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Dental pulp cells derived from permanent teeth express higher levels of R-cadherin than do deciduous teeth: Implications of the correlation between R-cadherin expression and restriction of multipotency in mesenchymal stem cells

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ABSTRACT

Objectives: The aim of this study was to characterize the expression status of cadherins in dental pulp-derived mesenchymal progenitor/stem cells from deciduous and permanent teeth, and to determine how cadherins affect the multipotency of the progenitor/stem cells.
Materials and methods: We evaluated and compared the expression status of cadherins in dental pulp-derived cells from deciduous teeth and in cells from permanent teeth by using an array of primers for amplification of RNA encoding human cell adhesion molecules and a real time PCR system. In order to elucidate how cadherins (which are differentially expressed in deciduous and permanent teeth) affect the multipotency of the dental pulp-derived progenitor/stem cells, the ability of the dental pulp cells to differentiate into adipocytes and osteoblasts was evaluated.
Results: R-cadherin was found to be vigorously expressed in the dental pulp cells derived from permanent teeth but not in the dental pulp cells derived from deciduous teeth. N-cadherin was found to be expressed essentially equally in both types of cells. The ability of the dental pulp cells of deciduous teeth to differentiate into adipocytes and osteoblasts was found to be much higher than that of cells obtained from permanent teeth.
Conclusion: R-cadherin may be a key molecule for providing control over the multipotency of the dental pulp-derived mesenchymal stem cells.

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1. Introduction

Cadherins are members of a family of transmembrane proteins involved in mediating homophilic adhesion in a Ca^{2+}-dependent manner. These proteins are major components of adherence junctions (AJs) in cells. E-cadherin is the main cadherin in the AJs of epithelial cells whereas other cadherins including N-cadherin, P-cadherin, R-cadherin, and VE-cadherin form AJs in other cell types. Cadherins are highly conserved transmembrane glycoproteins that mediate homotypic cell-cell adhesions through their extracellular domains. The cadherin cytoplasmic domains provide filamentous actin (F-actin) cytoskeleton attachment points via associations with catenins and other cytoskeletal-associated proteins.[1] These adhesive receptors regulate diverse functions beyond the basic adhesive process such as intracellular signalling events.[2] Whilst fibroblasts express several different cadherins including P-cadherin, R- cadherin, OB-cadherin, and fat-like cadherins,[3,4] N-cadherin is the predominant cadherin expressed by these cells.[3,5] N-cadherin-mediated AJs are of central importance in connective tissue physiology and are critical for the regulation of cell attachment and migration,[6] wound healing,[7] metastatic potential,[8] and embryonic development,[9,10] as well as differentiation and formation of numerous specialized tissues including fibrous connective tissues.[11–15]

Dental pulp (DP) tissue is a non-hematopoietic connective tissue that is almost completely surrounded by hard tissue.[16] Recently, DP-derived progenitor/stem cells have been shown to be capable of differentiating into osteoblasts, adipocytes and neural cells *in vivo* in a manner similar to that of undifferentiated mesenchymal cells derived from bone marrow.[17] Therefore, the DP tissue appears to be an excellent source of stem cells because it can be obtained from deciduous teeth requiring extraction after normal serial eruptions of corresponding permanent teeth. Intriguingly, Govindasamy et al. reported that the expression levels of several pluripotent markers such as OCT4, SOX2, NANOG, and REX1 were more than 2-fold higher in DP stem cells derived from deciduous teeth (SCDs) than in DP stem cells (DPSCs) derived from permanent teeth.[18] This indicates that the multipotency of DPSCs might be restricted relative to SCDs. It was found that SCDs retain their plasticity over the passages, whereas DPSCs lose their plasticity and tend to become increasingly committed towards the neuronal lineage. However, it remains to be clarified how the cell–cell adhesive molecules such as N-cadherin and other cadherins affect the status of multipotency in SCDs or DPSCs.

Here, we aimed to determine the profile of the expression status of adhesive molecules in fibroblast-like cells derived from either deciduous teeth DP (DDP cells) or permanent teeth DP (PDP cells). We found that R-cadherin is dominantly expressed in PDP cells but not in DDP cells, whereas the status of N-cadherin expression in DDP cells was comparable to that of PDP cells. Furthermore, we compared the ability of DDP cells to differentiate into osteoblasts or adipocytes with that of PDP cells.

2. Materials and methods

2.1. Cell culture

Dental pulp (DP) tissues were obtained from healthy human deciduous or permanent teeth from 6 donors, aged 7–8 years. Informed consent was obtained from the parents of the donors before tooth extraction, which was carried out in our hospital during the course of orthodontic treatment. The study protocol was approved by the Ethics Committee of Iwate Medical University, School of Dentistry (no. 01101).

A surgical blade was used to cut the DP tissues into pieces so that they could be digested with 2 mg/mL of collagenase (type I, Gibco BRL, Gaithersburg, MD, USA) at 37 °C for 30 min. The tissues were then washed with Dulbecco's phosphate-buffered saline (PBS), placed on culture dishes, and maintained in α-modified minimum essential medium (α-MEM; Gibco BRL) supplemented with 10% foetal bovine serum (FBS; Gibco BRL). Fibroblast-like cells that grew out of the DP tissue from 3 deciduous teeth or 3 permanent teeth were used as DDP1, DDP2, and DDP3 or PDP1, PDP2, PDP3 cells, respectively. When these cells reached confluence in 35-mm culture dishes, they were detached with 0.2% trypsin and 0.02% EDTA 4Na in PBS and subcultured at a 1:4 split ratio.

PDP1 cells were seeded into each well of a 96-well plate at a density of 1 cell/well. Each cells was expanded to a subconfluent culture on a 90-mm dish. The single cell-derived cultures from each well of the 96-well plate were named as PDP1.1, 1.3, 1.4, 1.7 and 1.9 cells, respectively.

The human bone marrow-derived mesenchymal stem cells, UE7T-13 cells, the life span of which was prolonged by infecting retrovirus encoding human papillomavirus E7 and hTERT,[19,20] and human acute myeloblastic leukemic cell line, HL60 cells, were purchased from Health Science Research Resources Bank (Japan Health Sciences Foundation, Japan).

All cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2.

2.2. Expression profiling of cell adhesion molecules

Gene expression profiling was performed using a PrimerArray of human cell adhesion molecules (PH003, Takara) in combination with a Thermal Cycler Dice Real Time System (Takara) according to the manufacturer's instructions. This PrimerArray is a set of real-time reverse transcription-polymerase chain reaction (RT-PCR) primers used for the analysis of RNA expression of cell adhesion molecules. The array contains a mixture of 96 primer pairs for 88 cell adhesion molecule genes and 8 housekeeping genes. Quantification of gene expression was performed using a PrimerArray Analysis Tool Ver. 2.0 (Takara).

2.3. Gene expression analysis with RT-PCR

Total RNA extracted from DDP and PDP cells was isolated with ISOGEN reagent (Nippongene) according to the manufacturer's instructions. First-strand cDNA was synthesized from total RNA using the PrimeScript RT reagent Kit (Takara).

Table 1 – Sequence of PCR primers used in this study.

Gene	Primer sequences
R-cadherin	Forward: ATCGTGGCCATCCTCATCTG; Reverse: CCTCGTCATACTTGAGGATGTTGTC
N-cadherin	Forward: CGAATGGATGAAAGACCCATCC; Reverse: GGAGCCACTGCCTTCATAGTCAA
Thy-1	Forward: ATCTCCAGCATTCTCAGCCACA; Reverse: CCTGGTCAAACCTGCATCTTCA
endoglin	Forward: TCATGCGCTTGAACATCATCAG; Reverse: TGCGAGTAGATGTACCAGAGTGCAG
CD73	Forward: GAGCCTGCTCAGCTCTGCATAA; Reverse: CCTCTAGCTGCCATTTGCACAC
osterix	Forward: CCTGGCTGCGGCAAGGTGT; Reverse: GATCTCCAGCAAGTTGCTCTGC
osteocalcin	Forward: CAGCAAAGGTGCAGCCTTTGT; Reverse: TCCTGAAAGCCGATGTGGTC
GAPDH	Forward: GCACCGTCAAGGCTGAGAAC; Reverse: ATGGTGGTGAAGACGCCAGT
β-actin	Forward: CTGGCACCACACCTTCTACAATG; Reverse: AATGTCACGCACGATTTCCCGC

Quantitative RT-PCR (qRT-PCR) was performed for RNA encoding R-cadherin, N-cadherin, Thy-1, endoglin and CD73 with a Thermal Cycler Dice Real Time System (Takara) using SYBR Premix Ex Taq II (Takara) with the specific oligonucleotide primers listed in Table 1. The mRNA expression levels were normalized to those obtained for glyceraldehyde adenosine-phosphate dehydrogenase (GAPDH). For expression analysis of osterix, osteocalcin, and β-actin, the PCR conditions were set at 30 cycles at 98 °C for 10 s, 55 °C for 30 s, and 72 °C for 30 s using the EmeraldAmp PCR Master Mix (Takara). PCR products were resolved on a 2% agarose gel and visualized with ethidium bromide. All the primer sequences used are shown in Table 1.

2.4. *Flow cytometry (FCM) analysis*

DDP1 and PDP1 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 anti-N-cadherin antibody (clone: GC-4; Abcam) and anti-R-cadherin antibody (clone: N-19; Santa Cruz) for 1 h at room temperature. The cells were then incubated with PE- and FITC-conjugated secondary antibodies for 1 h. The acquisition was performed using an EPICS XL ADC System (Beckman Coulter).

2.5. *Confocal fluorescence microscopy*

PDP1 cells were seeded onto 8-well culture slides (BD Biosciences). After 24 h, the cells were fixed with 4% paraformaldehyde and washed 5 times with PBS. For detection of N- and R-cadherin, cells were incubated with anti-N-cadherin (clone: GC-4; Abcam) and anti-R-cadherin antibody (clone: N-19; Santa Cruz), respectively, for 1 h at room temperature. The both primary antibodies were used in 1:100 dilution rates. The cells were then incubated with Alexa Fluor 488- or Alexa Fluor 633-conjugated secondary antibodies and DAPI. Fluorescence was examined by confocal laser scanning microscopy.

2.6. *Osteogenic and adipogenic differentiation*

DDP and PDP cells were cultured onto 60-mm culture dishes with osteogenic differentiation medium (α-MEM containing 50 μg/mL ascorbic acid (Nacalai Tesque) with 6 mM β-glycerophosphate (Sigma) and 10% FBS) or adipogenic differentiation medium (DMEM supplemented with 0.5 mM 3-isobutyl-1-methylxanthine (Sigma), 1 μM hydrocortisone (Sigma), 0.1 mM indomethacin (Sigma), and 10% FBS). Half of the medium in each dish was exchanged every 2–3 days. After 4

Table 2 – Expression status of cell adhesion molecules for the DDP and PDP cells.[#]

Official symbol	Official name	Ct value (mean ± SD)		-fold
		DDP cells	PDP cells	
CDH4	Cadherin 4, type 1, R-cadherin (retinal)	36.51 ± 0.33	29.46 ± 1.97	83.29
NCAM1	Neural cell adhesion molecule 1	32.57 ± 2.49	28.19 ± 1.62	13.09
HLA-DRA	Major histocompatibility complex, class II, DR alpha	28.10 ± 0.34	31.84 ± 0.13	8.63
SDC1	Syndecan 1	28.77 ± 0.72	25.27 ± 0.82	7.11
VCAM1	Vascular cell adhesion molecule 1	33.06 ± 2.84	29.66 ± 1.05	6.63
ITGB2	Integrin, beta 2	35.91 ± 2.15	32.52 ± 2.55	6.59
ITGA6	Integrin, alpha 6	27.36 ± 0.28	24.04 ± 1.47	6.28
CD274	CD274 molecule, programmed cell death 1 ligand 1	29.31 ± 0.80	26.08 ± 1.27	5.90
CLDN1	Claudin 1	30.34 ± 1.85	27.13 ± 0.83	5.82
CDH15	Cadherin 15, type 1, M-cadherin (myotubule)	33.33 ± 4.32	35.33 ± 2.61	0.15
ESAM	Endothelial cell adhesion molecule	30.46 ± 0.63	32.70 ± 1.91	0.13
NRXN2	Neurexin 2	31.77 ± 0.09	34.10 ± 1.65	0.13
NRXN3	Neurexin 3	28.18 ± 1.15	30.70 ± 0.70	0.11
ICAM1	Intercellular adhesion molecule 1	32.33[##]	36.04 ± 1.32	0.05

[#] Some of the genes expressed in PDP cells are listed. The expression levels of these genes are 5-fold higher or lower than those of the same genes in the DDP cells.

[##] The SD was not calculated because a Ct value was detected only in a single sample.

weeks of culture, the cells were evaluated for expression of osteogenic differentiation marker genes (osterix and osteocalcin) or for adipogenic differentiation as indicated by oil-red stain.

2.7. Statistics

Data are presented as the mean ± standard deviation (SD). Statistical analysis was performed by using the Student's *t*-test, and values of $p < 0.05$ were considered to be significant.

3. Results

3.1. Expression profile of cell adhesion molecules in DP cells

In order to identify the highly expressed cell adhesion molecules in DP cells, we performed primer array analyses as described in Section 2. We then compared the expression status of cell adhesion molecules of the DDP and PDP cells. Differentially expressed genes in the DDP and PDP cells were identified and are shown in Table 2. Amongst 88 target genes, 9 were identified as highly expressed genes in the PDP cells. In particular, the expression level of R-cadherin in the PDP cells was found to be more than 83-fold higher than that of the DDP cells.

3.2. R- and N-cadherin expression levels in DP cells

We examined the expression levels of R-cadherin and N-cadherin mRNA in DP cells by qRT-PCR analysis. The R-cadherin mRNA expression level was found to be significantly higher in the PDP cells compared to the DPD cells (Fig. 1A). On the other hand, the level of N-cadherin expression did not differ between the PDP and DDP cells (Fig. 1B).

3.3. PDP cells expresses both R- and N-cadherin

To determine whether the PDP cells express both R- and N-cadherin, we performed FCM analysis. As shown in Fig. 2, PDP cells express both R- and N-cadherin on the cell surface. The expression of these cadherins on the cell surface enables the cells to perform their intended functions (Fig. 2B). On the other hand, the DDP cells were found to only express N-cadherin (Fig. 2C). Furthermore, the results of confocal fluorescence microscopy analysis also indicate that PDP cells express both R- and N-cadherin (Fig. 3).

3.4. DDP cells maintains multipotency than PDP cells

We investigated osteogenic and adipogenic differentiation for the purpose of examining the multipotency of the DP cells. As shown in Fig. 4A, we performed qRT-PCR analysis in the DP cells in order to identify expression levels of mesenchymal stem cells marker genes. The expression levels of the typical marker genes such as Thy-1, endoglin and CD73 were detected in the both of DDP and PDP cells same as UE7T13 cells, but there were very low levels in HL60 cells. In the osteogenic differentiation of the DP cells, the expression of osterix was

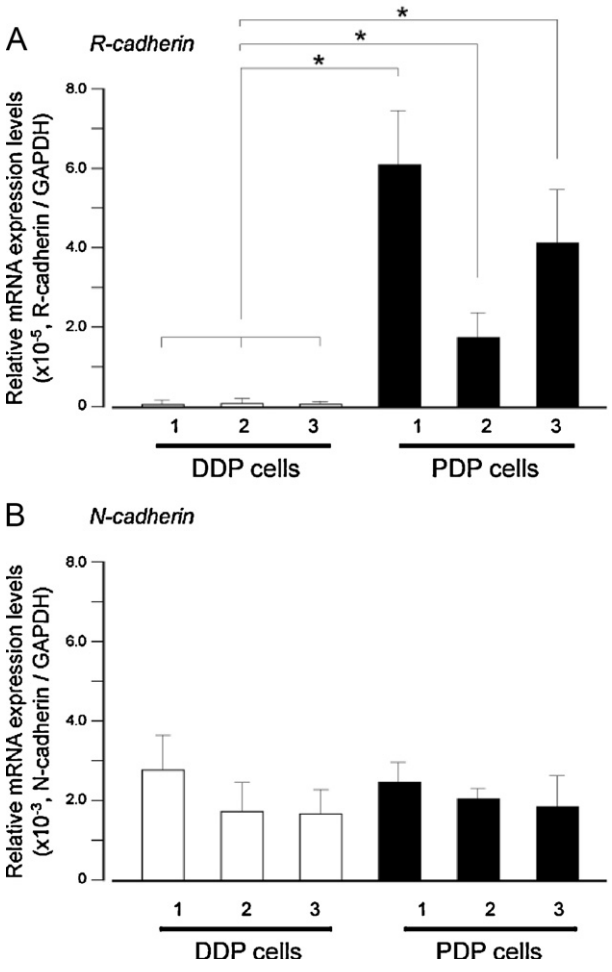

Fig. 1 – R- and N-cadherin expression levels in DP cells. Relative mRNA expression levels of R-cadherin (A) and N-cadherin (B) were analysed by qRT-PCR. Data represent the mean of 3 individual experiments (*n*) ± SD. *$p < 0.05$ was considered significant (in comparison with 3 DDP1, DDP2, and DDP3 cells).

detected in DDP cells cultured in both normal–medium and in osteogenic-differentiation medium. On the other hand, the expression of osteocalcin was only detected when DDP cells were cultured in the osteogenic-differentiation medium for 4 weeks (Fig. 4B). Furthermore, the DDP cells were shown to have the ability to undergo adipogenic differentiation in oil-red stain to a greater extent than do the PDP cells (Fig. 4C).

3.5. R-cadherin expression restricts osteogenic differentiation

We established several single cell-derived cultures from PDP cells (PDP1.1, 1.3, 1.4, 1.7 and 1.9 cells) expressing different levels of R-cadherin, respectively (Fig. 5A). As shown in Fig. 5A and B, the level of osteocalcin expression in the 1.7 and 1.9 cells which expressed high amount of R-cadherin was lower than 1.1 cells which expressed low amount of that. Furthermore, the expression level of osteocalcin in the R-cedherin-overexpressed UE7T13 cell (pRcad cells) was much lower than

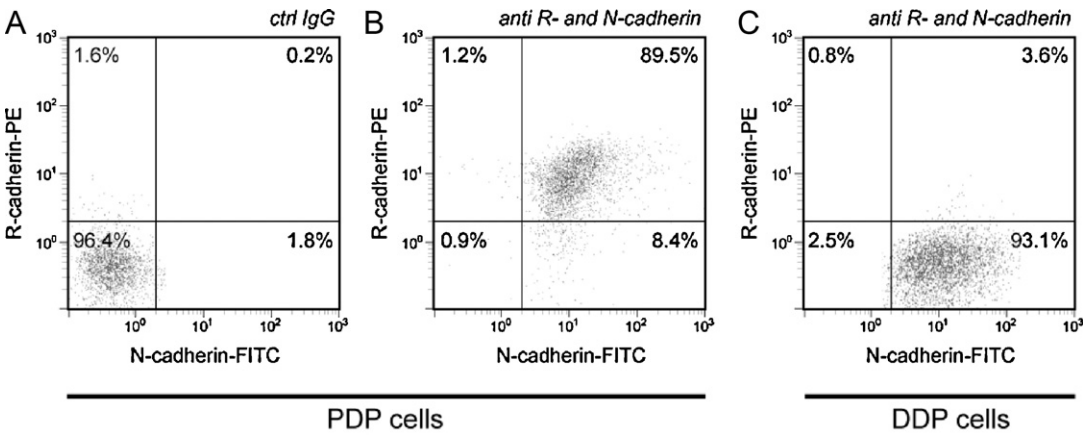

Fig. 2 – PDP cells express both R- and N-cadherin. PDP1 (A and B) and DDP1 (C) cells were incubated with control (ctrl) IgG (A), anti-R-cadherin and anti-N-cadherin antibody (B) in PDP1 cells, and anti-R-cadherin and anti-N-cadherin antibody (C) in DDP1 cells. The cells were then incubated with PE- and FITC-conjugated secondary antibodies. The acquisition was performed using the EPICS XL ADC system.

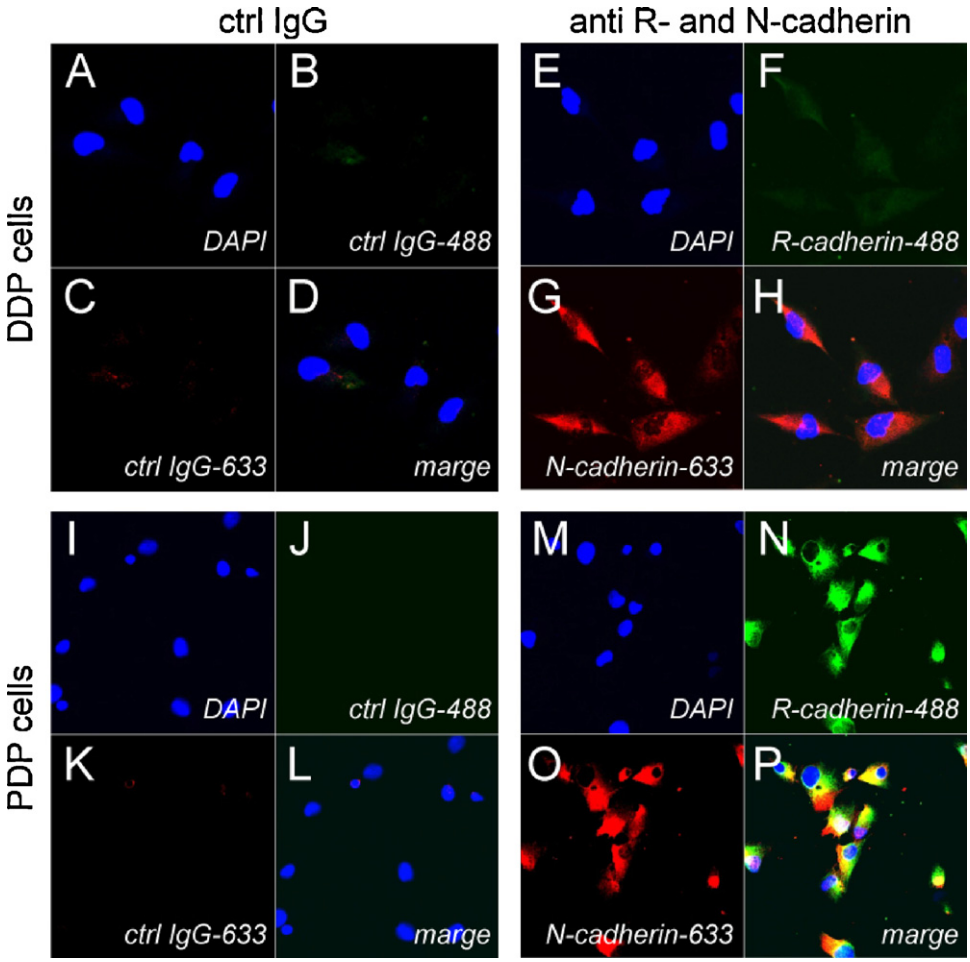

Fig. 3 – PDP cells express both R- and N-cadherin. DDP and PDP1 cells were incubated with control (ctrl) IgG (A–D, I–L) and anti-R-cadherin and anti-N-cadherin antibody (E–H, M–P). The cells were then incubated with Alexa Fluor 488- or 633-conjugated secondary antibodies and DAPI. Fluorescence was examined by confocal laser scanning microscopy.

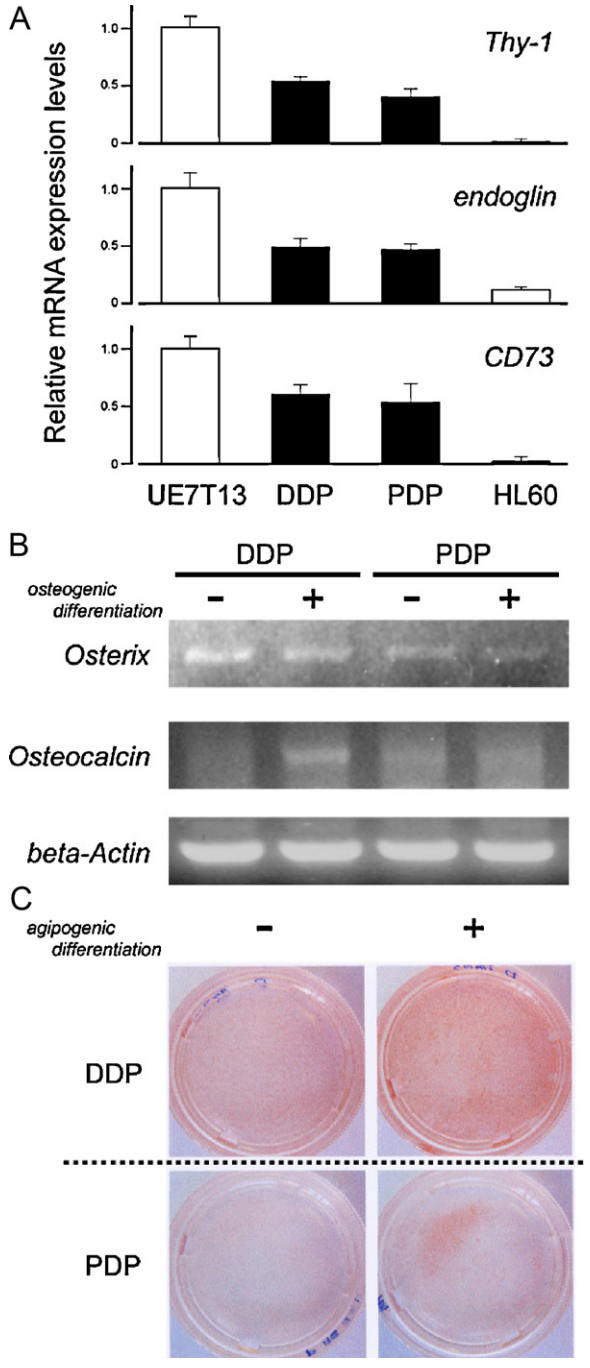

Fig. 4 – Osteogenic and adipogenic differentiation of DP cells. (A) Relative mRNA expression levels of Thy-1, endoglin and CD73 were analysed by qRT-PCR. Data represent the mean of 3 individual experiments (n) ± SD. (B and C) DDP1 and PDP1 cells were cultured onto 60-mm culture dishes with osteogenic differentiation medium (B) or adipogenic differentiation medium (C). After 4 weeks of culture, the cells were evaluated for the expression of osteogenic differentiation marker genes (osterix and osteocalcin) or for adipogenic differentiation as indicated by oil-red stain. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

that of pCTRL cells transfected with control vector or that of non-transfected UE7T13 cells (Fig. 5C and D).

4. Discussion

Here, a PrimerArray and qRT-PCR analysis of human cell adhesion molecules revealed that R-cadherin was vigorously expressed in the PDP cells but not in the DDP cells (Table 2 and Fig. 1A), whereas N-ceadherin was almost equally expressed in the both cells (Fig. 1B).

R-cadherin is a closely related member of the classical (type I) cadherins.[21] This protein structurally resembles E- and N-cadherin and is 74% identical to N-cadherin. R-cadherin appears to play important roles in the formation of blood vessels and the neuronal network. R-cadherin has also been shown to be involved in retinal angiogenesis during development. The neutralization of R-cadherin by antibodies or peptides was found to prevent the proper formation of a vascular network in the retina of newborn mice.[22] It was also reported that R-cadherin might affect cell fates of several kinds of progenitor/stem cells. R-cadherin expression in C2C12 myoblasts inhibits the induction of myogenesis,[23] suggesting that R-cadherin affects the pattern of commitment to differentiation of undifferentiated mesenchymal cells. In addition, Rosenberg et al. found that striated muscle differentiation was not observed in *E-cadherin−/−* embryonic stem cells. However, constitutive expression of R-cadherin in the cells was observed to exclusively rescue the formation of striated muscle.[24] Intriguingly, R-cadherin expression was almost completely shut off during the osteoblastic differentiation of C2C12 cells induced by treatment with bone morphogenetic protein 2. This indicates that R-cadherin might negatively affect osteoblastic differentiation of undifferentiated mesenchymal cells.[25] Intriguingly, Shan et al. showed that N-cadherin can form both *trans* and *cis* heterodimers with R-cadherin.[26] In addition, the overall tissue distribution of R-cadherin is similar to that of N-cadherin,[27] suggesting that the 2 cadherins generate functional units to mediate cell–cell adhesion. In fact, both N- and R-cadherin were detected in single PDP cells by FCM analysis and by fluorescence microscopy (Figs. 2 and 3). However, it remains to be clarified how R-cadherin affects the commitment to differentiation of adult stem cells into mesenchymal stem cells.

Govindasamy et al. reported that several pluripotent markers such as OCT4, SOX2, NANOG, and REX1 are more highly expressed in dental pulp stem cells derived from deciduous teeth than in stem cells derived from permanent teeth.[28] Moreover, DP-derived progenitor/stem cells have been shown to be capable of differentiating into osteoblasts, adipocytes and neural cells *in vivo* in a manner similar to that of undifferentiated mesenchymal cells derived from bone marrow.[17] In fact, the expression levels of typical mesenchymal stem cell marker genes such as Thy-1, endoglin and CD73 were detected in the both of DDP and PDP cells as similarly detected in the bone marrow-derived mesenchymal stem cells, UE7T13 cells (Fig. 4). Intriguingly, R-cadherin-expressing PDP cells were found to significantly suppress the ability of the cells to differentiate into adipocytes and osteoblasts. This was

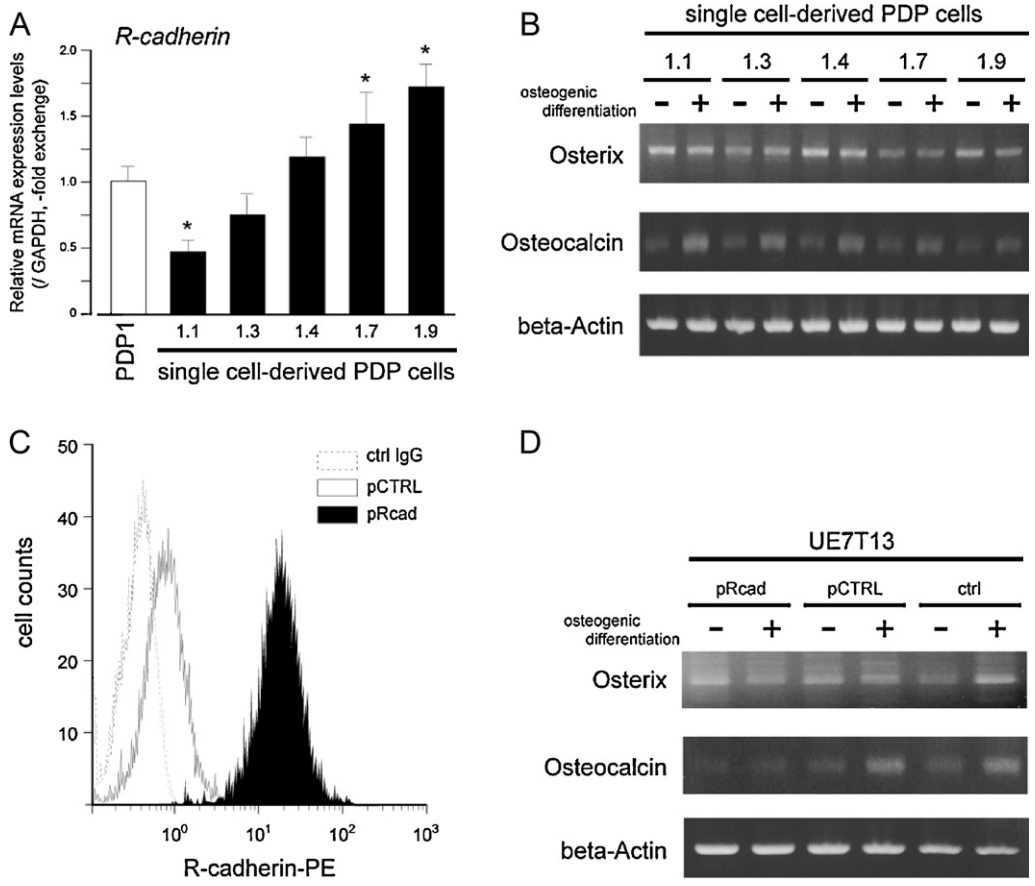

Fig. 5 – R-cadherin expression restricts osteogenic differentiation. (A) Expression level of R-cadherin in each single-cell derived culture established from PDP cells (PDP1.1–1.9) was evaluated by using qRT-PCR. (C) Cell surface expression of R-cadherin in UE7T13 cells transfected with control vector (pCTRL) or R-cadherin expression vector (pRcad) were incubated with control (ctrl) IgG or anti-R-cadherin. The cells were then incubated with PE-conjugated secondary antibodies. The acquisition was performed using the EPICS XL ADC system. (B and D) PDP1.1–1.9 cells or R-cadherin overexpressed UE7T13 cells were seed onto 6-well culture dishes. After 2 weeks of the culture with osteogenic differentiation medium, the cells were evaluated for the expression of osteogenic differentiation marker genes (osterix and osteocalcin). *$p < 0.05$ was considered significant (in comparison with PDP1 cells).

not observed for the DDP cells (Fig. 4). Moreover, the status of osteogenic differentiation of PDP cells seemed to be closely related to the expression level of R-cadherin in the cells: the cells vigorously expressing R-cadherin did not express osteoblast-specific marker osteocalcin (Fig. 5A and B). Furthermore, R-cadherin-overexpression clearly suppressed the expression of osteoblast-specific marker osteocalcin in mesenchymal stem cell line UE7T13 (Fig. 5C and D). These results strongly suggest that R-cadherin expression restricts the multipotency of DP cells. In addition, these results suggest that the ability of DDP cells to differentiate into osteoblasts and adipocytes is significantly higher than that of the PDP cells because R-cadherin is abundantly expressed in the PDP cells but not in the DDP cells.

In conclusion, R-cadherin is vigorously expressed in the DP cells of permanent teeth but not in the DP of deciduous teeth. N-cadherin is essentially equally expressed in both types of cells. R-cadherin-expressing DP cells derived from permanent teeth were found to suppress the ability of the cells to differentiate into adipocytes and osteoblasts. This indicates that R-cadherin might restrict the multipotency of the DP cells. It is possible that R-cadherin functions as a key molecule in controlling the multipotency of the DP-derived mesenchymal stem cells. This report provides the first evidence that R-cadherin restricts the multipotency of DP-derived mesenchymal stem cells.

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REFERENCES

1. Kemler R. From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion. *Trends Genet* 1993;**9**(9):317–21.
2. Fukata M, Kaibuchi K. Rho-family GTPases in cadherin-mediated cell-cell adhesion. *Nat Rev Mol Cell Biol* 2001;**2**(12):887–97.
3. Matsuyoshi N, Imamura S. Multiple cadherins are expressed in human fibroblasts. *Biochem Biophys Res Commun* 1977;**235**(2):355–8.
4. Simonneau L, Kitagawa L, Suzuki S, Thiery JP. Cadherin 11 expression marks the mesenchymal phenotype: towards new functions for cadherins? *Cell Adhes Commun* 1995;**3**(2):115–30.
5. Hatta K, Takeichi M. Expression of N-cadherin adhesion molecules associated with early morphogenetic events in chick development. *Nature* 1986;**320**(6061):447–9.
6. Akitaya T, Bronner-Fraser M. Expression of cell adhesion molecules during initiation and cessation of neural crest cell migration. *Dev Dyn* 1992;**194**(1):12–20.
7. De Wever O, Westbroek W, Verloes A, Bloemen N, Bracke M, Gespach C, et al. Critical role of N-cadherin in myofibroblast invasion and migration in vitro stimulated by colon-cancer-cell-derived TGF-beta or wounding. *J Cell Sci* 2004;**117**(20):4691–703.
8. Kashima T, Nakamura K, Kawaguchi J, Takanashi M, Ishida T, Aburatani H, et al. Overexpression of cadherins suppresses pulmonary metastasis of osteosarcoma in vivo. *Int J Cancer* 2003;**104**(2):147–54.
9. Radice GL, Rayburn H, Matsunami H, Knudsen KA, Takeichi M, Hynes RO. Developmental defects in mouse embryos lacking N-cadherin. *Dev Biol* 1997;**181**(1):64–78.
10. Garcia-CastroMI. Vielmetter E, Bronner-Fraser M. N-cadherin, a cell adhesion molecule involved in establishment of embryonic left-right asymmetry. *Science* 2000;**288**(5468):1047–51.
11. Hinz B, Pittet P, Smith-Clerc J, Chaponnier C, Meister JJ. Myofibroblast development is characterized by specific cell–cell adherens junctions. *Mol Biol Cell* 2004;**15**(19):4310–20.
12. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodeling. *Nat Rev Mol Cell Biol* 2002;**3**(5):349–63.
13. Marthiens V, Gavard J, Lambert M, Mege RM. Cadherin-based cell adhesion in neuromuscular development. *Biol Cell* 2002;**94**(6):315–26.
14. Krauss RS, Cole F, Gaio U, Takaesu G, Zhang W, Kang JS. Close encounters regulation of vertebrate skeletal myogenesis by cell–cell contact. *J Cell Sci* 2005;**118**(11):2355–62.
15. Marie PJ. Role of N-cadherin in bone formation. *J Cell Physiol* 2002;**190**(3):297–305.
16. Orchardson R, Cadden SW. An update on the physiology of the dentine–pulp complex. *Dent Update* 2001;**28**(4). p. 200–206; 208–209.
17. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: stem cells from human exfollated deciduous teeth. *Proc Natl Acad Sci USA* 2003;**100**(10):5807–12.
18. Govindasamy V, Abdullah AN, Ronald VS, Musa S, Ab Aziz ZA, Zain RB, et al. Inherent differential propensity of dental pulp stem cells derived from human deciduous and permanent teeth. *J Endod* 2010;**36**(9):1504–15.
19. Mori T, Kiyono T, Imabayashi H, Takeda Y, Tsuchiya K, Miyoshi S, et al. Combination of hTERT and bmi-1, E6, or E7 induces prolongation of the life span of bone marrow stromal cells from an elderly donor without affecting their neurogenic potential. *Mol Cell Biol* 2005;**25**(12):5183–95.
20. Shimomura T, Yoshida Y, Sakabe T, Ishii K, Gonda K, Murai R, et al. Hepatic differentiation of human bone marrow-derived UE7T-13 cells: effects of cytokines and CCN family gene expression. *Hepatol Res* 2007;**37**(12):1068–79.
21. Nollet F, Kools P, van Roy F. Phylogenetic analysis of the cadherin superfamily allows identification of six major subfamilies besides several solitary members. *J Mol Biol* 2000;**299**(3):551–72.
22. Dorrell MI, Aguilar E, Friedlander M. Retinal vascular development is mediated by endothelial filopodia: a preexisting astrocytic template and specific R-cadherin adhesion. *Invest Ophthalmol Vis Sci* 2002;**43**(11):3500–10.
23. Kucharczak J, Charrasse S, Comunale F, Zappulla J, Robert B, Teuron-Navvaro I, et al. R-cadherin expression inhibits myogenesis and induces myoblast transformation via Rac1 GTP ase. *Cancer Res* 2008;**68**(16):6559–68.
24. Rosenberg P, Esni F, Sjödin A, Larue L, Carlsson L, Gullberg D, et al. A potential role of R-cadherin in striated muscle formation. *Dev Biol* 1997;**187**(1):55–70.
25. Shin CS, Lecanda F, Sheikh S, Weitzmann L, Cheng SL, Civitelli R. Relative abundance of different cadherins defines differentiation of mesenchymal precursors into osteogenic: myogenic, or adipogenic pathways. *J Cell Biochem* 2000;**78**(4):566–77.
26. Shan WS, Tanaka H, Phillips GR, Arndt K, Yoshida M, Colman DR, et al. Functional cis-heterodimers of N- and R-cadherins. *J Cell Biol* 2000;**148**(3):579–90.
27. Inuzuka H, Redies C, Takeichi M. Differential expression of R- and N-cadherin in neural and mesodermal tissues during early chicken development. *Development* 1991;**113**(3):959–67.
28. Govindasamy V, Abdullah AN, Ronald VS, Musa S, Aziz ZA, Zain RB, et al. Inherent differential propensity of dental pulp stem cells derived from human deciduous and permanent teeth. *J Endod* 2010;**36**(9):1504–15.