The use of primary dermal fibroblast cultures to evaluate type I collagen expression in the oim model mouse
Kristin Rolwes, Stephanie Carleton and Charlotte Phillips

Osteogenesis imperfecta type III is a heritable disorder leading to impaired connective tissue function in type I collagen containing tissues including bone fragility, blue-grey sclera, short stature, and hearing loss. Normal type I collagen is a heterotrimeric molecule containing two proα1(I) collagen chains and a similar but genetically distinct proα2(I) collagen chain. The osteogenesis imperfecta murine (oim) model mouse produces only homotrimeric type I collagen due to a single nucleotide deletion in the COL1A2 gene resulting in a non-functional proα2(I) collagen chain. The result is expression of an abnormal type I collagen molecule which leads to the above phenotype. This study is aimed at developing a methodology whereby dermal fibroblast cultures can be utilized for a variety of assays, including type I collagen RNA and protein quantification. For genotype identification, primers flanking the site of the single nucleotide deletion allow for differentiation of wild type, heterozygous and homozygous animals at the genomic level. Upon confirmation, skin was removed from both oim and wildtype mice and the dermal layer harvested. Fibroblasts originating from the dermal layer of each genotype were then cultured and grown to confluence as separate cultures and the RNA and/or protein harvested from both cell types. Total RNA was harvested using the Qiagen RNeasy kit and used to make cDNA, which was then used in conjunction with specific PCR primers to differentiate between wildtype and oim transcripts. For protein studies, the cells were treated with ascorbic acid to maximize the production of collagen prior to harvesting. Type I collagen expression was then confirmed via a western blot using a type I collagen-specific antibody. Initial results indicate the presence of both proα1(I) and proα2(I) collagen chains from harvested wildtype dermal fibroblasts, while media harvested from oim dermal fibroblasts indicated the presence of only the proα1(I) collagen chains. These results confirm the in vivo protein expression profile seen in skin of both oim and wildtype mice is exhibited in vitro in the respective dermal fibroblast cultures. These results will allow us to quantitate proα1(I) and proα2(I) collagen mRNA and protein expression levels. Future experiments will include the quantitation of type I collagen mRNA levels using RT-PCR, as well as the use of densitometry to quantitate type I collagen protein levels by western blot analysis. This in turn promises to provide insight into the mechanism of formation of abnormal type I collagen in the oim model mouse.