EXPRESSION AND REGULATION OF BONE MATRIX COMPONENTS IN OSTEOBLASTS FROM PATIENTS WITH OSTEOGENESIS IMPERFECTA

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Osteogenesis imperfecta is a genetic disorder of connective tissue which is caused by defects in either the α1 or α2 chain of type I collagen, resulting in bone fragility and deformity. Mutations and collagen structural defects are well detected in patients dermal fibroblasts. However, additional studies are required to understand the OI bone phenotype. The complex structure of bone matrix is made up almost entirely of type I collagen (90%), with the remaining 10% representing the non-collagenous proteins of bone (NCP). Among the major NCPs, one can distinguish both proteoglycans decorin and biglycan, bone sialoprotein, osteonectin, osteopontin and osteocalcin. Since the matrix provides the underlying organization of bone architecture, alterations in its major components, in this case type I collagen, could lead to a pathological phenotype/condition. Indeed, previous studies have indicated a decrease in both decorin and biglycan, as well as osteonectin, protein content in OI bone. Such changes in matrix composition and structure might disrupt interactions between cell and matrix, as well as the responses of osteoblasts to growth stimulants.

We have analyzed the expression of matrix constituents in OI osteoblasts from patients affected with moderately severe OI (type IV). This study has three goals: (1) to compare collagen type I synthesized by osteoblasts versus fibroblasts, from the same patient; (2) to determine the level expression of the major NCPs of bone; (3) to examine the response of these transcripts in osteoblasts treated with transforming growth factor b (TGF-b). TGF-b is a potent regulator of cell proliferation and synthesis of extracellular matrix: cell response to the growth factor may depend on the specific cell and the composition of its surrounding matrix.

We have analyzed type I collagen synthesized by osteoblasts and fibroblasts in 10 patients affected with type IV OI, and compared it with collagen synthesized by normal fibroblasts and osteoblasts. In 4 out of the 10 cases, we detected a dramatic baseline shift only in osteoblasts, whereas fibroblasts type I collagen showed only normal or slightly delayed baselines. Analysis of an additional 4 patients showed osteoblasts collagen with less dramatic baseline shift and a broader α1(I), when compared to their fibroblasts on SDS-urea PAGE. Of the two remaining patients, one displayed only a broader α1(I) in osteoblasts, and the other did not show any difference between fibroblasts and osteoblasts. As shown in a patient with a new point mutation resulting in α1(I) Gly 349 -> Cys substitution, the difference in migration was caused by increased over-modification of type I collagen in both osteoblasts and fibroblasts. Following treatment of cells with α1-α2-dipyridyl, collagen from both tissues comigrated with control. We are investigating the thermal stability of [3H]-Pro and [35S]-Cys labelled collagen in both tissues of this proband. The Tm of osteoblasts collagen is decreased by not more than one degree, when compared to fibroblasts collagen.

To determine the level of expression of bone matrix constituents, we compared transcripts synthesized by TGF-b stimulated fibroblasts and osteoblasts of normal adult individuals. We found that, under our experimental conditions, both α1 and α2 chains of type I collagen were upregulated in fibroblasts. α1(I) showed 3.2±0.57 x increase (p=0.01) and α2(I) was stimulated 6.5±0.88, (p=0.004) in fibroblasts treated with 5ng/ml of TGF-b. Decorin showed a 17±11fold increase, (p=0.05), when fibroblasts were treated with 1 ng/ml TGF-b, whereas osteoblast decorin did not respond to the growth factor. Only biglycan was increased 4.5±1.1, (p=0.002) or 7.6±2.0 (p=0.009) fold in the bone derived cells, following stimulation with 1 or 5 ng/ml of TGF-b, respectively.

OI osteoblast expression of matrix components was detected by Northern Blot analysis of total RNA from a 9 year old proband carrying a mutation in α1(I), resulting in a Gly 222 -> Cys substitution. We compared the response to TGF-b of patient osteoblasts and age-matched control cells. Transcripts for both chains of type I collagen showed a 2 to 3 x increase in control and OI osteoblasts. However, peak response was at 4 to 8 hours for the control and at 24 to 48 hours for OI cells, when cells were treated at 1 ng/ml of TGF-b for 48 hours. A more
A dramatic difference was observed when analyzed expression of the proteoglycans decorin and biglycan. In cells treated with 5 ng/ml of TGF-b for up to 24 hours, decorin transcripts were upregulated 4-fold in control osteoblasts at 8 to 24 hours. However, in OI osteoblasts only a mild transient stimulation of decorin mRNA was detected, which was significantly (p<0.04) less than the control response. Biglycan message was stimulated in both control and OI osteoblasts treated with 5 ng/ml of TGF-b over 48 hours. However, OI osteoblasts experienced about half the maximum stimulation of age-matched control at 4 to 24 hours treatment (p=0.25 - 0.05). Thus, prior OI bone tissue results with a decrease in biglycan and decorin appear to be a reflection of osteoblast down-regulation of bone proteoglycans.

We are currently analyzing the expression of those matrix components in additional OI patients. These results will increase our knowledge of expression of both collagenous and non-collagenous proteins and our understanding of the condition bone matrix structure to OI phenotype and pathology.

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