5. SUMMARY

To decipher the molecular mechanisms involved in endogenous skin aging human sebocytes and fibroblasts were maintained with GH, IGF-I, 17β-estradiol, progesterone, testosterone and DHEA at levels corresponding to average serum levels of males and females from 20 to 60 years of age. First, the expression of estrogen, androgen, GH and IGF-I receptors were identified by highly sensitive RT-PCR and Western blotting and their cellular localization was detected by immunocytochemistry. SZ95 sebocytes incubated with the cocktail of hormones circulating in 60-y-old individuals showed significantly lower content of neutral lipids than cells treated with hormones of 20-y-old ones. Polar lipids, cell proliferation, and cell viability remained unchanged. On the other hand, proliferation of the fibroblasts was significantly affected by the hormone mixture, and fibroblasts incubated with hormones of 60-y-old individuals showed lower content of neutral and polar lipids than cells treated with hormones of 20-y-old ones. Increased mRNA and protein levels of c-Myc and increased protein levels of fibronectin, which are genes already associated with cell aging, were detected in SZ95 sebocytes at 60-y-old hormone levels compared to those detected at 20-y-old hormone levels after 5 d of treatment. Expression profiling employing a cDNA microarray composed of 15,529 cDNAs from known and novel genes identified age-related genes with altered expression levels at 20 and 60 years. The functional classification of these genes were related to several biological processes such as in mitochondrial function, oxidative damage and stress, ubiquitine-mediated proteolysis, cell cycle, immune responses, organization of the extracellular matrix, steroid biosynthesis and phospholipid degradation, which are all hallmarks of the aging process.

Furthermore, the effects of GH, IGF-I, 17β-estradiol, progesterone, testosterone and DHEA were tested as single agents. In SZ95 sebocytes, while IGF-I and GH were shown to amplify neutral and polar lipid synthesis, 17β-estradiol and testosterone showed mostly an effect on polar lipid production. After treatment with progesterone and DHEA no effect was detected. Proliferation and cytotoxicity remained by all hormones tested unchanged. On the other hand, IGF-I and 17β-estradiol enhanced fibroblasts proliferation and could also amplify lipid synthesis in a dose-dependent manner.

In addition, in the experiments presented here, an interaction between IGF-I and estradiol signaling pathways was documented in SZ95 sebocytes and fibroblasts, which also gave

evidence for a paracrine interaction between both cell types *in vivo*. 17β-estradiol synthesis was enhanced after treatment with IGF-I in SZ95 sebocytes and fibroblasts, while IGF-I synthesis was not affected by 17β-estradiol in SZ95 sebocytes but was increased by 17β-estradiol in fibroblasts. Moreover, IGF-IR and ERα expression in SZ95 sebocytes and IGF-IR expression in fibroblasts was shown to be influenced by 17β-estradiol and IGF-I in a time-dependent manner.

These data indicate how important hormones can be for the development of human cells, as they can regulate their biological activity and the pattern of their gene expression. Moreover, this *in vitro* model on endogenous skin aging may serve in the identification of skin aging- but also global aging-associated genes facilitating future studies on molecular aging.