DENTIN MATRIX PROTEIN-1 ISOFORMS PROMOTE DIFFERENTIAL CELL ATTACHMENT AND MIGRATION

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Running title: DMP1 mediates cellular response via αVβ3

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Supplementary Data

Legend to Supplementary Figure 1

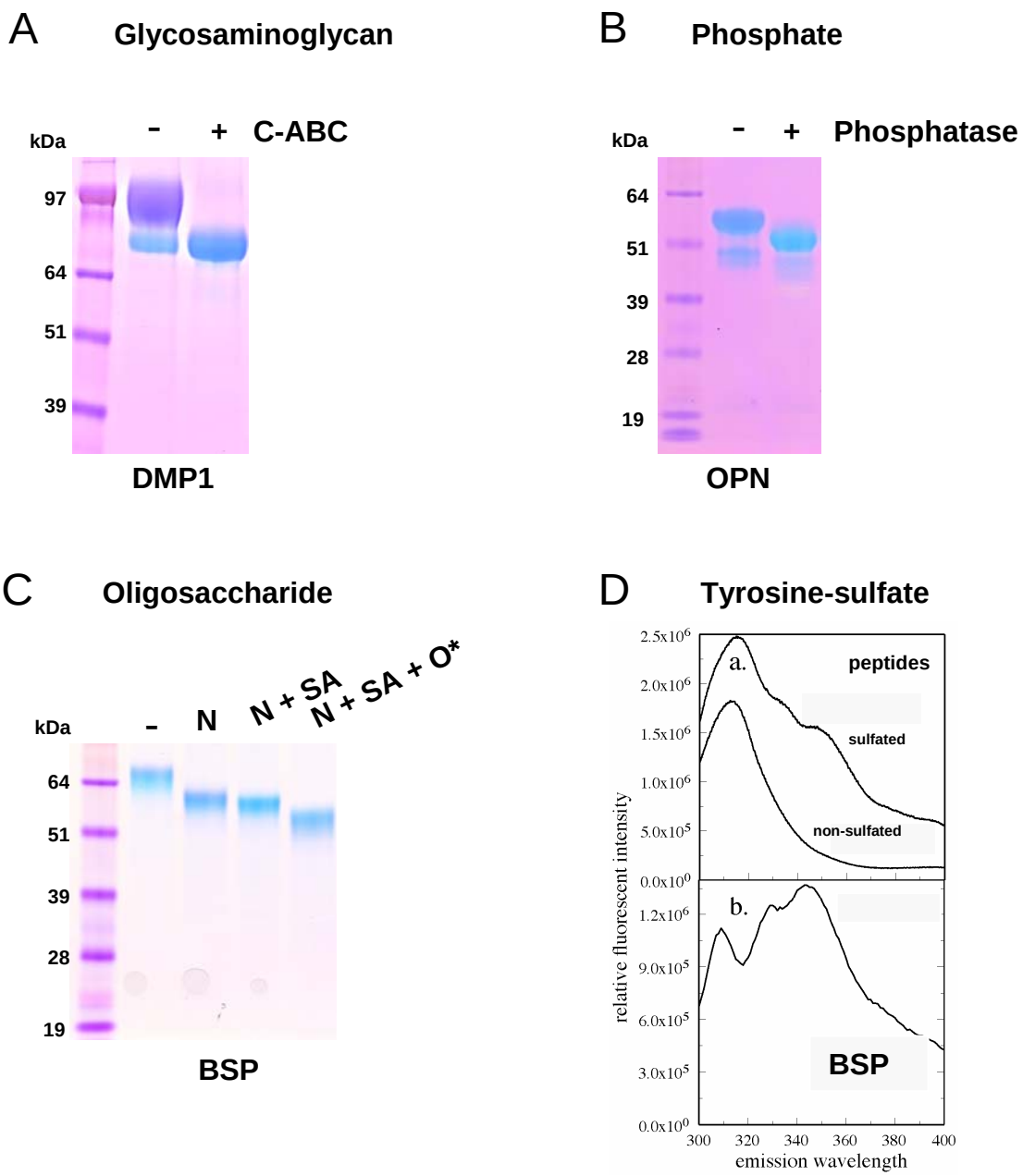
Bone marrow stromal cells (BMSCs) have the capacity to add the known post-translational modifications to SIBLINGs. **A**) Chondroitinase-ABC (C-ABC, 20 mU for 2 h at 37^{o}C) treatment of a proteoglycan-rich fraction of DMP1ΔBMP1 (10 μg) removed the GAG chain from the proteoglycan form (second lane, ~ 95 kDa M_r purple band) increasing its mobility to that of the full length DMP1ΔBMP1 (~ 80 kDa M_r cyan band). Note that the digested band electrophoreses at approximately the same mobility as the non-proteoglycan DMP1 in the untreated lane. Non-treated and C-ABC-treated protein samples were electrophoresed on a reducing SDS 4-12% NuPage gel and were detected by StainsAll. **B**) Dephosphorylation of 10 μg OPN with Antarctic Phosphatase (New England Biolabs, Ipswich, MA) changed the M_r of the StainsAll-positive band on the SDS 4-12% NuPage gel. **C**) BSP aliquots were digested for 3 days at 37^{o} C with a series of deglycosylation enzymes (Prozyme, San Leandro, CA) to illustrate the presence of both N- and O-linked oligosaccharides. Each lane contains 5 μg of BSP treated with: no enzymes (-); PNGase F (N); PNGase F + Sialidase A (N + SA, note the small decrease in M_r and the subtle change in color from a deeper blue (more acidic) to a cyan (less acidic) color with the removal of sialic acid); PNGase F + Sialidase A + three enzyme to remove various O-linked oligosaccharide groups, β(1-4)-Galactosidase + β-N-Acetylglucosaminidase + O-Glycanase (N + SA + O*). Buffers and procedures were used according to the manufacture's instructions for non-denaturing conditions. The decrease in M_r on the SDS 4-12% NuPage gel caused by each addition shows that the cells can add both N- and O-linked oligosaccharides during recombinant protein synthesis. **D**) Tyrosine sulfation: Two peptides corresponding to the tyrosine-rich domain N-terminal to BSP's RGD motif [(C)T_{256}VE**Y**EGE**Y**E**Y**TGANE**Y**DNG**Y**EI**Y**ESEN_{282}] were synthesized with and without sulfate groups covalently attached to all tyrosines (90% and 99% pure respectively). (The cysteine is not in the natural BSP sequence and was used in other experiments.) The fluorescence spectrum of the sulfated tyrosine groups in the peptide excited at 280 nm has additional emission peaks at ~335 and ~348 nm (top) that are also present in the BSP purified from the BSP-adenovirus infected BMSCs suggesting that the recombinant protein contains significant number of Tyr-sulfate as previously shown for rat BSP isolated from UMR-106 cell culture media (Midura, R. J., McQuillan, D. J., Benham, K J., Fisher, L. W., and Hascall, V. C. *J. Biol. Chem.* (1990) **265**:5285-5291).

Fluorscence analysis was kindly provided by Dr. N.S. Fedarko, Johns Hopkins University on a PTI Series M Fluorimeter (Monmouth Junction, NJ).

Legend to Supplementary Figure 2

Adhesion of αVβ3 over-expressing HSG cells on DMP1 and OPN is RGD dependent and can be inhibited by a blocking anti-αVβ3 antibody. Single-cell suspension of HSG cells expressing adenovirus-transduced αVβ3 integrins were plated on wells coated with DMP1, OPN, or their respective KAE variants. Noted cells were preincubatd with 1 mM GRGDS competing peptide for 15 min at RT (A) or with 10 μg/ml of an anti-αVβ3 (LM609) function-blocking antibody (B and C) prior to being added to wells. Bars show the mean ± SEM from at least 3 independent experiments, each performed in triplicate. **P<0.001, *P<0.01, as compared with IgG control by means of t-test.

Supplementary Figure 1



Supplementary Figure 2