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Research Article

High-cell density-induced VCAM1 expression inhibits the migratory ability of mesenchymal stem cells

Soko Nishihira*,†, Naoto Okubo*,‡, Noriko Takahashi*, Akira Ishisaki*, Yoshiki Sugiyama† and Naoyuki Chosa[1]*

* Division of Biochemistry and Molecular Genetics, Department of Oral Biology, Iwate Medical University School of Dentistry, Morioka, Iwate 020-8505, Japan

† Division of Oral Surgery, Department of Oral and Maxillofacial Surgery, Iwate Medical University School of Dentistry, Morioka, Iwate 020-8505, Japan

‡ Open Research Project, Advanced Oral Health Research Center, Iwate Medical University, Morioka, Iwate 020-8505, Japan

Abstract

MSCs (mesenchymal stem cells) migrate into damaged tissue and then proliferate and differentiate into various cell lineages to regenerate bone, cartilage, fat and muscle. Cell–cell adhesion of MSCs is essential for the MSC-dependent tissue regeneration after their homing into a damaged tissue. However, it remains to be elucidated what kinds of adhesion molecules play important roles in the cell–cell communication between MSCs. In order to identify adhesion molecules that facilitate mutual contact between MSCs, a comprehensive analysis of mRNA expression in adhesion molecules was performed by comparing profiles of expression status of adhesion molecules in MSCs at low- and high-cell density. We found that the expression level of VCAM1 (vascular cell adhesion molecule-1)/CD106 was clearly up-regulated in the human bone marrow-derived MSCs–UE7T-13 cells – under a condition of high cell density. Intriguingly, the migratory ability of the cells was clearly accelerated by a knockdown of VCAM1. Furthermore, the migratory ability of UE7T-13 cells was decreased by the over expression of exogenous VCAM1. In addition, the high cell density-induced expression of VCAM1 was clearly suppressed by NF-κB (nuclear factor-κB) signalling-related protein kinase inhibitors such as an IKK-2 (IκB kinase-2) inhibitor VI. In conclusion, the high cell density-induced VCAM1 expression through the NF-κB pathway inhibits the migratory ability of human bone marrow-derived MSCs.

Keywords: cell density; mesenchymal stem cell; migration; nuclear factor-κB pathway; vascular cell adhesion molecule-1

1. Introduction

The multipotency of bone marrow-derived MSCs (mesenchymal stem cells) is characterized by their ability of self-renewal and their capacity to develop into a variety of mesenchymal tissues such as bone, cartilage, fat and muscle (Prockop, 1997; Pittenger et al., 1999; Docheva et al., 2007). The expansion of human bone marrow-derived MSCs *in vitro* and their subsequent autoimplantation is expected to be a potential stem cell therapy without the risk of immune rejection. In fact, in recent years, MSCs have attracted significant attention from basic and clinical investigators for their usefulness in the treatment of immune disorders, such as GVHD (graft-versus-host disease) and autoimmune diseases (Le Blanc et al., 2004).

The bone marrow cells contain MSCs as well as HSCs (haematopoietic stem cells) (Pittenger et al., 1999). No single antigenic marker has been shown to be specific for MSCs; however, it was found that MSCs are positive for CD29, CD44, CD90, CD73, CD105, CD106/VCAM1 (vascular cell adhesion molecule-1), CD117 and CD177 (Majumdar et al., 2003; Bobis et al., 2006). In addition, STRO-1 is a classical candidate for an MSC-related cell surface marker, and the fraction of bone marrow cells sorted with anti-STRO-1 antibody was shown to be rich in MSC-like cells (Gronthos and Simmons, 1995). On the other hand, MSCs are negative for haemopoietic cell markers, such as CD45, CD34, CD3 and CD14.

MSCs are known to express various adhesion molecules, and their roles have begun to be elucidated: Pittenger et al. (1999) demonstrated the expression of adhesion molecules ICAM1 (intercellular adhesion molecule-1, also known as CD54), ICAM3, CD105, VCAM1 and ALCAM on MSCs alongside that of integrins α1, α2, α3, αV, β1, β3 and β4. Current experimental results confirmed that MSCs can migrate and home to the wounded tissue and participate in wound healing (Pittenger and Martin, 2004; Satake et al., 2004). Intriguingly, it has been hypothesized that circulating MSCs in the vasculature (Kuznetsov et al., 2001) can serve as a source of cells for the regeneration of damaged myocardial cells. An important first step required for cardiac regeneration by circulating stem cells involves adhesion of these cells to CMVE (cardiac microvascular endothelium). Segers et al. (2006) demonstrated that VCAM1, which interacts with integrin α4, appeared to be the dominant adhesion molecule in the cytokine-induced adhesion of MSCs to CMVE. The mutual interaction between MSCs and CMVE can be activated by certain cytokines:

[1] To whom correspondence should be addressed (email nchosa@iwate-med.ac.jp).

Abbreviations: CMVE, cardiac microvascular endothelium; DMEM, Dulbecco's modified Eagle's medium; ECs, endothelial cells; FBS, fetal bovine serum; FCM, flow cytometry; GFP, green fluorescent protein; HSCs, haematopoietic stem cells; ICAM1, intercellular adhesion molecule-1, also known as CD54; IKK-2, IκB kinase-2; IL-1, interleukin-1; MSCs, mesenchymal stem cells; NF-κB, nuclear factor-κB; PE, phycoerythrin; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; qRT-PCR, quantitative RT-PCR; RTK, receptor tyrosine kinase; RT-PCR, reverse transcription-PCR; siRNA, small interfering RNA; TNF-α, tumour necrosis factor-α; VCAM1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4.

inflammatory cytokines [TNF-α (tumour necrosis factor-α) and IL-1 (interleukin-1)] enhanced adhesion of MSCs to CMVE *in vitro* and in the intact animal. Thus, VCAM1 was inducible in both CMVE and MSCs. On the other hand, the integrin $\alpha4\beta1$/very late antigen-4 (VLA-4), the most important ligand of VCAM1, was also expressed in both MSCs and CMVE. VLA-4 expression was not inducible by TNF-α and IL-1, suggesting that MSC adhesion to CMVE may be controlled by variations in VCAM1 expression levels. However, it remains to be elucidated how the multiple adhesion molecules work in each step of the tissue regeneration process mediated by MSCs. Especially after homing to the wounded tissue, MSCs proliferate and differentiate into various types of lineages under the preservation of cell–cell contact, resulting in the formation of aggregates composed of MSC-derived cells. In fact, a spheroidal culture system, in which cells made tight contacts with each other, greatly improved differentiation efficiency of MSCs (Wang et al., 2009). However, it is still unclear what types of adhesive molecules participate in the preservation of cell–cell contact in MSCs.

Herein, to identify adhesion molecules related to the preservation of cell–cell contact in MSCs, we compared expression profiles of adhesion molecules in MSCs under various conditions of cell density. In addition, we investigated what kinds of signal transducers relay mechanosensitive signals induced by the up-regulation of cell–cell adhesion molecules in MSCs.

2. Materials and methods

2.1. Reagents

The SCADS inhibitor kit including various protein kinase inhibitors was generously supplied by the Screening Committee of Anticancer Drugs supported by Grant-in-Aid for Scientific Research on Priority Area 'Cancer' from The Ministry of Education, Culture, Sports, Science and Technology, Japan.

2.2. Cell culture

The human bone marrow-derived MSCs, UE7T-13 cells, the lifespan of which was prolonged by infecting retrovirus encoding human papillomavirus E7 and hTERT (human telomerase reverse transcriptase) (Mori et al., 2005; Shimomura et al., 2007), were purchased from Health Science Research Resources Bank (JCRB no. 1154, Japan Health Sciences Foundation). UE7T-13 cells were cultured in DMEM (Dulbecco's modified Eagle's medium, Sigma–Aldrich) supplemented with 10% FBS (fetal bovine serum, Hyclone) at 37°C in a humidified incubator with an atmosphere of 5% CO_2 (3.0×10^3 cells/cm^2, 'low' cell density; 2.0×10^4 cells/cm^2, 'medium' cell density; 1.0×10^5 cells/cm^2, 'high' cell density).

2.3. Comprehensive expression profiling of cell adhesion molecules

Gene expression profiling was performed using a PrimerArray of human cell adhesion molecules (PH003, Takara) with a Thermal Cycler Dice Real Time System (Takara) according to the manufacturer's instructions. This PrimerArray is a set of real-time RT-PCR (reverse transcription-PCR) primers used for the analysis of gene expression associated with cell adhesion molecules. The array contains a mixture of 96 primer pairs for 88 cell adhesion molecule genes and eight housekeeping genes. Quantification of gene expression was performed using a PrimerArray Analysis Tool Ver. 2.0 (Takara).

2.4. RNA isolation and qRT-PCR (quantitative RT-PCR)

Total RNAs from low-, medium- and high-density cultured UE7T-13 cells were isolated with ISOGEN reagent (Nippongene) according to the manufacturer's instructions. First-strand cDNA was synthesized from total RNA by using the PrimeScript RT reagent Kit (Takara). qRT-PCR was performed on a Thermal Cycler Dice Real Time System (Takara) using SYBR Premix Ex Taq II (Takara) with specific oligonucleotide primers (presented in Supplementary Table S1 at http://www.cellbiolint.org/cbi/035/cbi0350475add.htm). mRNA expression levels for VCAM1 and ICAM1 were normalized to those obtained for GAPDH (glyceraldehyde-3-phosphate dehydrogenase), and the relative expression levels were shown as fold increase or decrease relative to the control.

2.5. Western blot analysis of VCAM1 expression

UE7T-13 cells were seeded at low, medium, and high cell density of DMEM containing 10% FBS and cultured for 24 h. The cells were washed twice with PBS and then lysed in RIPA buffer (50 mM Tris/HCl, pH 7.2, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS) containing protease inhibitor cocktail (Sigma). Protein content of the samples was measured using the BCA reagent (Pierce). Each sample containing equal amounts of protein was separated by 12.5% SDS/PAGE and transferred to a PVDF membrane (Millipore). After being blocked with 5% non-fat dry milk in T-TBS (50 mM Tris/HCl, pH 7.2, 150 mM NaCl and 0.1% Tween-20), the membrane was incubated with a primary anti-VCAM1 (clone 4B2, R&D Systems) and anti-β-actin (ACTB, clone C4, Santa Cruz Biotechnology) antibody as a loading control for normalization. The blots were then incubated with alkaline phosphatase-conjugated secondary antibody and developed using a CDP-Star chemiluminescence detection system (Roche Diagnostics).

2.6. FCM (flow cytometry) analysis of cell surface VCAM1 expression

UE7T-13 cells were seeded at low- and high-cell density of DMEM containing 10% FBS and cultured for 24 h. Before FCM, cells were stripped with Cell Dissociation Buffer (Invitrogen) and washed with PBS containing 0.1% FBS. The cells (1.0×10^5) were incubated with PE (phycoerythrin)-conjugated VCAM1 antibody (clone E1, Santa Cruz) or PE-conjugated non-immune mouse IgG (Santa Cruz). The acquisition was performed in EPICS XL ADC System (Beckman Coulter).

2.7. siRNA (small interfering RNA) transfection

Three sets of Stealth siRNA oligonucleotide duplexes targeting genes VCAM1 were designed using the online BLOCK-iT RNAi Designer software (Invitrogen). Sequences of the siRNA oligonucleotide duplexes are listed (Supplementary Table S2 at http://www.cellbiolint.org/cbi/035/cbi0350475add.htm). UE7T-13 cells were seeded in 24-well culture plates without antibiotic selection at a density of 2.0×10^4 cells/well, 24 h before siRNA transfection. Then, transcriptional knockdown was performed by transfection of the cells with siRNA oligonucleotide duplexes at a final concentration of 20 nM in DMEM using Lipofectamine RNAiMAX (Invitrogen) for 24 h according to the manufacturer's instructions. The effects of RNAi knockdown of target genes were assayed by qRT-PCR. Stealth siRNA Negative Control medium GC Duplex (Invitrogen) was also included as a control for sequence independent RNAi knockdown.

2.8. Overexpression of VCAM1

The full-length coding region of VCAM1 cDNA, which is amplified from UE7T-13 cells, was constructed with In-Fusion Advantage PCR Cloning Kit (Clontech) in pAcGFP1-Hyg-N1 plasmid vector (Clontech) according to the manufacturer's instructions (pVCAM1–GFP). UE7T-13 cells were seeded in 24-well culture plates without antibiotic selection at a density of 2.0×10^5 cells/well, 24 h before plasmid vector transfection. Then, overexpression of exogenous VCAM1 was performed by transfection of pVCAM1–GFP (green fluorescent protein) plasmid vector using Lipofectamine LTX (Invitrogen) for 48 h according to the manufacturer's instructions. The expression level of exogenous VCAM1 was assayed by FCM.

2.9. Transwell migration assay

The migratory ability of UE7T-13 cells was determined using Transwell cell culture inserts (BD Bioscience) that were 6.5 mm in diameter with 8-μm pore filters. UE7T-13 cells precultured at low, medium and high cell density and/or transfected with VCAM1 Stealth siRNA, or pVCAM1–GFP plasmid vector was stripped with Cell Dissociation Buffer (Invitrogen). After washing the cells with PBS twice, 2.0×10^4 cells were suspended in 350 μl of serum-free DMEM containing 0.1% BSA (Sigma) and seeded into the upper well; 600 μl of normal growth medium was placed in the lower well of the Transwell plate. After incubation for 6 h at 37°C, cells that had not migrated from the upper side of the filters were scraped off with a cotton swab, and filters were stained with the Three-Step Stain Set (Diff-Quik, Sysmex). The number of cells that had migrated to the lower side of the filter was counted under a light microscope with five high-power fields ($\times 400$). The experiment was performed in triplicate.

2.10. Statistics

Data were presented as the mean $\pm$ S.D. Statistical analysis was performed by using Student's t test, and values of $P<0.05$ were considered to be significant.

3. Results

3.1. Cell density-dependent expression of cell adhesion molecules

In order to identify cell adhesion molecules highly expressed in UE7T-13 cells grown at high cell density, we performed primer array analyses as described in the Materials and methods section. Then, we compared the expression status of adhesion molecules between the cells grown at high and low cell density. Differentially expressed genes in the cells grown at high and low cell density were identified (Table 1). Among 88 target genes, 7 genes were identified as highly expressed genes in the cells grown at high cell density. In particular, the mRNA expression level of VCAM1 in the cells grown at high cell density was more than 32-fold higher than that in the cells grown at low cell density.

3.2. High-cell density-induced expression of VCAM1

To confirm whether the VCAM1 expression was dependent on the cell density, we examined the expression levels of VCAM1 mRNA

Table 1 **Cell adhesion molecules expression status between high and low cell density**
Names of some genes expressed in UE7T-13 cells grown at high cell density are listed, expression levels of which were 2-fold higher or lower than those of the same genes in the cells grown at low cell density.

Gene symbol	Gene name	Fold change
VCAM1	Vascular cell adhesion molecule 1	32.3
NRXN2	Neurexin 2	3.34
SELL	Selectin L (lymphocyte adhesion molecule 1)	2.66
PTPRM	Protein tyrosine phosphatase, receptor type, M	2.35
NCAM2	Neural cell adhesion molecule 2	2.18
HLA-DRA	Major histocompatibility complex, class II, DR alpha	2.15
CLDN4	Claudin 4	2.04
HLA-DOA	Major histocompatibility complex, class II, DO alpha	−2.13
CD34	CD34 molecule	−2.22
PVR	Poliovirus receptor	−2.33
PDCD1LG2	Programmed cell death 1 ligand 2	−2.70
CD86	CD86 molecule	−3.23
NRXN3	Neurexin 3	−4.55
CADM1	Cell adhesion molecule 1	−5.88
CD274	CD274 molecule	−5.88

and protein in UE7T-13 cells grown at low, medium and high cell density. qRT-PCR analysis revealed that VCAM1 mRNA expression level was up-regulated 10-fold in the medium cell density culture and 7-fold in high cell density culture compared with the low cell density culture, respectively (Figure 1B, left panel). On the other hand, the level of ICAM1 expression, which is regulated by NF-κB (nuclear factor κB) signalling, the same as VCAM1 (Kim et al., 2001; Yang et al., 2005), was up-regulated only 3-fold in the high cell density culture than in the low cell density culture (Figure 1B, right panel). The high cell density-induced VCAM1 expression was confirmed at the protein level by Western blot analysis (Figure 1C). Furthermore, FCM analysis revealed the presence of VCAM1 protein on the cell surface, which represented a functional property of VCAM1 (Figure 1D).

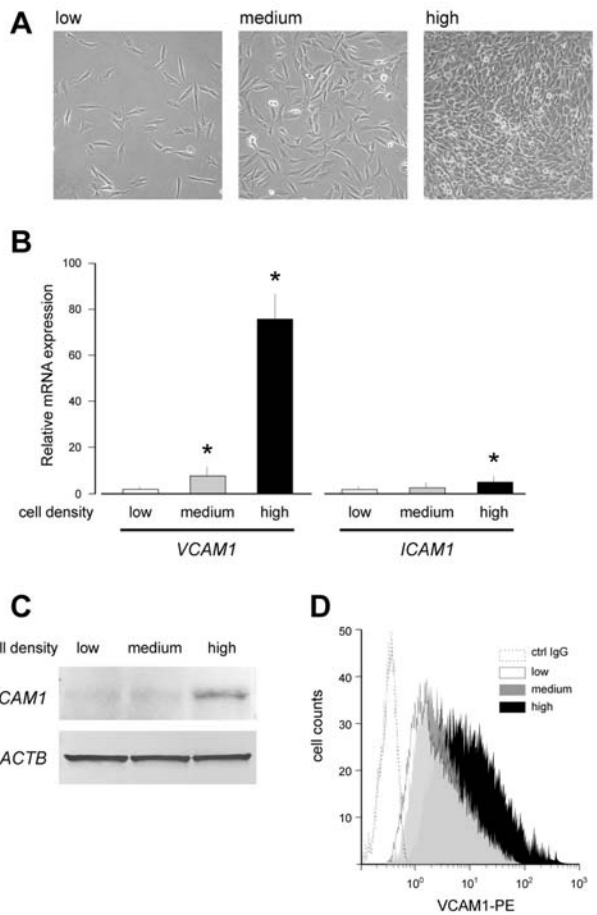

Figure 1 High cell density-induced expression of VCAM1 in MSCs
UE7T-13 cells were seeded at various cell densities as indicated and maintained for 24 h. Then, morphological and molecular biological studies were performed. (**A**) Appearance of each cell culture was viewed by phase-contrast microscopy. Relative mRNA expression levels of VCAM1 and ICAM1 (**B**) were analysed by qRT-PCR. Data represent the mean of three individual experiments (n) $\pm$ S.D. *$P<0.05$ was considered significant (in comparison with low cell density culture). (**C**) VCAM1 protein expression in total cell lysate was analysed by Western blotting. (**D**) Cell surface expression of VCAM1 was analysed by FCM.

3.3. High cell density-induced expression of VCAM1 inhibits Transwell migration of MSCs

In order to obtain a new insight into the function of VCAM1 on UE7T-13 cell surface, we investigated the relationship between the status of VCAM1 expression and the migratory ability of UE7T-13 cells. The migratory ability of cells precultured at high cell density, namely the cells with high expression levels of VCAM1, was compared with that of cells precultured at low cell density, namely the cells with low expression levels of VCAM1. We found that the migratory ability of the cells precultured at high cell density was much lower than that of the cells precultured at low cell density (Figure 2A). Furthermore, we investigated the ability of VCAM1 knockdown, through its siRNA transfection, to affect the migratory ability of UE7T-13 cells. The migratory ability of UE7T-13 cells was increased by the knockdown of VCAM1 expression in the cells precultured at each cell density (Figures 2B, 2C and Supplementary Figure S1 at http://www.cellbiolint.org/cbi/035/cbi0350475add.htm). In addition, the migratory ability of UE7T-13 cells grown at low, medium, and high cell density were decreased by the overexpression of exogenous VCAM1 by transfection with pVCAM1–GFP (Figure 3).

3.4. Effect of protein kinase inhibitors on the high cell density-induced VCAM1 expression

The expression of VCAM1 in the high cell density culture was significantly inhibited by treatment with IKK-2 (IκB kinase 2) inhibitor VI, PI3K (phosphoinositide 3-kinase) inhibitor (LY-294002), Src inhibitor (PP2 analogue) and PKC (protein kinase C) inhibitor (Gö7874) (Figure 4). In particular, the IKK-2 inhibitor decreased the VCAM1 mRNA expression more than 10-fold.

4. Discussion

The expression level of VCAM1 was up-regulated in bone marrow-derived MSCs cultured to overconfluency (Lee et al., 2008). However, a comprehensive analysis of the types of adhesion molecules that play important roles in cell–cell contact between MSCs has not been performed. Herein, we compared the expression status of adhesion molecules between MSCs grown at high and low cell densities by using primer array analyses. Among 88 target genes coding adhesion molecules, the expression level of VCAM1 was most strongly up-regulated in response to high cell density cultures (Table 1); but the extent of up-regulation of other gene expression was 3.34-fold at the most.

In the present study, we first demonstrated that high cell density-dependent VCAM1 expression inhibited the migratory ability of MSCs (Figures 1 and 2A). In fact, the migratory ability of UE7T-13 cells was increased during knocking down of VCAM1 expression by gene specific siRNA; alternatively, it was decreased by overexpression of exogenous VCAM1 (Figures 2B, 2C and 3). These facts suggested that the migration of the MSCs is VCAM1-dependent in manner. However, in the experiments of siRNA transfection, the migratory ability in precultured with high cell

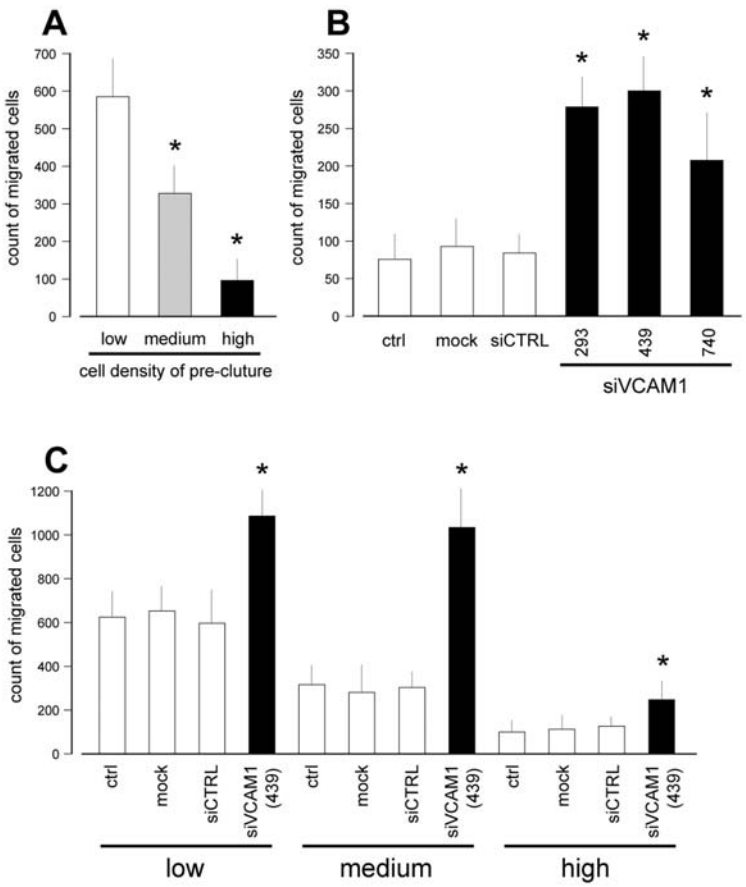

Figure 2 Roles of VCAM1 in the migratory ability of MSCs

UE7T-13 cells pre-cultured at low, medium and high cell densities (**A**) or the cells transfected with VCAM1-specific siRNA (siVCAM1, **B** and **C**) were seeded on 8-μm pore size Transwell cell culture inserts. After incubation for 6 h at 37°C, the cells that migrated from the upper side to the lower side were stained with Diff-Quik. The number of cells that had migrated to the lower side of the filter was counted under a light microscope using five fields. Data represent the mean of three individual experiments (n) ± S.D. *$P<0.05$ was considered significant in comparison with low-density preculture in (**A**) or untransfected control (ctrl) in (**B**) and (**C**).

density condition was not more remarkable than low and medium cell density conditions (Figure 2C). Because mRNA expression of endogenous VCAM1 is produced abundantly in high cell density in comparison with low and medium cell density conditions, the effect of siRNA knockdown efficiency may be limited. In addition, the various molecules in relation to migration as well as cell adhesion molecules such as VCAM1 may influence migration ability in high cell density condition.

VCAM1 is an endothelial ligand for VLA-4/integrin $\alpha4\beta1$ and lymphocyte Peyer's patch adhesion molecule (LPAM/integrin $\alpha4\beta7$). The interaction between VCAM1 expressed on ECs (endothelial cells), and integrins, such as VLA-4 and LPAM expressed on leucocytes, is thought to be involved in the extravasation of leucocytes through the endothelium to sites of inflammation (Petruzzelli et al., 1999; Kobayashi et al., 2007). In addition, Petty et al. (2009) suggested that a cross-talk between VCAM1 and VLA-4, which is expressed on marrow endothelium and stroma, regulated neutrophil retention in the bone marrow. Intriguingly, a previous study demonstrated that the integrin $\alpha4$ was expressed in MSCs (Segers et al., 2006). In this study, we demonstrated through FCM analysis that integrin $\alpha4$ was expressed on the cell surface of UE7T-13 cells (Supplementary Figure S2 at http://www.cellbiolint.org/cbi/035/cbi0350475add.htm). Therefore, the VCAM1-induced inhibition of MSC migration may depend on cell–cell adhesion caused by the binding between integrin $\alpha4$ and VCAM1. Alternatively, a VCAM1-dependent activation of the integrins may result in the activation of an intracellular signal resulting in the inhibition of MSC migration.

Expression of VCAM1 is regulated by several cytokines (Collins et al., 1995), and some of them are produced by HSCs in the bone marrow. The expression level of TNF-α, which is known to activate the NF-κB pathway, increased in plasma in response to various human tissue injuries (Collins et al., 1995; Jiang et al., 1997). In addition, TNF-α also plays an important role in tissue repair (Gerstenfeld et al., 2003). NF-κB-related signals play important roles in cell growth. NF-κB belongs to a family of pleiotropic transcription factors that control the expression of numerous genes involved in migration, growth, tumour genesis, tumour metastasis, differentiation, embryonic development, apoptosis and inflammation (Ghosh and Karin, 2002; Karin and Lin, 2002; Li and Verma, 2002). In most cell types, NF-κB proteins are sequestered in the cytoplasm by the inhibitor IκB in their inactive

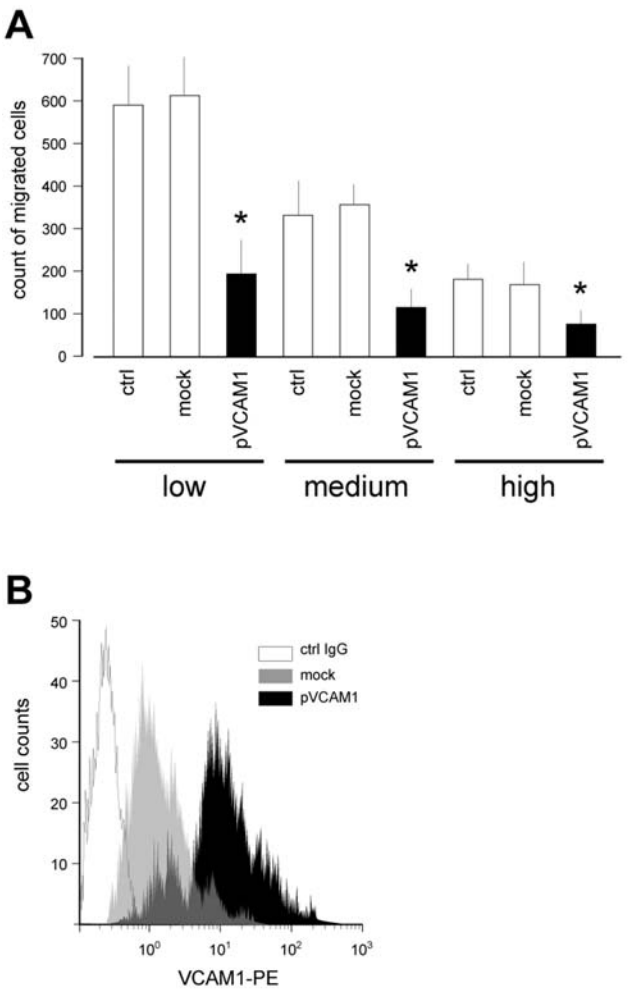

Figure 3 Overexpression of exogenous VCAM1 inhibits migratory ability in MSCs

(**A**) UE7T-13 cells transfected with pVCAM1–GFP were cultured at low, medium and high cell density conditions for 24 h and then seeded on 8-μm pore size Transwell cell culture inserts. After incubation for 6 h at 37°C, the cells that migrated from the upper side to the lower side were stained with Diff-Quik. The number of cells that had migrated to the lower side of the filter was counted under a light microscope using five fields. Data represent the mean of three individual experiments (n) ± S.D. *P<0.05 was considered significant in comparison with untransfected control (ctrl). (**B**) The expression level of cell surface exogenous VCAM1 was assayed by FCM.

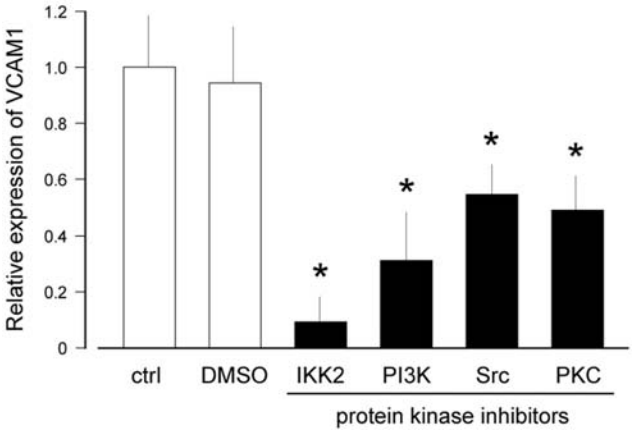

Figure 4 Effect of protein kinase inhibitors on cell density-dependent VCAM1 expression

UE7T-13 cells were seeded on a 24-well dish in high cell density condition. After 6-h cultures, cells were treated with 10 μM of various protein kinase inhibitors and then maintained for 18 h. Relative mRNA expression levels of VCAM1 were analysed by qRT-PCR. Inhibitors used were IKK-2 inhibitor VI (IKK), LY-294002 (PI3K), PP2 analogue (Src) and Gö7874 (PKC). *P<0.05 was considered significant compared with untreated control (ctrl).

form (Sen and Baltimore, 1986; Baeuerle and Baltimore, 1996; Li and Verma, 2002). Upon stimulation, IκB is phosphorylated by IKK, and subsequently polyubiquinated, which triggers its rapid degradation by proteasomes (Chen et al., 1995; Baeuerle and Baltimore, 1996). Consequently, the released NF-κB proteins translocate into the nucleus, where they activate the expression of target genes, such as VCAM1 (Ghosh and Karin, 2002; Li and Verma, 2002). In this study, the high cell density-induced expression of VCAM1 in UE7T-13 cells was clearly suppressed by IKK-2 inhibitor VI, suggesting that the high cell density-induced signal was relayed by a pathway similar to the TNF-α-induced NF-κB-related signalling. In addition, ICAM1 expression level, which was up-regulated by NF-κB signalling in a manner similar to VCAM1 expression (Kim et al., 2001; Yang et al., 2005), was up-regulated only 3-fold in response to high cell density cultures (Figure 1B). These results suggested that intracellular signals other than the NF-κB signal positively controlled the status of VCAM1 expression in MSCs. Interestingly, inhibitors of PI3K, Src and PKC significantly inhibited the high cell density-induced expression of VCAM1 in UE7T-13 cells (Figure 4).

Intriguingly, a recent report revealed that a mechanostress-induced intracellular signal was mediated by a RTK (receptor tyrosine kinase) in ECs, even if the receptor was not stimulated with any ligand. Vascular endothelial–cadherin forms a mechanosensory complex with VEGFR2 [VEGF (vascular endothelial growth factor) receptor 2] in ECs. Then, the complex responds to a subset of endothelial shear stresses, resulting in the activation of NF-κB-mediated Src and Shc signalling to up-regulate VCAM1 expression, even if VEGFR2 was not stimulated with VEGF (Liu et al., 2008). It is now under the investigation in our laboratory whether RTK mediates the cell–cell contact-induced signal responsible for VCAM1 expression in MSCs.

In conclusion, the high cell density-induced intracellular signalling mediated by NF-κB pathway inhibits the migratory ability of human bone marrow-derived MSCs through an up-regulation of VCAM1 expression. These findings could eventually lead to the development of MSC-based new cell therapies in regenerative medicine, though it is necessary to confirm how these phenomena affect MSCs *in vivo*.

Author contribution

Soko Nishihira was responsible for the design, analysis and writing of the manuscript. Naoto Okubo and Noriko Takahashi took charge of data collection and analysis. Akira Ishisaki and Yoshiki Sugiyama undertook an analysis and discussion of the study. Naoyuki Chosa contributed to the design, analysis and discussion of the study and acknowledges all individuals who have contributed to this work.

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