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Erratum

Erratum to Effect of cartilage oligomeric matrix protein on mesenchymal chondrogenesis *in vitro* [Osteoarthritis Cartilage 11 (6) (2003) 442–454]

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The publisher regrets that in the above article Figs. 1, 2, 3, 5 and 8 were not published in their correct format. These figures and the figure legends are reprinted correctly below.

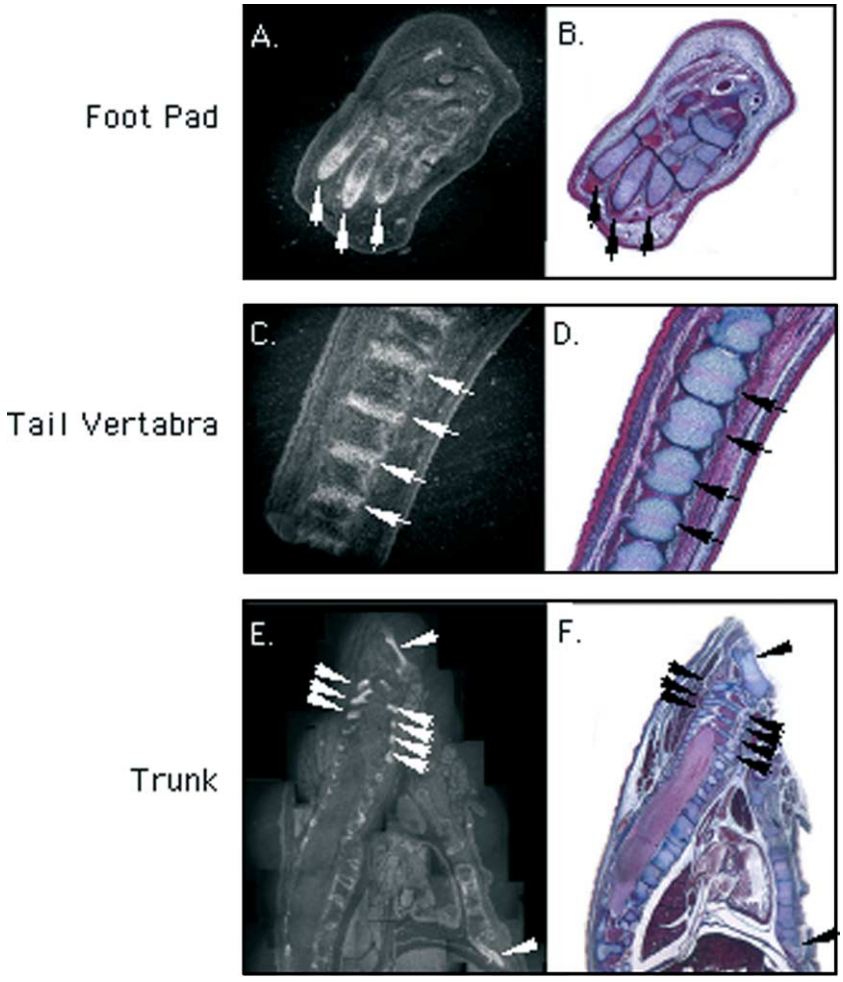

Fig. 1. *In situ* hybridization demonstrates widespread COMP expression in the developing cartilage of Day 16.5 embryonic mouse. Sagittal sections of embryonic mice (day 16.5 post coitus dpc) were processed for *in situ* hybridization for COMP mRNA, as described in the Methods section. The sections were observed by darkfield optics to visualize the *in situ* hybridization signal (left column), or by bright field optics to view the histology by Mallory's Trichrome stain (right column). Three regions of the embryo are shown: (A,B) footpad, (C,D) tail vertebrae and (E,F) trunk. Positive hybridization signals (white grains indicated by the arrows) were localized to sites of active chondrogenesis in the developing skeletal elements of the digits of the paw (A), the tail (C) and the vertebral column and sternum (E). The data show that COMP expression is extensive in the regions undergoing chondrogenesis. The black arrows (B, D and F) indicate the corresponding regions in the control, stained sections. Controls using radiolabeled sense probe showed no detectable hybridization signal (data not shown).

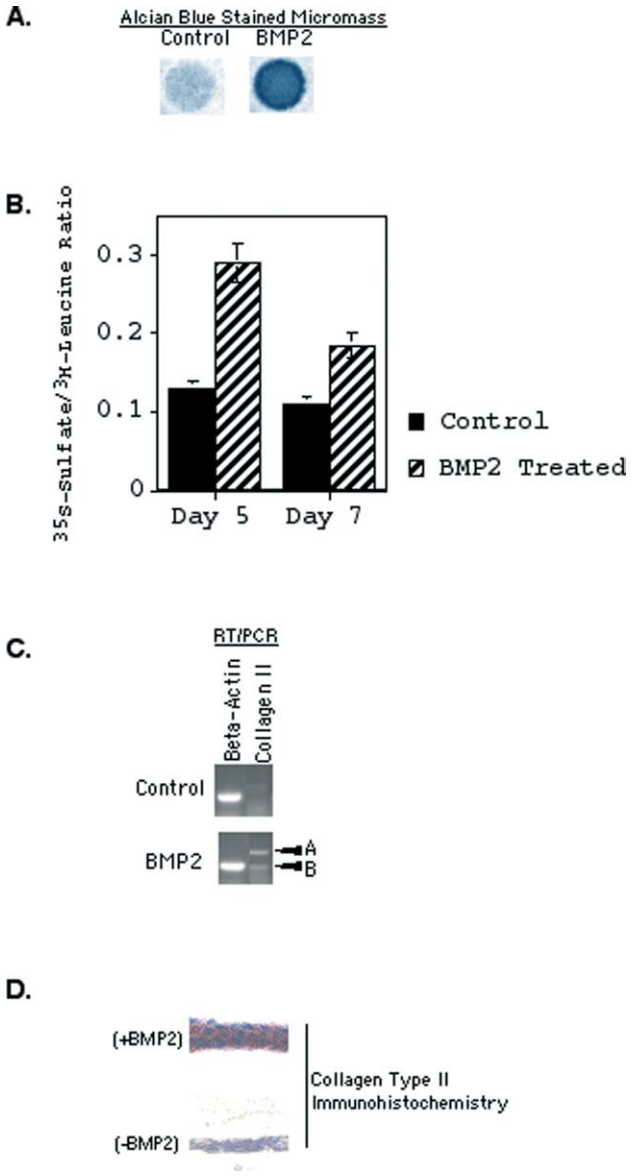

Fig. 2. BMP-2 treatment of micromass cultures of C3H10T1/2 cells leads to induction of chondrogenesis. C3H10T1/2 cells were cultured in a micromass environment in the presence or absence of 100 ng/ml of BMP-2, as described in the Methods section. (A) Alcian Blue stain. Alcian Blue staining of paraformaldehyde fixed, Day 5 cultures reveals a significant level of chondrogenesis in the BMP-2-treated micromasses. (B) Metabolic sulfate incorporation. $^{35}SO_4$ was added to the micromass cultures (5 μCi/ml final concentration) for a 24 h period ending at Day 5 or 7. ^{3}H-leucine at 1 μCi/ml (final concentration) was also added as a control to standardize for differences in protein levels. At the end of the labeling period, the cultures were washed extensively in PBS to remove any unincorporated label and then fixed in 4% paraformaldehyde. The amount of radioactive incorporation in each micromass was determined by scintillation counting. The ratio of $^{35}SO_4$ to ^{3}H-leucine incorporation on Days 5 and 7 is shown. The data show higher sulfate incorporation in the BMP-2-treated cultures (slashed bars) compared with controls (solid). (C) RT-PCR analysis. Total RNA isolated on Day 5 of culture was processed for RT-PCR using primers specific for mouse collagen type II and mouse β-actin. The primers for type II collagen differentiate between the 'A' and 'B' forms of the transcript (the 'B' form is cartilage specific). The reaction products, as shown, were electrophoresed on an agarose gel, which was stained with ethidium bromide. The stained gel shows a higher level of expression of both collagen type II transcripts in the BMP-2-treated cultures relative to the levels of β-actin mRNA. (D) Immunohistochemistry. Day 7 micromass cultures were processed for immunohistochemistry of collagen type II and revealed a significant level of this cartilage matrix protein in the BMP-2-treated cultures, compared with untreated controls. Collagen type II is detected by HRP staining (shown as red), and the cells were counterstained with hematoxylin (blue).

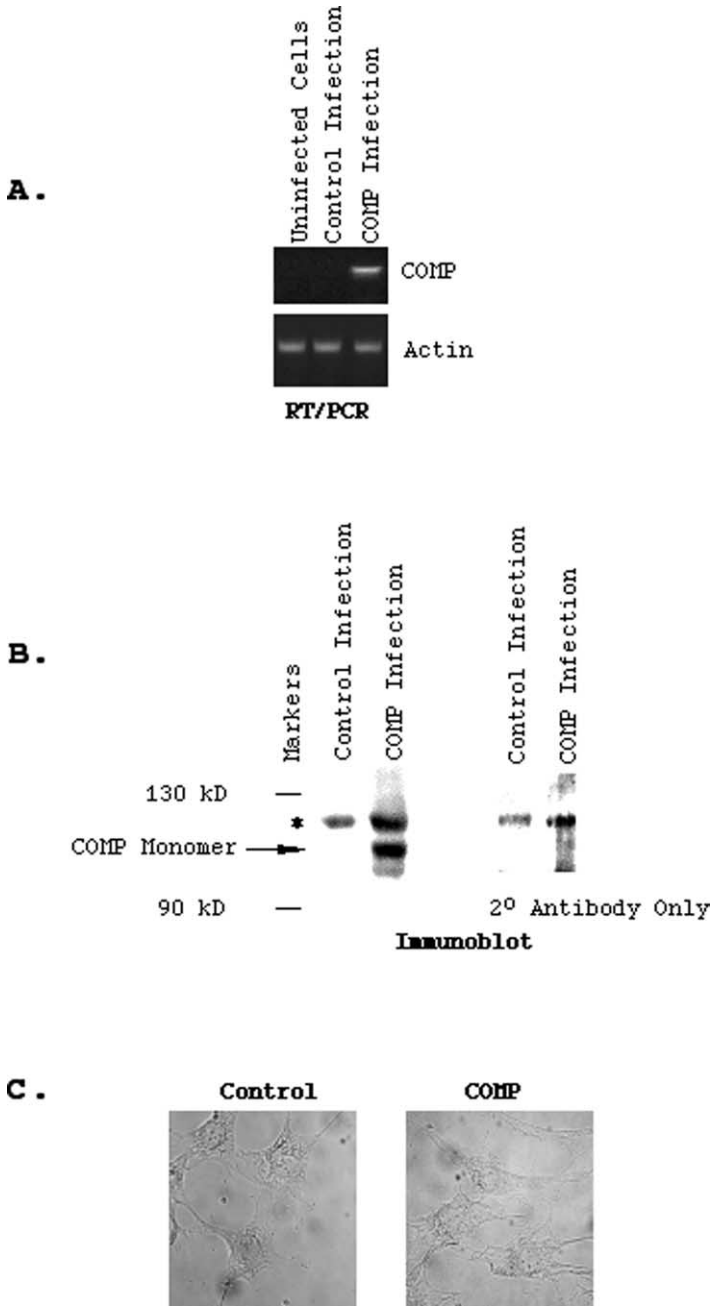

Fig. 3. Ectopic expression of COMP by retroviral gene transfer in cultures of C3H10T1/2 mesenchymal cells. (A) RNA analysis. RT-PCR was performed on 1 μg of total RNA isolated from confluent monolayer cultures of murine C3H10T1/2 cells at 7 days postinfection with either the control (pBABE) or the COMP expressing (pBABE-COMP) retrovirus. Also included was RNA from uninfected control C3H10T1/2 cells. The RT-PCR reaction was performed with primers specific for either COMP or β-actin and the reaction products were electrophoresed on an agarose gel, stained with ethidium bromide. The bands corresponding to COMP and β-actin are indicated and show a significant level of ectopic COMP expression in the pBABE-COMP infected culture but no detectable expression in the control. (B) Immunoblot analysis. The cells at 7 days postinfection with either the control or COMP retrovirus, as in (A), were cultured in serum-free media for 12 h. The media were harvested, the protein precipitated in cold acetone, which were analyzed by SDS-PAGE in the presence of reducing agent (DTT). The SDS-PAGE gel was immunoblotted and the blot incubated with an antibody generated against human COMP (the secondary antibody was conjugated to alkaline phosphatase). (Left side) The band corresponding to the COMP monomer protein is evident only in the extracts of the pBABE-COMP infected cells. (Right side) An identical immunoblot was incubated with secondary antibody only. The asterisk denotes a protein detected by the secondary antibody. (C) Phase contrast microscopy. Monolayer cells infected with control and COMP retrovirus (7 days postinfection), as in (A), were photographed by phase contrast microscopy. The cells infected with each retrovirus are indicated. The results revealed no significant differences in the phenotype between the control and COMP expressing cells.

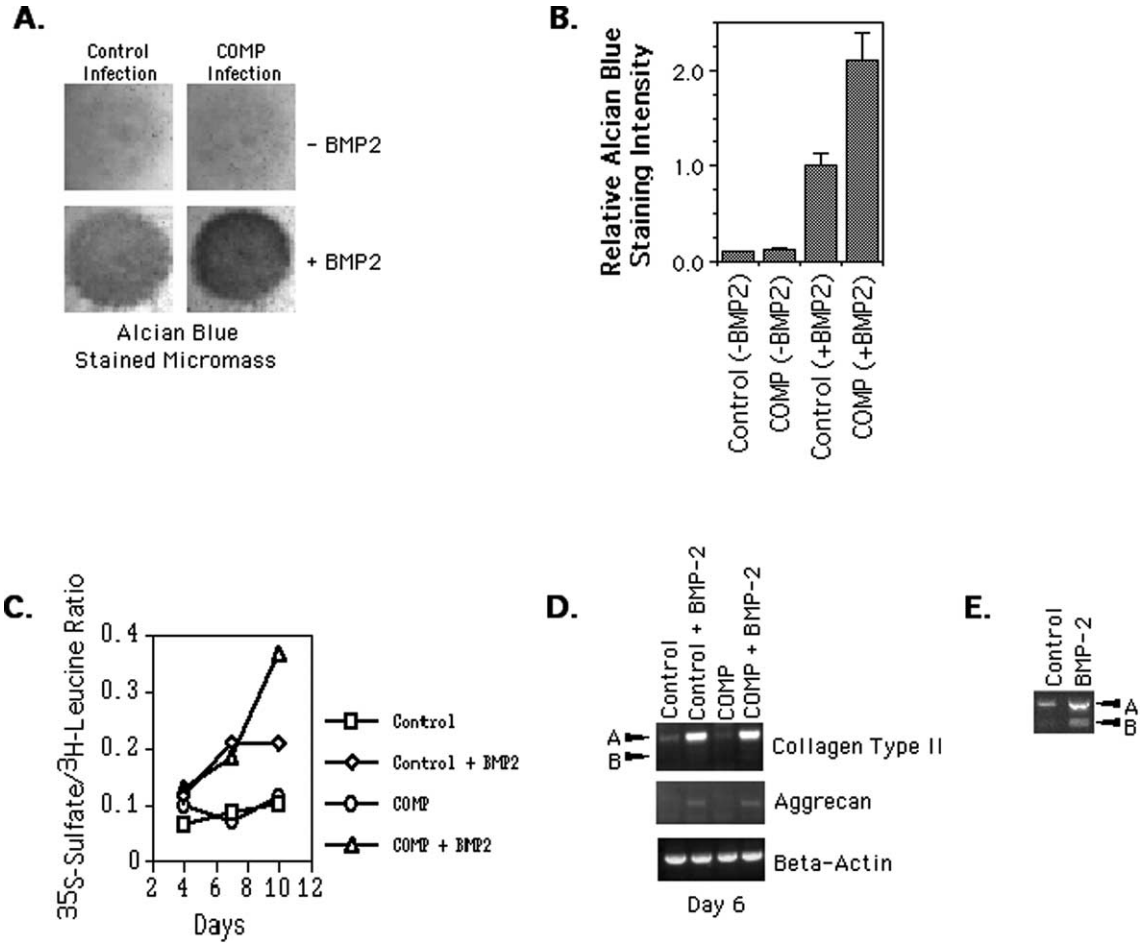

Fig. 5. Retroviral expression of COMP in cultures of BMP-2-treated C3H10T1/2 mesenchymal cells stimulates chondrogenesis. C3H10T1/2 cells infected with either control or COMP expressing retrovirus were cultured in micromass, then treated with or without BMP-2 (100 ng/ml) as described earlier. (A) Alcian Blue staining. The micromass cultures were fixed on Day 10 and stained with Alcian Blue. The data show that COMP expressing cells treated with BMP-2 have a higher level of Alcian Blue staining than the control cultures treated with BMP-2. (B) Quantification of Alcian Blue staining. The micromasses in (A) were scanned digitally and the staining intensity was measured. The levels were set relative to the control (control set equal to 1). As shown, the staining intensity in the COMP expressing cells treated with BMP-2 is significantly greater than the control cells treated with BMP-2. The error bars indicate the standard deviation of the mean. (C) Metabolic sulfate incorporation. The cultures, as in (A), were radiolabeled with $^{35}SO_4$ (5 μCi/ml final concentration) for a 24-h period ending on Days 4, 7 or 10. ^{3}H-leucine at 1 μCi/ml (final concentration) was also added as a control to standardize for differences in protein levels. The cultures were washed, fixed and the amount of radiolabel incorporation was determined. The ratios of $^{35}SO_4$ to ^{3}H-leucine incorporation on Days 4, 7 and 10 reveal that the COMP expressing cells treated with BMP-2 demonstrate a significantly greater sulfate incorporation on Day 10, compared with the control. Symbols: pBABE alone (open square), pBABE+BMP-2 (open diamond), COMP alone (open circle) and COMP+BMP-2 (open triangle). (D) RT-PCR analysis. Total RNA was isolated from the control and COMP micromass cultures (with and without BMP-2 treatment) on Day 6. One microgram of RNA was processed for RT-PCR using primers specific for mouse collagen type II, collagen, aggrecan and β-actin. The products were electrophoresed on agarose gels stained with ethidium bromide. The stained products reveal that BMP-2 stimulation leads to increased expression of aggrecan and collagen type II. The primers for type II collagen differentiate between the 'A' and 'B' forms of the transcript (the 'B' form is cartilage specific). The reaction products, as shown (left side), were electrophoresed on an agarose gel, which was stained with ethidium bromide. The stained gel shows a higher level of expression of both collagen type II transcripts in the BMP-2-treated cultures relative to the levels of β-actin mRNA. However, expression of COMP had only a minor effect on enhancing the expression of these matrix genes. (E) As a control, RNA was isolated from Day 10 micromass cultures (with and without BMP-2 treatment), and processed for RT-PCR for collagen type II. Shown are the 'A' and 'B' forms of type II collagen. The 'B' form is expressed only in the BMP-2-treated micromass condition.

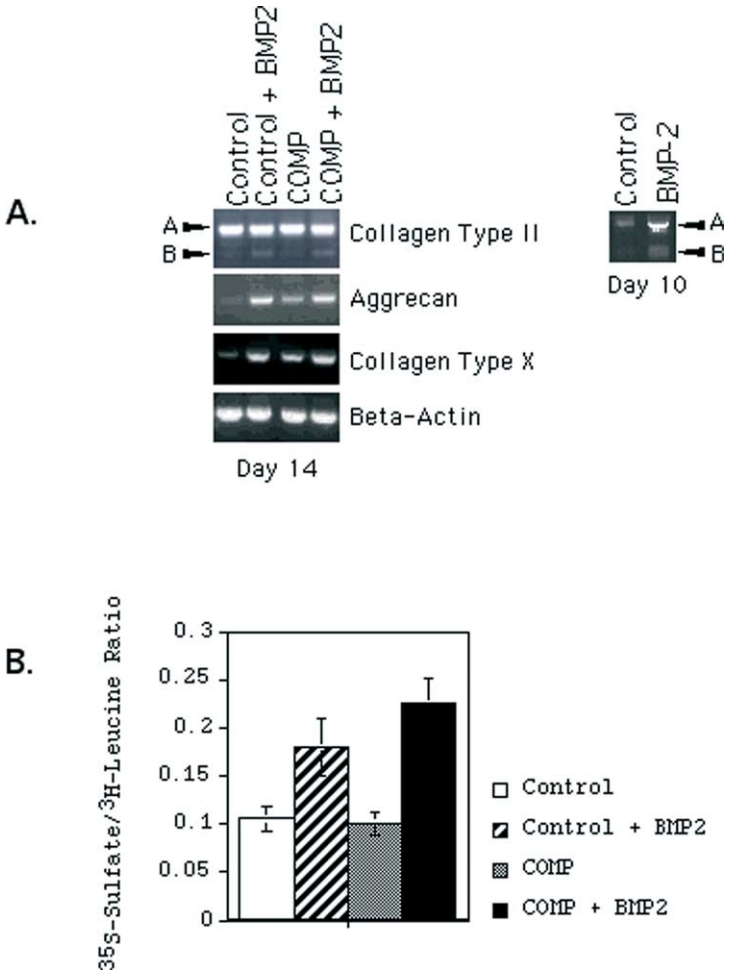

Fig. 8. Markers of chondrogenesis and sulfate incorporation are maintained in long-term micromass cultures of COMP expressing cells treated with BMP-2. C3H10T1/2 cells infected with either control or COMP expressing retrovirus were cultured in micromass, and then treated with or without BMP-2 (100 ng/ml). (A) RT-PCR analysis. (Left side) Total RNA was isolated from the control and COMP expressing micromass cultures (with and without BMP-2 treatment) on Day 14. One microgram of RNA was processed for RT-PCR using primers specific for mouse collagen type II, aggrecan, collagen X and β-actin. The primers for type II collagen differentiate between the 'A' and 'B' forms of the transcript (the 'B' form is cartilage specific). The products were electrophoresed on agarose gels, which were subsequently stained with ethidium bromide. The stained products are shown. The data show that in the cells remaining in the COMP+BMP-2 conditions, expression of aggrecan as well as collagen type II are elevated compared with the controls. (Right side) As a control, RNA was isolated from Day 10 micromass cultures (with and without BMP-2 treatment), and processed for RT-PCR for collagen type II. Shown are the 'A' and 'B' forms of type II collagen. The 'B' form is expressed only in the BMP-2-treated micromass condition. (B) Metabolic sulfate incorporation. $^{35}SO_4$ was added to the culture (5 μCi/ml final concentration) for a 24-h period ending at Day 16 (^{3}H-leucine at 1 μCi/ml final concentration was also added as a control to standardize for differences in protein levels). The ratios of $^{35}SO_4$ to ^{3}H-leucine incorporation on Day 16 are shown. The error bars represent the standard deviation of the mean. The data reveal that the COMP expressing cells treated with BMP-2 demonstrate a significantly enhanced incorporation of sulfate relative to the negative controls.