Effects of Lysyl Oxidase Overexpression on Collagen Cross-linking and Mineralization

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Fibrillar type I collagen controls spatial aspects of mineralization by providing a stable template in most mineralized tissues. The stability of the fibril is primarily derived from covalent intermolecular cross-linking initiated by lysyl oxidase (LOX). OBJECTIVE: The aims of this study were to establish MC3T3-E1 cell-derived clones expressing higher levels of LOX, and to partially characterize the effects on collagen cross-linking and mineralization in vitro.

METHODS: MC3T3-E1 cells were transfected with pcDNA3.1-V5His containing coding sequence of mouse LOX and stable cell clones expressing higher levels of LOX (S clones) were generated. Three S clones and control (MC3T3-E1 cells) were cultured in α-MEM in the presence of 10% fetal calf serum, 1mM β-glycerophosphate and 50 µg/ml ascorbic acid for up to 4 weeks. At the end of each week (week 1, 2, 3 and 4), cell-matrix were collected, reduced with standardized NaB3H4, hydrolyzed with 6N HCl and subjected to quantitative cross-link analysis.

The in vitro mineralization was also evaluated at the same time points by Alizarin-red S staining.

RESULTS: The S clones showed significant increases in collagen cross-links, dihydroxylysinonorleucine (1.3-2 fold) and pyridinoline (2-3 fold), as the culture period progressed. At week 4, the total aldehydes, i.e. free and those involved in cross-linking, in S clones was 1.5-2 fold of the control. The onset of mineralization was delayed in the S clones in comparison with the control.

CONCLUSION: These preliminary results indicate that the level of lysyl oxidase correlates with that of collagen cross-links, and that an alteration in the cross-linking may be detrimental to mineralization. Supported by NIH grant DE10489 and NASA grant NAG2-1596.