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Mesenchymal Stem Cell Modification of Endothelial Matrix Regulates Their Vascular Differentiation

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ABSTRACT

Mesenchymal stem cells (MSCs) respond to a variety of differentiation signal provided by their local environments. A large portion of these signals originate from the extracellular matrix (ECM). At the same time, MSCs secrete various matrix-altering agents, including proteases, that alter ECM-encoded differentiation signals. Here we investigated the interactions between MSC and ECM produced by endothelial cells (EC-matrix), focusing not only on the differentiation signals provided by EC-matrix, but also on MSC-alteration of these signals and the resultant affects on MSC differentiation. MSCs were cultured on EC-matrix modified in one of three distinct ways. First, MSCs cultured on native EC-matrix underwent endothelial cell (EC) differentiation early during the culture period and smooth muscle cell (SMC) differentiation at later time points. Second, MSCs cultured on crosslinked EC-matrix, which is resistant to MSC modification, differentiated towards an EC lineage only. Third, MSCs cultured on EC-matrix pre-modified by MSCs underwent SMC-differentiation only. These MSC-induced matrix alterations were found to deplete the factors responsible for EC-differentiation, yet activate the SMC-differentiation factors. In conclusion, our results demonstrate that the EC-matrix contains factors that support MSC differentiation into both ECs and SMCs, and that these factors are modified by MSC-secreted agents. By analyzing the framework by which EC-matrix regulates differentiation in MSCs, we have uncovered evidence of a feedback system in which MSCs are able to alter the very matrix signals acting upon them. J. Cell. Biochem. 107: 706–713, Published 2009 Wiley-Liss, Inc.†

KEY WORDS: PERIVASCULAR NICHE, MESENCHYMAL STEM CELLS, ENDOTHELIAL CELLS, EXTRACELLULAR MATRIX, EXTRACELLULAR MATRIX MODIFICATIONS

Mesenchymal stem cell (MSC) differentiation has been shown to be heavily influenced by cell–matrix interactions [Bradham et al., 1995; Mizuno et al., 1997, 2000; Heng et al., 2004; Qian and Saltzman, 2004; Salasznyk et al., 2004; Klees et al., 2005, 2007; Bosnakovski et al., 2006; Hashimoto et al., 2006]. Separate studies have demonstrated MSC-expression of molecules, including proteases, that degrade and alter matrix molecules. Proteases secreted by MSCs include plasmin and matrix metalloproteinases (MMPs)-2, -3, -9, -10, -11, -13, and -14 [Coussens et al., 2000; Annabi et al., 2003; Gruber et al., 2005; Ho et al., 2006; Ries et al., 2007; Kasper et al., 2007a,b]. In yet another set of studies, protease degradation has been shown to alter the biological activity of a variety of matrix molecules. These alterations reveal cryptic domains [Giannelli et al., 1997; Xu et al., 2001; Schenk and Quaranta, 2003; Hallmann et al., 2005; Rodenberg and Pavalko, 2007], release bioactive fragments [Limper et al., 1991; Fukai et al., 1993; Ramchandran et al., 1999; Amano et al., 2000; Colorado et al., 2000; Ferreras et al., 2000; Marneros and Olsen, 2001; Kalluri, 2003; Mongiat et al., 2003; Schenk and Quaranta, 2003; Ambesi et al., 2005; Gonzalez et al., 2005; Klees et al., 2005, 2007; Magnon et al., 2005; Hashimoto et al., 2006], and liberate stores of matrix-bound and matrix-regulated growth factors [Paralkar et al., 1990, 1991, 1992; Yamaguchi et al., 1990; Fukai et al., 1993; Jones et al., 1993; Aviezer et al., 1994; Zhu et al., 1999; Firth and Baxter, 2002; Annes et al., 2003; Kalluri, 2003; Mongiat et al., 2003; Chen et al., 2004].

In this study we attempt to combine these separate observations

This manuscript is Part 2 of 2. Part 1 is described in the manuscript entitled "Human Mesenchymal Stem Cells Express Vascular Cell Phenotypes Upon Interaction With Endothelial Cell Matrix," which has also been submitted to the *Journal of Cellular Biochemistry* under reference number JCB-09-0120.

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by demonstrating that MSCs alter the very matrix signals acting upon them, thereby affecting their own differentiation. To put this feedback mechanism into a physiological context, we chose to study MSC interactions with a perivascular matrix. We focused on the interactions between MSC and matrix produced by endothelial cells (ECs), the dominant vascular cell type. We have shown that MSCs directly cultured on EC-matrix exhibit enhanced expression of vascular cell markers (see accompanying report). Particularly interesting were the obvious differences in the temporal mRNA expression profiles between the different types of markers tested; MSC expression of EC markers peaked early and then tapered off, while expression of smooth muscle cell (SMC) markers did not increase until after the EC markers had begun to decline. Here we investigated the cause for these changes in MSC marker expression. We considered whether MSCs altered the biological activity of EC-matrix and whether MSC-altered EC-matrix provided different differentiation signals than unaltered matrix.

The main goal of this study was to determine whether changes to EC-matrix or changes to MSCs themselves are responsible for the shift in vascular cell markers expressed by MSCs cultured on EC-matrix. Our first aim was to study the effects of artificial changes in matrix supply on MSC differentiation. Our second aim was to investigate how alterations (i.e., crosslinking, pre-exposure to MSCs) affected the biological activity of EC-matrix. Our third aim was to determine whether physical contact was required for the interactions between MSCs and EC-matrix.

EXPERIMENTAL

CELL CULTURE

The macrovascular EC (macroEC) line HUV-EC-C (American Type Culture Collection, Manassas, VA) was cultured in EC medium (EGM-2-MV medium (Cambrex, East Rutherford, NJ) supplemented with 5% fetal bovine serum (FBS) and proprietary amounts of hEGF, hFGF-B with heparin, VEGF, R^3-IGF-1 hydrocortisone, ascorbic acid, and GA-1000). Human bone marrow-derived MSCs obtained with IRB approval (University of Washington) were isolated as tissue culture plastic (TCP) adherent cell populations. MSCs were expanded in MSC medium (high-glucose DMEM (Invitrogen, Carlsbad, CA) containing 10% FBS) and used between passages 3 and 5. Insulin-transferrin-selenium-x (Invitrogen) replaced FBS in serum-free (SF) culture conditions.

NATIVE MATRIX PRODUCTION

EC-matrix was derived from macroECs using the following method adapted from Gospodarowicz et al. [1983] (Fig. 1A). ECs were seeded on TCP at a density of 2×10^4 cells per cm^2 and cultured for 3 days in full EC medium. The EC monolayers were then washed with HBSS and cultured for 7 days in SF EC medium. After culture, the cells were lysed for 15 min in H_2O, washed with 0.02 M NH_4OH in H_2O to remove remaining cell debris, and washed 3–6 times with PBS. The resulting surface is referred to here as EC-matrix.

MATRIX PROCESSING

To produce crosslinked matrix, EC-matrix was crosslinked with formalin (1% in PBS) for 15 min and washed 5× with HBSS. The resulting surface is referred to here as crosslinked EC-matrix (Fig. 4A).

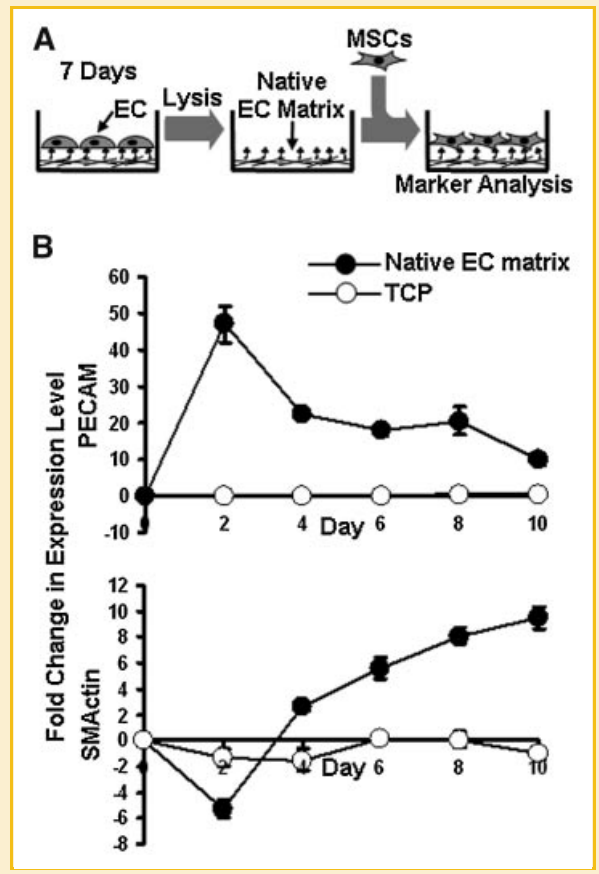

Fig. 1. Culture of MSCs on EC-matrix induces vascular phenotype. A: MSCs were cultured on de-cellularized EC-matrixes after ECs had been removed via hypotonic lysis in water. B: Real-time RT-PCR analysis of PECAM and SMActin expression for MSCs cultured on (●) EC-matrix or (○) TCP over 10 days. Error bars represent 95% confidence intervals.

To produce MSC-modified EC-matrix, MSCs were seeded on EC-matrix at 1×10^4 cells per cm^2 and cultured in SF MSC media for 4 days. Afterwards the MSCs were removed using the previously described method (wash with HBSS, 15 min in H_2O, wash with 0.02 M NH_4OH, wash with PBS). The resulting surfaces are referred to here as MSC-modified EC-matrix (Fig. 6A).

To produce crosslinked MSC-modified EC-matrix, MSC-modified EC-matrix was crosslinked with formalin (1% in PBS) for 15 min and washed 5× with HBSS. The resulting surfaces are referred to here as crosslinked MSC-modified EC-matrix.

MATRIX CULTURE

MSCs were seeded on EC-matrix (either native, crosslinked, MSC-modified, or crosslinked MSC-modified) at 1×10^4 cells per cm^2 and cultured in SF MSC media for 10 days. MSCs seeded on identically treated TCP were used as controls. MSC mRNA samples were collected every 2 days and analyzed via real-time RT-PCR for expression of EC and SMC markers. In addition, every 2 days samples were fixed (10% formalin in PBS, 15 min) and

stained for PECAM expression using the Blood Vessel Staining Kit (Millipore, Billerica, MA) according to the manufacture's instructions.

CHANGING MATRIX SUPPLY

After 10 days of culture on EC-matrix (either native or crosslinked), MSCs were processed in one of three ways: (1) The MSCs were cultured on the same surface, (2) the MSCs were removed (trypsonized) and re-seeded on fresh EC-matrix (native or crosslinked), or (3) MSCs were re-seeded on TCP. The MSCs were cultured on each of the three surfaces for 20 days. mRNA samples were collected at days 2, 4, 6, 8, 10, and 20.

INDIRECT MSC/EC-MATRIX CO-CULTURES

EC-matrix (either native or crosslinked) were prepared in the wells of TCP six-well dishes. MSCs were seeded in overhanging tissue-culture treated Transwell inserts (24 mm, 0.4 μm pore polyester membrane) (Corning, Corning, NY) at 1×10^4 cells per cm^2. Co-cultures in which MSC-containing Transwells were suspended over empty TCP were used as controls (Fig. 7A). The indirect co-cultures were maintained in SF MSC medium for 10 days, and MSC mRNA samples were collected every 2 days.

REAL-TIME RT PCR

MSC RNA samples were isolated using TRIzol Reagent (Invitrogen), treated with DNase using the Turbo DNA-free kit (Ambion, Austin, TX), and converted to cDNA with Superscript III First-Strand Synthesis Kits (dT primer) (Invitrogen). Real-time PCR was performed on a BioRad iCycler using SYBR green detection (BioRad, Hercules, CA). MacVector (Cary, NC) was used to design primers for the EC markers PECAM, KDR, and VECAD and the SMC markers smooth muscle α-actin (SMActin), smooth muscle 22α, and smoothelin. For each experimental condition, mRNA levels of EC and SMC markers were normalized to those of GAPDH for each time point and are presented as fold changes to day 0 values.

RESULTS

DIRECT CULTURE WITH NATIVE EC-MATRIX

MSCs cultured on de-cellularized EC-matrix exhibited enhanced mRNA expression of the EC markers and SMC tested compared to control groups cultured on TCP (Figs. 1B and 2). The temporal expression profiles of these two types of EC markers were distinct. The EC markers increased early in response to culture on EC-matrix. However, these heightened expression levels were not maintained and were followed immediately by sharp decreases. The SMC markers increases began later, after the EC markers had peaked, and were more gradual and consistent.

We next examined the effects of changes in EC-matrix supply on the vascular cell expression profiles described above. (Did replenishment or removal of the matrix affect EC and SMC marker expression in MSCs?) Native EC-matrix cultures were set up just as before, and, after the usual 10-day culture, the MSCs were processed in one of three ways. One group of MSCs was left on the same native matrix for continued culture. The second group of MSCs was removed and re-seeded on fresh native EC-matrix, while the final group was re-seeded on TCP without matrix. All three groups of MSCs were then cultured for another 20 days on their respective surfaces. MSCs left on the same native EC-matrix did not maintain their elevated EC marker expression levels (Fig. 3). MSCs re-seeded on fresh matrix temporarily recovered EC marker expression, but this again was not sustained. This loss of EC marker expression was accelerated when MSCs were re-seeded without matrix. Interestingly, SMC-differentiation was found to follow very different trends compared to EC-differentiation (Fig. 3). MSCs cultured on the same native EC-matrix or re-seeded on fresh matrix exhibited maintained elevated SMC marker mRNA expression levels. Furthermore, SMC marker expression was sustained even when the matrix was removed.

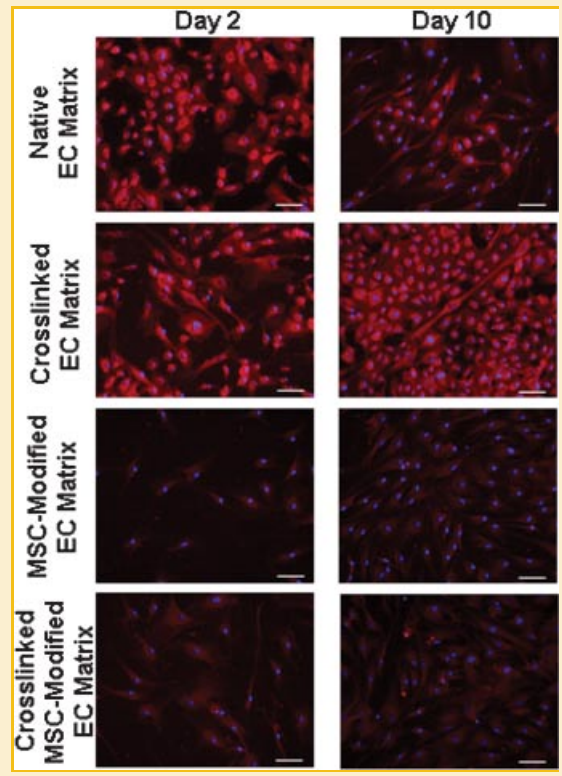

Fig. 2. Immunohistological PECAM-staining of MSCs cultured on variably-processed EC-matrixes. MSCs were directly cultured on either native EC-matrix, crosslinked EC-matrix, MSC-modified EC-matrix, or crosslinked MSC-modified EC-matrix. After days 2 and 10, the cultures were then fixed and stained for PECAM (TRITC/red) and DAPI (blue) (Bar = 20 μm). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

DIRECT CULTURE WITH CROSSLINKED EC-MATRIX

Crosslinking EC-matrix changed its biological activity. Crosslinked EC-matrix was produced by formaldehyde fixation of native EC-matrix (Fig. 4A), rendering it resistant to modification and factor release [Folkvord et al., 1989]. In this manner, the dependence of the biological activity of EC-matrix on modification and factor release could be assessed. As with native matrix, MSCs cultured on crosslinked matrix exhibited increases in mRNA expression of

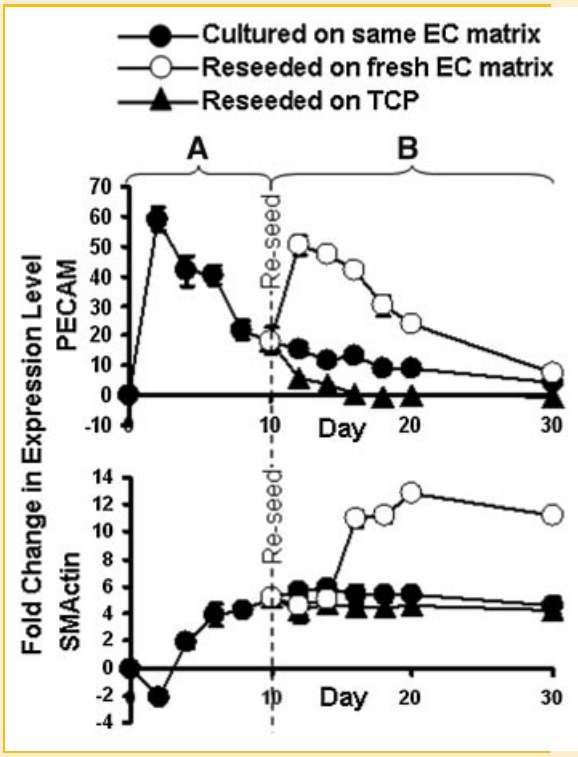

Fig. 3. Changing matrix supply affects MSC vascular-gene expression. A: MSCs were cultured on (●) EC-matrix for 10 days. B: Afterwards, MSCs were either (●) left on the same EC-matrix, or re-seeded on (○) fresh EC-matrix or (▲) TCP. PECAM and SMActin expression was analyzed via real-time RT-PCR every 2 days. Error bars represent 95% confidence intervals.

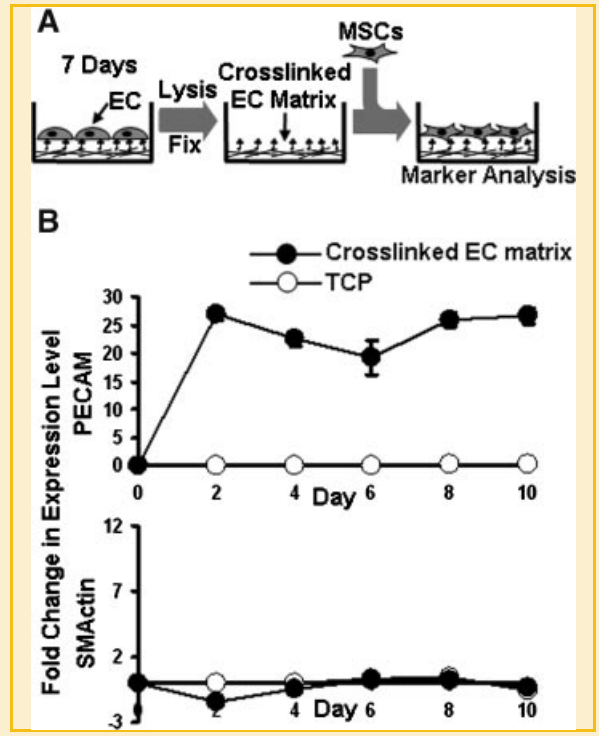

Fig. 4. Effects of crosslinked EC-matrix on MSCs. A: MSCs were seeded on crosslinked EC-matrixes that had been fixed in 1% formaldehyde for 15 min. B: Real-time RT-PCR analysis of MSC PECAM and SMActin expression in response to culture on (●) crosslinked EC-matrix or (○) TCP over 10 days. Error bars represent 95% confidence intervals.

EC markers (Fig. 4B). However, crosslinking greatly influenced the temporal profiles of EC marker expression; sustained EC marker expression replaced the transient peak observed with native EC-matrix. Similarly, PECAM-staining revealed a large population of PECAM-positive cells on crosslinked matrix that were maintained during the course of the experiment (Fig. 2). In marked contrast to MSCs cultured on native matrix, MSCs cultured on crosslinked EC-matrix did not exhibit increases in SMC marker expression (Fig. 4B).

After 10 days of culture on crosslinked EC-matrix, MSCs were either left to culture on the same crosslinked EC-matrix or were re-seeded on fresh crosslinked EC-matrix or TCP. MSCs on the same crosslinked EC-matrix sample exhibited elevated EC marker levels that were sustained (Fig. 5), while MSCs provided with fresh crosslinked EC-matrix exhibited further increases in EC marker expression, which were also sustained. However, EC marker levels were not sustained if the MSCs were re-seeded without matrix. Increased expression of SMC makers was not observed for any of the experimental conditions (Fig. 5).

DIRECT CULTURE WITH MSC-MODIFIED EC-MATRIX

Pre-exposure to MSCs changed the biological activity of EC-matrix. MSC-altered EC-matrix was produced by pre-exposing native EC-matrix to MSCs for 4 days, after which MSCs were removed, and a new set of MSCs were seeded on the MSC-modified EC-matrix (Fig. 6A). Culture on pre-modified EC-matrix induced this second set of MSCs to exhibit enhanced expression of SMC markers only (Fig. 6B). Neither mRNA analysis nor immunohistochemistry revealed detectable increases in EC marker expression (Figs. 6B and Fig. 2). Furthermore, MSCs cultured on MSC-modified EC-matrix exhibited earlier increases in SMC marker expression compared to those observed with native EC-matrix (day 1 vs. day 4).

DIRECT CULTURE WITH CROSSLINKED MSC-MODIFIED EC-MATRIX

MSC-modified EC-matrix was formaldehyde crosslinked. MSCs cultured on this type of matrix exhibited increases in neither EC nor SMC marker expression (data not shown). The lack of EC marker expression was confirmed by the absence of PECAM-immunostaining in MSCs cultured on crosslinked MSC-modified EC-matrix (Fig. 2).

INDIRECT CO-CULTURE WITH EC-MATRIX

Transwells, which allow exchange of soluble factors but prevent direct interactions, were used to test the effects of indirect co-culture of MSCs with EC-matrix. MSCs were seeded in Transwell inserts positioned over wells containing de-cellularized native EC-matrix that had been produced by 1-week SF cultures of ECs (Fig. 7A). Interestingly, these MSCs exhibited the same temporally distinct

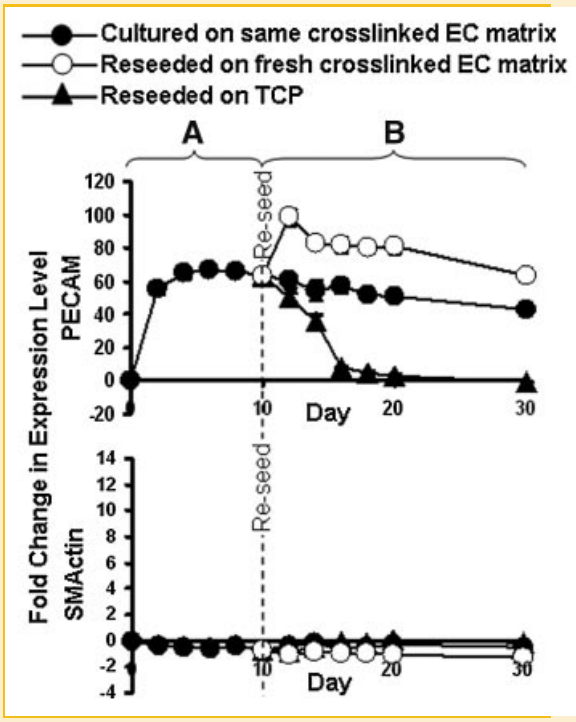

Fig. 5. Effects of changing crosslinked matrix supply on MSCs. A: MSCs were cultured on (●) crosslinked EC-matrix for 10 days. B: Afterwards, MSCs were either (●) left on the same crosslinked EC-matrix, or re-seeded on (○) fresh crosslinked EC-matrix or (▲) TCP. PECAM and SMActin expression was analyzed via real-time RT-PCR every 2 days. Error bars represent 95% confidence intervals.

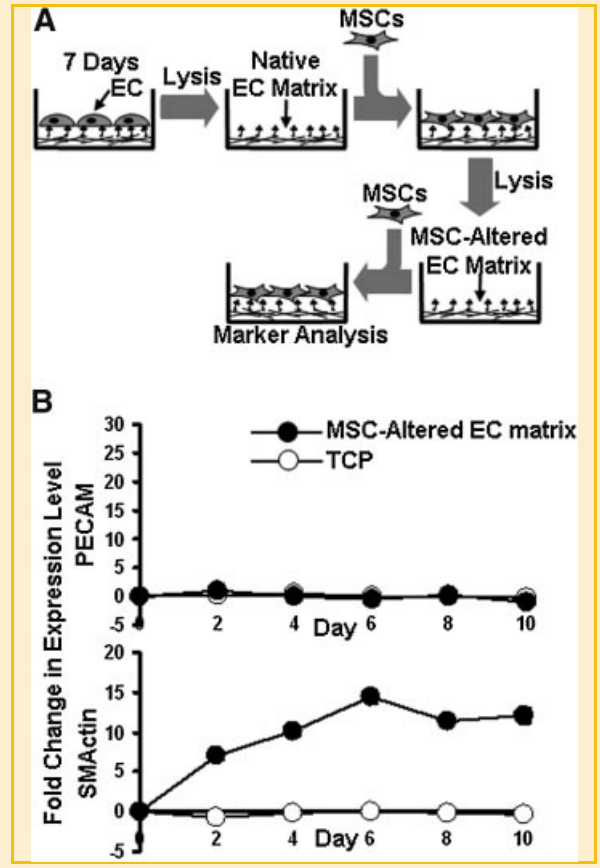

Fig. 6. Effects of MSC-altered EC-matrix on MSCs. A: MSCs were cultured on EC-matrixes that had been pre-altered by a separate group of MSCs over 4 days. B: Real-time RT-PCR analysis of PECAM and SMActin expression by MSCs cultured on MSC-altered macroEC-matrix or (○) TCP over 10 days. Error bars represent 95% confidence intervals.

increases in EC and SMC marker levels as observed with direct culture with matrix (Fig. 7B).

The above experiment was repeated by indirectly culturing MSCs with crosslinked EC-matrix. Under these conditions, neither EC nor SMC-differentiation was observed (data not shown).

DISCUSSION

MSC differentiation is guided by interactions with ECM. MSCs also secrete matrix-altering factors that change the nature of these interactions. Here we offer evidence that MSCs alter matrix-derived differentiation signals, thereby affecting their own differentiation. This study focused on the interactions between MSCs and EC-matrix. MSCs have recently been shown to occupy a perivascular niche [Crisan et al., 2008], making studies investigating the interactions between MSCs and EC-matrix particularly poignant. The same sample of EC-matrix was shown to support both EC and SMC-differentiation, but these differentiation processes took place at different times (early EC-differentiation vs. later SMC-differentiation). By artificially modifying EC-matrix, this shift in matrix differentiation capacity was linked to MSC-supported matrix alterations.

For example, MSCs cultured on EC-matrix that had been crosslinked with formaldehyde, making it resistant to modification, exhibited sustained EC-differentiation and no SMC-differentiation. The change in biological activity of EC-matrix in response to chemical crosslinking suggested that matrix modifications were taking place and that EC-matrix, in its original state, supports EC-differentiation and not SMC-differentiation. The divergent effects on EC and SMC marker expression suggested that modifications to EC-matrix are not required for EC-differentiation, but are required for SMC-differentiation. In fact, these results strongly suggest that alterations to EC-matrix are responsible for (1) the reduction of EC-differentiation capacity and (2) the shift to and activation of SMC-differentiation. Thus, when these alterations were prevented from taking place, such as by formaldehyde fixation, EC-matrix remained supportive of EC-differentiation, but was not transformed to one that supports SMC-differentiation.

To further demonstrate that the MSC differentiation program is regulated by alterations to EC-matrix, and not by changes in the MSCs themselves, we tested the effects of artificial changes to the supply of EC-matrix on MSC expression of vascular cell markers. MSCs grown on the same EC-matrix lost their markers for EC-differentiation as culture continued. This loss of EC-differentiation was exacerbated if the MSCs were removed from EC-matrix

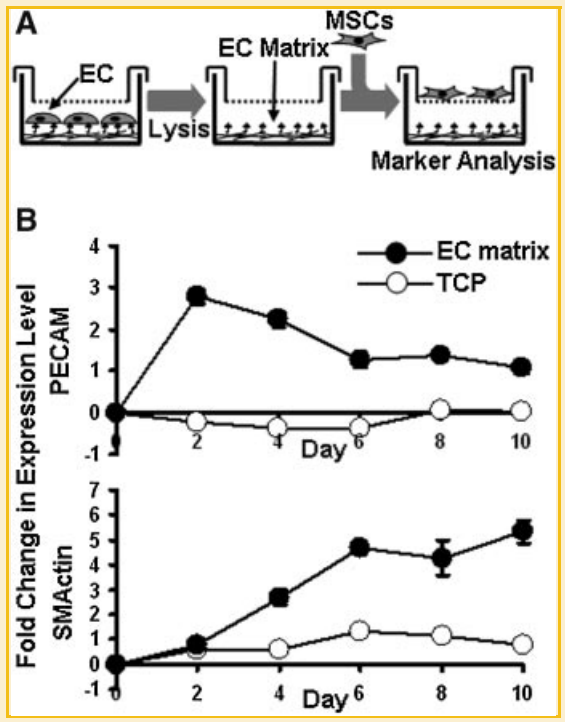

Fig. 7. Indirect culture of MSCs and EC-matrix. A: MSCs were cultured in Transwell inserts suspended over de-cellularized EC-matrixes. B: Real-time RT-PCR analysis of PECAM and SMActin expression for MSCs indirectly cultured with (●) EC-matrix or (○) TCP over 10 days. Error bars represent 95% confidence intervals.

and cultured without EC-matrix. However, if MSCs cultured on EC-matrix were removed and re-seeded on fresh EC-matrix, EC-differentiation quickly recovered. Again, however, EC-differentiation began to wane after this initial spike in renewed activity. These results indicated that MSCs were inefficient at maintaining EC-differentiation. They also indicated that EC-differentiation in MSCs was linked to the availability of some factor(s) provided by EC-matrix. After MSCs were initially exposed to this factor, EC-differentiation rapidly proceeded. However, this supply was depleted as it was used, and EC-differentiation waned. When MSCs were abruptly removed from this supply completely, the loss of EC-differentiation was accelerated. However, if MSCs were supplied with a fresh supply of factors, such as through re-seeding on a new matrix, EC-differentiation was reactivated. Again, this supply of factors was depleted as it was used, so the cycle of EC-differentiation wax and wane repeated. This cycle could be disrupted if matrix alterations were suspended, such as by crosslinking. MSCs re-seeded on crosslinked matrix exhibited sustained EC-differentiation. Again, EC-differentiation was lost if the MSCs were re-seeded without matrix. These results indicated that the loss of EC-differentiation was due to an alteration-induced depletion of the supply of EC-differentiation factor contained in EC-matrix. That the EC-matrix retained its ability to support EC-differentiation when crosslinked indicated that these EC-differentiation factors did not require matrix alterations for activity.

Interestingly, SMC-differentiation was found to follow very different trends compared to EC-differentiation. MSCs exhibited maintained SMC-differentiation in response to culture on EC-matrix even after the matrix was removed. This indicated that SMC-differentiation, once activated, would proceed in the absence or depletion of EC-differentiation factors. Since crosslinked EC-matrix did not support SMC-differentiation, matrix modifications were likely to be required for the activation of SMC-differentiation factors.

We also showed that the MSCs themselves were responsible for the alterations to EC-matrix. When MSCs were cultured on modified matrix that had been pre-exposed to MSCs, the characteristic early EC-differentiation was absent and was replaced by SMC-differentiation. This provided further evidence that MSC-modified EC-matrix was capable of supporting SMC-differentiation but not EC-differentiation. Finally, MSCs cultured on EC-matrix that had been MSC-modified and then crosslinked underwent neither EC-differentiation nor SMC-differentiation. These results indicated that the shift in differentiation capacity caused by MSC-modifications was not a single event. Instead, multiple alterations of EC-ECM by MSCs were required to support SMC-differentiation. These results also showed that MSC-altered matrix was unable to support EC-differentiation even if modifications had ceased.

Furthermore, we demonstrated that both MSCs and EC-matrix can interact purely through soluble factors. When MSCs were Transwell-cultured with native EC-matrix, a familiar pattern of early EC-differentiation and later SMC-differentiation was observed. As discussed above, the wane of EC-differentiation and the support of SMC-differentiation involved modifications to EC-matrix. Since these processes were observed during culture with MSCs without physical contact, MSC-secreted soluble factors were implicated in the matrix alterations responsible for the depletion of EC-differentiation factors and the activation of SMC factors. Previous reports have shown that MSCs secrete factors, such as MMPs, which act on vascular matrix [Coussens et al., 2000; Annabi et al., 2003; Gruber et al., 2005; Ho et al., 2006; Kasper et al., 2007a; Ries et al., 2007]. In separate studies, we identify the specific MSC-secreted factors responsible for altering the differentiation capacity of EC-matrix.

MSCs Transwell-cultured with crosslinked EC-matrix did not exhibit EC or SMC-differentiation, indicating that the EC and SMC-differentiation factors themselves are released through matrix modifications. This was expected in the case of SMC-differentiation, which, as discussed above, was shown to be negatively affected by crosslinking. EC-differentiation factors, on the other hand, were shown to be functional despite being fixed when they were allowed to interact with MSCs directly. In the case of Transwell culture, however, crosslinked EC-differentiation factors could not interact with MSC directly or through soluble means, and EC-differentiation was not induced.

Thus, we propose the following scenario to summarize our results (Fig. 8): EC-matrix contains supplies of factors that support both EC and SMC-differentiation in MSCs. In a feedback mechanism, MSCs secrete matrix-altering factors that release EC-differentiation factors from the matrix. In doing so, the matrix-altering factors

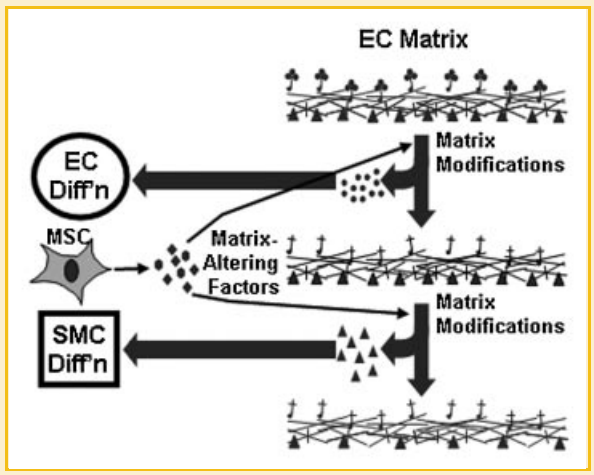

Fig. 8. Proposed mechanism of MSC/EC-matrix interactions. MSCs secrete matrix-altering factors that induce modifications to the EC-matrix, including the release of EC-differentiation factors (●). In doing so, the supply of these factors is depleted, so that the modified matrix no longer supports EC-differentiation. However, these matrix-altering factors also release and activate SMC-differentiation factors (▲). Thus, the modified matrix supports SMC-differentiation, but not EC-differentiation.

deplete the supply of EC-differentiation factors contained in EC-matrix, and the altered matrix no longer supports EC-differentiation. MSC-secreted factors also modify and release SMC-differentiation factors from the EC-matrix. Unlike the EC-differentiation factors, these factors require modification to become active. EC-matrix has been shown to bind many biological factors, such as growth factors, including VEGF, PDGF, and FGF [Kalluri, 2003], and the specific identities of the EC-differentiation and SMC-differentiation factors are the focus of separate studies.

In conclusion, this study presents evidence identifying EC-matrix as a critical regulator of vascular cell differentiation in MSCs. The same MSCs were found to manipulate EC-matrix differentiation signals with secreted factors. Instead of a unidirectional scenario in which stem cells act as a mere target for a barrage of matrix signals, a more complex situation exists in which the stem cells themselves alter the very matrix signals acting upon them.

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REFERENCES

Amano S, Scott IC, Takahara K, Koch M, Champliaud MF, Gerecke DR, Keene DR, Hudson DL, Nishiyama T, Lee S, Greenspan DS, Burgeson RE. 2000. Bone morphogenetic protein 1 is an extracellular processing enzyme of the laminin 5 gamma 2 chain. J Biol Chem 275:22728–22735.

Ambesi A, Klein RM, Pumiglia KM, McKeown-Longo PJ. 2005. Anastellin, a fragment of the first type III repeat of fibronectin, inhibits extracellular signal-regulated kinase and causes G(1) arrest in human microvessel endothelial cells. Cancer Res 65:148–156.

Annabi B, Lee YT, Turcotte S, Naud E, Desrosiers RR, Champagne M, Eliopoulos N, Galipeau J, Beliveau R. 2003. Hypoxia promotes murine bone-marrow-derived stromal cell migration and tube formation. Stem Cells 21:337–347.

Annes JP, Munger JS, Rifkin DB. 2003. Making sense of latent TGFbeta activation. J Cell Sci 116:217–224.

Aviezer D, Hecht D, Safran M, Eisinger M, David G, Yayon A. 1994. Perlecan, basal lamina proteoglycan, promotes basic fibroblast growth factor-receptor binding, mitogenesis, and angiogenesis. Cell 79:1005–1013.

Bosnakovski D, Mizuno M, Kim G, Takagi S, Okumura M, Fujinaga T. 2006. Chondrogenic differentiation of bovine bone marrow mesenchymal stem cells (MSCs) in different hydrogels: Influence of collagen type II extracellular matrix on MSC chondrogenesis. Biotechnol Bioeng 93:1152–1163.

Bradham DM, Passaniti A, Horton WE, Jr. 1995. Mesenchymal cell chondrogenesis is stimulated by basement membrane matrix and inhibited by age-associated factors. Matrix Biol 14:561–571.

Chen XD, Fisher LW, Robey PG, Young MF. 2004. The small leucine-rich proteoglycan biglycan modulates BMP-4-induced osteoblast differentiation. FASEB J 18:948–958.

Colorado PC, Torre A, Kamphaus G, Maeshima Y, Hopfer H, Takahashi K, Volk R, Zamborsky ED, Herman S, Sarkar PK, Ericksen MB, Dhanabal M, Simons M, Post M, Kufe DW, Weichselbaum RR, Sukhatme VP, Kalluri R. 2000. Anti-angiogenic cues from vascular basement membrane collagen. Cancer Res 60:2520–2526.

Coussens LM, Tinkle CL, Hanahan D, Werb Z. 2000. MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. Cell 103:481–490.

Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, Andriolo G, Sun B, Zheng B, Zhang L, Norotte C, Teng PN, Traas J, Schugar R, Deasy BM, Badylak S, Buhring HJ, Giacobino JP, Lazzari L, Huard J, Peault B. 2008. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 3:301–313.

Ferreras M, Felbor U, Lenhard T, Olsen BR, Delaisse J. 2000. Generation and degradation of human endostatin proteins by various proteinases. FEBS Lett 486:247–251.

Firth SM, Baxter RC. 2002. Cellular actions of the insulin-like growth factor binding proteins. Endocr Rev 23:824–854.

Folkvord JM, Viders D, Coleman-Smith A, Clark RA. 1989. Optimization of immunohistochemical techniques to detect extracellular matrix proteins in fixed skin specimens. J Histochem Cytochem 37:105–113.

Fukai F, Iso T, Sekiguchi K, Miyatake N, Tsugita A, Katayama T. 1993. An amino-terminal fibronectin fragment stimulates the differentiation of ST-13 preadipocytes. Biochemistry 32:5746–5751.

Giannelli G, Falk-Marzillier J, Schiraldi O, Stetler-Stevenson WG, Quaranta V. 1997. Induction of cell migration by matrix metalloprotease-2 cleavage of laminin-5. Science 277:225–228.

Gonzalez EM, Reed CC, Bix G, Fu J, Zhang Y, Gopalakrishnan B, Greenspan DS, Iozzo RV. 2005. BMP-1/Tolloid-like metalloproteases process endorepellin, the angiostatic C-terminal fragment of perlecan. J Biol Chem 280:7080–7087.

Gospodarowicz D, Gonzalez R, Fujii DK. 1983. Are factors originating from serum, plasma, or cultured cells involved in the growth-promoting effect of the extracellular matrix produced by cultured bovine corneal endothelial cells? J Cell Physiol 114:191–202.

Gruber R, Kandler B, Holzmann P, Vogele-Kadletz M, Losert U, Fischer MB, Watzek G. 2005. Bone marrow stromal cells can provide a local environment that favors migration and formation of tubular structures of endothelial cells. Tissue Eng 11:896–903.

Hallmann R, Horn N, Selg M, Wendler O, Pausch F, Sorokin LM. 2005. Expression and function of laminins in the embryonic and mature vasculature. Physiol Rev 85:979–1000.

Hashimoto J, Kariya Y, Miyazaki K. 2006. Regulation of proliferation and chondrogenic differentiation of human mesenchymal stem cells by laminin-5 (laminin-332). Stem Cells 24:2346–2354.

Heng BC, Cao T, Stanton LW, Robson P, Olsen B. 2004. Strategies for directing the differentiation of stem cells into the osteogenic lineage in vitro. J Bone Miner Res 19:1379–1394.

Ho W, Tawil B, Dunn JC, Wu BM. 2006. The behavior of human mesenchymal stem cells in 3D fibrin clots: Dependence on fibrinogen concentration and clot structure. Tissue Eng 12:1587–1595.

Jones JI, Gockerman A, Busby WH, Jr., Camacho-Hubner C, Clemmons DR. 1993. Extracellular matrix contains insulin-like growth factor binding protein-5: Potentiation of the effects of IGF-I. J Cell Biol 121:679–687.

Kalluri R. 2003. Basement membranes: Structure, assembly and role in tumour angiogenesis. Nat Rev Cancer 3:422–433.

Kasper G, Dankert N, Tuischer J, Hoeft M, Gaber T, Glaeser JD, Zander D, Tschirschmann M, Thompson M, Matziolis G, Duda GN. 2007a. Mesenchymal stem cells regulate angiogenesis according to their mechanical environment. Stem Cells 25:903–910.

Kasper G, Glaeser JD, Geissler S, Ode A, Tuischer J, Matziolis G, Perka C, Duda GN. 2007b. Matrix metalloprotease activity is an essential link between mechanical stimulus and mesenchymal stem cell behavior. Stem Cells 25:1985–1994.

Klees RF, Salasznyk RM, Kingsley K, Williams WA, Boskey A, Plopper GE. 2005. Laminin-5 induces osteogenic gene expression in human mesenchymal stem cells through an ERK-dependent pathway. Mol Biol Cell 16:881–890.

Klees RF, Salasznyk RM, Vandenberg S, Bennett K, Plopper GE. 2007. Laminin-5 activates extracellular matrix production and osteogenic gene focusing in human mesenchymal stem cells. Matrix Biol 26:106–114.

Limper AH, Quade BJ, LaChance RM, Birkenmeier TM, Rangwala TS, McDonald JA. 1991. Cell surface molecules that bind fibronectin's matrix assembly domain. J Biol Chem 266:9697–9702.

Magnon C, Galaup A, Mullan B, Rouffiac V, Bouquet C, Bidart JM, Griscelli F, Opolon P, Perricaudet M. 2005. Canstatin acts on endothelial and tumor cells via mitochondrial damage initiated through interaction with alphavbeta3 and alphavbeta5 integrins. Cancer Res 65:4353–4361.

Marneros AG, Olsen BR. 2001. The role of collagen-derived proteolytic fragments in angiogenesis. Matrix Biol 20:337–345.

Mizuno M, Shindo M, Kobayashi D, Tsuruga E, Amemiya A, Kuboki Y. 1997. Osteogenesis by bone marrow stromal cells maintained on type I collagen matrix gels in vivo. Bone 20:101–107.

Mizuno M, Fujisawa R, Kuboki Y. 2000. Type I collagen-induced osteoblastic differentiation of bone-marrow cells mediated by collagen-alpha2beta1 integrin interaction. J Cell Physiol 184:207–213.

Mongiat M, Sweeney SM, San Antonio JD, Fu J, Iozzo RV. 2003. Endorepellin, a novel inhibitor of angiogenesis derived from the C terminus of perlecan. J Biol Chem 278:4238–4249.

Paralkar VM, Nandedkar AK, Pointer RH, Kleinman HK, Reddi AH. 1990. Interaction of osteogenin, a heparin binding bone morphogenetic protein, with type IV collagen. J Biol Chem 265:17281–17284.

Paralkar VM, Vukicevic S, Reddi AH. 1991. Transforming growth factor beta type 1 binds to collagen IV of basement membrane matrix: Implications for development. Dev Biol 143:303–308.

Paralkar VM, Weeks BS, Yu YM, Kleinman HK, Reddi AH. 1992. Recombinant human bone morphogenetic protein 2B stimulates PC12 cell differentiation: Potentiation and binding to type IV collagen. J Cell Biol 119:1721–1728.

Qian L, Saltzman WM. 2004. Improving the expansion and neuronal differentiation of mesenchymal stem cells through culture surface modification. Biomaterials 25:1331–1337.

Ramchandran R, Dhanabal M, Volk R, Waterman MJ, Segal M, Lu H, Knebelmann B, Sukhatme VP. 1999. Antiangiogenic activity of restin, NC10 domain of human collagen XV: Comparison to endostatin. Biochem Biophys Res Commun 255:735–739.

Ries C, Egea V, Karow M, Kolb H, Jochum M, Neth P. 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.

Rodenberg EJ, Pavalko FM. 2007. Peptides derived from fibronectin type III connecting segments promote endothelial cell adhesion but not platelet adhesion: Implications in tissue-engineered vascular grafts. Tissue Eng 13:2653–2666.

Salasznyk RM, Williams WA, Boskey A, Batorsky A, Plopper GE. 2004. Adhesion to vitronectin and collagen I promotes osteogenic differentiation of human mesenchymal stem cells. J Biomed Biotechnol 2004:24–34.

Schenk S, Quaranta V. 2003. Tales from the crypt[ic] sites of the extracellular matrix. Trends Cell Biol 13:366–375.

Xu J, Rodriguez D, Petitclerc E, Kim JJ, Hangai M, Moon YS, Davis GE, Brooks PC. 2001. Proteolytic exposure of a cryptic site within collagen type IV is required for angiogenesis and tumor growth in vivo. J Cell Biol 154:1069–1079.

Yamaguchi Y, Mann DM, Ruoslahti E. 1990. Negative regulation of transforming growth factor-beta by the proteoglycan decorin. Nature 346:281–284.

Zhu Y, Oganesian A, Keene DR, Sandell LJ. 1999. Type IIA procollagen containing the cysteine-rich amino propeptide is deposited in the extracellular matrix of prechondrogenic tissue and binds to TGF-beta1 and BMP-2. J Cell Biol 144:1069–1080.