The skin as a mirror of the aging process in the human organism – State of the art and results of the aging research in the German National Genome Research Network 2 (NGFN-2)

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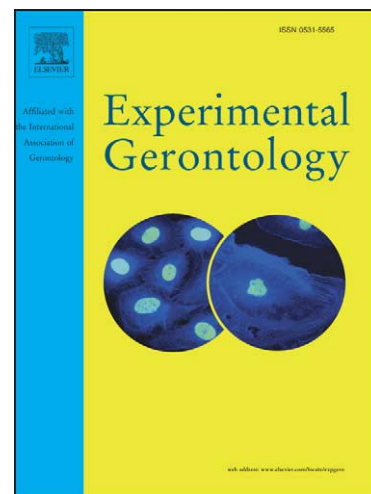Evgenia Makrantonaki, Christos C. Zouboulis

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Abstract

As our society is growing older, the consequences of aging have begun to gain particular attention. Improvement of quality of life at old age and prevention of age-associated diseases have become the main focus of the aging research. The process of aging in humans is complex and underlies multiple influences, with the probable involvement of heritable and various environmental factors. In particular, hormones are decisively involved in the generation of aging. Over time, important circulating hormones decline due to a reduced secretion of the pituitary, the adrenal glands and the gonads or due to an intercurrent disease. Among them, serum levels of growth factors and sexual steroids show significant aging-associated changes. Within the scope of the Explorative Project 'Genetic aetiology of human longevity' supported by the German National Genome Research Network 2 (NGFN-2) an *in vitro* model of human hormonal aging has been developed. Human SZ95 sebocytes were maintained under a hormone-substituted environment consisting of growth factors and sexual steroids in concentrations corresponding to those circulating in 20- and in 60-year-old women. 899 genes showed a differential expression in SZ95 sebocytes maintained under the 20- and 60-year-old hormone mixture, respectively. Among them genes were regulated which are involved in biological processes which are all hallmarks of aging. The most significantly altered signaling pathway identified was that of the transforming growth factor-β (TGF-β). A disturbed function of this cascade has been associated with tumorigenesis, i.e. in pancreatic, prostate, intestine, breast, and uterine cancer. Interestingly, genes expressed in signaling pathways operative in age-associated diseases such as Huntington's disease (HD), dentatorubral-pallidoluysian atrophy (DRPLA), and amyotrophic lateral sclerosis (ALS) were also identified. These data demonstrate that skin and its appendages may represent an adequate model for aging research. Hormones interact in a complex fashion, and aging may be partly attributed to the changes in their circulating blood levels. Furthermore, a disturbed hormone status may partially act towards the manifestation of neurodegenerative diseases. Thus, these results could be a basis for an integrated and interdisciplinary approach to the analysis of the aging process.

1. Aging -Epidemiology

As we enter the third millenium, we are witnessing an unprecedented rapid expansion of the population of elderly people both in the developed and developing world (Diczfalusy, 1996). Since 1840, life expectancy has increased at a rate of about three months per year (Birg, 2004; Oeppen and Vaupel, 2002), whereas the total worldwide aged population is expected to rise from 605 million in 2000 to 1.2 billion in 2025 and to nearly 2 billion in 2050 (Aleksandrova and Velkova, 2003).

In Germany an industrialized country with a high population density, the median life expectancy increased during the last century by about 30 years. According to the World Health Organization's (WHO) estimates, only between 1990 and 2001, Germans gained 3.2 years in life expectancy, with men showing a greater gain than women: 3.6 years and 2.9 years, respectively. In parallel, Germany has the second lowest birth rate in Europe, which has dropped by 21% since 1990. Consequently, as the large birth cohorts of the late 1940s approach retirement age, the number of Germans aged 65 and over is expected to grow from about 17.5% of the population in 2003 (Council of Europe, 2003) to 26.4% in 2030. In the year 2025 the median life expectancy will be about 83 years for women and 76 years for men (Diepgen, 2003) and by 2030, one person out of every four is expected to be aged 65 or over.

Such a rapid and ubiquitous change has never been seen in the history of civilization. Among multiple challenges, our society has to deal with important socio-economic, political and health-economic consequences and particularly with the maintenance of the quality of life of the aging people. It is well established that the prevalence of many chronic and degenerative diseases increases with advancing age, probably because of long-term exposure to exogenous factors (Diepgen, 2003). Nowadays, physicians have to confront with age-associated diseases, which were almost unknown some centuries ago.

The main focus of the Explorative Project 0313359 "Genetic Etiology of Human Longevity" supported by the German National Genome Research Network 2 (NGFN-2) is to map and characterize genetic susceptibility factors that predispose to healthy longevity in humans as well as to identify molecular pathways that are associated with the physiology of aging and/or age-related disorders and diseases. It is a multi-center study with contributions by the following institutes: Clinical Molecular Biology,

University Hospital Schleswig-Holstein, Kiel, Laboratory for Biogerontology, Dermato-Pharmacology and Dermato-Endocrinology, Institute of Clinical Pharmacology and Toxicology, Charité Universitaetsmedizin Berlin and Department of Vertebral Genetics, Max Planck Institute for Molecular Genetics, Berlin.

2. Skin

Since the collection of specimens from bones, internal glands and brain throughout life for experimental research purposes is associated with major practical and ethical obstacles in humans, interspecies research and the use of skin, the largest and heaviest organ of the body as a common research tool offer promising alternative approaches.

The skin exhibits multiple functions, among them it serves as a protective barrier between internal organs and the environment, but is also a complex organ with multiple cell types and structures. It is divided into four major compartments: epidermis, dermis, appendages and subcutaneous tissue.

The epidermis is the most superficial layer of the skin and is approximately 100 µm thick. It is a keratinized stratified squamous epithelium and its main function is to protect the body from harmful stimuli of the environment and diminish fluid loss. The principal cells of this region are keratinocytes which make up 95% of the epidermal cells. The epidermis is subdivided into five layers or strata, the stratum germinativum, the stratum spinosum, the stratum granulosum, the stratum lucidum, in which a keratinocyte gradually migrates to the surface and is detached in a process called desquamation, and the stratum corneum. The epidermis also consists of 1-2% pigment-producing cells called melanocytes, Langerhans cells, which are the single most important antigens presenting cell population in the skin and Merkel cells, which may act as mechanoreceptors and are thought to have APUD (amino precursor uptake and decarboxylation)-like activity (Kanitakis, 2002).

The dermis is the layer of connective tissue to which the epidermis is attached and is approximately 1-2 mm thick. The dermis assumes the important functions of thermoregulation and supports the vascular network to supply the epidermis with nutrients. It is typically subdivided into two zones, a papillary dermis and a reticular layer. It contains mostly fibroblasts, which are responsible for secreting collagen, elastin,

glycosaminoglycans, proteoglycans, fibronectin and other extracellular matrix proteins that provide the support and elasticity of the skin. Collagen makes up to 70-80% of the dermal weight. It is composed mainly of glycine, proline and hydroxyproline and is one of the strongest proteins in nature. Type I collagen is the most abundant protein in skin connective tissue, which also contains other types of collagen (III, V, VII). Newly synthesized type I procollagen is secreted into the dermal extracellular space, where it undergoes enzymatic-processing arranging itself into a triple helix configuration. The triple helix complexes associate with other extracellular matrix proteins, such as leucine-rich small proteoglycans, to form regularly arranged fibrillar structures. This process, called fibrillogenesis, results in formation of collagen bundles (Bateman and Chothia, 1996) that are responsible for the strength and resiliency of the skin (Uitto, 1986). Type III collagen is the second major fibrillar collagen found in the skin. It is also known as fetal collagen because of its abundance in fetal tissues. Although it is found in equal amounts to type I collagen in fetal skin, in adult skin there is a greater production of type I collagen, which results in a final ratio 6:1 type I : type III (Burgeson et al., 1994). Elastic fibers constitute less than 1-2% of the weight of the dermis, but they play an enormous functional role by resisting deformational forces and returning the skin to its resting shape. Elastic fibers are mainly composed of two distinct proteins, elastin and fibrillin, both produced by resident fibroblasts. Amorphous, hydrophobic, cross-linked elastin constitutes the central core of the fibers, which are surrounded by fibrillin-rich microfibrils. Fibrillin microfibrils are also found as free microfibrils arranged in bundles in the superficial dermis. Elastic fibers form a fine network that extends vertically in the dermal papillae and surrounds dermal blood vessels, while in the reticular dermis they build fibers which are much thicker and run parallel to the epidermis surrounding the larger collagen fibers. They also surround the adnexal structures (Holbrook et al., 1982; Kielty and Shuttleworth, 1997). In the dermis, immune cells that are involved in defence against foreign invaders passing through the epidermis are also present.

The dermo-epidermal junction is an undulating basement membrane that adheres the epidermis to the dermis. It is composed of two layers, the lamina lucida and lamina densa. The junction is characterized by downward folds of the epidermis called epidermal ridges or rete ridges, which interdigitate with upward projections of the dermis called

dermal papillae. This structure of the dermo-epidermal junction contributes to minimizing the risk of dermo-epidermal separation by shearing forces (Bruckner-Tuderman, 1993).

Epidermal appendages are intradermal epithelial structures lined with epithelial cells with the potential for division and differentiation. These are important as a source of epithelial cells, which accomplish re-epithelialization should the overlying epidermis be removed or destroyed in situations such as partial thickness burns, abrasions, or split-thickness skin graft harvesting. Epidermal appendages include sebaceous glands, sweat glands, apocrine glands, mammary glands and hair follicles. They often are found deep within the dermis, and in the face may even lie in the subcutaneous fat beneath the dermis. This accounts for the remarkable ability of the face to re-epithelialize even the deepest cutaneous wounds.

The subcutaneous tissue consists of fat cells that underline the connective tissue. This layer connects loosely the skin to the underlying fascia. The fat cells insulate the organism and provide energy.

With increasing age the epidermis, the dermis and the skin appendages progressively lose their youthful characteristics and abilities; the skin gradually loses its structural and functional characteristics (Braverman and Fonferko, 1982; Fisher et al., 2002; Ghadially et al., 1995; Moragas et al., 1993; Raine-Fenning et al., 2003; Wulf et al., 2004). Consequently, the skin becomes more fragile and vulnerable to damage which may lead to major aging-associated diseases [e.g. ulcera crurum, herpes zoster, bullous pemphigoid, epithelial tumors, lichen sclerosus et atrophicus, atrophic vulvovaginitis/ vulvodynia etc.] (Zouboulis Ch, 2003).

Skin aging can be classified into light-induced aging (photoaging, extrinsic aging) and intrinsic aging. The latter occurs in non-exposed areas, which are not in direct contact with environmental factors such as ultraviolet (UV) irradiation (e.g. the inner side of the upper arm) (Makrantonaki and Zouboulis, 2007) and is mainly attributed to genetic factors, and alterations of the endocrine environment. In contrast to photoaging, intrinsically aged skin reflects degradation processes of the entire organism.

3. The sebaceous gland - a model of aging

Sebaceous glands are holocrine glands found over the entire surface of the body except the palms, soles and dorsum of the feet. They are largest and most concentrated in the face and scalp, which are the sites of origin of acne. The normal function of sebaceous glands is to produce and secrete sebum, a group of complex oils including triglycerides and fatty acid breakdown products, wax esters, squalene, cholesterol esters and cholesterol (Downing et al., 1987; Nikkari et al., 1974; Ramasastry et al., 1970; Thody and Shuster, 1989). Sebum lubricates the skin to protect it against friction and makes it more impervious to moisture.

Furthermore, the sebaceous gland transports antioxidants in and on the skin and exhibits a natural light protective activity (Zouboulis, 2004). It possesses an innate antibacterial activity and has a pro- and anti-inflammatory function. It can regulate the activity of xenobiotics and is actively involved in the wound healing process. It possesses all enzymes required for the intracellular androgen metabolism and confers upon the skin an independent endocrine function (Fritsch et al., 2001).

With advancing age the size of sebaceous gland cells tends to decrease, while their number remains approximately the same throughout life (Zouboulis and Boschnakow, 2001). Sebaceous gland cells show an age-related reduced secretory output, which results in a decrease in the surface lipid levels and skin xerosis (Engelke et al., 1997; Pochi et al., 1979) - a major characteristic of aged skin. Hormone substitution with estrogens *in vivo* could significantly reverse skin xerosis indicating a hormone-dependent function of the sebaceous gland cells (Dunn et al., 1997).

Human SZ95 sebocytes are sebaceous gland cells derived from facial skin and transfected with the SV-40 large T antigen and offer a unique model for investigations on the physiology of aging (Zouboulis et al., 1999) [Figure 1]. They constitute a better alternative to animal research and they functionally behave in a manner concominant to non-transfected human sebocytes. They show a similar epithelial morphology and they can produce squalene and wax esters, as well as triglycerides and free fatty acids, even after 25-40 passages (Patents and patent applications: EP1151082, DE59913210D, AU770518B AT319813T, CA2360762, CN1344314T, DK1151082T, HU0200048, IL144683D, JP2002535984, KR31762, PL350191, US2002034820, WO0046353).

Several of the data reported have only been obtained by the application of the human SZ95 sebocyte aging model.

4. Hormone decline and aging

Hormones are decisively involved in intrinsic aging. Over time important circulating hormones decline due to a reduced secretion of the pituitary, adrenal glands and the gonads or due to an intercurrent disease. Among them, growth factors [i.e. growth hormone (GH) and insulin-like growth factor-I (IGF-I)), and sex steroids (e.g. androgens and estrogens) show significant changes in their blood levels and play a distinct role in the generation of the aging phenotype.

4.1 GH and IGF- I

GH secretion is relatively stable during childhood, increases during adolescence and decreases gradually during adulthood. This age-related decline in GH secretion involves a number of changes in the GH axis, including decreased serum levels of IGF-I and decreased secretion of GH-releasing hormone from the hypothalamus. The cause of the normal age-related decrease in GH secretion is not well understood, but is thought to result in part from increased secretion of somatostatin, the GH-inhibiting hormone, or from an age-related decrease in the number and size of somatotrophs. Moreover, the GH response to GH-releasing hormone is attenuated with advancing age.

GH acts on virtually all tissues of the body. GH stimulates epiphyseal bone growth and also maintenance of bone after epiphyseal closure, is anabolic for muscles (Kostyo et al., 1959), stimulates lipolysis (Fain et al., 1965), decreases body fat (Salomon et al., 1989) and increases gluconeogenesis. It can also cause resistance to the action of insulin (Weaver et al., 1995). Decline of GH with aging may impair all the functions mentioned above.

Serum levels of IGF-I have also been reported to increase from birth to puberty, followed by a slow decline through adulthood. This reduction is correlated with the progressive decline of GH with advancing age (Bennett et al., 1984). The reduction of GH and IGF-I with aging is also called somatopause.

4.2. Sexual steroids

Among the numerous endocrine signals that affect the skin, sexual steroids - androgens and estrogens - play a predominant role. The secretion of sexual steroids is under the stimulatory influence of luteinizing hormone (LH) and follicle stimulating hormone (FSH), both derived from the pituitary and regulated by a decapeptide, the gonadotropin-releasing hormone (GnRH), which is synthesized in the hypothalamus. Sexual steroids can be distinguished by the carbon numbers, C-19 being androgens, C-18 being estrogens and C-21 being progestenoids.

4.2.1 Androgens

Androgens can be classified into two categories: adrenal androgens [androstenedione and 11β-hydroxyandrostenedione, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEA-S)] and gonadal androgens (testosterone).

The direct biological activity of the adrenal androgens is minimal, and they function primarily as precursors for peripheral conversion to the active androgen hormones testosterone and 5α-dihydrotestosterone (5α-DHT). In males with normal gonadal function, the conversion of adrenal androstenedione to testosterone accounts for less than 5% of the production rate of this hormone and thus the physiologic effect is negligible.

In females, the adrenal substantially contributes to total androgen production by the peripheral conversion of androstenedione. In the follicular phase of the menstrual cycle, adrenal precursors account for two/thirds of testosterone production and one-half of 5α-DHT production. During midcycle, the ovarian contribution increases and the adrenal precursors account for only 40% of testosterone production.

Skin and its appendages, including hair follicles, sebaceous glands and eccrine/apocrine glands have been shown to possess all the necessary enzymes required for androgen synthesis and metabolism and skin can be responsible for the development of hyperandrogenism-associated conditions and diseases, such as seborrhea, acne, hirsutism and androgenetic alopecia (Rossouw et al., 2002). Furthermore, several functions of the human skin can be affected by androgens, such as sebaceous gland growth and differentiation, hair growth, epidermal barrier homeostasis and wound healing.

Dehydroepiandrosterone (DHEA)

DHEA and DHEA-S are the most abundant steroids in the human plasma having serum concentrations of the order of 10^{-8} and 10^{-6} M, respectively. In adult men and women, serum DHEA-S levels are 100 to 500 times higher than those of testosterone and are 1,000 to 10,000 times higher than those of estradiol (Labrie et al., 1998). The concentrations in serum reach a peak between the ages of 25 and 30 years and thereafter decline steadily, so that by the age of 60, serum concentrations are only 5-10% of corresponding values in young adults (Orentreich et al., 1984). This decline in women appears to be primarily a function of age and seems to be unrelated to menopause status (Burger et al., 2000). In addition, the maximal responses of DHEA to adrenocorticotropin hormone (ACTH) or corticotropin releasing hormone (CRH) in older adults are also significantly lower than they are in young men and women, whereas the secretory response of glucocorticoids to these modulators is not reduced in aging (Parker et al., 2000).

Changes of DHEA with age have been related with altered immune response (Lucas et al., 1985), declines in cognitive ability (Flood and Roberts, 1988), bone mass density (Hollo et al., 1970), libido (Bachmann et al., 2002) and increase in the incidence of cardiovascular diseases and atherosclerosis in men (Ishihara et al., 1992).

Testosterone

Serum testosterone levels in men decrease with age (Corpas et al., 1993; Gray et al., 1991). It has been shown that 20% of healthy men in their sixties and 30% of men in their seventies have lower testosterone levels than 97.5% of healthy 20 to 45-year-old men (Corpas et al., 1993). Feldman et al. found that total testosterone levels decreased by 0.8% per year, while bioavailable testosterone fell by 2% per year and sexual hormone-binding globulin (SHBG) levels increased by 1.6% per year (Gray et al., 1991). These changes are due in part to a reduction in the number of Leydig cells in the testes that produce testosterone.

Symptoms and findings of testosterone deficiency are similar to those associated with aging. They include loss of energy, depressed mood, decreased libido, erectile

dysfunction, decreased muscle mass and strength, increased fat mass, frailty, osteopenia, and osteoporosis (Hijazi and Cunningham, 2005). The decline of testosterone in men, which is accompanied by androgen deficiency symptoms and signs is called partial androgen deficiency in the aging male (PADAM), androgen deficiency in the aging male (ADAM), or aging-associated androgen deficiency (AAAD).

4.2.2 Estrogens

Skin is one of the peripheral endocrine organs also responsible for the estrogen production in both genders. After immunohistochemical examination it was shown that aromatase, which catalyzes three consecutive hydroxylation reactions converting C-19 androgens to C-18 estrogens is expressed in the outer rooth sheath of anagen and terminal hair follicles, in sebaceous glands, in keratinocytes and fibroblasts (Rossouw et al., 2002).

Estrogens are derived from androstenedione and testosterone. Estrone is obtained from androstenedione and estradiol from testosterone. Estradiol has approximately ten times greater estrogenic activity than estrone and is bound in plasma by SHBG and albumin.

In women, estrogen levels decline rapidly at menopause as a result of the loss of ovarian follicles (Smyth et al., 1994), whereas in men the levels of estrogens remain unchanged. The fall-off in ovarian production of estrogens tends to accelerate skin aging, bone and vascular aging. Low levels of estrogens have been also correlated with profound effects on various compartments of the skin (Castelo-Branco et al., 1996; Uitto, 1986). There is also evidence, that lack of estrogens plays an important role in the osteoporosis in men (Eastell and Lambert, 2002) and systemic application of phyto-estrogens has been shown to reduce the clinical symptoms of the benign prostate hypertrophy (Gambacciani et al., 2001). Furthermore, decline of estrogens has been associated with alterations of brain functions such as cognition, learning and memory, neuroprotection, mood and affective behavior, and locomotor activity (Chakraborty and Gore, 2004).

5. *In vitro* model of human hormonal aging

Despite the progress in elucidating the role of hormones in the aging process of model organisms like flies, nematodes, and mammalians, a systematic approach for the exploration of hormonal aging in humans has not yet been undertaken.

Within the scope of the Explorative Project 'Genetic aetiology of human longevity' supported by the German National Genome Research Network 2 (NGFN-2) an *in vitro* model of human hormonal aging has been developed. Human SZ95 sebocytes were maintained under a hormone-substituted environment consisting of GH, IGF-I, estrogens, androgens and progesterone in concentrations corresponding to those circulating in 20- and in 60-year-old women (Makrantonaki et al., 2006). Not only the effects of one hormone as a single agent were observed but the effects of a group of hormones in an effort to represent as much as possible the *in vivo* conditions and to give an insight into the resulting cellular and molecular processes, also altered by interactions of the corresponding hormonal signaling pathways. Upon 15,529 tested genes 899 genes showed a differential expression between SZ95 sebocytes under the 20- and 60-year-old hormone mixture, respectively. This result demonstrates that hormones interact in a complex fashion, and changes in their circulating blood levels may significantly alter the development of cells by regulating their transcriptome. Among the 899 genes, genes were regulated, which are involved in cholesterol biosynthesis (*DHCR7, FDFT1, MVD, PMVK* and *LYPLA1*) and fatty acid metabolism (*HADA2, ACOX3, GCDH, NQO1*), eicosanoid biosynthesis, synthesis of extracellular matrix (e.g. *MFAP1, ITGB2, NTN4, MMP2* and *NECL*), stress response (e.g. *TXN2* and *ESTs*), chaperone activity (e.g. *CCT2* and *HSP27/HSPB1*), ubiquitine-proteosome activity (*UBE2G2, UBE2M,* and *UBE3A*), nucleotides and ATP metabolism (e.g. *ACVR1, ALS2CR2* and *CAMK2G*) and DNA repair mechanisms (e.g. *VCP* and *TREX1*).

Many of these biological processes have been already implicated in the generation of aging. A global reduction in skin surface lipids and a profound abnormality in cholesterol synthesis have been described in aged skin (Elias and Ghadially, 2002). In addition, alterations of the extracellular matrix components have been correlated with premature skin aging (Labat-Robert, 2003).

Oxygen radicals are increasingly considered as the major contributors to aging and the protective mechanism against oxidative stress is observed as an indispensable function (Barja, 2004). It has been shown that oxygen radicals levels rise and anti-oxidant activity declines with advancing age (Hu et al., 2000; Kohen, 1999). A disturbed stress response during aging is also known to be associated with a defect in proteolytic sytems such as lysosomal activity and ubiquitine-proteosome pathway in somatic cells (Cuervo and Dice, 1998). As a consequence altered proteins cannot be eliminated resulting in accumulation of misfolded and damaged proteins in the cells.

With age the nuclear and mitochondrial genome are more susceptible to DNA damage. One of the major reasons are the impaired DNA repair mechanisms which have been described in several studies and have been associated with the initiation of age-associated diseases and progeroid syndromes (Hasty et al., 2003; Lieber and Karanjawala, 2004). Furthermore, dysregulated immune and inflammatory responses have been already documented both in humans and mouse with increasing age (Badawi et al., 2004; Kovaiou et al., 2007).

In order to identify pathways that were altered due to hormonal induction, we computed a statistical analysis of entire pathways, with pathway annotation taken from the KEGG database. The most significantly altered signaling pathway identified was that of transforming growth factor-β (TGF-β) (Makrantonaki et al., 2006). The TGF-β signaling pathway is involved in different biological processes during embryonic development and plays a distinct role in adult organisms in tissue homeostasis (Massague, 1998). In human skin, the TGF-β signaling pathway has been shown to regulate many cellular processes, such as differentiation and proliferation of keratinocytes and fibroblasts and the synthesis of extracellular matrix proteins (Massague et al., 1983). In addition, a disturbed function of this cascade has been associated with tumorigenesis, i.e. in pancreatic, prostate, intestine, breast, and uterine cancer (Levy and Hill, 2006). A differential expression of TGF-β isoforms, activins, BMPs, MADHs/SMADs, and other components of the TGF-β signaling cascade was shown at SZ95 sebocytes under the 60-year-old hormone mixture. These data suggest that age-specific hormonal changes are likely to play a determining role not only in the healthy aging process, but also in tumorigenesis.

Interestingly, genes expressed in signaling pathways operative in age-associated diseases such as Huntington's disease (Luthi-Carter et al., 2002; Sipione et al., 2002), dentatorubral-pallidoluysian atrophy (Luthi-Carter et al., 2002), and amyotrophic lateral sclerosis (Jiang et al., 2005) were also identified. According to these results, a disturbed hormone status may act a part into the generation of neurodegenerative diseases.

6. Concluding remarks

As the modern population is rapidly greying, the consequences of aging have begun to gain particular attention. The main focus of the aging research is the better understanding of the mechanisms involved and the prevention of age-associated diseases by early identification of individual molecular risk profiles. Recent data suggest that skin represents an adequate model for aging research and that changes of hormone levels occurring with age play a major role in the generation of aging. Thus, these results could be a basis for an integrated and interdisciplinary approach to the analysis of aging.

Acknowledgements

Legends to the figures

Fig. 1 Human immortalized SZ95 sebaceous gland cell line. Confluent culture under light microscopy [A], electron microscopy, metabolically active SZ95 sebocytes with abundant endoplasmatic reticulum and numerous intracellular droplets of neutral lipids (arrows) [B], fluorescence microscopy, double labeled SZ95 sebocytes with the nuclear dye DAPI (blue) the lipid dye nile red (yellow-red) detecting abundant intracellular, mostly perinuclear lipid droplets [C; courtesy of S. Schagen, Ph.D. and Pentapharm Ltd., Basel, Switzerland] and abundant nuclear labeling with an antibody against the human androgen receptor ((N-20; Santa Cruz, Heidelberg, Germany) [D]

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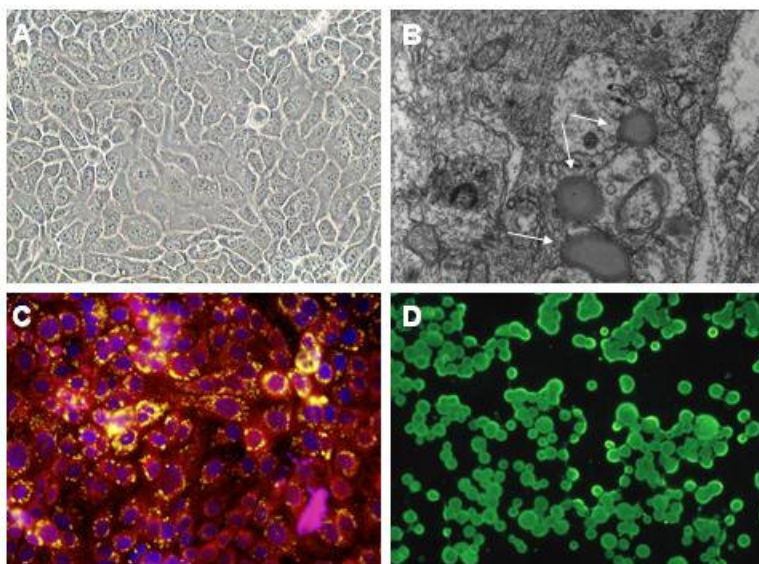
Fig. 1