estrogen receptor alpha in rat bone marrow mesenchymal stem cells. *Biochem Biophys Res Commun* 347: 117-123.
 31. Sinha-Hikim I, Taylor WE, Gonzalez-Cadavid NF, Zheng W, Bhasin S (2004) Androgen receptor in human skeletal muscle and cultured muscle satellite cells: up-regulation by androgen treatment. *J Clin Endocrinol Metab* 89: 5245-5255.
 32. Sharifi N, Hurt EM, Farrar WL (2008) Androgen receptor expression in prostate cancer stem cells: is there a conundrum? *Cancer Chemother Pharmacol* 62: 921-923.
 33. Tomchuck SL, Zwezdaryk KJ, Coffelt SB, Waterman RS, Danko ES, et al. (2008) Toll-like receptors on human mesenchymal stem cells drive their migration and immunomodulating responses. *Stem Cells* 26: 99-107.
 34. Sun Y, Li H, Liu Y, Mattson MP, Rao MS, et al. (2008) Evolutionarily conserved transcriptional co-expression guiding embryonic stem cell differentiation. *PLoS One* 3: e3406.
 35. Dahéron L, Opitz SL, Zaehres H, Lensch MW, Andrews PW, et al. (2004) LIF/STAT3 signaling fails to maintain self-renewal of human embryonic stem cells. *Stem Cells* 22: 770-778.
 36. Taga T, Fukuda S (2005) Role of IL-6 in the neural stem cell differentiation. *Clin Rev Allergy Immunol* 28: 249-256.
 37. Katoh M, Katoh M (2007) STAT3-induced WNT5A signaling loop in embryonic stem cells, adult normal tissues, chronic persistent inflammation, rheumatoid arthritis and cancer (Review). *Int J Mol Med* 19: 273-278.
 38. Liang J, Wan M, Zhang Y, Gu P, Xin H, et al. (2008) Nanog and Oct4 associate with unique transcriptional repression complexes in embryonic stem cells. *Nat Cell Biol* 10: 731-739.
 39. Pricola KL, Kuhn NZ, Haleem-Smith H, Song Y, Tuan RS (2009) Interleukin-6 maintains bone marrow-derived mesenchymal stem cell stemness by an ERK1/2-dependent mechanism. *J Cell Biochem* 108: 577-588.
 40. Sherry MM, Reeves A, Wu JK, Cochran BH (2009) STAT3 is required for proliferation and maintenance of multipotency in glioblastoma stem cells. *Stem Cells* 27: 2383-2392.
 41. Zhang M, Atkinson RL, Rosen JM (2010) Selective targeting of radiation-resistant tumor-initiating cells. *Proc Natl Acad Sci U S A* 107: 3522-3527.
 42. Das R, Jahr H, van Osch GJ, Farrell E (2010) The role of hypoxia in bone marrow-derived mesenchymal stem cells: considerations for regenerative medicine approaches. *Tissue Eng Part B Rev* 16: 159-168.
 43. Keith B, Simon MC (2007) Hypoxia-inducible factors, stem cells, and cancer. *Cell* 129: 465-472.
 44. Glinsky GV (2008) "Stemness" genomics law governs clinical behavior of human cancer: implications for decision making in disease management. *J Clin Oncol* 26: 2846-2853.
 45. Glinsky GV (2007) Stem cell origin of death-from-cancer phenotypes of human prostate and breast cancers. *Stem Cell Rev* 3: 79-93.
 46. Gebauer G, Krones-Herzig A, Glinskii AB, Glinsky GV (2005) Identification of genes associated with tumor progression using microarrays. *Anticancer Res* 25: 1477-1482.
 47. Kamminga LM, Bystrykh LV, de Boer A, Houwer S, Douma J, et al. (2006) The Polycomb group gene Ezh2 prevents hematopoietic stem cell exhaustion. *Blood* 107: 2170-2179.
 48. Yeh HY, Cheng SW, Lin YC, Yeh CY, Lin SF, et al. (2009) Identifying significant genetic regulatory networks in the prostate cancer from microarray data based on transcription factor analysis and conditional independency. *BMC Med Genomics* 2: 70.