INTRODUCTION: Both Demineralized Bone Powder (DBP) and BMP-2 stimulate endochondral osteogenesis in vivo. Nevertheless, the relationship between BMP-2 and skeletal induction by DBP is unknown. When human dermal fibroblasts (hDFs) are cultured with DBP in a porous collagen sponge for 7 days, cartilage-specific genes, aggrecan and collagen II, are upregulated and cartilage matrix accumulates around the cells in proximity to the DBP [1]. We reported that this chondroinduction is preceded by shifts in some signaling genes [2]. This study tests the hypothesis that DBP and BMP-2 affect similar signal pathways prior to chondrotypic changes in target cells.

MATERIALS AND METHODS: A chondroinduction model was used in which hDFs were cultured in a total of 32 porous collagen sponges containing 0 or 3 mg DBP, or 0 or 6 µg/µL rhBMP-2 in PBS/BSA solution (manufacturer’s recommended concentration). The rhBMP was delivered in 4x4x2 mm coupons of absorbable collagen felt and inserted into the centers of porous collagen sponges. The absorbable collagen and rhBMP-2 were generously provided by Wyeth, Cambridge, MA. One million hDFs were cultured for 3 days in each of the sponge types. After culture, some specimens were prepared for histology and others were used for isolation of RNA for targeted cDNA macroarrays (Human Signal Transduction PathwayFinder, Q Series; SuperArray Inc., Bethesda MD). In addition, effects of DBP and rhBMP-2 were compared by macroarray for Human TGF-β/BMP Signaling Pathway genes and selected RT-PCR analysis of genes in the TGF-β/BMP signaling pathway.

RESULTS: Histological evaluation of cultured sponges 3 days after seeding with hDFs showed that cells were evenly distributed throughout the lattice of the porous collagen. Sponges that contained particles of demineralized bone showed many hDFs attached to the particles. As expected, there was no evidence of metachromatic extracellular matrix at this early time. There was little evidence of migration of hDFs into the insert of ACS felt. In contrast, some hDFs were seen within the ACS felt insert that contained rhBMP-2, but at a sparser density than in the collagen lattice of the sponge.

First, in the global macroarray analysis of 18 signal pathways, the following pathways were found to be modulated in hDFs by DBP: TGF-β pathway, hedgehog pathway, insulin/LDL pathway, PI3 kinase/akt pathway, NFAT pathway, NFκB pathway, retinoic acid pathway, androgen pathway, and phospholipase C pathway.

Because of the importance of the TGF-β/BMP signaling pathways, differentially expressed genes were further examined by macroarray analysis of those pathways for hDFs cultured with and without DBP or rhBMP-2. Similarities and differences in gene expression were observed (Figure). Smad target genes were the predominant group of DBP or rhBMP-2 regulated genes. Several of the Smad target genes, such as IGF-BP3, ID2, and ID3, showed similar responses to DBP and rhBMP-2. This finding suggested that the dosing and timepoint were apt. In contrast, some of the genes that were most dramatically increased by DBP, such as TGF-β-induced protein (βig-h3, 1160%) and Collagen 3A1 (1300%), were barely affected by rhBMP-2. Although there was concordance with ID2 and ID3, ID4 was decreased by DBP by 60% and by rhBMP-2 by only 10%. Both PAI-1 and TIMP were greatly increased by DBP, but PAI-1 was decreased by rhBMP-2 (30%) and TIMP was not at all changed. DBP increased cyclin-dependent kinase inhibitor P21/Waf1/Cip1 by 160%, whereas it was decreased (30%) by rhBMP-2. It is possible that DBP’s inhibition of proliferation may contribute to its effects to promote differentiation. Cbfa1 was highly expressed in targeted hDFs but was moderately decreased by both DBP (10%) and rhBMP-2 (20%). In these cells, rhBMP-2 was a stronger stimulus than DBP for a number of genes: TGFβ1, endoglin, gremlin, Smad 6, and Smad 7. Smad 2-5 and 9 were low in control hDFs and were not modulated by either BMP-2 or DBP. (This method does not measure Smad activation.)

The RT-PCR analysis of selected genes showed similar changes as in the arrays but with different magnitudes. DBP up-regulated TGFβ-Induced (βig-h3, by 130% more than control), Col3A1 (by 90%), IGFBP3 (by 130%), and ID2 (by 100%). In contrast, rhBMP-2 upregulated TGFβ-Induced (βig-h3, by 40%), IGFBP3 (by 250%), and ID2 (by 280%). Col3A1 was not significantly modulated by rhBMP-2.

DISCUSSION: This analysis showed that multiple signaling pathways are altered in fibroblasts exposed to DBP for 3 days. Among its effects, DBP down-regulated the insulin-pathway marker gene fatty acid synthase and the LDL-pathway marker gene ELAM1/ε-selectin. Those findings imply that DBP may decrease fibroblasts’ potential for adipogenesis.

These results indicate that both DBP and rhBMP-2 affect many of the same SmadTarget genes. The different effects of DBP and BMP provide information about the mechanisms by which DBP acts on target cells. Scrutiny of the pattern of downstream targets indicates that DBP signals two paths of Smad activation, BR Smads, the one responsive to BMP, as well as AR Smads, the one responsive to activin/TGFβ (Disc Figure). This classification of responses was intended to show how the many members in the TGF-β/BMP superfamily have such a broad array of biological activities with only a finite number of signaling receptors [3]. In this study, DBP regulated many of the Smad target genes that are known to respond specifically to BMP via BR Smads 1, 5, and 8; namely, ID 2-4, and STAT1. In addition, DBP regulated many of the Smad-target genes that are known to respond specifically to activin/TGFβ via AR Smads 2 and 3: TGFβ-Ind/βig-h3, COL1A2, COL3A1, IGFBP3, TIMP1, PAI-1, and p21. Thus, with respect to these signaling molecules, DBP affects genes that are targets of both subgroups of regulatory Smads. Because many bioactive factors are present in bone matrix, it is reasonable to expect that multiple responses would be elicited by demineralized bone powder. Although BMP-2 was originally isolated as a putative inductive factor in DBP, rhBMP-2 alone and DBP do not affect all the same genes in the same ways.

Discussion Figure. Effects of different families of ligands on Smads. TGFβ/Activin members bind to receptors Aβ and activate AR Smads (2,3) whereas the BMP/GDF factors activate BR Smads [3]. On the basis of Smad-target gene changes, we conclude that DBP acts through both AR and BR Smads.

REFERENCES: