Investigating the Role of Lysine Post-Translational Modifications in Type I collagen for Bone Mineralization

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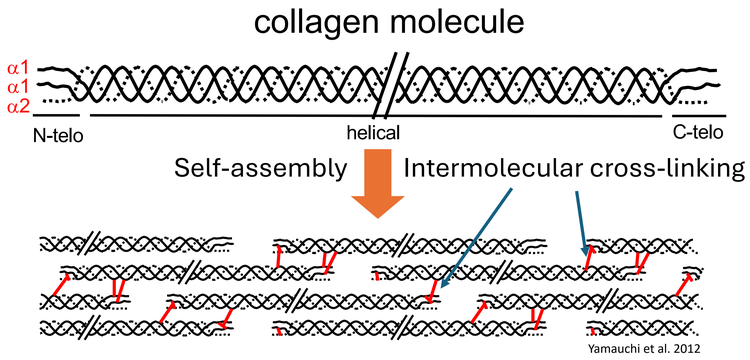
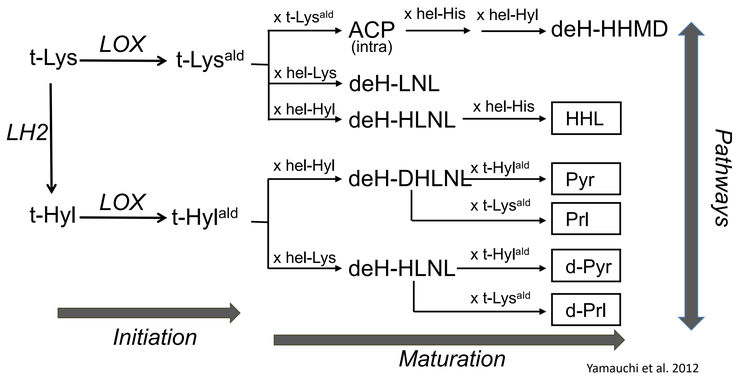
References

Introduction

- Type I collagen is the most abundant protein in our body, including bones and teeth.
- It is a triple-helical molecule composed of two α1 chains and one α2 chain which associate to form a central helical region with short non-helical domains at N and C-termini (telopeptides).



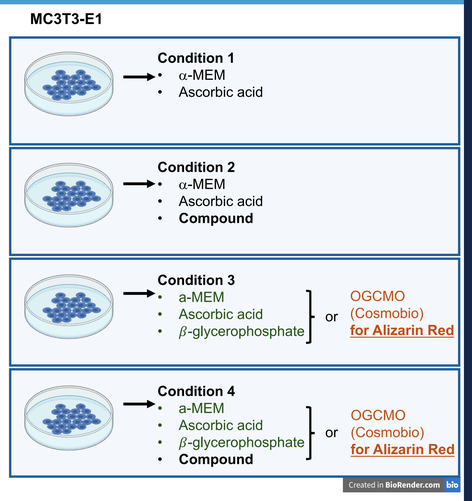
- Lysyl hydroxylase 2 (LH2) is an ER-resident enzyme that catalyzes the hydroxylation of telopeptidyl lysine (Lys) residues on type I collagen to yield hydroxylysine (Hyl) which is critical for the formation of stable collagen cross-links, and thus, the mechanical functions of bone.



- Dysfunction of LH2 causes Bruck syndrome, a recessive osteogenesis imperfecta with joint contracture. However, nothing has been done to find LH2-targeted therapeutics.
- In collaboration with Dr. Uzawa's group (Chiba Univ, Japan), we developed a potential LH2 activator**. The objective of this study is to investigate the effect of this compound on collagen cross-linking and mineralization in vitro.** Such study may provide insights into the development of therapeutic strategies for bone diseases.

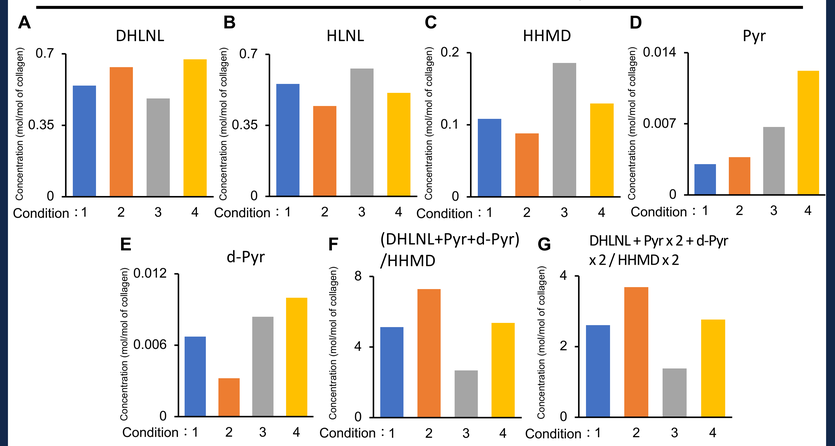
Materials and Methods

- Cell culture: Mouse-derived osteoblastic cells, MC3T3-E1.
- Culture cells for 2 weeks under 4 different medium conditions using a novel LH2 activator compound.
- Quantify the extent of Lys^{ald} and Hyl^{ald} derived cross-links via HPLC.
- Evaluate the extent of mineralization using Alizarin Red staining with OGCMO medium.



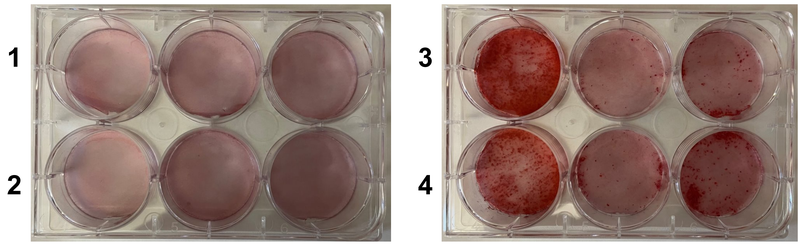
Results

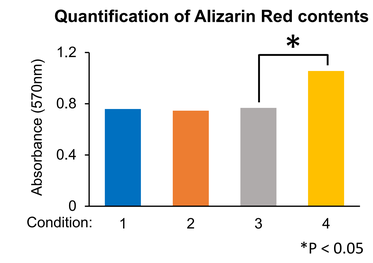
Results Part 1: Cross-Link Analysis



- DHLNL cross-links, derived from Hyl^{ald} pathways, were found to be higher in the groups where LH2 activator was added (conditions 2 and 4) **(Fig. A)**.
- HHMD cross-links, which are derived from Lys^{ald} pathways, were found to be lower in conditions 2 and 4 where LH2 activator was added **(Fig. C)**.
- When comparing the ratios of Lys^{ald} and Hyl^{ald} derived cross-links, we found that the ratio of Hyl^{ald} derived cross-links were higher in conditions 2 and 4, despite all conditions having the same level of total cross-links/aldehydic residues **(Fig. F-G)**.

Results Part 2: Mineralization Assay using Alizarin Red





- The extent of mineralization was higher in condition 4 where LH2 activator was added.
- Thus, LH2 activation led to increased levels of in vitro mineralization.

Discussion

- LH2 proves to be a crucial determinant of telopeptidyl Lys hydroxylation, as confirmed by our collagen cross-linking profiles. This is in line with previously published studies.
- LH2 activation causes increased Hyl^{ald} derived cross-links with concomitant decreases in Lys^{ald} derived cross-links.
- LH2 activation created observable differences in the extent of type I collagen mineralization under the conditions employed.
- These mineralization differences due to higher LH2 activity could provide insights into potential therapeutics for bone-related diseases.
- Future studies should confirm and expand the in vitro mineralization assay, examine the dose-effect relationship of the LH2 activator, and ultimately its effect on bone mineralization in vivo.

Acknowledgements

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