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Peptide array-based screening of human mesenchymal stem cell-adhesive peptides derived from fibronectin type III domain

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ABSTRACT

Human mesenchymal stem cell-adhesive peptides were screened based on the amino acid sequence of fibronectin type III domain 8–11 (FN-III $_{8-11}$) using a peptide array synthesized by the Fmoc-chemistry. Using hexameric peptide library of FN-III$_{8-11}$ scan, we identified the ALNGR (Ala-Leu-Asn-Gly-Arg) peptide that induced cell adhesion as well as RGDS (Arg-Gly-Asp-Ser) peptide. After incubation for 2 h, approximately 68% of inoculated cells adhere to the ALNGR peptide disk. Adhesion inhibition assay with integrin antibodies showed that the ALNGR peptide interacts with integrin β1 but not with αvβ3, indicating that the receptors for ALNGR are different from RGDS. Additionally, the ALNGR peptide expressed cell specificities for adhesion: cell adhesion was promoted for fibroblasts but not for keratinocytes or endotherial cells. The ALNGR peptide induced cell adhesion and promoted cell proliferation without changing its property. It is therefore useful for the construction of functional biomaterials.

Human mesenchymal stem cells (hMSCs) are multipotent cells that are able to differentiate at least to osteoblast, adipocytes, chondrocytes *in vitro* and *in vivo* [1–3]. Since hMSCs can be obtained by bone marrow aspiration, they are regarded as an important source that may provide new regenerative treatments for human tissues. Thus, the expansion of hMSCs *in vitro* is necessary for clinical applications, since a large number of hMSCs is required for the regeneration of injured tissues.

Most eukaryotic anchorage-dependant cells, including hMSC, recognize and interact with extracellular matrices (ECM) and set off cell adhesion before cell migration, proliferation, and differentiation. Fibronectin (FN) is one of the major ECM proteins that play an important role in cell adhesion, spreading, migration, wound repair, and angiogenesis. It is reported that FN contains binding sites for different molecules, including sulfated glycosaminoglycans, DNA, gelatin, heparin, and fibrin [4], and also for various integrin receptors [5]. In particular, the FN-III$_{10}$ domain contains an RGDS (Arg-Gly-Asp-Ser) site that can bind the α5β1, αvβ1, αvβ3, αvβ5, αvβ6, α8β1, and αIIbβ3 integrins [6,7]. In recent years, the RGDS received much attention as a useful biomaterial for the cell scaffold [8]. Besides RGDS, short peptides found from the ECM, such as LDV (Leu-Asp-Val) [9], YIGSR (Tyr-Ile-Gly-Ser-Arg) [10], and PHSRN (Pro-His-Ser-Arg-Asn) [11,12], were also reported to enhance cell adhesion. However, these cell-adhesive peptides do not express cell specificity.

We have recently developed a peptide array-based cell assay system that allows the screening of peptides directly through their interaction with cell surface receptors. Peptide arrays, developed as the SPOT method by Frank, are a designable peptide library that is covalently synthesized on a cellulose support and has been applied to various interaction assays [13,14]. Peptides interacting with cell surface receptors, may contribute greatly to the development of drugs and biomaterials. By the cell assay system, we have screened cell adhesion peptides [15] and cell death inducible peptides [16,17].

In this study, high-throughput screening of cell-adhesive peptides was performed with FN-III$_{8-11}$ using hMSCs. The ALNGR (Ala-Leu-Asn-Gly-Arg) peptide, identified by peptide screening, showed high-cell adhesion effect and induced cell-specific interaction. Peptides that can selectively induce cell-specific adhesion would be promising targets for developing functionalized biomaterials in tissue engineering.

Materials and methods

Cell culture. Normal human mesenchymal stem cells (hMSCs) were purchased from Cambrex-Biowhittaker (Walkersville, MD) at passage 2, and were routinely cultured and expanded in MSCGM at 37 °C under 5% CO_2. All experiments were conducted using the cells under passage 5.

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Normal human dermal fibroblasts (NHDFs), normal human epidermal keratinocytes (NHEKs), and normal human umbilical vein endothelial cells (HUVECs) (Kurabo Industries Ltd.) were cultured in commercially available growth media (Medium 106S for NHDF, HuMedia-KG2 for NHEK, and HuMedia-EG2 for HUVEC; Kurabo).

Preparation of peptide arrays. A hexamer peptide array covering the FN-III$_{8-11}$ (No. 1267–1541, Swiss-Prot No. P02751) was constructed on a cellulose membrane. The peptide array was prepared by Fmoc chemistry using the SPOT-synthesis method, employing a peptide auto-spotter (ASP222, IntavisAG, Köln, Germany) as described previously [13,14]. According to this method, the C-terminal end of the peptide was anchored on the surface of a cellulose membrane. Positive and negative control sequences were RGDS and AAAAAA, respectively.

Anchorage-dependant cell assay on a peptide spot. The basic scheme of the anchorage-dependant cell assay on a peptide array was performed as described previously [15]. Each peptide spot was punched out as a peptide disk and embedded in the 96-well plate followed by the cells' seeding and culture (5000 cells/well) directly in serum-free medium (MSCBM containing L-glutamine and penicillin/streptomycin) for 5 h at 37 °C under 5% CO_2. After washing the non-adhered cells with phosphate-buffered saline (PBS), calcein AM solution (Molecular Probes, Leiden, The Netherlands) was added to each well to the final concentration of 20 μg/ml, and the fluorescence intensity was measured after 1 h using a fluorescence plate reader (Fluoroskan Ascent, Labsystems, Helsinki, Finland). The relative cell adhesion ratio was defined as the ratio of the number of viable cells remaining on the peptide disk as compared to that with no peptide; this was calculated to be 1.0.

Cell attachment/spreading assay by electrical cell substrate impedance sensing (ECIS). The attachment and spreading of hMSCs were analyzed by ECIS (ECIS-1600, Bio-Physics, Inc., Troy, NY, USA). The ECIS device is based on AC impedance measurements using weak and non-invasive AC signals [18]. For the immobilization of the peptide on the Au electrode that was set at the bottom of the electronic sensor plate, the self-assembled monolayer (SAM) of 4 mM 11-amino-1-undecanthiol, hydrochloride in pure water was formed on Au surfaces by incubating overnight at room temperature. The peptides (1 mM) were immobilized covalently on the SAM-modified surface using the dehydration coupling reagent, DMT-MM (2 mM, Kokusan Chemical Co. Ltd., Tokyo, Japan). hMSCs were seeded at a density of 5000 cells/well in 200 μl of the serum-free media.

Proliferation assays on glass slide. hMSCs were seeded at a density of 1.0×10^5 cells/well on an ALNGR-immobilized slide glass in 4-well plates in a serum-free medium. After 24 h, the MSCGM was changed, and then changed again every 3 days thereafter until day 7. At day 7, the cells were detached using trypsin–EDTA and counted using a hemocytometer.

Flow cytometric analysis of CD105 expression. The cell surface expression of CD105 was analyzed by a flow cytometer (Partec, Münster, Germany). Briefly, hMSCs were harvested on a peptide-immobilized slide from their culture environment via routine techniques, counted, and re-suspended in BSA solution (PBS + 0.1% BSA) to yield 1.0×10^6 cells/ml. The suspensions were incubated with FITC anti-human CD105 (Biolegend, San Diego, CA, USA) diluted 20-fold at room temperature for 60 min under rotation. Following centrifugation and washing with a BSA solution to remove unbound antibodies, the stained cells were then analyzed using a flow cytometer. Non-specific background staining was determined by non-stained cell samples (negative control). A minimum of 5000 cells was counted in each sample.

Immunofluorescence and microscopy. Synthesis of the peptides on microscopic slides (type I high density amine coated slides, Matunami Glass Ind. Ltd., Osaka, Japan) was performed as described in the previous section except for the blocking of amino groups remaining on the slides by succinic anhydride after the synthesis of the first amino acids. hMSCs were seeded on the peptide-immobilized slide in a serum-free medium and incubated for 5 h at 37 °C under 5% CO_2. The unadhered cells were washed twice with PBS and were subsequently fixed with 4% paraformaldehyde in PBS for 20 min (Wako Pure Chemical Industries, Japan). For immunofluorescent staining of F-actin and vinculin, MSCs were permeabilized with 0.1% Triton X-100 in PBS for 3 min and rinsed with PBS, followed by soaking with Block Aces (Dainippon Sumitomo Pharma Co., Ltd., Japan) at room temperature for blocking non-specific binding. Then, the cells were incubated overnight with an anti-vinculin primary antibody at 4 °C. Subsequently, the cells were washed twice with PBS and once with Tris-buffered saline (Dako, CA, USA). The slides were then incubated in the presence of Alexa 488 goat anti-mouse IgG secondary antibody (Molecular Probes, OR, USA) for 1 h. F-actin was visualized by staining the cells with Alexa Fluor 568-conjugated phalloidin (Molecular Probes) for 40 min. The stained cells were observed using a fluorescence microscope (model IX81, Olympus, Tokyo, Japan).

Adhesion inhibition assay. Monoclonal antibodies (mAbs) against the human integrin αv, β1, and β3 subunits, and human vinculin were purchased from Chemicon (Temecula, CA, USA). Cell suspensions in a serum-free medium (2×10^5 cells/ml) were pre-incubated with 2.5 μg/ml integrin mAbs for 1 h at 37 °C under 5% CO_2 prior to their seeding onto the peptide disks. After 5 h incubation under the same conditions, non-adherent cells were removed by gently rinsing several times with PBS. The fluorescence intensities of adhered cells were estimated by staining with calcein AM.

Results and discussion

Peptide array-based screening of cell-adhesive peptides obtained from fibronectin

The screening of cell-adhesive peptides was performed by a peptide array-based interaction assay. The average relative cell adhesion ratio of 198 peptides was 1.29 ± 0.22. Six peptides that revealed above a 1.8-fold increase in cell-adhesive effect were found (Table 1); peptide number 83 at FN$_{1430-1435}$ (ALNGRE), No. 129 at FN$_{1522-1527}$ (TGRGDS), Nos. 74 and 75 at FN$_{1412-1419}$ (SITLTNLT), No. 157 at FN$_{1578-1582}$ (PKNGPG) and No. 151 at FN$_{1566-1571}$ (SSPVTG). The strongest effects were observed with peptide No. 83 ALNGRE (2.48 ± 0.16). To identify the functional peptide sequence, FN$_{1428-1437}$ (IVALNGREEES) was divided into 3-, 4-, 5-, and 6-mer peptides (Fig. 1A) and cell adhesion assay was performed. Since the 5-mer peptide sequence of ALNGR showed the strongest effect, the active core of this peptide was determined to be ALNGR.

The effect of single amino acid substitutions in the ALNGR peptide on a cell adhesion was also investigated. Almost all analog peptides inhibited cell adhesion. In contrast, the analog peptides with Lys-substitutions showed as strong an effect as the ALNGR peptide. Four analogue peptides which induced cell adhesion along with ALNGR were AANGR, KLNGR, AKNGR, and ALNGK. Also, the ALHGR and ALKGR increased cell adhesion by 1.18- and 1.09-fold, respectively, as compared to the ALNGR. A stronger cell adhesion effect was observed by substituting the third amino acid residue with a higher charged residues.

Attachment and spreading of hMSCs and ECIS analysis

The attachment and spreading of hMSCs on a peptide-immobilized slide glass were studied (Fig. 1B). After 5 h of incubation, approximately 90% of the inoculated cells were adhered to the peptide-immobilized slide. The rate of cell spreading on an ALNGR-immobilized slide increased gradually and was 10% and 28% after 2 h and 5 h, respectively. Alternatively, the rate on an RGDS peptide-immobilized slide reached 40% already after 2 h. These results show that the cell spreading rate on ALNGR was slower than that of RGDS.

Next, we monitored cell attachment and spreading by ECIS using peptide-immobilized electrodes. As the cells attach and spread on the electrode surface, the impedance increases due to the electrical resistance properties of cells and the change in signal can be used to analyze the cell behavior [19]. Fig. 1C shows the time course of the impedance measurement when hMSCs were inoculated at 255 cells/mm^2 on peptide-immobilized electrodes. The attachment and spreading of cells on an RGDS substrate were observed at 60 min, and the signal was saturated. In contrast, it took more than 5 h for an ALNGR substrate. In order to describe

Table 1
Data of cell adhesion peptides obtained by the screening of fibronectin type III domain (Nos. 1267–1541)

Peptide number	Peptide sequence	Relative cell adhesion ratio[a] (−)
83	ALNGRE	1.98 ± 0.20
129	TGRGDS	1.95 ± 0.08
75	TLTNLT	1.93 ± 0.72
74	SITLTN	1.92 ± 0.48
157	PKNGPG	1.87 ± 0.32
151	SSPVTG	1.81 ± 0.70

Relative cell adhesion of RGDS was 2.20 ± 0.26.
Peptide sequence with relative cell adhesion ratio of above 1.8 was shown.
[a] Relative cell adhesion is the average ratio of fluorescent intensity of three copies of disks standardized with the values of control, i.e., membrane only. This was calculated to be 1.0.

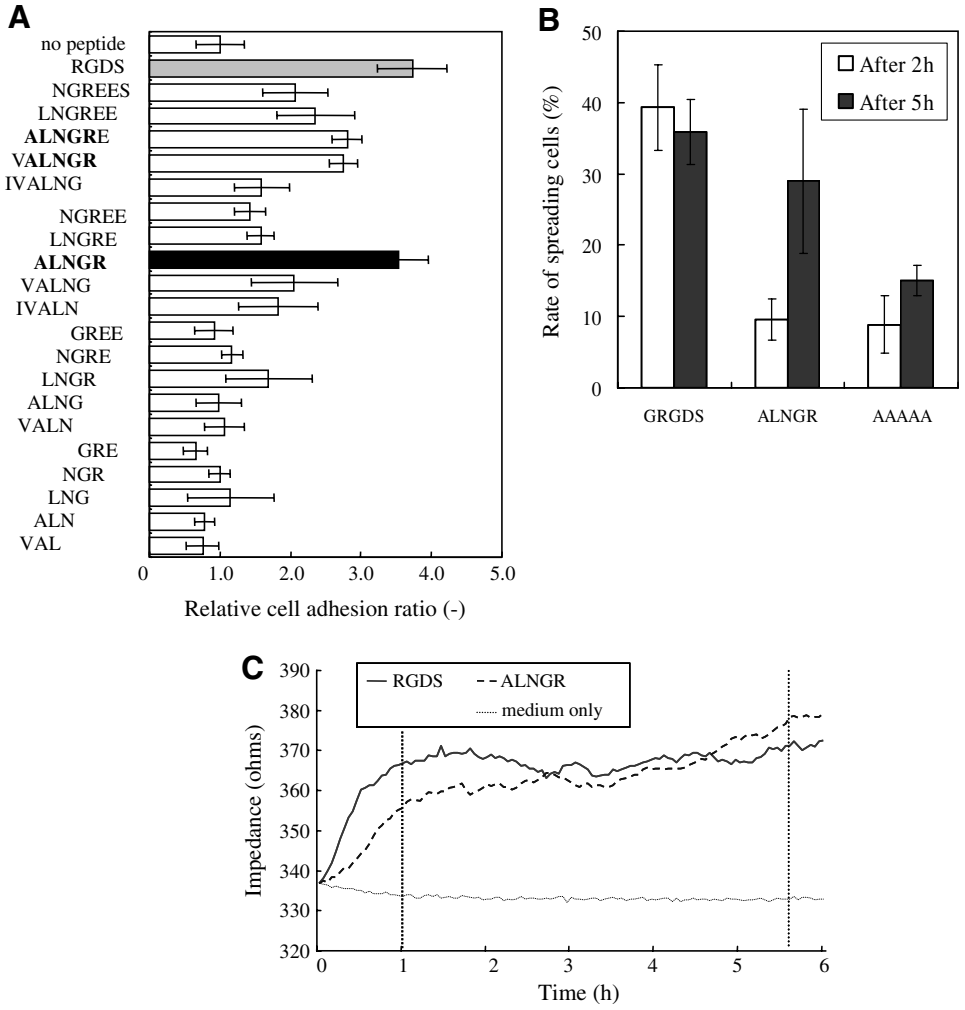

Fig. 1. (A) Screening for a cell adhesion site using 3-, 4-, 5-, and 6-mer peptides of IVALNGREES. The fluorescence intensity of a membrane with no peptide bound was set to be 1.0. (B) The rate of cell spreading of hMSCs on the ALNGR and RGDS peptides measured by morphological analysis. (C) Time course of the impedance measurement at 40 kHz when hMSCs were seeded into the ECIS arrays. The peptides were immobilized individually on Au electrodes; RGDS (—); ALNGR (- -); medium only (·····).

the individual spreading behavior, we extracted an index from the measurement: $t_{1/2}$, time to produce an half-maximum impedance (=368 Ω) for the peptide-immobilized electrodes. Theoretically, $t_{1/2}$ means the time required for the cells to adhere on 50% of the available electrode area. Spreading time (*ST*) signifies the time required for maximum impedance. The term *ST* means the time period from cell adhesion to cell spreading, corresponding to the speed of cell spreading. In Fig. 1C, $t_{1/2}$ was 0.45 h for RGDS and 0.92 h for ALNGR, this means cell adhesion on an ALNGR substrate took approximately twice of that for RGDS. The *ST* for ALNGR also showed 4.4 h, which is above 4-fold *ST* for RGDS (=1.0 h).

These data indicate that the ALNGR peptide induce adhesion of hMSCs with cell spreading rate slower than that of RGDS peptide.

Cell proliferation assay

The undifferentiated state of hMSCs cultured on the ALNGR peptides was confirmed by flow cytometry using CD105 Ab. As shown in Fig. 2A, the peak shift in the flow cytometric analysis revealed that the cell state after cultivation on an ALNGR-immobilized slide was not changed on days 1 and 7. Cell morphology on ALNGR was similar to that on no peptide (Fig. 2B). Cell growth rate with 7 days of an ALNGR-immobilized slide showed a 3.38 ± 0.14-fold increase, which is higher than that of no peptide (2.78 ± 0.24). These results show that the ALNGR peptide promotes the cell proliferation of hMSCs and does not influence the cell differentiation state.

The ALNGR peptide induced focal adhesion-associated with vinculin

hMSCs seeded on an ALNGR-immobilized slide were incubated for 3 h, and the adherent cells were fixed and double-stained for F-actin (red) and vinculin (green). The hMSCs which adhered to the ALNGR peptide displayed a well-developed actin cytoskeleton with numerous stress fibers running across the cell body and enriched with vinculin at the ends (Fig. 2C). The attachment form on the RGDS peptide was spindle-shaped while that on the ALNGR peptide was one of multidirectional spreading. The hMSCs adhering to AAAAA peptide were not spreading and did not express vinculin (data not shown).

Cell attachment assay with various cell lines

Fig. 3 shows the effect of the ALNGR peptide on cell adhesion with various cell lines. The RGDS peptide induced cell adhesion with all cell lines, hMSCs, NHDFs, NHEKs, and HUVECs. In contrast, the ALNGR peptide induced cell adhesion with hMSCs and NHDFs, but not NHEKs and HUVECs. NHDFs possess a strong adhesive capacity because they produce ECM by themselves. Since the ALNGR peptide had a little cell-adhesive effect on epidermal cells

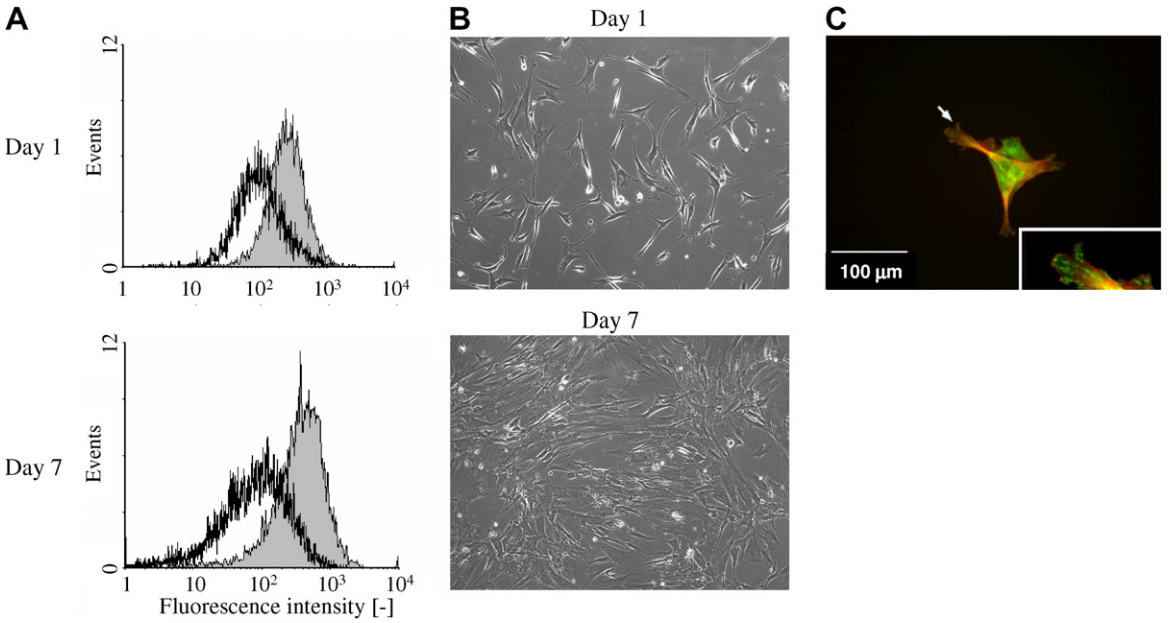

Fig. 2. Proliferation of hMSC on the ALNGR-immobilized slide glass. (A) Flow cytometry measurement of cell surface expression of CD105, undifferentiated marker for hMSC, at days 1 and 7. The hatched pattern represents a cell counts with the peptide. (B) Microscopic observation of hMSC on a peptide-modified slide glass after 1 and 7 days. (C) Fluorescence microscopic observation of focal adhesions and actin stress fibers in hMSCs on ALNGR-modified glass slide after 3 h incubation. Actin stress fiber and vinculin straining was performed with Alexa-568 and Alexa-488, respectively.

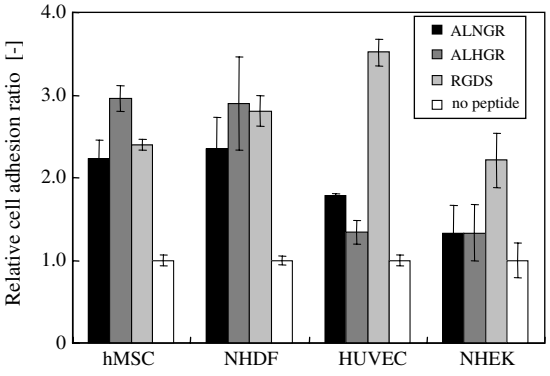

Fig. 3. The effect of the ALNGR and ALHGR peptide on cell adhesion with various cell lines. The RGDS peptide was used as the positive control. The negative control was with no peptide-bound membrane; this was set to be 1.0.

such as NHEKs and HUVECs, it was concluded that the ALNGR peptide has cell specificity.

Adhesion inhibition assays

An adhesion inhibition assay was performed to identify the integrins interacting with the ALNGR peptide (Fig. 4A). When the cells were blocked by the soluble RGDS peptide, cell adhesion on the ALNGR and RGDS peptides was inhibited. Alternatively, when cells were blocked by the soluble ALNGR peptide, cell adhesion to the ALNGR peptide was inhibited, while no inhibition was observed with the RGDS peptide. These results suggest that the receptors interacting with the ALNGR peptide can interact with the RGDS peptide as well.

Next, we performed a cell adhesion inhibition assay by blocking cells with the antibodies to the integrin αv, β1, and β3 subunits (Fig. 4B). Cell adhesion to the ALNGR peptide was blocked by the antibodies to integrin β1 by 60%, but not with the antibodies to integrin αv or β3. On the other hand, antibodies to the integrins

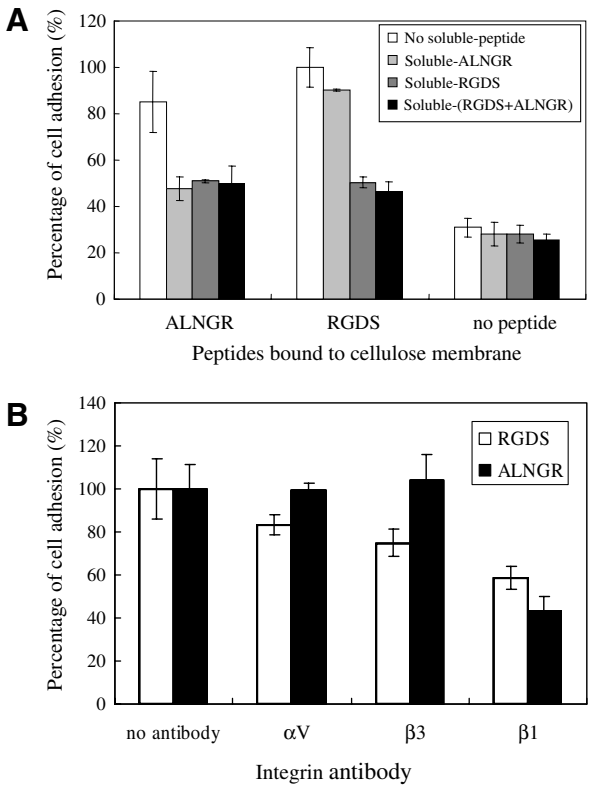

Fig. 4. Inhibition assays. (A) Inhibition assay using cells after pre-incubation with the soluble ALNGR, RGDS, and RGDS + ALNGR peptides. The hMSCs were pre-incubated with 0.5 mM soluble peptides in a serum-free medium (2×10^5 cells/ml) for 30 min at 37 °C under 5% CO_2. Experiment data represents means ± SD from the assay results of three different disks. (B) The inhibition of cell adhesion on a peptide array by mAbs to integrin αV, β1, and β3 subunits. To confirm the interaction receptor of cell adhesion peptides, 2.5 μg/ml of mAbs were pre-incubated individually with hMSCs in a 0.5 ml serum-free medium (2×10^5 cells/ml) for 30 min at 37 °C under 5% CO_2.

β1, αv, and β3 reduced cell adhesion to RGDS peptide, by 40, 20, and 25%, respectively. This means that RGDS peptide interacts with integrins αv, β3 and β1, whereas the ALNGR peptide interacts with integrin β1 dominantly, but not with integrins αv and β3. It was suggested that the integrin receptors for the ALNGR peptide are different from those for the RGDS peptide, and the ability to induce adhesion with cell specificity would be attributed to this difference.

The proliferation and differentiation of MSCs have attracted attention for their potential clinical applications in tissue engineering. In the present study, we focused on the identification of hMSCs-adhesive peptides. The NGR motif as cell-adhesive peptide has been reported in the literature through mutagenesis and phage display studies [20,21]. Koivunen et al. have shown that the NGR motifs, such as NGRAHA and DGRAHA, have affinity for the α5β1 and αvβ3 receptors three orders of magnitude ($IC_{50} = 10^{-5}$) lower than the RGD peptide. Since, these receptors play a crucial role in cell migration, angiogenesis, and consequently in tumor invasion and metastasis formation, Colombo et al. have used cyclic and linear peptides containing the NGR motif as a tumor-homing motif for the delivery of various anti-tumor compounds and viral particles to tumor vessels [22–24]. Thus, most reports of the NGR motif were related to the tumor studies. In this study, we focused on the adhesion of hMSCs since cell scaffolding might be able to regulate cellular behavior such as cell adhesion, migration, proliferation, and differentiation.

According to our data, the ALNGR peptide has affinity for integrin α5β1, but not for αvβ3 (Fig. 4B). The NGR motifs found in other regions of fibronectin: FN-I_5 (GNGRG), FN-II_1 (YNGRT), FN-I_7 (GNGRG) [25], and cell-adhesive peptides screened by phage display with NGR motif: NGRAHA, NGRAHA and DGRAHA, showed smaller cell adhesion effects than ALNGR (data not shown). Therefore, it was suggested that the ALNGR peptide has a stronger cell adhesion activity compared to other NGR motifs. Fig. 2 illustrates the role of the ALNGR peptide in cell proliferation; promoting cell proliferation without influencing undifferentiated state. This result indicates that the ALNGR peptides will be applicable for use in the proliferation of hMSC. Generally, it was reported that the proliferation of hMSCs decreases as the expression of differentiation markers increases [26]. Based on this knowledge, it is necessary to study whether the proliferation effect of the ALNGR peptide can decrease the differentiation of hMSCs. Moreover, McBeath et al. reported that the differentiation of hMSCs is regulated by the cell shape, for example, hMSCs which are allowed to adhere, flatten, and spread undergo osteogenesis, while unspread, round cells become adipocyte [27]. In our study, the cell shape on ALNGR peptide was different from that on RGDS. Therefore, further experiments are necessary to investigate the differentiation of hMSCs using the cell-adhesive peptides identified in this study.

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References

[1] R.F. Pereira, K.W. Halford, M.D. O'Hara, D.B. Leeper, B.P. Sokolov, M.D. Pollard, O. Bagasra, D.J. Prockop, Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage, and lung in irradiated mice, Proc. Natl. Acad. Sci. USA 92 (1995) 4857–4861.
[2] M.F. Pittenger, A.M. Mackay, S.C. Beck, R.K. Jaiswal, R. Douglas, J.D. Mosca, M.A. Moorman, D.W. Simonetti, S. Craig, D.R. Marshak, Multilineage potential of adult human mesenchymal stem cells, Science 284 (1999) 143–147.
[3] K. Shimizu, A. Ito, T. Yoshida, Y. Yamada, M. Ueda, H. Honda, Bone tissue engineering with human mesenchymal stem cell sheets constructed using magnetite nanoparticles and magnetic force, J. Biomed. Mater. Res. B. Appl. Biomater. 82 (2007) 471–480.
[4] K.M. Yamada, Fibronectins: structure, functions and receptors, Curr. Opin. Cell. Biol. 1 (1989) 956–963.
[5] S. Johansson, G. Svineng, K. Wennerberg, A. Armulik, L. Lohikangas, Fibronectin-integrin interactions, Front. Biosci. 2 (1997) d126–d146.
[6] M.D. Pierschbacher, E. Ruoslahti, Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule, Nature 309 (1984) 30–33.
[7] R.O. Hynes, Integrins: bidirectional, allosteric signaling machines, Cell 110 (2002) 673–687.
[8] U. Hersel, C. Dahmen, H. Kessler, RGD modified polymers: biomaterials for stimulated cell adhesion and beyond, Biomaterials 24 (2003) 4385–4415.
[9] A. Komoriya, L.J. Green, M. Mervic, S.S. Yamada, K.M. Yamada, M.J. Humphries, The minimal essential sequence for a major cell type-specific adhesion site (CS1) within the alternatively spliced type III connecting segment domain of fibronectin is leucine-aspartic acid-valine, J. Biol. Chem. 266 (1991) 15075–15079.
[10] J. Graf, R.C. Ogle, F.A. Robey, M. Sasaki, G.R. Martin, Y. Yamada, H.K. Kleinman, A pentapeptide from the laminin B1 chain mediates cell adhesion and binds the 67,000 laminin receptor, Biochemistry 26 (1987) 6896–6900.
[11] R. Pytela, M.D. Pierschbacher, E. Ruoslahti, Identification and isolation of a 140 kd cell surface glycoprotein with properties expected of a fibronectin receptor, Cell 40 (1985) 191–198.
[12] S. Aota, M. Nomizu, K.M. Yamada, The short amino acid sequence Pro-His-Ser-Arg-Asn in human fibronectin enhances cell-adhesive function, J. Biol. Chem. 269 (1994) 24756–24761.
[13] R. Frank, The SPOT-synthesis technique. Synthetic peptide arrays on membrane supports—principles and applications, J. Immunol. Methods 267 (2002) 13–26.
[14] A. Kramer, J. Schneider-Mergener, Synthesis and screening of peptide libraries on continuous cellulose membrane supports, Methods Mol. Biol. 87 (1998) 25–39.
[15] R. Kato, C. Kaga, M. Kunimatsu, T. Kobayashi, H. Honda, Peptide array-based interaction assay of solid-bound peptides and anchorage-dependant cells and its effectiveness in cell-adhesive peptide design, J. Biosci. Bioeng. 101 (2006) 485–495.
[16] M. Okochi, M. Nakanishi, R. Kato, T. Kobayashi, H. Honda, High-throughput screening of cell death inducible short peptides from TNF-related apoptosis-inducing ligand sequence, FEBS Lett. 580 (2006) 885–889.
[17] C. Kaga, M. Okochi, M. Nakanishi, H. Hayashi, R. Kato, H. Honda, Screening of a novel octamer peptide, CNSCWSKD, that induces caspase-dependent cell death, Biochem. Biophys. Res. Commun. 362 (2007) 1063–1068.
[18] J. Wegener, C.R. Keese, I. Giaever, Electric cell-substrate impedance sensing (ECIS) as a noninvasive means to monitor the kinetics of cell spreading to artificial surfaces, Exp. Cell. Res. 259 (2000) 158–166.
[19] I. Giaever, C.R. Keese, A morphological biosensor for mammalian cells, Nature 366 (1993) 591–592.
[20] E. Koivunen, D.A. Gay, E. Ruoslahti, Selection of peptides binding to the alpha 5 beta 1 integrin from phage display library, J. Biol. Chem. 268 (1993) 20205–20210.
[21] E. Koivunen, B. Wang, E. Ruoslahti, Isolation of a highly specific ligand for the alpha 5 beta 1 integrin from a phage display library, J. Cell Biol. 124 (1994) 373–380.
[22] K.R. Gehlsen, W.S. Argraves, M.D. Pierschbacher, E. Ruoslahti, Inhibition of in vitro tumor cell invasion by Arg-Gly-Asp-containing synthetic peptides, J. Cell Biol. 106 (1988) 925–930.
[23] G. Colombo, F. Curnis, G.M. De Mori, A. Gasparri, C. Longoni, A. Sacchi, R. Longhi, A. Corti, Structure–activity relationships of linear and cyclic peptides containing the NGR tumor-homing motif, J. Biol. Chem. 277 (2002) 47891–47897.
[24] H.M. Ellerby, W. Arap, L.M. Ellerby, R. Kain, R. Andrusiak, G.D. Rio, S. Krajewski, C.R. Lombardo, R. Rao, E. Ruoslahti, D.E. Bredesen, R. Pasqualini, Anti-cancer activity of targeted pro-apoptotic peptides, Nat. Med. 5 (1999) 1032–1038.
[25] F. Curnis, R. Longhi, L. Crippa, A. Cattaneo, E. Dondossola, A. Bachi, A. Corti, Spontaneous formation of L-isoaspartate and gain of function in fibronectin, J. Biol. Chem. 281 (2006) 36466–36476.
[26] J.B. Lian, G.S. Stein, Concepts of osteoblast growth and differentiation: basis for modulation of bone cell development and tissue formation, Crit. Rev. Oral Biol. Med. 3 (1992) 269–305.
[27] R. McBeath, D.M. Pirone, C.M. Nelson, K. Bhadriraju, C.S. Chen, Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment, Dev. Cell 6 (2004) 483–495.