SEP, 7TH, 2012

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The Effect of Coactivator-associated Arginine Methyltransferase 1 (CARM1) Protein on Differentiation Potential of Human Mesenchymal Stem Cells

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조 중 현

BACKGROUND

Mesenchymal stem cells(MSCs)

A. Promising tool for cellular therapy in clinical use

- Proliferative capacity
- Ability to differentiate into multiple lineages
- Ability to migrate into target organ

B. The weak point of hMSCs for clinical applications

- Inferior plasticity to embryonic stem cells
- Replicative senescence
- Decreasing of differentiation potential during *in vitro* culturing

Critical to develop methods that improve the differentiation potential of hMSCs

BACKGROUND (continue)

Differentiation potency of hMSC correlate epigenetic regulation

A. Epigenetic regulation

- DNA methylation
- Histone acetylation
- Histone methylation

DNA methylation of the *Trip10* promoter accelerates mesenchymal stem cell lineage determination

Shu-Huei Hsiao[a,1], Kuan-Der Lee[b,1], Chia-Chen Hsu[a], Min-Jen Tseng[a], Victor X. Jin[c], Wei-Sheng Sun[a], Yi-Chen Hung[a], Kun-Tu Yeh[d], Pearlly S. Yan[e], Yen-Yu Lai[f], H. Sunny Sun[g], Yen-Jung Lu[h], Yu-Sun Chang[h], Shaw-Jenq Tsai[f], Tim H.-M. Huang[e], Yu-Wei Leu[a,*]

Biochem Bioph Res Co (2010)

Histone deacetylase inhibitors decrease proliferation potential and multilineage differentiation capability of human mesenchymal stem cells

S. Lee*,†,‡,§, J-R. Park*,†, M-S. Seo*,†,§, K-H. Roh*,†,§, S-B. Park*,†,§, J-W. Hwang*,†, B. Sun*,‡, K. Seo*,§, Y-S. Lee*,†,‡,§, S-K. Kang¶, J-W. Jung*,‡ and K-S. Kang*,†,‡,§

***Cell Proliferation* (2009)**

Histone H3 Modifications Associated With Differentiation and Long-Term Culture of Mesenchymal Adipose Stem Cells

Agate Noer, Leif C. Lindeman, and Philippe Collas

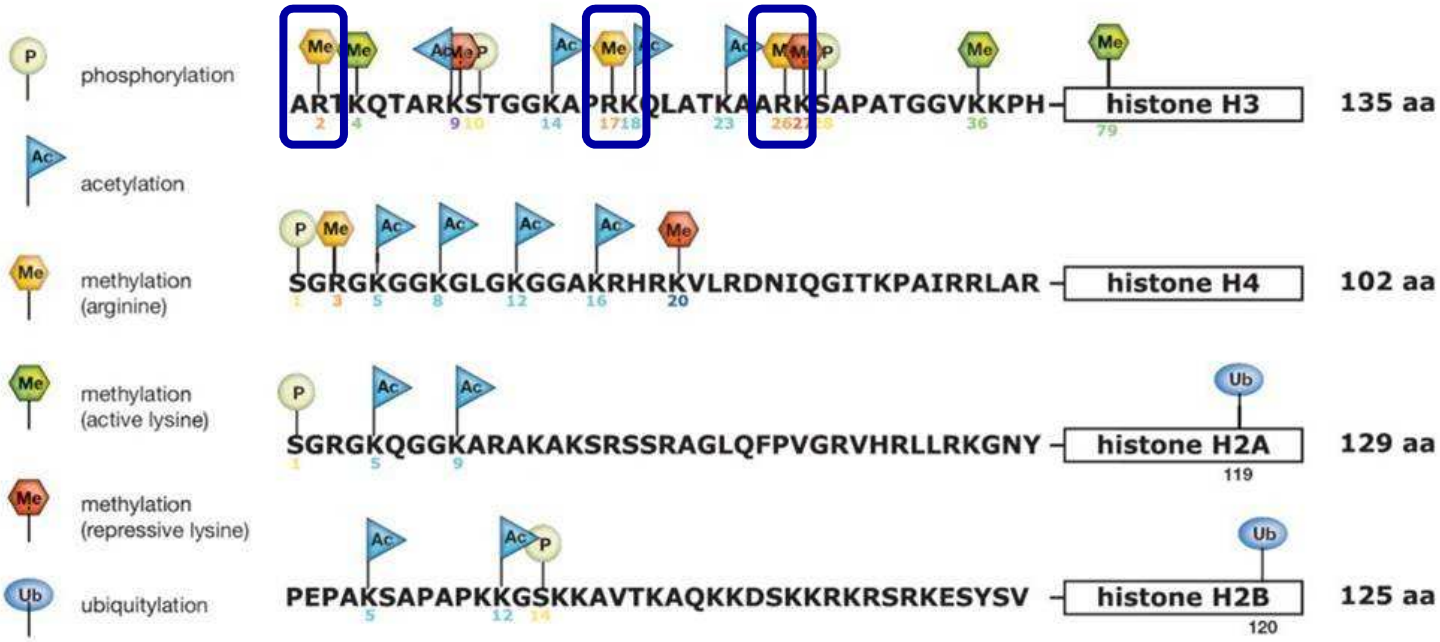
Stem Cells Dev (2009)

BACKGROUND (continue)

Coactivator-associated arginine methyltransferase 1 (CARM1)

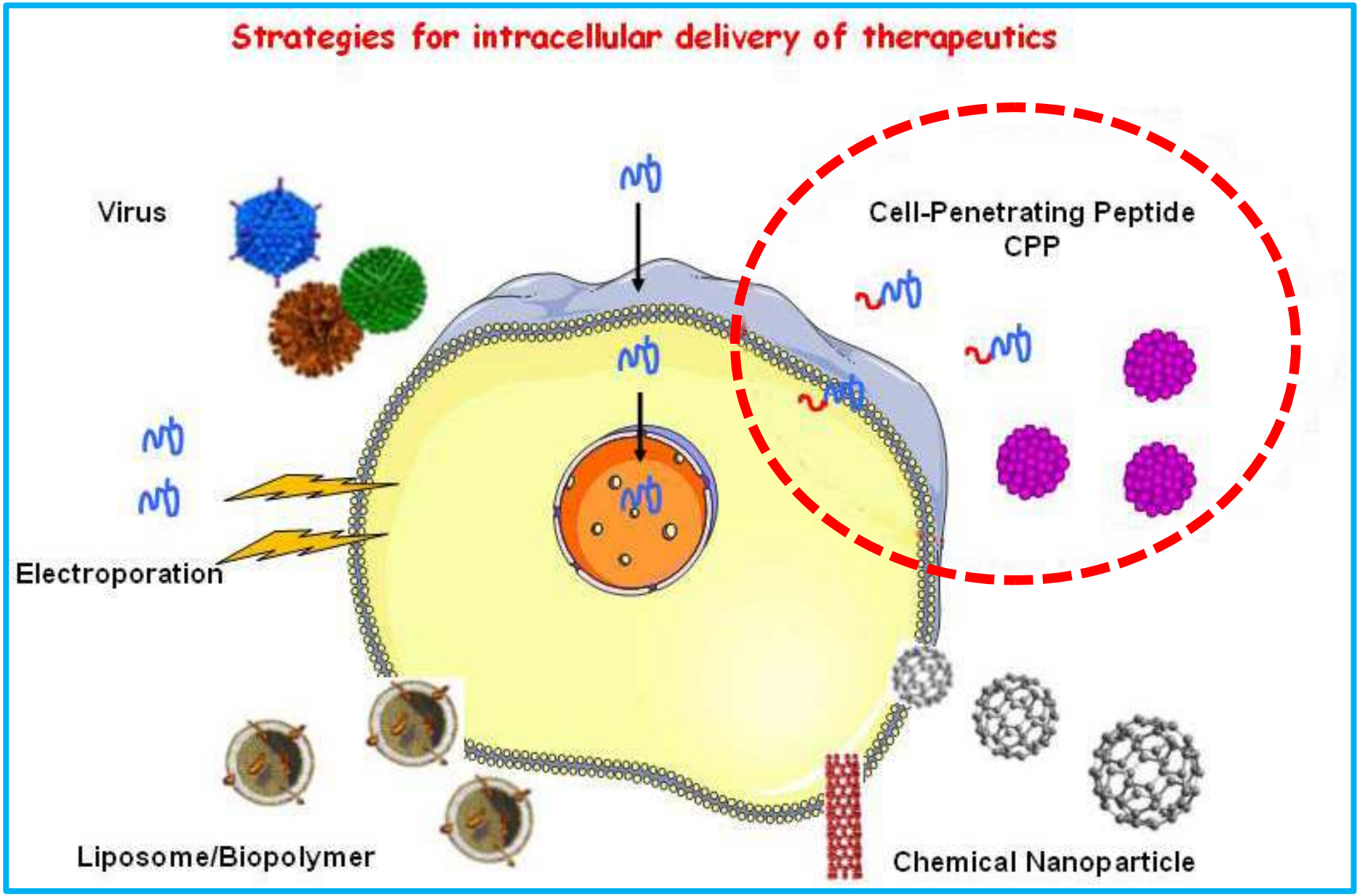
A. One of arginine methyltransferase (PRMT) family

- **Methyl group addition : Histone H3 Arg-2, -17, -26**
  - **Chromatin remodeling**
  - **Coactivation for many nuclear receptors, and transcription factor**



Allis et al., Epigenetics. (2006)

BACKGROUND (continue)



PURPOSE

The aim of this study was

A. Establishment of protein direct delivery system

- **To examined that CPP-CARM1 is delivered to appropriate location in nucleus and CPP facilitate cellular uptake of CARM1**
- **To transfer methyl group to histone H3 arginine 17 residue by the recombinant CPP-CARM1 *in vitro* culture system of hMSCs**

B. Overcome a weak point of hMSCs for clinical applications

- **To increase to efficacy of differentiation into multilineage of hMSCs, according to methylation of histone H3R17 by CPP-CARM1**

PROCEDURE

CPP-CARM1 protein

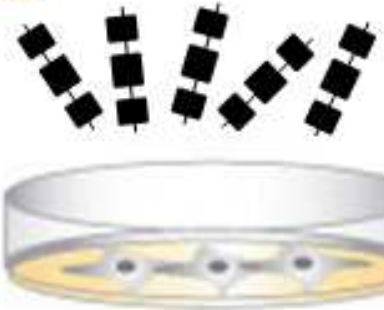
Construction of vector

Purification of CPP-CARM1

Treatment of protein

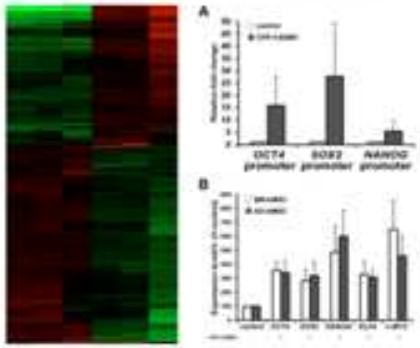
Cultivation of hMSC

Treatment of CPP-CARM1 twice

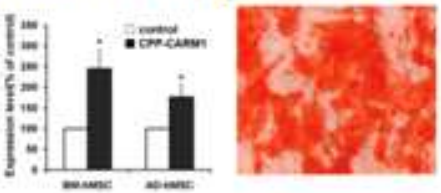


Analysis

WB



Microarray ChIP



qRT-PCR Differentiation assay

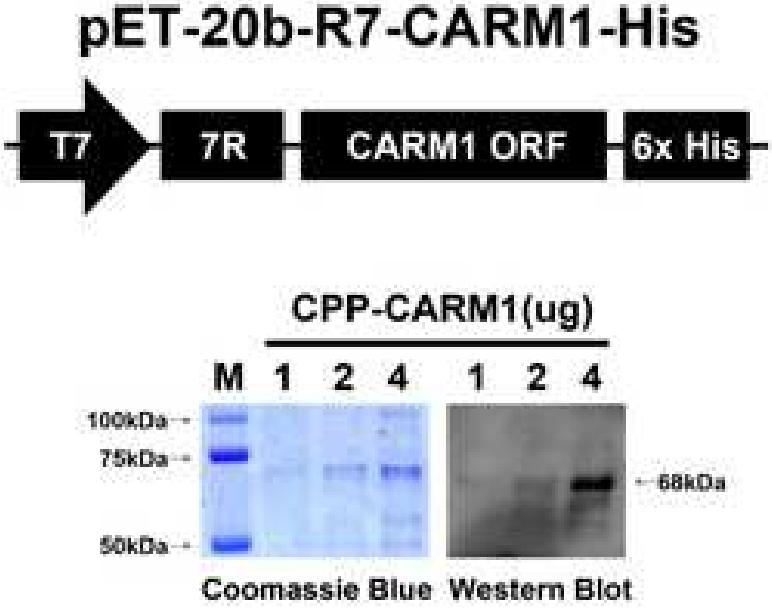
RESULTS

A. CPP-CARM1 vector construction

- Used pET expression vector
- 5’-7x arginine- cDNA CARM1-6x His-taq-3’

B. Western blot of CPP-CARM1 protein

- Antibody : CARM1
- Protein size : 68kDa

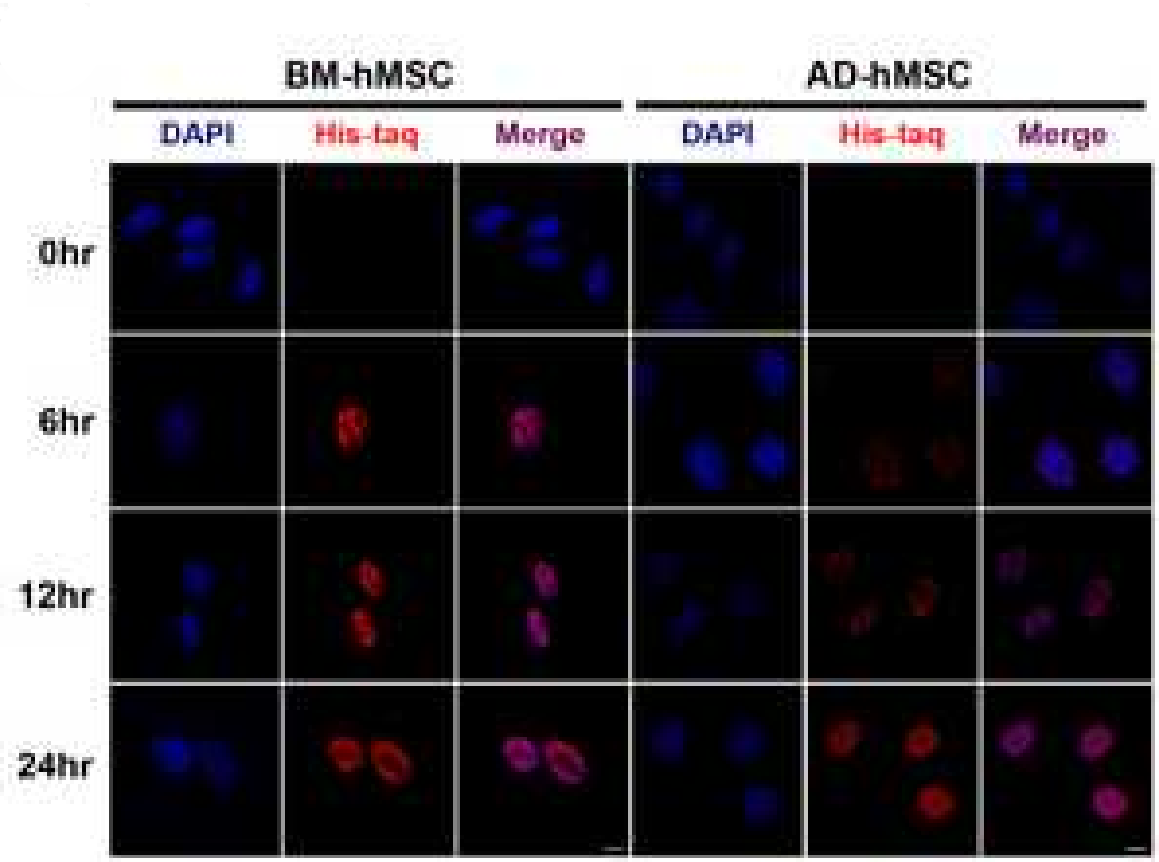


Jo et al., Stem Cells. (2012)

RESULTS (continue)

C. Localization of CPP-CARM1 in hMSCs

- Immunocytochemistry
- Antibody : 6x His-taq
- BM, AD-hMSC
- 6hr : cytoplasm
  and nucleus
- 12hr : nucleus
- 24hr : nucleus

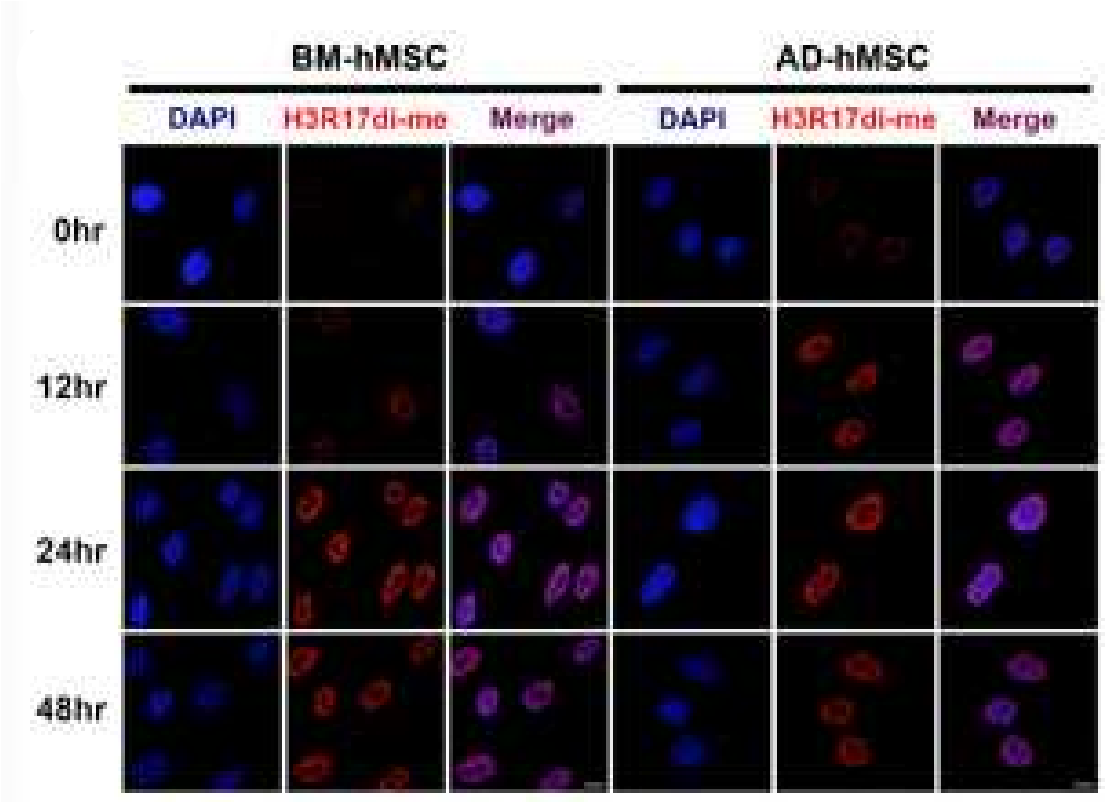


Jo et al., Stem Cells. (2012)

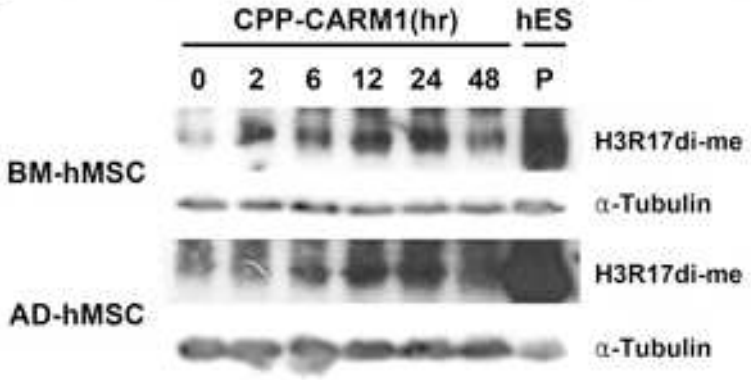
RESULTS (continue)

A. Methylation of CPP-CARM1 in hMSCs

- Immunocytochemistry, Western blot
- Antibody : H3R17di-me
- BM, AD-hMSC



- The highest methylation level : 12 ~ 24hr
- After 24hr : reduced methylation



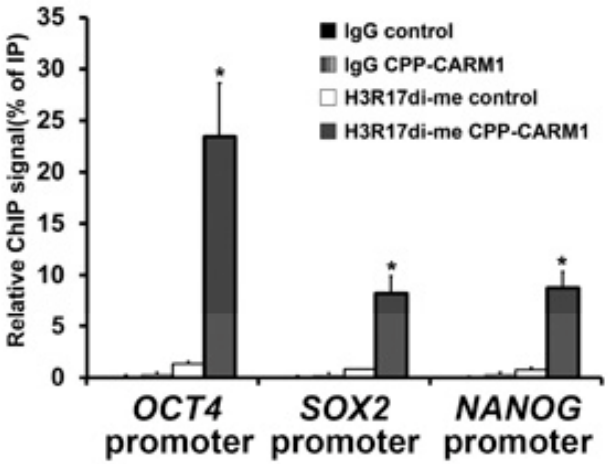
Jo et al., Stem Cells. (2012)

RESULTS (continue)

Alteration of gene expression level

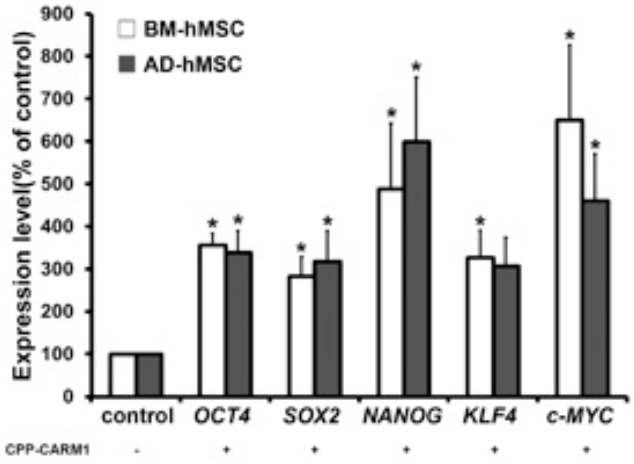
A. Chromatin Immunoprecipitation (ChIP) assay

- pluripotent-related genes promoter
  - : *OCT4*, *SOX2*, *NANOG* promoter



B. Realtime RT-PCR

- pluripotent-related genes
  - : *OCT4*, *SOX2*, *NANOG*, *KLF4*, *c-MYC*



Jo et al., Stem Cells. (2012)

RESULTS (continue)

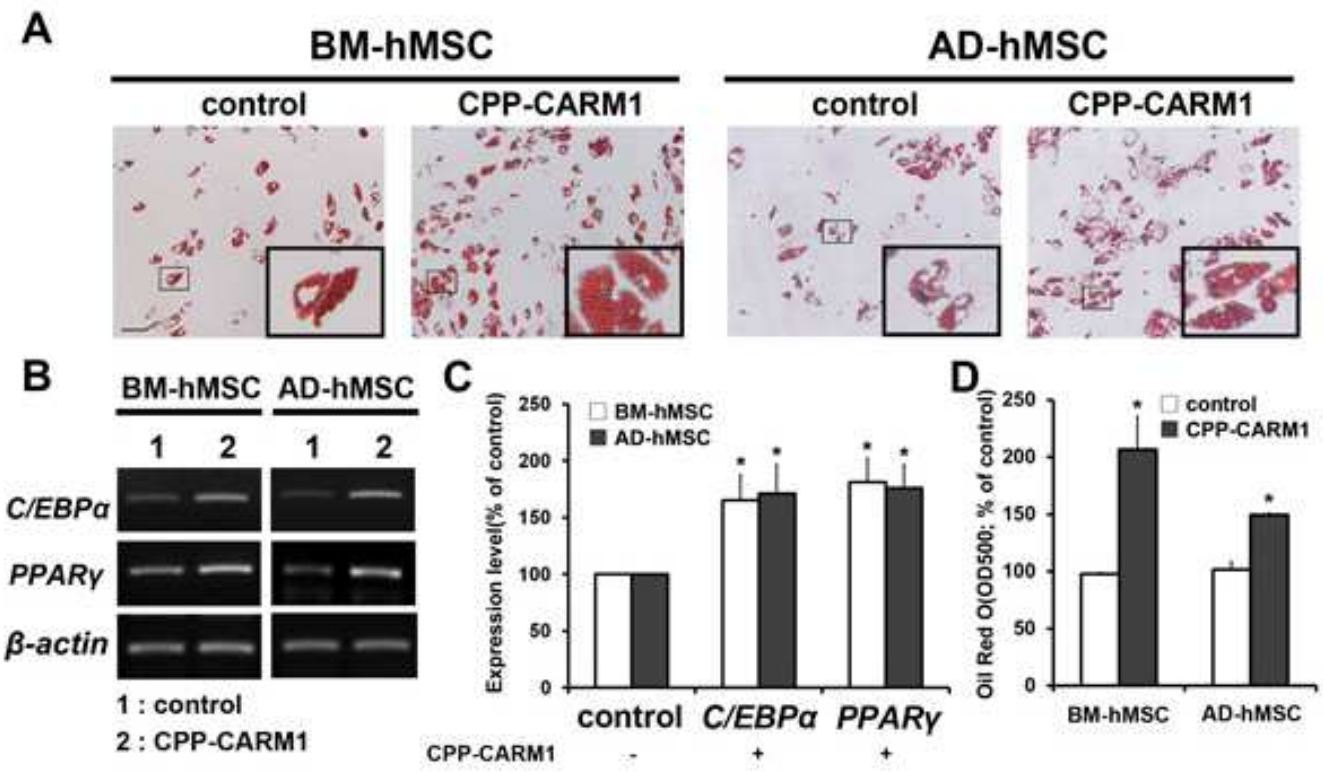
Differentiation into adipogenic lineage

A. Oil Red O staining

B. RT-PCR

C. Realtime PCR

- ***C/EBPα***
  : 1.5 ~ 2 fold
- ***PPARγ***
  : 1.5 fold



Jo et al., Stem Cells. (2012)

RESULTS (continue)

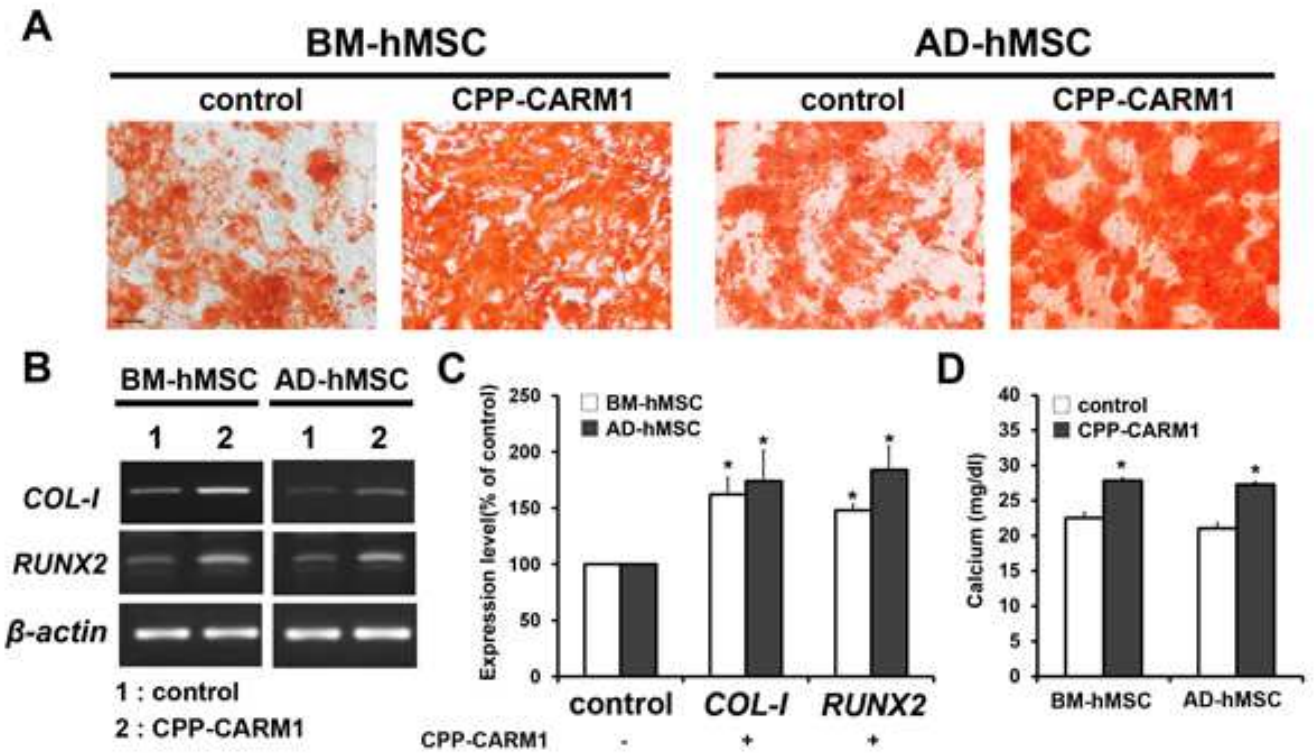
Differentiation into osteogenic lineage

A. Alizarin Red S staining

B. RT-PCR

C. Realtime PCR

- ***COL-I***
  : 2 ~ 2.5 fold
- ***RUNX2***
  : 2 fold



Jo et al., Stem Cells. (2012)

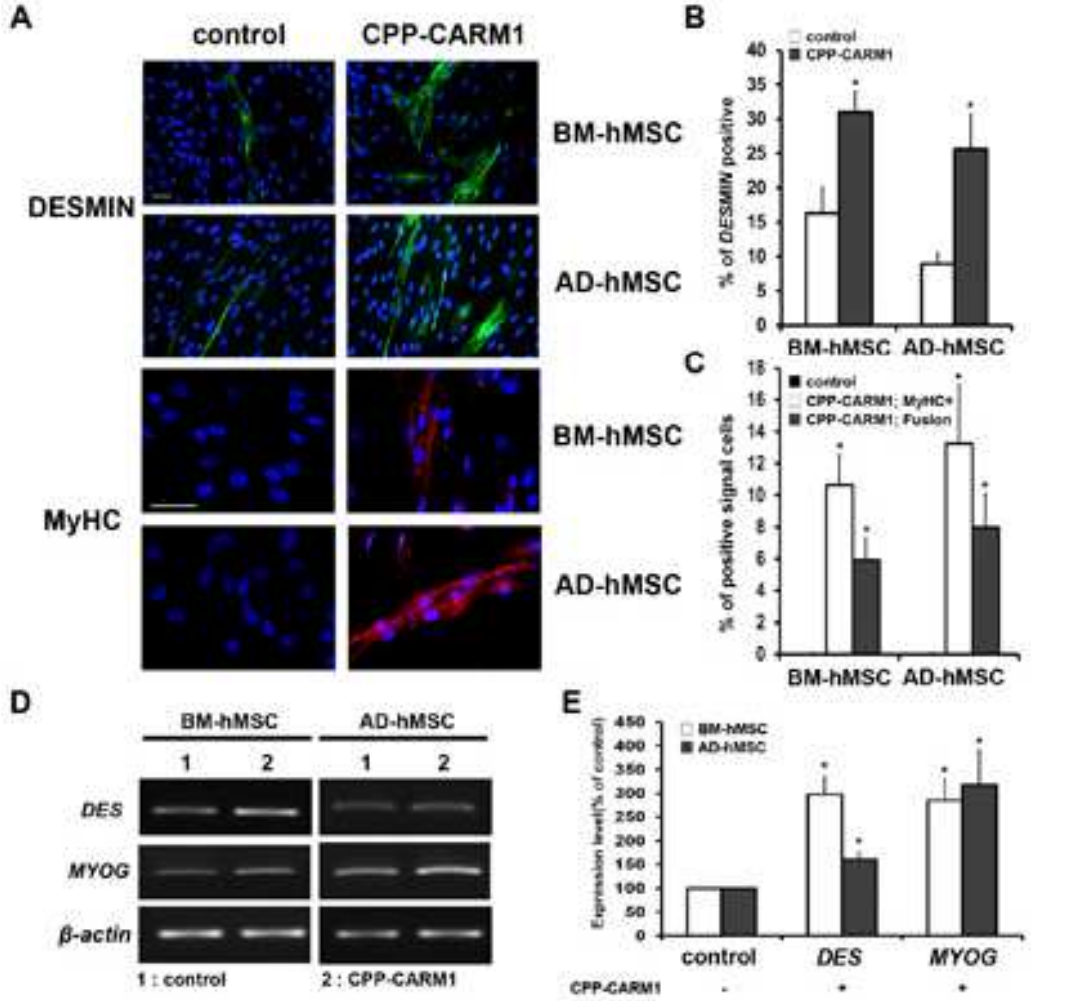
RESULTS (continue)

Differentiation into myogenic lineage

A. Immunocytochemistry
- DESMIN
- Myosin Heavy Chain

B. RT-PCR

C. Realtime PCR
- ***DESMIN***
  : 1.5 ~ 3 fold
- ***MYOGENIN***
  : 3 fold



Jo et al., Stem Cells. (2012)

SUMMARY & CONCLUSION

1. Successfully purified CPP-CARM1 with biological activity

2. Establishment of a protein direct delivery system using cell-penetrating peptide

3. CPP-CARM1 protein translocalized within 12hr, and highly methylated on H3R17 at 12 ~ 24hr in hMSCs

4. Alteration of gene expression pattern by histone modification

5. Elevated the differentiation abilities of hMSCs

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STEM CELLS

STEM CELL TECHNOLOGY: EPIGENETICS, GENOMICS, PROTEOMICS, AND METABONOMICS

Regulation of Differentiation Potential of Human Mesenchymal Stem Cells by Intracytoplasmic Delivery of Coactivator-Associated Arginine Methyltransferase 1 Protein Using Cell-Penetrating Peptide

JUNGHYUN JO,[a] HAENGSEOK SONG,[a] SANG GYU PARK,[a] SOO-HONG LEE,[a] JUNG-JAE KO,[a] JONG-HYUK PARK,[b] JAEMIN JEONG,[c] YONG-PIL CHEON,[b] DONG RYUL LEE[a,d]

Jo et al., Stem Cells. 30:1703-1713. (2012)