Dermatotoxicological and other Safety Testing Methods without Animals - State November 2013

Session 2: Susceptibility factors and disease models in reconstructed human skin

3D KSS model representing accelerated skin aging

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In previous studies we and others have shown that (i) photoaged human skin, in comparison with intrinsically aged skin, contains increased amounts of the most frequent large scale deletion of human mitochochondrial (mt) DNA, ie. the common deletion (CD) and (ii) that repetitive UV exposure leads to an accumulation of the CD in cultured primary human skin fibroblasts in-vitro as well as in-vivo in human skin. In order to assess whether the accumulation of the CD in skin fibroblasts is causally related to skin aging, we next developed a 3-dimensional dermis model, in which primary human skin fibroblasts from patients with the mitochondriopathy Kearns Sayre Syndrome (KSS) were seeded into a collagen matrix. These cells constitutively carry large amounts of the photoaging-associated CD and thus functional consequences can be studied without the need for UV irradiation, which might cause multiple biological effects independent of mtDNA mutagenesis. We observed that KSS cell containing dermal equivalents (KSS DE) contained increased amounts of the CD over a 6 week culture period, when compared to DE which had been generated with normal human skin fibroblasts matched for donor age, skin site and cell passage number (NHF DE). Interestingly, KSS DE showed an increased expression of "senescence associated secretory phenotype (SASP)-associated genes" including VEGF, MMP-1, IL-8 and IL-6, which are typically found to be increased in vivo in photoaged human skin. By focusing on MMP-1 we next showed that expression of this SASP-like phenotype was of functional relevance for dermal photoaging. Accordingly, in contrast to NHF DE, in KSS DE increased MMP-1 activity was detected which was associated with an increased breakdown and rarefication of collagen fibers and a concomitant increase in collagen fiber fragments, ie structural alterations which are a hallmark and strongly reminiscent of photoaged human skin. Importantly, this phenotype developed within 1 – 6 weeks and thus in a greatly accelerated fashion, as compared to photoaging of human skin, which usually takes decades to develop.