Analysis of COL6A proteins as a potential therapeutic marker for Ullrich muscular dystrophy and Bethlem myopathies

Sunhee Jung[1], Valeria Vasta[1], and Si Houn Hahn[1,2]

[1]Seattle Children's Hospital Research Institute, Seattle, WA 98101

[2]Department of Pediatrics, University of Washington School of Medicine, Seattle, WA 98195

Introduction: Abnormalities in the extracellular matrix are common in human muscular dystrophies. Mutations in *COL6A* (*COL6A1*, *COL6A2* and *COL6A3*) are prevalent causes of congenital muscular dystrophy such as Ullrich muscular dystrophy, Bethlem myopathies and yet poorly defined intermediate form. Most of the *COL6A* mutations lead to altered extracellular deposition of COL6A[1-4]: thus COL6A protein markers have enormous potential in monitoring and evaluating efficacy of new therapeutic trials for COL6A-related muscular dystrophies. Approaches to accurately access the level of COL6A in cell-based assays are critically needed. Here, we propose that a LC-SRM-MS assay can detect the cellular level and extracellular release of COL6A proteins in human fibroblasts and cultured media. This assay will facilitate the study of the efficacy of drugs that are being developed.

Methods: Candidate peptides for COL6A1 were screened by *In Silico* trypsin digestion modeling followed by BLAST search to insure the sequences are unique within the human genome. The final "signature" peptides were selected by evaluating the SRM chromatogram for the isotopically labeled peptides and the digest peptides of fibroblasts and cultured media digest peptides. The eluates were analyzed using UPLC system coupled with a Waters Quattro Premier XE mass spectrometer. The amount of the signature peptides in the fibroblast extracts was determined by taking the ratio of the peak areas for the signature peptide to the heavy isotope-labeled peptide. Actin and Fibronectin were used for normalization.

Preliminary Data: Severe mutations in *COL6A* genes produce proteins that remain entrapped within the endoplasmic reticulum or cause complete absence or reduction of the protein, resulting in altered levels of intra and extra-cellular protein. We hypothesize that these mutations cause altered level of COL6A peptides compared to normal and that the difference between cells and media will reflect defective extracellular deposition. This altered

abundance/distribution of COL6A can be followed as a characteristic biomarker of disease and of therapeutics efficacy. In an initial approach, we selected proteotypic candidate peptides for COL6A1 based on specific criteria, including proper physiochemical properties for MS detection, uniqueness to the protein of interest, and no known modifications or single nucleotide polymorphisms. Our preliminary analysis suggests that signature peptides for COL6A1 with a good signal to noise can be detected in both tryptic digests of human fibroblasts and their cultured media. The identity of these peptides was validated with their heavy isotope labeled internal standards. Optimization of sample process including cell growth conditions, cell collection, and trypsin digestion has been completed. We are currently analyzing normal control fibroblast cell lines and positive cases with *COL6A* related muscular dystrophies.

Reference

1. Allamand V, Brinas L, Richard P, Stojkovic T, Quijano-Roy S, Bonne G. ColVI myopathies: where do we stand, where do we go? *Skelet Muscle* 2011;1:30.
2. Butterfield RJ, Foley AR, Dastgir J, Asman S, Dunn DM, Zou Y, Hu Y, Donkervoort S, Flanigan KM, Swoboda KJ, Winder TL, Weiss RB, Bonnemann CG. Position of Glycine Substitutions in the Triple Helix of COL6A1, COL6A2, and COL6A3 is Correlated with Severity and Mode of Inheritance in Collagen VI Myopathies. *Hum Mutat* 2013;34(11):1558-67.
3. Jimenez-Mallebrera C, Maioli MA, Kim J, Brown SC, Feng L, Lampe AK, Bushby K, Hicks D, Flanigan KM, Bonnemann C, Sewry CA, Muntoni F. A comparative analysis of collagen VI production in muscle, skin and fibroblasts from 14 Ullrich congenital muscular dystrophy patients with dominant and recessive COL6A mutations. *Neuromuscul Disord* 2006;16(9-10):571-82.
4. Baker NL, Morgelin M, Peat R, Goemans N, North KN, Bateman JF, Lamande SR. Dominant collagen VI mutations are a common cause of Ullrich congenital muscular dystrophy. *Hum Mol Genet* 2005;14(2):279-93.