Hepatocyte Growth Factor Suppresses Bone Morphogenic Protein-2 Induced Heterotopic Ossification

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INTRODUCTION
Bone morphogenic proteins (BMPs) are members of the transforming growth factor-β superfamily that possess the remarkable ability to induce heterotopic ossification in vivo and osteoblast differentiation in vitro. Skeletal muscle satellite cells are myogenic stem cells activated at injury sites, proliferating and differentiating into myoblasts during the course of muscle repair. Once satellite cells are exposed to BMPs, osteoblast differentiation of satellite cells may occur and represent an important process of heterotopic ossification in muscle tissue. Heterotopic ossification in muscle tissue is often a severe clinical complication following joint arthroplasty, neurological trauma and muscle injury, elucidating the mechanisms underlying heterotopic ossification should provide useful insights into clinical situations.

Hepatocyte growth factor (HGF) is known as a multifunctional cytokine involved in various physiological processes, including tissue development and regeneration. HGF is eventually and abundantly produced in muscle tissue and plays a central role in muscle satellite cell activation under certain situations [1]. Several investigators have proposed close relationships between HGF and muscle regeneration after traumatic or operative injury. Inhibition of heterotopic ossification in muscles might be one of the important biological effects of HGF, according to a recent report dealing with inhibitory effects on BMP-induced osteoblast differentiation in vitro [2]. The purpose of this study was to analyze the effects of HGF on heterotopic ossification in muscles, both in vitro and in vivo.

MATERIAL AND METHODS
In vivo analysis
To examine the effects of HGF on BMP-induced heterotopic ossification in muscles, 8-week-old male C57BL/6J mice were used. Gelatin sponges (Gelfoam®) containing constant amounts of BMP-2 (2 µg) and varying amounts of HGF (0, 2, 20, 200 or 2000 ng) were implanted into an intramuscular pocket in the thigh (n=10). On postoperative day 14, radiographs were taken using a soft X-ray instrument (CMB-2; SOFTEX) for the assessment of heterotopic ossification in the transplanted site. Radiographic images were captured and the area of heterotopic ossification was measured by a planimetric method using ImageJ software. Implanted Gelfoam® with adjacent tissues was harvested to examine changes in gene expression of HGF and its receptor, c-Met, using real-time RT-PCR.

In vitro analysis
C2C12 cells were used to examine the effects of HGF on BMP-induced osteoblast differentiation in vitro. C2C12 cells were seeded at 1 ×104 cells/well in D-MEM containing 15% FBS. On achieving confluence, cells were cultured in D-MEM containing 2.5% FBS with or without BMP-2 (1000 ng/ml) and HGF (20 ng/ml) for 3 or 5 days (n=4). Alkaline phosphatase (ALP) activity was assayed using p-nitrophenylphosphate as a substrate. ALP activity was normalized by the amount of protein. Results are expressed as mean ± SD. Statistical significance was determined by paired two-tailed Student’s t-tests. All experiments were performed according to the protocol approved by the Laboratory Animal Care and Use Committee of Keio University School of Medicine.

RESULTS
Implantation of gelatin sponges containing BMP-2 consistently induced heterotopic ossification in muscles at post-implantation day 14. HGF and its receptor, c-Met, were highly expressed at sites of BMP-2-induced heterotopic ossification (Fig. 1). Quantitative measurement on radiographs revealed that HGF suppressed BMP-2-induced heterotopic ossification. Low-dose HGF exerted no significant effects, whereas high-dose HGF significantly inhibited heterotopic ossification (Fig. 2). ALP activity was used as a marker of osteoblast differentiation in vitro. C2C12 cells normally differentiated into myoblasts, but differentiated into osteoblasts when cells were treated with BMP-2. ALP activity of C2C12 cells was inhibited by addition of HGF, indicating inhibitory effects of HGF on BMP-2-induced osteoblast differentiation of C2C12 cells (Fig. 3).

DISCUSSION
HGF and c-Met are reportedly expressed at muscle injury sites and continuously expressed at sites of heterotopic ossification. However, the effect of endogenous HGF on heterotopic ossification remains unclear. The present results clearly indicate that exogenous recombinant HGF suppresses BMP-2-induced heterotopic ossification by inhibiting osteoblast differentiation of muscle satellite cells in an in vivo model of heterotopic ossification. Other investigators have reported contrary results that HGF accelerates osteoblast differentiation in vitro, which might encourage the use of HGF for bone tissue engineering.

We believe that HGF/c-Met signals increase during muscle repair and play a key role in inhibiting heterotopic ossification by interfering with BMP signals for the maintenance of homeostasis within muscle tissues. Further analyses are warranted to provide a better understanding of the role of HGF in muscle repair, particularly regarding detailed mechanisms underlying the inhibitory effects of HGF on BMP signaling.

References