

DIFFERENTIAL ROLES FOR SMALL LEUCINE RICH PROTEOGLYCANS IN BONE FORMATION

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INTRODUCTION: The small leucine-rich proteoglycans (SLRPs), decorin and biglycan have been identified as matrix components in many connective tissues. Within soft connective tissues, such as skin, these macromolecules are primarily substituted with a dermatan sulphate (DS) glycosaminoglycan (GAG) chain, whilst in mineralized matrices of bone, the chondroitin sulphate (CS) substituted form predominates. The ability of the protein and GAG moieties to interact with collagen and growth factors have led to proposed functions in matrix assembly and modulation of cellular behaviour. Studies have also indicated that these macromolecules are also capable of interacting with calcium and hydroxyapatite surfaces, suggesting roles in either inhibiting or regulating mineral crystal growth and controlling crystal morphology. In order to further assess the role of SLRPs during bone formation, the present paper discusses the temporal expression of decorin and biglycan during bone formation, examining the biochemical nature and potential modification to structure during synthesis of the premineralised and mineralising matrices.

METHODS: A model system of primary bone cell culture models derived from marrow stromal cells and alveolar bone supporting teeth was used. We have identified periods relating to cell proliferation and development of the osteoblast phenotype associated with matrix deposition, remodelling of the osteoid, prerequisite to mineral deposition. Temporal expression of decorin and biglycan was assessed by RT-PCR. SLRP components were chaotrophically extracted from the matrix synthesized at various time points described above. SLRPs were separated by immunoprecipitation using the monoclonal antibody CS56 (Sigma), immunoreactive for CS. The GAG moiety within each fraction was examined by cellulose acetate electrophoresis whilst the proteoglycan species were identified by Western blot analysis using polyclonal antibodies against decorin and biglycan.

RESULTS: From these studies we have shown that decorin and biglycan exhibit differences in their patterns of expression and the nature of their glycosylation. Biglycan is expressed in two distinct phases relating to cell proliferation, after which it is removed from the matrix and appears to be re-

expressed at a time point relating to the onset of mineralization. Of significance, biglycan expressed during cell proliferation was substituted with DS chains, whilst biglycan synthesized during mineralization carried CS chains. Decorin was expressed later than biglycan, associated with early matrix deposition, with significant mRNA levels continuing to the mineralization stages. Again, DS-decorin prevailed with osteoid, whilst CS-decorin predominated within the mineralizing matrix. During remodeling of the osteoid, the GAG chain appears to be selectively removed from decorin, with the protein core persisting within the matrix.

DISCUSSION: The nature of the GAG chain conjugated to SLRP and the timing of its expression would seem to dictate the function decorin and biglycan play in bone formation. The role of DS-SLRP may correlate with those present in soft tissues such as skin. The role of biglycan is unclear but it has been implicated in regulating cell attachment, migration and angiogenesis (1,2). Within bone, our results would suggest a role in controlling cell proliferation. Conversely, expression of decorin arrests the growth of tumour cells (3) and the delayed expression within our bone culture model may indicate a role in down-regulating cell proliferation. Decorin also has potentially differing roles in collagen fibrillogenesis during matrix formation. DS-decorin appears to inhibit fibrillogenesis (4), whilst CS-decorin promotes fibrillogenesis (5) and biglycan would seem to display no effect (5). Both CS-decorin and CS-biglycan are capable of binding hydroxyapatite and influencing crystal growth, even when bound to collagen (5), suggesting roles in regulating mineral deposition. Overall the varied regulatory activity of decorin and biglycan is an important consideration for bone formation and tissue engineering.

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ACKNOWLEDGEMENTS: Dr LW Fisher for the provision of antibodies and the Medical Research Council, UK for financial assistance.