OSTEOBLAST METABOLISM OF MUTANT TYPE I COLLAGEN: INTRACELLULAR SURVIVAL, SECRETION AND INCORPORATION INTO MATRIX.

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Purpose: In previous comparisons of collagen synthesis by OI osteoblasts and fibroblasts, we reported that the proportion of overmodified alpha chains, as well as their relative electrophoretic delay, was greater in osteoblasts than fibroblasts (Hum Mut 11:395, 1998). Further comparisons of mutant collagen metabolism were conducted in paired primary osteoblasts and fibroblasts to determine the bases of the differences in cell-specific processing and their effect on matrix composition.

Methods: For intracellular processing and secretion studies, we selected non-lethal OI cases in which normal and mutant type I chains from fibroblasts were electrophoretically well-resolved. Mutations were identified using RNase A digestion of RNA hybrids , then subcloned and sequenced. The products of steady-state and continuous pulse labelling of osteoblast and fibroblast collagen were compared electrophoretically. For studies of mutant collagen incorporation into matrix deposited in culture, we selected cells with a known alpha2(I) Exon 16 skipping defect (Hum Mut 2: 230, 1993).

Results: We identified point mutations resulting in glycine substitutions in the three cases with extensively overmodified collagen: alpha2(I) gly703-arg (GGA-AGA) and gly922-ser (GGT-AGT), and alpha1(I) gly997-ser (GGT-AGT). For each proband, we detected differences in collagen electrophoretic patterns between osteoblasts and fibroblasts. For steady-state labelling, the intracellular and secreted proportion of electrophoretically delayed alpha chains was greater in osteoblasts than fibroblasts. Continuous pulse-labelling showed overmodified chains appear rapidly in osteoblasts, about 2 hours sooner than in fibroblasts. Overmodified collagen chains are present in higher proportion and with greater electrophoretic delay than in fibroblasts. Further, the overmodified chains in osteoblast samples have a gradually increasing electrophoretic delay with time, attaining the steady-state pattern in about 4 hrs. The proportion of secreted mutant chains was greater in osteoblasts than fibroblasts, where only a low proportion of overmodified form is detected in media samples. In matrix incorporation experiments using cells with alpha2(I) Exon16 skipping, a small amount of mutant chain was found in immaturely crossed linked fibroblast matrix. In long term osteoblast cultures, the mutant chain is abundant in immature and stably cross-linked collagen. Conclusions: In these OI cases, osteoblasts are more permissive than fibroblasts for intracellular survival and secretion of mutant collagen. Greater matrix incorporation of mutant collagen may reflect availability while extent of cross-linking may be influenced by mutation location. Cell- specific differences between fibroblasts and osteoblasts in processing / secretion of collagen may be a crucial part of the pathophysiology of OI.

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