OSTEOPROGENITOR CELLS IN SKELETAL MUSCLE

*Bosch, P.., Musgrave, DS., Shuler, FS., Menetrey, J. , Lowenstein, JE., Evans, CH., and +Huard, J.
*Growth & Development Lab, Musculoskeletal Research Center, Dept. of Orthopaedic Surgery, Children's Hospital of Pittsburgh and University of Pittsburgh, Pittsburgh, PA 15213. (+)Johnny Huard, Growth & Development Lab, Musculoskeletal Research Center, Dept. of Orthopaedic Surgery, Children's Hospital of Pittsburgh and University of Pittsburgh, Pittsburgh, PA 15213, Phone: 412-692-7822, Fax: 412-692-7095, Email: jhuard+@pitt.edu

Introduction: Extraskeletal bone formation is known to occur in muscle. Experiments establishing bone morphogenic proteins' (bmp) bioactivity demonstrate that bone is capable of forming in skeletal muscle in response to an external signal. (1) Pathological bone formation also occurs in muscle in heterotopic ossification and other disorders such as fibrodysplasia ossificans progressiva. Controversy exists as to whether the cells responsible for the bone formation originate in the muscle or from distant sites via the blood stream. In order to confirm the presence of a population of cells within skeletal muscle capable of differentiating into bone, we performed a series of *in vitro* experiments in which variable populations of cells derived from muscle were stimulated by bmp-2 protein and analyzed for osteogenic differentiation. Furthermore, the population most responsive to the bmp-2 *in vitro* was tested for *in vivo* bone forming potential in an *ex-vivo* gene therapy animal model.

Materials and Methods: Primary cell cultures were obtained from an adult mdx (muscular dystrophy model) mouse by a described technique (2). Briefly, cells were separated according to cell adhesion characteristics into variable populations of cells. These cells were then stimulated by media containing 50, 200, or 600ng/ml of bmp-2 protein. Recombinant bmp-2 was obtained from the Genetics Institute (Cambridge, MA). After a 5-day incubation in bmp containing media, cell lysates were analyzed for alkaline phosphatase (ALP) activity by a commercially available assay (Sigma). A population of stromal cells known to respond to bmp-2 stimulation was included as a positive control (3). The most responsive muscle derived cell population was selected for *in vivo* bone formation. These cells were transduced by adenoviral-bmp-2 (ad-bmp-2; m.o.i. 50:1) constructed in our institution with a plasmid also from the Genetics Institute. These same cells were concomitantly transduced by retroviral-β-galactosidase (rv-lacZ), a marker gene. 10^6 of these cells were injected into the hindlimb of adult severe combined immunodeficient (SCID) mice. Mice were sacrificed at 2 weeks and analyzed histologically by 1-hour incubation in lacZ substrate and standard hemotoxylin and eosin staining.

Results: No muscle-derived cells demonstrated ALP activity without bmp-2 stimulation. One subpopulation of muscle derived cells and the positive control demonstrated a statistically significant ($p<.05$) induction of ALP activity after 5-day bmp-2 stimulation (Figure 1). Furthermore, that subpopulation of muscle derived cells response appeared to be dose dependent (Figure 2).

When these cells were injected into scid mice after transduction with both ad-bmp-2 and rv-lacZ we were able to follow their fate in a bone forming *ex-vivo* gene therapy model. Histological analysis of the extraskeletal bone induced by the gene delivery of bmp-2, revealed incorporation of β-galactosidase positive cells within forming osteoid. (Figure 3)

Discussion: Our *in vitro* experiment demonstrates that a subpopulation of muscle derived cells can be identified which responds to bmp-2 with increased ALP activity. We infer from this that they are capable of osteogenic differentiation. This contention was strengthened by our *in vivo* experiment. The transduced cells produced sufficient bmp-2 in the SCID mouse muscle to induce bone formation. Based on the histological appearance, the injected muscle-derived cells not only delivered bmp, but may also actively participate in the bone formation. Some of the cells are entrapped within lacunae-like structures implying that they formed the surrounding osteoid. We cannot rule out, however, that the cells were passively trapped within bone formed exclusively by stem cells originating from host muscle or distant sites. Ongoing projects within our laboratory to address these limitations include: additional staining of these cells to confirm osteoblastic differentiation; seeking markers for the osteoprogenitor cell within skeletal muscle; and ultimately developing techniques to further purify these pluripotent cells from muscle samples.

References:
(1) Urist, M.R. Science 150:893-899, 1965
(2) Rando, T.A., and Blau, H.M., J Cell Biology 125(6): 1275-80, 1994
(3) Balk, ML. et al, Bone 21(1):7-15, 1997.

Figure 1. ALP activity induced by bmp-2 stimulation of stromal cell control (SC) and variable populations of myoblasts, designated as "preplate 1, preplate 2, etc..."(PP1, PP2, …).

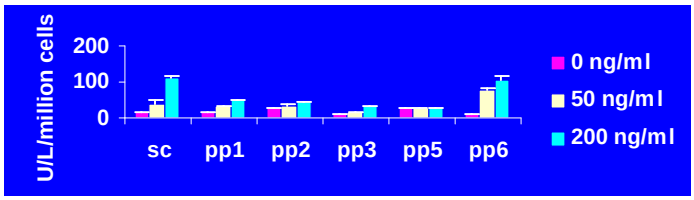


Figure 2. The dose dependence of the muscle derived cells' response to bmp-2 is illustrated.

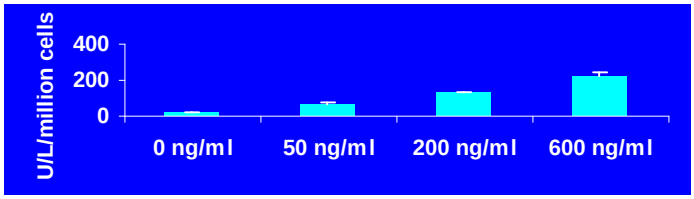
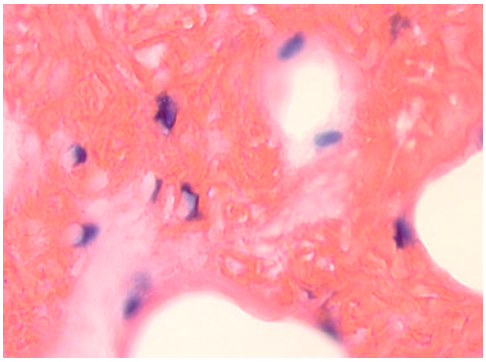


Figure 3. Hemtoxylin and eosin staining after incubation in β-galactosidase substrate demonstrates injected muscle-derived cells co-transduced with ad-bmp-2 and rv-lacZ within newly formed extraskeletal osteiod in SCID mouse muscle.



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