Effects of adenovirus expressing bone morphogenetic protein-4 on different cell types of osteoblastic differentiation

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Abstract

Background
Bone morphogenetic proteins(BMPs) play a pivotal role in inducing undifferentiated cells into osteoblastic cells that induce bone formation.

Aim/Hypothesis
In the present study, we investigated the effect of gene delivery of adenovirus expressing BMP-4 on osteoblastic differentiation of different cell types.

Materials and methods
An adenovirus expressing BMP-4(Ad5BMP4) was constructed by in vivo homologous recombination and transduced MC3T3-E1(mouse osteoblastic cell line), C2C12(mouse myoblastic cell line), NHDF(normal human dermal fibroblast-adult) to induce cell transformation. We assessed BMP-4 expression by ELISA for evaluation of gene expression activity of Ad5BMP4. MTT assay was performed at 3, 7, 15days to assess proliferation of cells that transduced with Ad5BMP4 and alkaline phosphatase activity was measured at 3, 7, 15days to assess osteoblastic differentiation of cells. The effect of Ad5BMP4 on mineralization was determined using alizarin red S staining after 14 days.

Results
At 3days, all three different cell types that transduced with Ad5BMP4 were observed BMP-4 expression and increased in proportion to the increase of concentration of Ad5BMP4. Up to 14days, level of BMP-4 was maintained on MC3T3-E1, but remarkably decreased on C2C12 and NHDF compared with 7days. When transduced with Ad5BMP4, the cell proliferation was not inhibited on MC3T3-E1, but significantly decreased on C2C12 compared with not transduced cells at 14days. Cell proliferation of NHDF that transduced with 6.2 pfu/cell concentration of Ad5BMP4 was similar to that of not transduced NHDF, but decreased above the concentration after 7days. To observe the osteogenic abilities of cells that transduced with Ad5BMP4, we investigated alkaline phosphatase(ALPase) activity and mineralization formation. ALPase activities were significantly increased after 7days on MC3T3-E1 and C2C12 and after 14days on NHDF. Mineralization formation was observed on MC3T3-E1, but the other were not observed at 14days.

Conclusion and clinical implication
Present study demonstrated that gene delivery using adenovirus expressing BMP-4 facilitated osteoblastic differentiation of MC3T3-E1, C2C12, NHDF cells. Also we confirmed the possibility of ex vivo BMP-4 gene delivery that be able to apply to effective technique for regeneration of bone defects.

Background and Aim

- Objectives of the study :
to investigate the effect of gene delivery of adenovirus expressing BMP-4 on osteoblastic differentiation of different cell types.

Methods and Materials

- Cell culture
MC3T3-E1(mouse osteoblastic cell line), C2C12(mouse myoblastic cell line): DMEM supplemented with 10% fetal bovine serum(FBS) and 1% penicillin-streptomycin
NHDF(normal human dermal fibroblast-adult) : DMEM supplemented with 5% fetal bovine serum(FBS) and 1% penicillin-streptomycin
- Gene recombination
BMP-4 gene was inserted into pShuttle2 plasmid and amplified. pShuttle2 vector was cut by restriction enzyme *PI-Sce I* and *I-Ceu I*. The fragment was inserted into Adeno-X Viral DNA(BD Biosciences Clontech, San Jose, CA, USA) (Ad5BMP4). Recombinant adenoviral DNA were transduced MC3T3-E1, C2C12, and NHDF cells to induce cell transformation.
- Cell proliferation assay(MTT assay)
MC3T3-E1, C2C12, and NHDF cells were plated in 96-well plates (1×10^4 cells/well) and were cultured with various doses of Ad5BMP4 for 3, 7, 15 days. MTT assay (sigma, St. Louis, MO, USA) was performed to assess proliferation of cells
- Expression of BMP4 by transduced cells (ELISA)
ELISA (R&D system, Minneapolis, MN, USA) was used to verify the expression of BMP4. MC3T3-E1, C2C12, and NHDF cells were plated in 24-well plates (1×10^4 cells/well) for 3, 7, 15 days.
- Measurement of alkaline phosphatase activity
Alkaline phosphatase activity was measured at 3, 7, 15days to assess osteoblastic differentiation of cells.
- Mineralization activity
MC3T3-E1, C2C12, and NHDF cells were plated in 24-well plates (1×10^5 cells/well) and were cultured with various doses of Ad5BMP4 for 14 days. Dulbeccos Modified Eagle's Medium(DMEM) supplemented 15% FBS, 50 ascorbic acid, 10mM -glycerol phosphate, 1% penicillin-streptomycin was used. The cells were fixed in 4% formaldehyde for 1 hour and were rinsed in distilled water three times. The cells were stained in 1% alizarin red S solution.

Results

- Cell proliferation assay(MTT assay)

Fig1. MC3T3-E1

Fig2. C2C12

Fig3. NHDF

- Expression of BMP4 by transduced cells(ELISA)

Fig4. MC3T3-E1

Fig5. C2C12

Fig6. NHDF

- Measurement of alkaline phosphatase activity

Fig7. MC3T3-E1

Fig8. C2C12

Fig9. NHDF

- Mineralization activity

Fig10. Mineralization activity (M: MC3T3-E1, C: C2C12, H: NHDF (n=2), pfu: pfu/cell)

Fig11. Mineralization activity (X40) (M: MC3T3-E1, C: C2C12, H: NHDF, pfu: pfu/cell)

Conclusion

Present study demonstrated that gene delivery using adenovirus expressing BMP-4 facilitated osteoblastic differentiation of MC3T3-E1, C2C12, NHDF cells. Also we confirmed the possibility of ex vivo BMP-4 gene delivery that be able to apply to effective technique for regeneration of bone defects

References

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