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= Abstract =

The role of DNA methylation to type II collagen expression in dedifferentiation of articular chondrocytes

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Purpose: We explored the possibility of DNA methylation as a mechanism of loss of type II collagen expression in dedifferentiating chondrocytes by culturing in monolayer.

Materials and Methods: Dedifferentiation was induced by low density subculturing primary porcine chondrocytes in vitro. The mRNA expression of Type I collagen, Type II collagen and DNA methyltransferase (DNMT) was measured by RT-PCR. Induction of redifferentiation in dedifferentiated chondrocytes was performed in 3-dimensional alginate bead culture system. As stimulating factors for reexpression of genes in dedifferentiated chondrocytes, 10 ng/ml TGF- β 1 and 5 μ M 5-azacytidine were used.

Results: Type II collagen mRNAs was expressed strongly in freshly isolated cells but had decreased in monolayer cultured cells after 3 weeks up to 40%. In contrast, type I collagen expression was increased from 21 days and kept increasing during the 86 days of study. After treatment of 5 μ M 5-azacytidine, fibroblast like morphology was changed to round shape such as traditional chondrocyte morphology at day 4. At day 10, type II collagen expression was increased by 5-azacytidine and TGF- β 1 marginally and also integrin α 1 expression was increased in all groups. RT-PCR analysis demonstrated that DNMT3A expression increased in dedifferentiating chondrocytes when compared with control cells for 40 days.

Conclusion: Loss of type II collagen mRNA expression and increase of DNMT 3A expression were showed similar patterns during dedifferentiation. These results suggest that type II collagen gene expression may be influenced by DNA methylation. As stimulating factors, TGF- β 1 and 5-azacytidine have potential activity to increase the type II collagen expression in alginate culture system.

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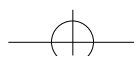
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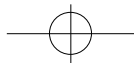
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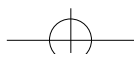


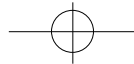
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Key Words: Chondrocyte dedifferentiation, Type II collagen, DNA methylation, Demethyl agent, DNA methyltransferase

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3. Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR)





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primer (Table 1). 103mM CaCl₂ 19 gauge
 DNA methyl- , bead
 transferase (DNMT 1, 3A, 3B) 0.15M NaCl , 0.5%
 . 90 50 ug/ml ascorbic acid†
 RNeasy mini kit (OIA GEN, Hilden, DMEM . 10ng/ml
 Germany) total RNA TGF- 1 (R&D system, Minneapolis,
 . 1 µg total RNA Oligo d(T)₁₂₋₁₈ MN, USA) 5 µM 5-azacytidine (Sigma,
 Ominiscript kit (QIAGEN) St. Louis, MO, USA)
 cDNA , 2 µ cDNA, 0.2 µM 5-azacytidine
 dNTP, 1.5 mM MgCl₂, 0.5 µM primer 10% DMEM
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 1.5% agarose gel electrophoresis
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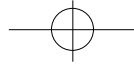
4. (5-azacytidine)

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 bead culture 3 (Fig. 1A).
 Alginate bead culture 2 3
 가 2×10⁶ cells/ml 40% , 50
 1.2% alginate .

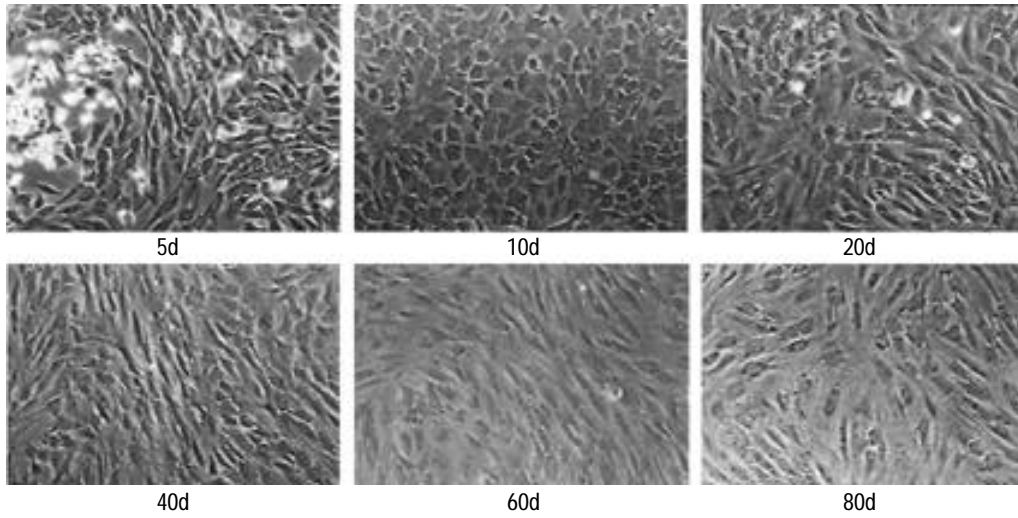
Table 1. RT-PCR primers

Genes	Primers
Type II Collagen	5 ' CCT GTT CTG AGA GGT CTT CC 3 ' 3 ' CTT CAC CAG GAA CGC CCT GA 5 '
Type I Collagen	5 ' AGA CAT CCC ACC AGT CAC CT 3 ' 3 ' GTG GGT GAC ACC TCG CCT TC 5 '
DNMT 1	5 ' ACC GCT TCT ACT TCC TCG AGG CCT A 3 ' 3 ' GTT GCA GTC CTC TGT GAA CAC TGT GG 5 '
DNMT3A	5 ' CAC ACA GAA GCA TAT CCA GGA GTG 3 ' 3 ' AGT GGA CTG GGA AAC CAA ATA CCC 5 '
DNMT3B	5 ' AAT GTG AAT CCA GCC AGG AAA GGC 3 ' 3 ' ACT GGA TTA CAC TCC AGG AAC CGT 5 '
Integrin 1	5 ' CAC TCA AAT CCA GCC ACA GCA GC 3 ' 3 ' CAA CCA CCT TAC ACT GTG CCG AC 5 '
GAPDH	5 ' ACC ACA GTC CAT GCC ATC AC 3 ' 3 ' ATG TCG TTG TCC CAC CAC CT 5 '

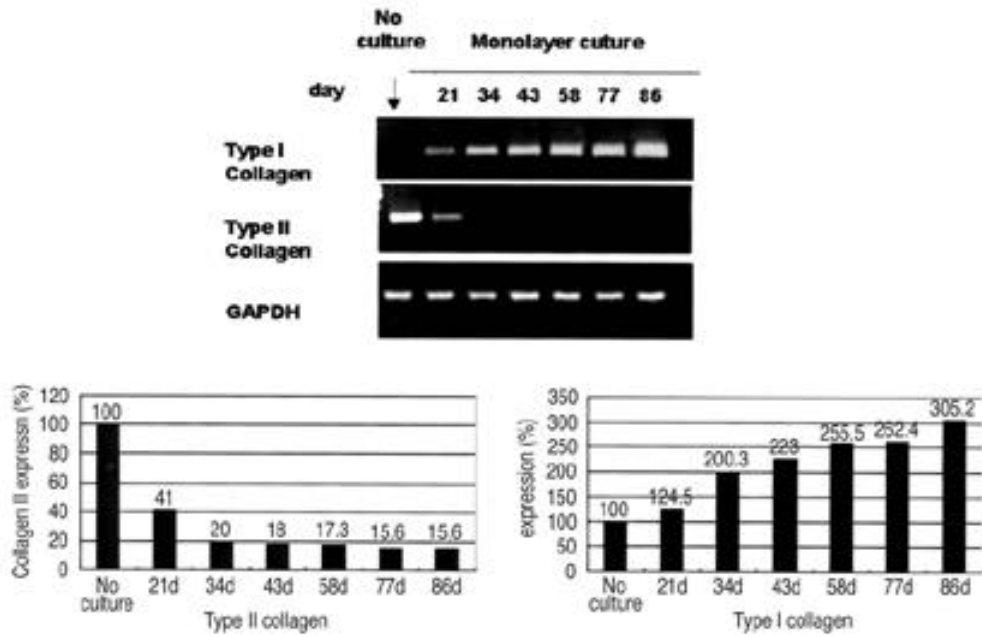




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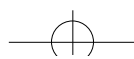


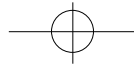
A



B

Fig. 1. (A) Cell morphology and collagen gene expression during chondrocyte dedifferentiation from 5 day to 80 day. Dedifferentiation was induced by subculturing in monolayer. Round shape of chondrocytes was changed to fibroblast like morphology. (B) Type I and II collagen expression were by RT-PCR analysis in freshly isolated chondrocytes or in chondrocytes subcultured on plastic for various days.





1. 가 (Fig. 1B). azacytidine 1 μ M 5-10 μ M 4 . 40 5 μ M 5-azacytidine 4

2. (DNA methyltransferase, DNMT) 가 10 μ M 5-azacytidine (Fig. 3). 4

3. (DNMT 1, 3A, 3B) RT-PCR DNMT 3A 5-azacytidine 가 , 10 μ M 4 5-azacytidine 가 . (data not shown).

4. (5-azacytidine) 5-azacytidine 2 40 alginate bead 3 . 2 , TGF- 1 . 40 2 3 10 ,

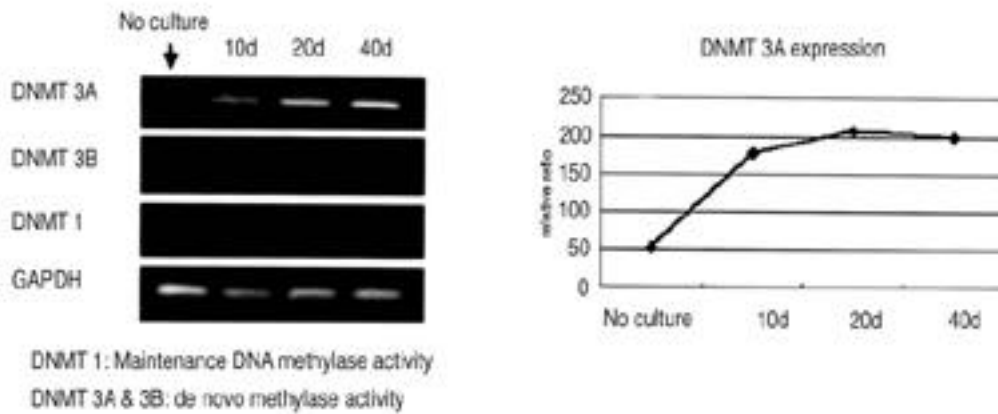
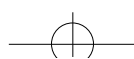
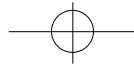


Fig. 2. DNMT expression in dedifferentiated chondrocytes. RT-PCR analysis demonstrated that DNMT3A expression increased in dedifferentiating chondrocytes when compared with control cells.





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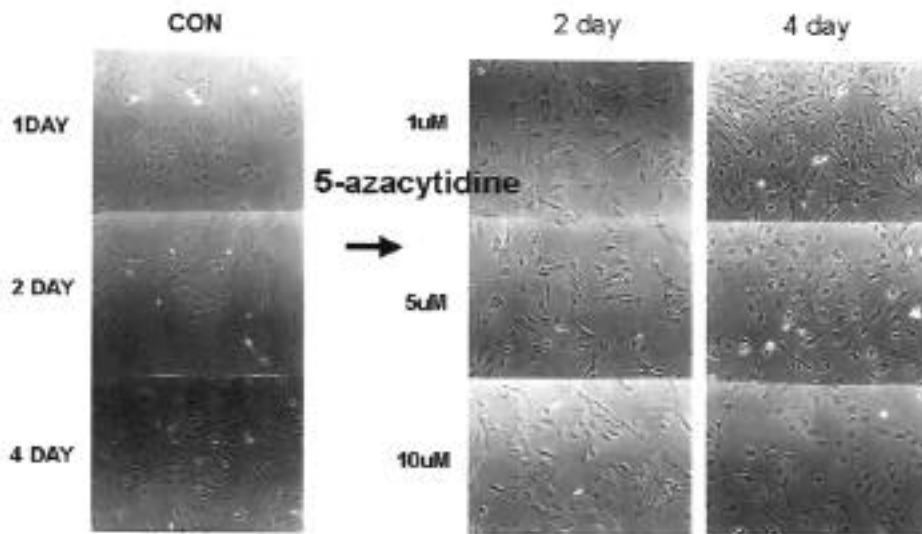


Fig. 3. Morphological changes by treatment in monolayer culture system. 5-azacytidine, a demethyl agent, induced a dose dependent changes of cell morphology in monolayer culture. The changes were started from 5 μ M of 5-azacytidine and maintained at 10 μ M of 5-azacytidine at day 4.

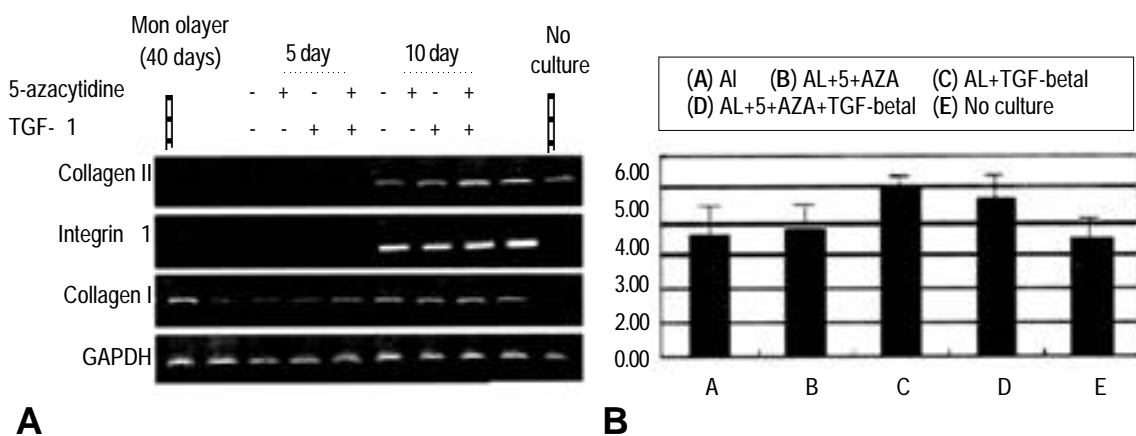
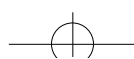
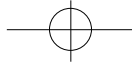


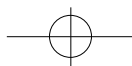
Fig. 4. (A) Reexpression of collagen genes by 5-azacytidine and TGF- 1 as stimulating factors in alginate bead culture system. (B) At day 10, type II collagen expression was analyzed with use of a densitometer .





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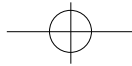
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