BIOPHYSICAL REGULATION OF STEM CELL DIFFERENTIATIN IS SURFACE DEPENDENT

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INTRODUCTION: Osteogenesis in vivo occurs on a bone surface, however in vitro models to assess the mechanisms involved in the differentiation of mesenchymal stem cells (MSCs) into osteoblasts use conventional cell culture methods, including a tissue culture plastic substrate. We and others have shown the importance of substrate surface chemistry on osteoblast differentiation [1], but less is known about the requirements for MSC differentiation and commitment to an osteoblast phenotype. The purpose of this study was to compare MSC differentiation on tissue culture plastic to differentiation on a calcium phosphate ceramic substrate when cultured under osteogenic conditions.

Typically, osteogenic assay conditions involve the use of a synthetic "osteogenic media" that contains dexamethasone and high levels of beta-glycerol phosphate. Addition of this media to confluent cultures of MSCs results in the formation of von Kossa positive nodules that express extracellular matrix proteins typical of bone. This is a time-dependent process that has been well characterized with respect to the inverse relationship between cell proliferation and differentiation, with the onset of an osteoblast phenotype at approximately 3 weeks post-confluence. The bone morphogen BMP-2, which induces osteogenesis in mesenchymal tissues in vivo, is used to stimulate osteoblastic differentiation in vitro. Pulsed electromagnetic fields (PEMF) are used clinically with the intent to stimulate MSCs, and studies using demineralized bone matrix to induce osteogenesis in mouse muscle support the contention that PEMF enhances osteogenesis. Recent studies indicate that PEMF also affects MSCs in vitro.

This study was based on the hypothesis that the osteoblastic differentiation of MSCs and the regulation of the process are substrate dependent. Accordingly, human MSCs were cultured on plastic or calcium phosphate disks and their response to osteogenic media determined in the presence and absence of a known activator of osteoblast differentiation, BMP-2, and a clinically used bone growth stimulator, PEMF.

METHODS: Human MSCs were purchased from Clonetics, Inc. and were grown in 24-well plates on tissue culture plastic or calcium phosphate disks (BD Bioscience, Inc.) in MSC growth medium. At confluence, growth media were replaced with osteogenic media containing dexamethasone and beta-glycerophosphate. To determine whether MSCs could be induced to express an osteoblast phenotype, BMP-2 was added to the medium (0, 10, 40, 70, 100 ng/ml) for 0 [confluence], 6, 12, 16, 20 or 24 days. At harvest, cultures were examined for expression of an osteoblastic phenotype by measuring cellular alkaline phosphatase specific activity and osteocalcin levels in the conditioned media. Based on these results, 40 ng/ml BMP-2 was selected to prime the MSCs along an osteoblast differentiation pathway. To determine if PEMF could induce a similar response or if it could enhance the effects of BMP-2, MSCs were cultured in growth media and at confluence, BMP-2 (40 ng/ml) was added to the media in one half of the cultures at each media change. PEMF was applied via Helmholtz coils specially configured to two matched incubators using a signal that is used clinically (EBI, LP). Coils were activated for 8 hours per day in one incubator only. All experiments were conducted twice, alternating the active coil incubator to account for any variations that might exist. At harvest (days 0 [confluence], 6, 12, 16, 20, 24), cultures were examined for expression of an osteoblastic phenotype based on alkaline phosphatase specific activity and production of osteocalcin. In each experiment, each experimental variable was tested in six separate cultures. Data were analyzed by ANOVA and significant differences between groups determined using the Bonferroni modification of Student's t-test. $P<0.05$ was considered significant. * v. cultures on plastic or calcium phosphate disk at each day; # BMP v. osteogenic media; ●v. PEMF. All experiments were repeated to ensure validity.

RESULTS: MSCs grown on plastic or calcium phosphate disks demonstrated minor increases in alkaline phosphatase activity during 24 days in culture and no change in osteocalcin. When the cells were grown in osteogenic medium, by day 6 there was an increase in osteocalcin. The magnitude of the increase was greater when the cells were cultured on the CaP disks. Treatment with PEMF had no additional effect at any time point. BMP-2 was stimulatory over the effect of the osteogenic media, but the increase in cultures grown on plastic was less than that seen in cultures grown on disks. PEMF did not affect osteocalcin in cultures grown on plastic, even in the presence of BMP-2. In contrast, when cells were grown on CaP discs, PEMF caused a synergistic increase in osteocalcin production.

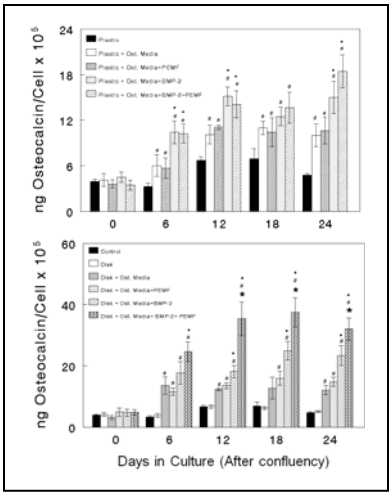

Fig 1. Human MSCs were grown on tissue culture plastic or CaP disks. At confluence, growth media were replaced with osteogenic media ± BMP-2. One half of the cultures were treated with PEMF. Data are means ± SEM for 6 cultures. $P<0.05$, *v. time 0 for each treatment; # v. control at each time point; ●v. osteogenic medium.

DISCUSSION: This study shows that response of MSCs to factors that regulate osteo-genesis depends on the substrate. Effects of BMP-2 were greater on disks and the stimulatory effect of PEMF was only evident in cultures grown on disks and treated with BMP-2. These results suggest that surface-dependent changes make cells more responsive to BMP-2 and surface-dependent differentiation induced by BMP-2 results in cells that are sensitive to PEMF.

REFERENCES

(1) Boyan et al., 2003 Eur Cells Materials 24:22-27.

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