Endothelial cell modulation of bone marrow stromal cell osteogenic potential

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SPECIFIC AIM

The aim of this study was to determine whether endothelial cells (ECs) directly modulate the osteogenic differentiation of bone marrow stromal cells (BMSCs), both in vitro in different coculture systems and in vivo using a tissue engineering model of ectopic bone formation.

PRINCIPAL FINDINGS

1. ECs enhance BMSC osteogenic differentiation only when cocultured in direct contact with BMSCs

To determine the effects of ECs on BMSC osteogenic differentiation, BMSCs were cultured in the presence of EC conditioned medium (CM), in medium shared with ECs, on EC extracellular matrix (ECM), and in coculture with ECs. In the presence of EC CM, shared media, and ECM there were no significant differences in BMSC osteogenic differentiation, as measured by Alk-P expression and osteocalcin production. Direct contact of ECs with BMSCs led to qualitative differences in BMSC Alk-P activity expression patterns. Quantitative analysis of Alk-P activity and osteocalcin production confirmed a statistically significant ($P<0.05$) increase in BMSC differentiation in BMSC/EC cocultures compared with BMSCs alone.

2. EC production of bone morphogenetic protein-2 (BMP-2) and its role in BMSC osteogenic differentiation

RNA and protein were isolated from ECs and subsequently analyzed for expression of BMP-2, -4, and -7. BMP-2 mRNA and protein were both present, as indicated through RT-PCR and Western blot analyses, but neither BMP-4 nor BMP-7 was detected. Inhibition of EC production of BMP-2 with siRNA largely abolished the effects of EC coculture on BMSC osteogenic differentiation (**Fig. 1*A***). Quantitative analysis of alkaline phosphatase activity confirmed the statistical significance of this effect (Fig. 1*B*). These findings indicate that BMP-2, produced by ECs, plays a role in osteogenic differentiation of BMSCs.

3. Cotransplantation of ECs with BMSCs increased bone formation

ECs and BMSCs were cotransplanted to determine whether ECs could enhance the osteogenic potential of BMSCs in vivo. Cells were transplanted subcutaneously onto the backs of SCID mice using biodegradable polymer scaffolds. The total number of new blood vessels formed in tissues, as measured through blood vessel analysis, was not significantly different between scaffolds containing BMSCs and ECs, and scaffolds containing only BMSCs. However, human CD31 immunostaining demonstrated a significantly higher number of vessels that were human-derived in scaffolds containing BMSCs and ECs, compared with scaffolds containing BMSCs alone. Gross qualitative histological analysis of whole sections showed that scaffolds cotransplanted with BMSCs and ECs formed more bone than scaffolds transplanted with BMSCs alone (**Fig. 2*A***). Bone tissue had a wavy, trabecular appearance with little to no presence of marrow or hematopoietic tissue formation. Immunostaining for bone sialoprotein confirmed the presence of bone in these tissues. Formation of a mineralized extracellular matrix, indicative of bone formation, was confirmed with Von Kossa staining of additional sections. Quantitative bone histomorphometry revealed a statistically significant ($P<0.05$) increase in engineered bone formation in implants containing both cell types compared with implants containing only BMSCs (Fig. 2*B*).

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CONCLUSIONS AND SIGNIFICANCE

These studies demonstrate that in vitro, ECs enhance the osteogenic potential of BMSCs only when cocultured in close proximity or direct contact, and documents that BMP-2 at least partially mediates the role of ECs in the osteogenic differentiation of BMSCs. In vivo, transplanted ECs enhance the bone forming capacity of transplanted BMSCs.

A key finding from our studies is the functional significance of BMP-2 expression by ECs. Previous studies demonstrate BMP-2 expression from ECs, although the effect of this EC BMP-2 production in osteogenic differentiation was not evaluated. To elucidate the role of BMP-2 in a coculture system, its production in ECs was inhibited. This resulted in a significant decrease in osteogenic differentiation of BMSCs cocultured with ECs, relative to coculture with ECs in which BMP-2 production was not inhibited. Cell-cell contact and/or the manner in which these two cell types associate with one another likely mediates the BMP-2 signaling.

ECs not only enhanced the osteogenic differentiation of BMSCs in vitro, but also increased the bone forming ability of BMSCs in vivo. There was no significant difference in the total angiogenic response comparing cotransplantation of ECs with BMSCs and transplantation of BMSCs alone. A combination of the relatively low numbers of transplanted ECs in these studies, and the ability of BMSCs to promote neovascularization through their production of angiogenic factors, likely underlies the lack of a net increase in blood vessel density with EC transplantation.

Although transplanted ECs did not significantly heighten the total angiogenic response, they increased bone tissue formation. This result suggests that the transplanted ECs had direct effects on BMSCs' ability to

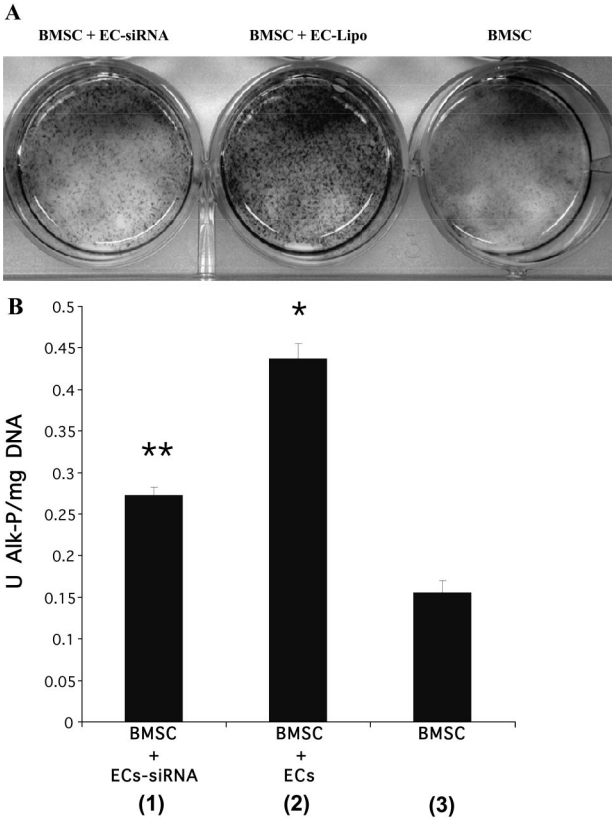

Figure 1. siRNA BMP-2 inhibition. *A)* Digital photographs showing osteogenic differentiation (Alk-P staining) of BMSCs cultured for 2 days with ECs transfected with BMP-2 siRNA, with ECs that were not transfected, and alone. *B)* Alk-P expression was measured from BMSCs cultured with ECs transfected with BMP-2 siRNA (1), BMSCs cultured with ECs (2), and BMSCs cultured alone (3). Values: mean ± SE (n=3). *$P < 0.05$ comparing condition 1 to condition 2; **$P < 0.05$ comparing condition 1 to condition 3.

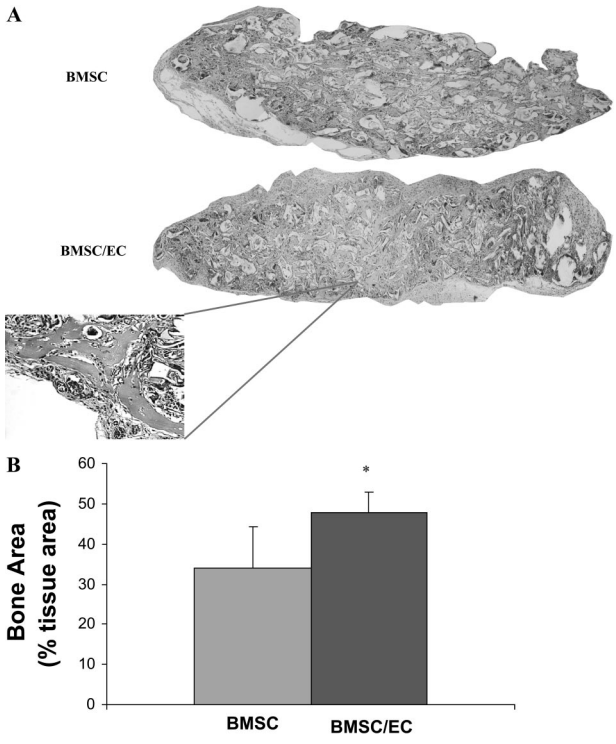

Figure 2. Bone analysis at 8 wk. *A)* Photomicrographs of entire scaffolds retrieved after 8 wk. Scaffolds were seeded with BMSCs alone or with ECs. Insert: high magnification area (×200) of bone tissue formation. *B)* Bone histomorphometric analysis of scaffolds retrieved 8 wk after implantation. Values: mean ± SE (n=5). *$P < 0.05$

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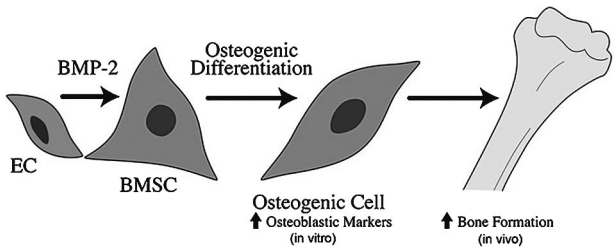

Figure 3. Schematic representation of EC modulation of BMSC osteogenic potential. BMP-2 synthesis by ECs plays a functional role in enhancing BMSC osteogenic differentiation (in vitro) and cotransplantation of ECs enhances the bone forming capacity of BMSCs (in vivo).

form bone, rather than indirect effects on bone formation through a heightened angiogenic response. The close proximity of these cell types to one another when seeded onto polymer scaffolds prior to transplantation lends itself to cell-cell interactions and communication between ECs and BMSCs, which could contribute to potentiating the bone forming ability of BMSCs. BMP-2 expression increases in ECs when in hypoxic conditions, and a hypoxic environment likely exists after transplantation of ECs with BMSCs. This may further enhance the effect of EC based production of BMP-2 on bone formation.

This work has shown that ECs produce a potent bone morphogen that affects osteogenic differentiation of BMSCs, and expands their function beyond their traditional role as structural components of blood vessels. Due to the close proximity and intimate association of ECs with cells of all tissues, they may prove to be instructive for a number of other different cell types and tissues as well. In the context of bone and blood vessel formation, the intricate interaction between BMSCs and ECs could potentially be exploited in tissue engineering applications for the therapeutic regeneration of bone. FJ