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Review

The hallmarks of fibroblast ageing

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ABSTRACT

Ageing is influenced by the *intrinsic disposition* delineating what is maximally possible and *extrinsic factors* determining how that frame is individually exploited. Intrinsic and extrinsic ageing processes act on the dermis, a post-mitotic skin compartment mainly consisting of extracellular matrix and fibroblasts. Dermal fibroblasts are long-lived cells constantly undergoing damage accumulation and (mal-)adaptation, thus constituting a powerful indicator system for human ageing. Here, we use the systematic of ubiquitous hallmarks of ageing (Lopez-Otin et al., 2013, *Cell* 153) to categorise the available knowledge regarding dermal fibroblast ageing. We discriminate processes inducible in culture from phenomena apparent in skin biopsies or primary cells from old donors, coming to the following conclusions: (i) Fibroblasts aged in culture exhibit most of the established, ubiquitous hallmarks of ageing. (ii) Not all of these hallmarks have been detected or investigated in fibroblasts aged *in situ* (in the skin). (iii) Dermal fibroblasts aged *in vitro* and *in vivo* exhibit additional features currently not considered ubiquitous hallmarks of ageing. (iv) The ageing process of dermal fibroblasts in their physiological tissue environment has only been partially elucidated, although these cells have been a preferred model of cell ageing *in vitro* for decades.

1. The crucial role of the dermal fibroblast in extrinsic skin ageing

Ageing is a somatic process entailing the progressive loss of maximal function, stress resistance, metabolic efficiency and adaptive potential. Ageing is associated with various diseases and delimits health-span in the absence of disease. Many signalling pathways, gene networks and organelle functions become altered in the course of normal ageing and/or have an impact on ageing trajectories upon genetic or pharmacological manipulation. Ageing can thus be considered a *syndrome*, in which various chronic molecular processes converge on a common, rather uniform set of

Abbreviations: 6-4PP, 6-4 pyrimidine–pyrimidone photoproducts; AHR, Aryl hydrocarbon receptor; AP-1, heterodimeric transcription factor belonging to the c-fos/c-jun families; ARNT, arylhydrocarbon receptor nuclear translocator; BER, base excision repair; CCN1, cysteine-rich, angiogenic inducer protein 61 also known as CYR61; CPD, cyclobutane pyrimidine dimers; CYP1A1, Cytochrome P450, family 1, member A1; DNA SCARS, DNA structures sustaining damage-induced senescence and growth arrest and inflammatory cytokine secretion; DSB, DNA double strand break; ECM, extracellular matrix; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ETC, electron transport chain; γH2AX, histone H2AX phosphorylated at serine 171; HA, hyaluronic acid; HAS, hyaluronic acid synthase; HGPS, Hutchinson-Gilford progeroid syndrome; HR, homologous recombination; IGF, insulin like growth factor; MMP, matrix metalloproteinase; MMR, mismatch repair; mtDNA, mitochondrial DNA; NER, nucleotide excision repair; NHEJ, non homologous end joining; NOX, NADPH oxidase; PAH, polycyclic aromatic hydrocarbon; PTPN6, tyrosine protein phosphatases non-receptor type 6 also known as SHP-1; RB, retinoblastoma susceptibility gene; ROS, reactive oxygen species; SAHF, senescence-associated heterochromatin foci; SASP, senescence-associated secretory phenotype; SHP-1, Src homology domain-containing phosphatase-1 also known as PTPN6; SSA, single strand annealing; TGFβ, transforming growth factor beta; TIMP1, metallopeptidase inhibitor 1; UV, ultraviolet light; UVA, long wavelength ultraviolet light; UVB, short wavelength ultraviolet light; WNT, pathway regulated by ligands of *Wingless* and *Int-1*.

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phenotypic changes denominated *frailty* (Kirkwood and Melov, 2011). Frailty is a clinical state in which there is an increase in an individual's vulnerability for developing increased dependency and/or mortality when exposed to a stressor. Frailty can occur as a result of ageing or diseases or both and is increasingly perceived as an important medical syndrome that should be diagnosed and subjected to medical treatment and prevention (Clegg and Young, 2011; Morley et al., 2013; Ruiz et al., 2012).

Ageing of different cell types, tissues and organs is associated with distinct patterns of altered gene expression and tissue function (Glass et al., 2013; Harries et al., 2011; Rodwell et al., 2004; Sundberg et al., 2011; Welle et al., 2003; Zahn et al., 2007, 2006), whereas isolated genetic defects in ageing-relevant pathways give rise to segmental, tissue-selective ageing phenotypes (Kipling et al., 2004). For most tissues it remains, however, unclear, which age-related alterations play a leading and causative role in the ageing process, and which ones are just epiphenomena.

It is generally accepted that ageing has two principal determinants: The *intrinsic disposition* (genetic make up, somatic capacity and composition) delineating what is maximally possible, and *extrinsic factors* (life style, nutrition, environmental influences) determining how the pre-set frame of opportunity is exploited in the course of the individual ageing trajectory. Extrinsic ageing is thus closely related to the quality, with which life-supportive tasks are adjusted to the environmental condition (Brink et al., 2009), and inseparably linked to mechanisms of stress response and adaptation. Insufficient adaptation and/or collateral maladaptation due to trade-offs with other probiotic or species-protective processes (*e.g.* fertility or tumour suppression) are thought to be major principles of extrinsic ageing (Campisi, 2005; Kirkwood, 2005; Kirkwood and Melov, 2011; Martins et al., 2011).

Human skin is particularly suited for discriminating extrinsic and intrinsic ageing processes, because the entire organ is subjected to intrinsic ageing, whereas extrinsic ageing is restricted to sites exposed to environmental factors such as sun light. Moreover, intrinsic and extrinsic skin ageing processes appear to involve different compartments of the organ. The upper compartment, the *epidermis*, is a stratified squamous epithelium, which provides the essential protective barrier. To maintain tissue integrity it continuously regenerates and plays a major role also in wound healing. This highly proliferative cell population has established many defence mechanisms. Most notably, the epidermis is able to eliminate extrinsic macromolecular damage by constant shedding of terminally differentiated keratinocytes, thus precluding damage accumulation and rendering the tissue compartment comparatively resistant to environmental stress. Age-related thinning of the epidermis and the associated decline of barrier function and wound healing capacity is ubiquitous and reflects an intrinsic process. Such alterations are commonly related to progressive dysfunction of stem cells. However, across an average mouse's life time, there was no measurable loss in the physiologic functional capacity of epidermal stem cells (Stern and Bickenbach, 2007) and their abundance, organisation, and proliferation did not change notably (Giangreco et al., 2008), prompting the notion that at least in mouse, epidermal stem cells are resistant to ageing.

In contrast, the lower compartment of the skin, the *dermis*, is a post-mitotic tissue relying on adaptation and damage repair for homeostasis. It mainly consists of extracellular matrix (ECM), which determines the structural and mechanical properties of the skin. The dermal matrix is made and controlled by fibroblasts, which scarcely proliferate and therefore are much less able to remove extrinsic damage by cell shedding. Dermal fibroblasts thus constitute a long-lived cell population undergoing continuous damage accumulation and – adaptation, processes typically associated with extrinsic ageing. In keeping with this notion, most phenotypic changes in extrinsically aged skin such as wrinkle formation are linked to dysfunctions of dermal fibroblasts and corresponding remodelling of the dermal ECM (Boukamp, 2005; Parrinello et al., 2005). These characteristics have made the dermal fibroblast a preferred model for the study of extrinsic ageing processes at the cellular level.

The major exogenous determinants of human skin ageing are photo-oxidative stress (mostly due to sun light/UV radiation) and the toxicity of polycyclic aromatic hydrocarbons (PAHs) contained in cigarette smoke and industrial waste (Daniell, 1971; Grady and Ernster, 1992; Krutmann et al., 2012; Schroeder et al., 2006). Chronically degenerative processes promoted by these noxae converge on the dermis and are associated with (mal-)adaptive stress-responses of dermal fibroblast, which in concert with ECM interactions and signals received from the epidermal compartment are thought to bring about the majority of extrinsic skin ageing phenomena (Boukamp, 2005; Parrinello et al., 2005). Here, we have used and adapted the recently proposed systematic of ubiquitous "*Hallmarks of Ageing*" (Lopez-Otin et al., 2013) to review and categorise what is known about these extrinsic ageing processes manifesting in the dermal fibroblast.

2. The hallmarks of dermal fibroblast ageing

2.1. DNA damage, genome instability

2.1.1. Irreparable double-strand breaks (DSB) and enhanced recombination

Human fibroblasts subjected to replicative or stress-induced premature senescence *in vitro* (see Section 2.7) accumulate γH2AX foci that contain DNA double strand break (DSB) repair complexes. Similar foci were also observed in various tissues (not including skin) of aged mice. It was suggested that these foci represent unrepaired DSB (Sedelnikova et al., 2004) and reflect an age-related increase in DNA damage and structural chromosomal aberrations, which was also observed in human blood lymphocytes from aged humans (Bolognesi et al., 1997; Fenech, 1998; Garm et al., 2013; Mayer et al., 1989; Singh et al., 1990), though not yet seen in hematopoietic stem cells (Wagner et al., 2009). The actual number of DSBs that accumulate in senescent cells could be far larger than deduced from γH2AX foci, because senescent fibroblasts are subjected to enhanced hetero-chromatinisation (Kreiling et al., 2011; Narita et al., 2003), and in heterochromatin DSB are not labelled by γH2AX and much slower repaired (Cann and Dellaire, 2011). There are indeed indications that the capacity of DSB-repair decreases with age (see Section 2.1.4). Furthermore, the observed γH2AX foci could reflect an increase in DNA segments with chromatin alterations reinforcing senescence (DNA SCARS), which consist of persistent γH2AX accumulation at PML bodies thought to support altered expression of secreted proteins in senescent fibroblasts (Rodier et al., 2011). Fibroblasts of patients with the hereditary progeroid laminopathy Hutchinson-Gilford Syndrome (HGPS) accumulate irreparable DSBs induced by reactive oxygen species (ROS) (Richards et al., 2011), and a characteristic of senescent fibroblasts is an abnormal (globulated) nuclear structure caused by changes in lamin A localisation (Shumaker et al., 2006). Such lamin A-dependent nuclear defects are also found in foreskin fibroblasts from very old normal human donors (Scaffidi and Misteli, 2006). Moreover, the genomes of fibroblasts subjected to replicative senescence *in vitro* undergo global epigenetic changes leading to the activation of transposable elements, which may be another plausible cause for increases in chromosome damage and recombination (De Cecco et al., 2013). In summary, these findings suggest that aged fibroblasts could acquire a hyper-recombinatorial state similar to the one associated with chronological ageing in yeast (McMurray and Gottschling,

2003), which is possibly triggered by an increase in DSB that are induced by ROS or senescence-associated epigenetic transposon activation, and inefficiently repaired due to lamin A dysfunction. However, whether this is indeed a ubiquitous phenomenon remains elusive (Figueroa et al., 2000).

2.1.2. Chromosome aberrations

There is a large body of evidence that base line frequencies of structural and numerical chromosome aberrations increase with age in peripheral nucleated blood cells and buccal epithelia (Bolognesi et al., 1997; Fenech, 1998; Jacobs et al., 2012; Ramsey et al., 1995; Thomas and Fenech, 2008), but also in hepatocytes (Curtis and Crowley, 1963), vascular smooth muscle cells (Jones and Ravid, 2004) and human brain cells (Faggioli et al., 2011; Fischer et al., 2012). In liver and brain, aneuploidisation is thought to be an orchestrated developmental process and not an indication of chromosomal instability (Ricke and van Deursen, 2013). In blood cells, the age-related increase in aneuploidy is thought to reflect chromosomal instability. It is associated with decreased levels of vitamins and increased levels of homo-cystein, suggesting that it is a consequence of age-associated malnutrition and insufficient folate intake (Fenech, 2007). A causal role of aneuploidy in mammalian ageing is suggested by a study of mice with low levels of the spindle assembly checkpoint protein BubR1, which develop progressive aneuploidy along with a variety of progeroid features, including short lifespan, cachectic dwarfism, lordokyphosis, cataracts, loss of subcutaneous fat and impaired wound healing (Baker et al., 2004). The latter two features may argue for a role of aneuploidy in skin ageing, but the occurrence of chromosomal instability in the ageing process of human fibroblasts is still controversial. While human fibroblasts subjected to replicative senescence *in vitro* exhibit increased chromosomal instability associated with de-methylation of satellite DNA (Suzuki et al., 2002), centrosome aberrations (Ohshima, 2012), and a decline in rapid DSB repair pathways (Seluanov et al., 2004), numerical and structural chromosomal abnormalities were rare (Figueroa et al., 2000). Furthermore, since dermal fibroblasts only rarely proliferate *in vivo*, it remains to be seen whether chromosomal instability observed in replicative fibroblast senescence *in vitro* reflects the situation in dermal fibroblasts in aged human skin. This said, the site of skin may be of particular importance, as structural chromosomal aberrations are long known to be induced by UV light (Popescu et al., 1986).

2.1.3. Accumulation of oxidative DNA damage and DNA photo-adducts

Results of more than 50 studies measuring the level of oxidative damaged nuclear DNA in organs of animals at various ages provide compelling evidence for ageing-associated accumulation of oxidative damaged DNA in organs with limited cell proliferation, such as liver, kidney, brain, heart, pancreas, and muscle (Moller et al., 2010). There seems to be no conclusive evidence of accumulation of oxidative damage in nuclear DNA of skin cells, although skin is particularly exposed to extrinsic photo-oxidative stress. It is well documented that mice defective in mechanisms involved in the repair of oxidative DNA damage age faster and live shorter (Hasty et al., 2003). However, it is still debated whether this effect is due to the accumulation of DNA damage *per se* or other effects such as persistent transcription blocking (Diderich et al., 2011; Garinis et al., 2009; Lans and Hoeijmakers, 2012). In DNA repair defective mice the level of oxidative DNA lesions (which is marginal to start with) is not enhanced and does not increase with age, suggesting that factors other than DNA damage (*e.g.* cellular responses to DNA damage) are involved in ageing process of these animals (Maslov et al., 2013). Photo-oxidative stress is well documented to play an established role in ageing-associated attrition of mitochondrial DNA (Krutmann and Schroeder, 2009) and protein oxidation in skin fibroblasts (Sander et al., 2002). Nevertheless conclusive evidence of accumulation of oxidative damage in nuclear DNA of dermal fibroblasts or other skin cells is still elusive.

2.1.4. Decline or altered usage of DNA repair systems

Nucleotide excision repair (NER) is the pathway that removes adducts and intra-strand crosslinks from DNA. Multiple mutations in NER genes result in dramatically accelerated ageing phenotypes in mouse and man (Hoeijmakers, 2009). The extent of repair deficiency and the acceleration of specific progeroid symptoms including the skin are closely correlated, and these effects are only seen for the part of the NER machinery that is involved in transcription-coupled repair and protects from cytotoxic as well as cytostatic effects of DNA damage (Diderich et al., 2011). NER capacity is subjected to circadian oscillation (Gaddameedhi et al., 2011) (see Section 2.11) and declines with increasing age in various cells and tissues (Garm et al., 2013; Gorbunova et al., 2007). NER plays a major role in the defence of skin against photo-stress as it removes the major photoproducts cyclobutane pyrimidine dimers (CPD) and 6-4 pyrimidine-pyrimidone photoproducts (6-4PP) from DNA (Moriwaki and Takahashi, 2008). Several studies show that in the epidermis the capacity to remove CPDs and 6-4PPs goes down with increasing donor age (Yamada et al., 2006). In contrast, in dermal fibroblasts, overall NER activity declines with donor age but the ability to remove CPDs and 6-4PPs stays the same. Gene expression studies suggest that only NER-components acting subsequent to the damage excision step are affected by age (Takahashi et al., 2005).

Base excision repair (BER) is the major pathway repairing oxidised DNA bases, most notably 8-oxoguanine and 7,8-dihydro-8-oxoadenine, both induced in skin cells by solar light (Moriwaki and Takahashi, 2008). BER is the major repair mechanism for oxidative damage also of mtDNA, which increases with age in various tissues including dermal fibroblast suggesting that mitochondrial BER possibly becomes insufficient during ageing (Druzhyna et al., 2008). Nuclear BER also undergoes age-related changes in various mouse tissues and cell models other than skin (Gorbunova et al., 2007). It is not known whether it declines in skin cells with increasing chronological donor age. However, there are several reports on an acute loss of function of various components of the BER machinery following exposure of dermal fibroblasts to ultraviolet light or oxidising substances, which could indicate a maladaptive response contributing to extrinsic skin ageing (Moriwaki and Takahashi, 2008).

DNA mismatch repair (MMR) removes miss-paired bases resulting from replication errors, recombination between imperfectly matched sequences and deamination of 5-methyl-cytosine. MMR is thought to also play a role in the repair of oxidative DNA damage (Skinner and Turker, 2005). MMR mutations in tumours are associated with a substantial destabilisation of microsatellites (Karran, 1996). Although humans defective in MMR have no progeroid phenotype (Hsieh and Yamane, 2008), microsatellite instability increases with ageing in human peripheral blood cells (Neri et al., 2005) and T-lymphocytes (Krichevsky et al., 2004), suggesting that a decline in MMR could be involved in the ageing process of these proliferating cells. It is not known whether MMR also declines during *in vivo* ageing in other, mostly non-proliferating human cell types, such as the dermal fibroblast.

DNA inter-strand crosslinks (ICL) are repaired by a group of proteins that belong to the complementation group of Fanconi anaemia (FA). FA is considered a segmental progeroid syndrome of ageing-associated progressive kidney failure. In mice, deletion of various ICL-repair genes provokes genome instability and accelerated ageing in various organs (Lans and Hoeijmakers, 2012).

However, these segmental progeroid phenotypes do not encompass accelerated skin ageing, and in human skin evidence for age-associated alterations of ICL-repair is lacking.

DSB repair is supported by two complementary systems: non-homologous end joining (NHEJ) and homologous recombination (HR). HR is a slow and precise mechanism that requires the sister chromatid as a template, and, therefore, is restricted to the G2/M phase and absent in fibroblasts locked in replicative senescence (Mao et al., 2008). NHEJ is a fast mechanism that does not require a template and appears to be the dominant DSB-repair mechanism in mammals. DSBs situated between two direct repeats can also be repaired by single-strand annealing (SSA), a highly mutagenic mechanism that deletes the sequence between the repeats. Mouse models and human syndromes deficient in various components of NHEJ and SSA exhibit accelerated ageing, but it is unclear how NHEJ-deficiency causes the progeroid phenotype (Lombard et al., 2005). It has been observed that NHEJ becomes less efficient and precise as human fibroblasts enter replicative senescence *in vitro* (Seluanov et al., 2004). NHEJ activity is also reduced in brains of old rats and Alzheimer disease patients, and declines in peripheral human lymphocytes with donor age (Garm et al., 2013; Gorbunova et al., 2007). It is not clear why NHEJ activity declines with age since the expression of the major NHEJ genes does not change. One possible explanation is that NHEJ complexes get increasingly sequestered at irreparable DSB (Sedelnikova et al., 2004) or damaged telomeres (Fumagalli et al., 2012). In male germ line cells of *drosophila*, the age-related decline of NHEJ and SSA is matched by a corresponding increase in HR (Preston et al., 2006), but it is unclear whether this is also the case in aged mammalian cells.

2.2. Telomere shortening and irreparable DNA damage at telomeres

Most of the current mechanistic understanding of cellular senescence of skin fibroblasts was obtained in cells having acquired senescence due to telomere shortening induced by continuous replication, *i.e.* replicative senescence (Hayflick, 1980; Hayflick and Moorhead, 1961). The same endpoint is also reached at by activation of ras oncogene associated with the accumulation of p53 and $p16^{INK4a}$ (oncogene-induced premature senescence) (Serrano et al., 1997), which is considered a powerful tumour suppressor mechanism (Bartek et al., 2007; Ramsey and Sharpless, 2006). A different type of cellular senescence can be induced in human diploid fibroblasts by oxidative stress or suboptimal cell culture conditions (*i.e.* stress induced premature senescence, SIPS), which is independent of telomere shortening and has a different proteomic profile (Dierick et al., 2002; Toussaint et al., 2002, 2000). Replicative senescence may not be a valid model for fibroblast ageing *in vivo*, since fibroblasts rarely proliferate and age-dependent telomere loss as measured in skin from different-age donors is minimal (Krunic et al., 2009 and references therein). In line with that, the replicative lifespan of skin fibroblasts analysed *ex vivo* does not correlate with chronological age, morbidity or mortality of the donor (Cristofalo et al., 1998; Maier et al., 2007). Furthermore, damage responses associated with telomere shortening were found in mitotic but not in post mitotic tissue of aged primates (Jeyapalan et al., 2007). Given that the dermal fibroblast is mostly a post mitotic cell, one may assume that replicative telomere shortening *per se* does not play role in dermal fibroblast ageing. However, this does not altogether exclude a role of telomere damage in extrinsic skin ageing. Extrinsic DNA strand breaks inflicted at the telomeres of dermal fibroblasts may be irreparable, providing a persistent DNA damage signal that induces cell cycle arrest in a similar manner as telomere shortening. While described for ionising radiation (Fumagalli et al., 2012), similar UV-dependent effects have yet to be shown. It is suggested that telomeres could be particularly susceptible to oxidative stress-induced damage (Gilchrest et al., 2009), which is repaired only slowly (Fumagalli et al., 2012; Von Zglinicki, 2003; von Zglinicki et al., 2005). Thus it was reported that deficiency in the glyoxylase Nth1 involved in repair of oxidative DNA lesions enhances telomere fragility in mice (Vallabhaneni et al., 2013). Moreover DNA damage foci, which to some extent were co-localised with telomeres, increased in the nuclei of dermal fibroblasts in aged primate skin (Herbig et al., 2006). These findings suggest that cellular senescence induced by DNA stress could be due to the persistence of irreparable damage in telomeric DNA (Gilchrest et al., 2009). Interestingly, several features of DNA-damage induced senescence have recently been observed to accumulate in postmitotic neurons of old mice (Jurk et al., 2012). This phenotype was aggravated in $TERC^{-/-}$ mice suggesting the involvement of telomere dysfunction and to some extent supporting the idea that DNA damage can persist in telomeric DNA of non-replicative cells. Regarding skin, we could identify a corresponding scenario only in the epidermis (keratinocytes) of skin from elderly human donors (Leufke et al., 2013). Therefore, a role for fibroblast senescence induced by telomere dysfunction or persistent damage in telomeric DNA in human skin ageing still remains to be shown. Irrespective of that, a self-amplifying cycle between mitochondrial and telomeric DNA damage has been proposed that possibly interlinks the dysfunction of the two genomes during cellular senescence (Passos et al., 2007).

2.3. Disruption of post-transcriptional pre-mRNA processing

It has been known for some time that the expression patterns of alternatively spliced mRNA and protein isoforms translated thereof change during development and ageing. A long standing example is alternative splicing of fibronectin, which is tightly controlled during development (Chauhan et al., 2004), and significantly changes in aged tissues of rat (Pagani et al., 1991) or during *in vitro* ageing of primary cell cultures obtained from various human tissues (Magnuson et al., 1991). Similar age-related changes of mRNA splicing patterns have been observed for the five functionally different isoforms of the gamma-subunit of the nicotinic acetylcholine receptor (Azim et al., 2012), and methionine synthase isoforms in human cerebral cortex (Muratore et al., 2013). It has also been postulated that τ protein, a microtubule-associated protein involved in ageing-associated neurodegenerative diseases including Alzheimer's disease, should be considered as a family of multiple isoforms rising by alternative mRNA splicing (Avila et al., 2013). In addition, alternative mRNA-splicing possibly also contributes to age-related changes in IGF-1 function (Oberbauer, 2013).

Genome wide analyses of murine (Dillman et al., 2013) and human brain (Mazin et al., 2013; Tollervey et al., 2011) tissues as well as human peripheral blood leukocytes (Harries et al., 2011) have revealed that many genes are affected by age-associated alterations in pre-mRNA splicing. These changes follow discrete patterns that can be linked to organ-specific gene functions and corresponding changes in the expression profiles of splicing factors. Age-related pre-mRNA splicing changes in human brain are consistent with increased polypyrimidine tract binding protein (PTB)-dependent splicing activity (Mazin et al., 2013; Tollervey et al., 2011), and have a high potential to target the transcripts to nonsense-mediated decay (Mazin et al., 2013). Interestingly, similar global patterns of altered pre-mRNA splicing as observed in aged brain are also present in brain affected by age-related neurodegenerative diseases (Tollervey et al., 2011). In peripheral human blood leukocytes, the most significant age-related disruptions of splicing patterns were found in transcription control pathways involved in proliferation control and stress response. These changes were again associated with significant up-regulation of genes involved in posttranscriptional procession of pre-mRNA

(Harries et al., 2011). In summary, these findings suggest ubiquitous ageing-related disruption of pre-mRNA splicing, which is associated with expressional changes in the splicing machinery.

There is compelling evidence that altered pre-mRNA splicing could also play a role in ageing of dermal fibroblasts. It has been observed that replicative and stress-induced senescence of mammary fibroblasts in culture is associated with the expression of a novel splice variant of STAC, a gene encoding for a protein involved in protein kinase C signalling *via* domains predicted to be lacking in the senescence-associated splice variant (Hardy et al., 2005). Moreover, alternative splicing of lamin A pre-mRNA is possibly involved in skin ageing (McClintock et al., 2007; Takeuchi and Runger, 2013). Most cases of the hereditary Hutchinson-Gilford Progeria Syndrome (HGPS) are due to a silent mutation (G608G) activating a cryptic splice site in exon 11 of the LMNA gene (Goldman et al., 2004), which leads to an internal deletion of 150 base pairs and thus to the expression of an alternative form of lamin A protein called progerin. Progerin not only lacks a farnesylation site crucial for the appropriate distribution of lamin A between nuclear lamina and nucleoplasm but also negatively effects the nuclear architecture and epigenetic chromatin control (Shumaker et al., 2006), and induces accumulation of irreparable oxidative DNA damage (Richards et al., 2011). In mice heterozygous for the HGPS-associated LMNA mutation, expression levels of progerin relative to wild type lamin A are directly correlated to life span abbreviation (Lopez-Mejia et al., 2011). The cellular HGPR phenotype can be rescued or ameliorated by antioxidants (Richards et al., 2011) and drugs interfering with protein farnesylation or histone acetylation (Columbaro et al., 2005). The onset of accelerated ageing in HPGS mouse models can be delayed by systemic application of antisense oligonucleotides (Osorio et al., 2011), farnesyl-transferase inhibitors (Yang et al., 2006), or combinations of statins and amino-bisphosphonates (Varela et al., 2008). Extremely low level usage of the cryptic splice site in absence of any mutation is found in foreskin fibroblasts from old normal human donors, which also exhibit a HGPS-like cellular phenotype (Scaffidi and Misteli, 2006). In aged human skin progerin accumulates in a subset of dermal fibroblasts and a few terminally differentiated keratinocytes (McClintock et al., 2007). Accumulation of progerin due to aberrant splice site usage and alternative splicing of LMNA mRNA in dermal fibroblasts is enhanced by irradiation with long wavelength UV light *in vitro*, in particular in fibroblasts subjected to extended culture splitting (Takeuchi and Runger, 2013). In line with this observation, production of progerin in normal human fibroblasts goes hand in hand with progressive telomere shortening in the course of replicative senescence (Cao et al., 2011). Therefore, it may not play a role for fibroblast ageing *in situ*, *as* fibroblasts rarely proliferate and do not exhibit age-related telomere shortening *in vivo* (see Section 2.2). In summary, the available data suggest (i) that aberrant splicing of LMNA pre-mRNA could play a role in photo-ageing of the skin, (ii) that other pre-mRNAs could be subjected to aberrant splicing in aged fibroblasts as well, and (iii) that genes and mechanisms involved in posttranscriptional procession of pre-mRNA could be important targets of extrinsic ageing.

2.4. Epigenetic alterations

Fibroblasts subjected to replicative senescence *in vitro* exhibit a loss of "open" regulatory genome regions (euchromatin) in promoters and enhancers of active genes, while the accessibility of gene poor, heterochromatic regions increases, which is accompanied by activation of major retrotransposon classes (ALU, SVA, L1) and enhanced transcription of microsatellites (De Cecco et al., 2013). It is therefore conceivable that activation of retrotransposons could contribute to age-related increases in DSB and chromosome instability. However, it is not known whether these effects also occur when fibroblasts age *in situ*. UVA- and UVB-exposure of keratinocytes *in vitro* induces hypermethylation and histone modifications that play a role in silencing of tumour suppressor genes (Cip1/p21/p16^{INK4a}) relevant for photo-carcinogenesis (Chen et al., 2012; Katiyar et al., 2012). DNA hypermethylation of the *CDKN2A/B* locus was also observed in dermal fibroblasts from old donors (Koch et al., 2013). These alterations are potentially interlinked with repression/derepression of the polycomb complex that plays a crucial role in skin development, skin regeneration, and skin carcinogenesis (Zhang et al., 2012). Genome-wide analysis of DNA methylation patterns obtained in epidermal suction blisters and whole skin punch biopsies from sun-protected and sun-exposed skin areas of young and old individuals indicates that chronic sun exposure (*i.e.* extrinsic ageing) results in global DNA hypo-methylation, whereas intrinsic ageing (of sun-protected skin) may cause widespread hypermethylation of CpG islands. These alterations were consistent with a role of DNA methylation in the silencing of cell type specific genes such as KRT5 (Gronniger et al., 2010). Interestingly, induced pluripotent stem cells generated from human fibroblasts lose the characteristics inherited from the parent cells and adapt to very closely resemble embryonic stem cells, indicating that altered DNA methylation signatures acquired during the ageing process are reversible (Nishino et al., 2011). A recent comprehensive survey of age-related changes in DNA methylation in 51 healthy tissues and cell models as well as 6000 cancer samples of human and chimpanzee origin (Horvath, 2013) revealed that DNA methylation is closely correlated with chronological age and reliably reflects heritable and cancer-related ageing acceleration. A set of 353 CpG sites was derived from these studies, the methylation state of which provides a reliable "epigenetic clock" indicating the biological age of most healthy human tissues studied. However, dermal fibroblasts formed an exception as they were poorly calibrated to that DNA methylation clock, which probably reflects opposing influences of chronological and extrinsic ageing on DNA methylation in skin (Gronniger et al., 2010). In summary, the available studies indicate that DNA methylation plays a role in intrinsic and extrinsic ageing of fibroblasts, however the influence of the two components of the ageing process on DNA methylation is divergent and precludes an conclusive interpretation of global pattern changes. Moreover, it remains unclear whether epigenetic alterations are an epiphenomenon or play a causal role in chronological and adaptation/maladaptation processes contributing to ageing of the dermal fibroblast.

2.5. Loss of proteostasis

The term proteostasis (protein homeostasis) encompasses mechanisms that preserve the stability of correctly folded proteins (*i.e.* chaperone mediated folding and unfolding) (Hartl et al., 2011), proteolytic systems that remove damaged proteins (*i.e.* the ubiquitin-proteasome system, the autophagy-lysosomal system and secreted proteases controlling ECM composition) (Brennan et al., 2003; Cuervo et al., 2005; Mizushima et al., 2008; Rubinsztein et al., 2011; Tomaru et al., 2012), and mechanisms that regulate the aggregation of misfolded proteins (van Ham et al., 2010). Proteostasis is impaired in aged organisms and cells. Experimental enhancement/impairment of proteostasis prolongs/shortens lifespan and proper cell- and organ function (reviewed in Lopez-Otin et al., 2013) and induces/prevents cellular senescence. Regarding the ageing process of the dermal fibroblasts and its role in skin ageing, progressive decline of proteasome function and altered protease secretion play established roles, and it is probable that decreased activity of autophagy is also involved. These three topics will be addressed in more detail including the related topic

of ageing-associated alterations of ECM-proteoglycane homeostasis. We will not address the involvement of chaperone-mediated protein folding, which may play a role in fibroblast ageing but there are no data available.

2.5.1. Decline of the proteasome

Skin ageing is associated with the accumulation of oxidised proteins, which need to be removed by the proteasome (Bulteau et al., 2007) and/or chaperone-assisted autophagy (Cuervo et al., 2005). Proteasome function in dermal fibroblasts decreases during ageing as well as upon UV irradiation, which has been attributed to a decreased expression or inactivation of proteasome subunits and the accumulation of endogenous inhibitors (Bulteau et al., 2007). Partial proteasome inhibition in young human fibroblasts triggers accelerated senescence by a p53/Rb-dependent pathway (Chondrogianni et al., 2008), whereas stimulation of proteasome activity by poleuropein (Katsiki et al., 2007) or quercetin (Chondrogianni et al., 2010) increases their replicative life span in culture. These findings suggest that the age-related decline in proteasome activity plays a causal role in human fibroblast senescence and possibly skin ageing. The age-related loss of proteasome activity in the dermal fibroblast is causally interlinked with the induction of MMP-1 (Catalgol et al., 2009) and a decrease of cell respiration (Koziel et al., 2011). However, levels of proteasome activity appear to be highly heterogeneous in fibroblasts of old donors (Koziel et al., 2011). Moreover, in mouse NIH3T3-fibroblasts, expression of the main proteasome subunits is subject to circadian regulation (Menger et al., 2007). It cannot be excluded that this is also the case in human skin, a consideration so far not taken into account in any of the studies (see Section 2.11).

2.5.2. Decreased autophagy

Autophagy is considered as a protective factor in the chronic degeneration of various organs (Cuervo et al., 2005; Gottlieb and Mentzer, 2010; Jiang et al., 2010). Three different forms of autophagy are discriminated – micro-autophagy, chaperone-assisted autophagy, and macro-autophagy. Chaperone-assisted autophagy clearing defective macromolecules (Cuervo et al., 2005) and macro-autophagy removing dysfunctional mitochondria are believed to be crucial in ageing (Lemasters, 2005; Yen and Klionsky, 2008). In keeping with this notion, autophagy can be pharmacologically stimulated to prolong the lifespan of various model organisms (Madeo et al., 2010; Morselli et al., 2009). Some of the life-prolonging effects of rapamycin are also attributed to a release of the repressive effect of mTOR on autophagy (Rubinsztein et al., 2011). Up regulation of autophagy is instrumental in adaptive prolongation of lifespan in response to sub-lethal mitochondrial dysfunction in *Caenorhabditis elegans* (Schiavi et al., 2013). Up-regulation of autophagy is also thought to be essential for the prolongation of lifespan by caloric reduction (Bergamini et al., 2007). Similar to proteasome activity, autophagy is subjected to circadian regulation in certain tissues (see Section 2.11), and decreases with age in many organs including brain, heart, muscle and kidney (Rubinsztein et al., 2011). The age-related decrease in autophagy seems to a large part due to posttranslational mechanisms, such as acetylation/deacetylation of autophagy proteins (Morselli et al., 2011). Moreover, in *C. elegans* (mitohormetic) pro-longevity responses to mild respiratory chain dysfunction are dependent on a p53-mediated increase in autophagy (Schiavi et al., 2013). If similarly accounted for dermal fibroblasts, autophagy could be a factor that influences the ageing process and thus may provide a potential intervention target.

2.5.3. Induction of secreted matrix metalloproteinases

UV radiation of the dermal fibroblast stimulates expression and secretion of matrix metalloproteinases *via* upregulation of the transcription factors AP-1 and NF-kappa B (Fisher et al., 1996). Recent evidence suggests that in aged dermal fibroblasts enhanced expression of cysteine-rich protein 61 (CCN1) also plays a role in the up-regulation of matrix metalloproteinase secretion (Quan et al., 2011). Enhanced expression of matrix metalloproteinase 1 (MMP1) and decreased expression of the endogenous inhibitor TIMP-1 is the main cause for the degeneration of the extracellular matrix in extrinsically aged skin (Brennan et al., 2003). AP-1/MMP1 up-regulation seems to be the common end stage of various pathogenic pathways. Cutaneous AP-1/MMP1 up-regulation and TIMP-1 down-regulation are triggered by retrograde signalling in response to mtDNA mutations and electron transport chain (ETC) dysfunction (Krutmann and Schroeder, 2009), and by the activation of the cytoplasmic arylhydrocarbon receptor (AHR) by tobacco smoke or UV-induced conversion of tryptophan to the agonist 6-formylindolo[3,2-b]carbazole (Fritsche et al., 2007; Ono et al., 2013). The role of the dermal fibroblast in enhanced secretion of MMPs is under debate, since dermal fibroblasts appear refractory to AHR-signalling (Tigges et al., 2013), and many of the MMPs and TIMPs playing a role in skin ageing are secreted by keratinocytes rather than fibroblasts (Tandara and Mustoe, 2011). Degradation of the extracellular matrix (ECM) by MMP1 is enhanced and possibly autonomously perpetuated in aged skin, as collagenolytic fragments inhibit type I pro-collagen synthesis (Varani et al., 2001). Moreover, expression of the arylhydrocarbon receptor nuclear translocator gene ARNT, the down-stream effector linking AHR to AP-1, is subject to circadian regulation in mouse fibroblasts (Menger et al., 2007), suggesting that the susceptibility to noxae driving skin ageing and ECM degradation through the AHR/AP1/MMP1-pathway could oscillate in a circadian manner. It should finally be noted that enhanced secretion of metalloproteinases is a feature of the altered secretory phenotype acquired in conjunction with cellular senescence (see Section 2.7.4).

2.5.4. Down-regulation of hyaluronic acid synthases

Chronic UV irradiation not only induces breakdown of collagen but also decreases other molecules in the dermal extracellular matrix ECM, most notably hyaluronan, and alters the dermal proteoglycan composition (Knott et al., 2009; Koshiishi et al., 1999; Stern and Maibach, 2008). UV-induced loss of dermal hyaluronan is attributed to the inhibition of hyaluronan synthesis by down-regulation of hyaluronic acid synthases (HAS) 1, -2 or -3. Several mechanisms have been identified that mediate UV-mediated inhibition of HAS expression. Loss of transforming growth factor beta (TGFβ) signalling as a result of down-regulation of both TGFβ and TGFβ receptors occurs in response to chronic UVB-irradiation (Dai et al., 2007), which subsequently leads to a long-termed decrease in HAS1 and HAS2 expression. The degradation of the collagenous matrix is also directly linked to the loss of HA through down-regulation of HAS2 in the dermal fibroblast by signalling of by collagen fragments (Dai et al., 2007; Rock et al., 2011). Recently it was discovered that loss of HA is counteracted by estrogen through the release of epidermal growth factor from keratinocytes, which in turn strongly induces HAS3 and versican, a HA binding proteoglycan, in dermal fibroblasts (Rock et al., 2012). Estrogen thus protects the dermal hyaluronan/versican matrix from UV-induced rarefication, which may explain some of the gender-specific differences in human skin and the notable acceleration of skin ageing upon the onset of menopause (Makrantonaki et al., 2010). It is not known whether estrogen or other gender-related factors influence other mechanisms of fibroblast ageing. Little is known about changes of proteoglycan composition during extrinsic ageing. However, in some studies evidence for remodelling of the proteoglycan matrix has been proposed (Knott et al., 2009; Koshiishi et al., 1999; Rock et al., 2012). The changes in proteoglycan matrix may be very important functionally, because

certain proteoglycans such as decorin and biglycan affect structure and function of collagen networks and others interact with hyaluronan matrices.

2.6. Mitochondrial damage and dysfunction

2.6.1. Decline in respiratory capacity

Mitochondrial dysfunction and a decline in respiratory chain activity is a hallmark of ageing in many tissues (Nunnari and Suomalainen, 2012). On the other hand, reducing the expression of electron transport chain (ETC) subunits has pro-longevity effects from yeast to mammals (Copeland et al., 2009; Dell'agnello et al., 2007; Dillin et al., 2002; Durieux et al., 2011; Kirchman et al., 1999; Lapointe et al., 2009; Lee et al., 2002a; Rea et al., 2007). Thus, it is not clear which role cell respiration plays in the ageing process of the dermal fibroblast. Studies on foreskin fibroblasts subjected to replicative ageing *in vitro* showed an age-related decrease in the coupling of electron transport with phosphorylation, while the resulting increase in proton leakage was fully compensated by enhanced electron-transport activity (Hutter et al., 2004). This finding was partially recapitulated in fibroblast of mixed tissue provenience that were established from human donors of different ages, *i.e.* had aged *in vivo*. These cells showed a progressive decrease in respiration rate above a donor age of 40 years, and progressive uncoupling of oxidative phosphorylation above a donor age of 60 years, collectively suggesting that aerobic ATP-production becomes inefficient with age (Greco et al., 2003). In another study on dermal fibroblasts presumably from sun-protected skin areas, respiratory activity was not found significantly different between young, middle-aged, and old healthy donors. However, in the samples from old donors there was a significant decrease in mitochondrial membrane potential, accompanied by a significant increase in ROS levels (Koziel et al., 2011).

2.6.2. Imbalanced ROS levels

One key player in the ageing process seems to be oxidative stress, which is defined as the imbalance between the production of reactive oxygen species (ROS) and their elimination. ROS generation by redox systems inside mitochondria (ETC) and in the outer cell membrane (NADP-oxidases, NOXs) is balanced by anti-oxidative defence systems. The balance may be tipped as ETC function becomes defective in the course of ageing in various model organisms (Balaban et al., 2005). However, the mechanistic link between mitochondrial ETC function, ROS levels and cell ageing remains unclear. There is considerable evidence that respiratory capacity and mitochondrial ROS-production are independently modulated (Barja, 2007). For instance ROS derived from the mitochondrial ECR are not relevant for oxidative modifications of DNA in the mammalian cell nucleus (Hoffmann et al., 2004), and enhanced mtDNA mutations leading to ETC dysfunction do not affect ROS production (Trifunovic et al., 2005). This said, mitochondrial ROS-levels are increased in dermal fibroblasts from old donors (Koziel et al., 2011). The latter finding was made using super-oxide sensitive fluorescent dyes that do not allow for a clear distinction between intra-mitochondrial ROS levels (related exclusively to ETC-function) and cytosolic ROS levels (also influenced by extra-mitochondrial and extracellular ROS-generating systems) (Zielonka and Kalyanaraman, 2010). Therefore, it seems unclear whether the increase in ROS-levels observed in dermal fibroblasts from old donors is related to alterations of ETC-function observed in the same cells (Koziel et al., 2011). Genetic screens suggest that ageing in certain mouse tissues (heart, kidney, brain) is correlated to an altered balance of dissipative gene networks involved in energy metabolism and stabilising networks involved in anti-oxidative defence, prompting the conclusion that contrary to the free radical theory of ageing (Harman, 1956) it may not be the rate of ROS production but the maintenance of stable ROS levels that plays a role in ageing (Brink et al., 2009). The conserved existence of NADPH oxidases (NOXs), which appear to be dedicated to the production of ROS, suggests that ROS play a biological role when present at physiological concentrations. The NOX family consists of the 'classical' NADPH oxidases NOX1-NOX5 and the dual oxidases Duox1 and Duox2 (Bedard and Krause, 2007). Though presently still largely elusive, the contribution of NOXs to ageing-associated increases in ROS levels is probably underestimated (Krause, 2007) and largely unclear. On the one hand decrease expression of Nox4 induced a senescence-like state in human thyroid cells (Weyemi et al., 2012), on the other hand increased expression of NOX 4 restricts the replicative life span of endothelial cells (Lener et al., 2009). Moreover, upregulation of matrix metalloproteinases in photoaged skin depends on ROS produced by NOXs (Shin et al., 2008). Age-related increases in ROS-levels could also be due to a reduction of the anti-oxidative capacity, since it has been demonstrated that glutathione and thioredoxin-1 systems are inactivated in dermal and/or endothelial cells during ageing (Altschmied and Haendeler, 2009; Rhie et al., 2001a, 2001b). In summary, there are at least three mechanisms that potentially affect the ROS balance in aged tissues: (i) enhanced ROS leakage from dysfunctional mitochondrial ETCs, (ii) induction and/or enhanced activity of NOXs, and (iii) inactivation of antioxidative systems. Currently, it is unclear to what extent these mechanisms individually contribute to the increased ROS levels observed in dermal fibroblasts from old donors.

2.6.3. Accumulation of mtDNA mutations

In mice, enhanced mtDNA mutations produce an accelerated ageing phenotype including the skin organ (Trifunovic et al., 2004). Levels of mtDNA mutations in these mice are at least 10-fold higher than in aged humans and their ageing-like features are shared with several other premature ageing mouse models, where no mtDNA mutations are involved, suggesting the ageing-like phenotype of the mtDNA mutator mouse does not necessarily imply the involvement of mtDNA mutations in natural mammalian ageing (Khrapko et al., 2006). A recent study demonstrates that premature ageing observed in the mutator mouse is probably due to stem cell dysfunction, since mtDNA mutations induced at similar levels in postmitotic cells by mutant mitochondrial helicase Twinkle did not induce a progeroid phenotype in mice (Ahlqvist et al., 2012). Current belief holds that mtDNA deletions are naturally acquired by faulty repair of damaged mtDNA molecules (Krishnan et al., 2008) and the accumulation of such acquired mtDNA mutations in aged tissues seems to be a consequence of clonal expansions of single founder molecules rather than on-going mutational events (Lane, 2012; Wiesner et al., 2006). This mechanism seems to hold some relevance for extrinsic ageing of the skin, where the persistence of UV radiation-induced mtDNA deletions entail inadequate energy production, which triggers retrograde mitochondrial signalling pathways that then transduce functional and structural alterations to the skin (Krutmann and Schroeder, 2009). This pathogenic cascade may even perpetuate itself independently of UV exposure as ROS-leakage from the defective ECR induces additional/further mtDNA damage (Krutmann and Schroeder, 2009). The rarely proliferating, long-lived cell population of the dermal fibroblast seems to be a major target of the above mtDNA-degenerative mechanisms, because it is less able to eliminate macromolecular damage by cell shedding as it is done by, *e.g.* the highly proliferative epidermal keratinocytes.

2.6.4. Altered mitochondrial biogenesis

It is a long-standing observation that mitochondrial content increases in fibroblasts subjected to replicative senescence in culture (Goldstein et al., 1984; Hayflick, 1980; Lee et al., 2002b).

Mitochondrial mass and biogenesis is also increased in primary cells retrieved from aged humans (Lezza et al., 2001). It is currently believed that the increase in mitochondrial mass in senescent and aged cells is due to retrograde triggering of a mito-biogenic stress response by increased ROS-production, decreased ATP-synthesis or persistent DNA damage signals caused by dysfunctional mitochondria (Butow and Avadhani, 2004; Finley and Haigis, 2009) that are not properly removed because autophagy is down-regulated (Yen and Klionsky, 2008). On the other hand senescence induced in human fibroblasts by DNA damage is maintained through an anterograde signalling loop in which p21 stimulates mitochondrial ROS production in order to replenish DNA damage foci and maintain ongoing DNA damage responses (Passos et al., 2010). While stimulation of mito-biogenesis is detectable in fibroblasts subjected to replicative or stress-induced senescence in culture (Lee et al., 2002b) and also in some aged human tissues (Gottlieb and Mentzer, 2010; Lezza et al., 2001), it is not clear whether the proposed cycle is also activated during ageing of human dermal fibroblasts *in situ*. It should also be noted that *in vivo* ageing of human skeletal muscle (a cell system sharing many similarities with the dermal fibroblast) encompasses the opposite mechanism, namely an increasing deficiency in the activation of mito-biogenic stress responses mediated by the mTOR pathway and the α_2-subunit of AMP-activated protein kinase (Li et al., 2012) as well as by a switch to AMPK-independent regulation of mito-biogenesis due to altered SIRT1-signalling leading to an pseudo-hypoxic state encompassing imbalanced transcription of mitochondrial genes encoded in the nucleus and by mtDNA (Gomes et al., 2013).

2.6.5. Altered mitochondrial fusion/fission equilibrium

Mitochondria are highly dynamic organelles that constantly undergo fusion and fission events to adapt their shape and number in order to preserve cellular homeostasis (Chan, 2012; Detmer and Chan, 2007). Mitochondrial fusion and fission help maintaining mtDNA integrity, regulate cellular redox status, cooperate in the elimination of damaged mitochondria through autophagy (mitophagy) and are directly involved in the execution of the apoptotic programme (Cho et al., 2010; Liesa et al., 2009). A number of age-associated neurodegenerative diseases are ascribed to direct or indirect alterations of the fusion/fission machinery (Chen and Chan, 2009). The mitochondrial fusion/fission equilibrium has a direct impact on life span in lower eukaryotes (Scheckhuber et al., 2012, 2011; Westermann, 2010) and significant alterations of mitochondrial dynamics are associated with cellular senescence of human vascular endothelial cells (Jendrach et al., 2005). Age-associated deceleration of mitochondrial fusion and fission is thought to serve the adaptation to an increased load of mitochondrial damage (Figge et al., 2013, 2012). The proteins crucial for mitochondrial fusion and fission are highly conserved and most certainly expressed in dermal fibroblasts, but their role in fibroblasts ageing has not been investigated.

2.7. Cellular senescence

2.7.1. Definition of cellular senescence

Cellular senescence is a genetic programme that limits the proliferation of cells. It is primarily activated by telomere shortening (d'Adda di Fagagna et al., 2003) but also by non-telomeric DNA damage and many other stressors (Campisi and d'Adda di Fagagna, 2007). Senescence is characterised by an irreversible cell cycle arrest triggered through pathways involving p53, pRB, $p16^{INK4A}$ (d'Adda di Fagagna et al., 2003) and p21(CIP1) (Herbig et al., 2004). The senescent phenotype encompasses the expression of a specific β-galactosidase (Dimri et al., 1995), enhanced hetero-chromatinisation (Kreiling et al., 2011; Narita et al., 2003) and increased secretion of cytokines and other biologically active proteins (Coppe et al., 2008). In diploid human fibroblasts, cellular senescence can be induced by replicative exhaustion (replicative senescence) (Hayflick, 1980; Hayflick and Moorhead, 1961), leading to telomere shortening (Harley et al., 1990) and the activation of p53/pRB pathways (d'Adda di Fagagna et al., 2003). The same endpoint is also reached along the p53 and $p16^{INK4a}$ pathway following activation of ras oncogene (oncogene-induced premature senescence) (Serrano et al., 1997). Alternatively, cellular senescence can be induced in culture by oxidative stress (stress induced premature senescence, SIPS) (Toussaint et al., 2000), which gives rise to a distinct phenotype (Dierick et al., 2002). Senescent dermal fibroblasts accumulate in skin of ageing baboons (Herbig et al., 2006). It has also been shown that $p16^{INK4A}$ positive, non-proliferative cells accumulate in aged human dermis suggesting that senescence of dermal fibroblasts is induced *in vivo* even though the cells do not proliferate (Jeyapalan et al., 2007; Ressler et al., 2006). Enhanced clearance of $p16^{INK4A}$-positive senescent cells has been demonstrated to protect progeroid mouse models from ageing-associated disorders (Baker et al., 2011). Thus, it is conceivable that accumulation of $p16^{INK4A}$-positive, senescent dermal fibroblasts plays a causal role in skin ageing, although an aged skin phenotype was not among the disorders suppressed by enhanced clearance of these cells in progeroid mice (Baker et al., 2011). However, it was recently reported that the number of p16-positive cells in the epidermis as well as in the dermis increase with age. Actually, the authors not only established a correlation of human familial longevity with fewer p16INK4a-positive skin cells *in situ*, but also an association with fewer senescent cells after a cellular stressor *in vitro* (Waaijer et al., 2012).

2.7.2. Heterochromatinisation and activation of transposons

In senescent fibroblasts there is an accumulation of distinct heterochromatin structures designated senescence-associated heterochromatin foci (SAHF). SAHF-formation in human fibroblasts undergoing replicative senescence *in vitro* is associated with the recruitment of heterochromatin proteins to E2F-promoters and the irreversible repression of the retinoblastoma (RB) pathways leading to suppression of the numerous E2F-traget genes involved in DNA replication and cell cycle progression, DNA damage repair, apoptosis, differentiation and development (Bracken et al., 2004; Narita et al., 2003). Moreover, it is associated with epigenetic changes leading to gene silencing and the activation of retrotransposons (De Cecco et al., 2013). Increased expression of heterochromatin proteins was also observed in various tissues of mice and baboons, but SAHP were not detectable. Age-related increase in diffuse nuclear heterochromatin protein expression was most prominent in post-mitotic tissues commonly thought to be not susceptible to replicative senescence (Kreiling et al., 2011). These findings suggest that during ageing, dermal fibroblasts could undergo progressive hetero-chromatinisation leading to increased DSB formation and DNA recombination due to the activation of transposable elements.

2.7.3. DNA-damage foci and DNA-SCARS

In skin biopsies of baboons the percentage of fibroblasts exhibiting γH2AX-positive DNA damage foci in their nuclei increase exponentially with age (Herbig et al., 2006). These persistent DNA damage foci are thought to be an indicator of cellular senescence. They are either co-localised with telomeric DNA indicating telomere dysfunction (Herbig et al., 2006) or with PML nuclear bodies delineating nuclear structures that sustain damage-induced senescence growth arrest and inflammatory cytokine secretion (DNA SCARS). DNA SCARS are thought to be the transcription factories where the genes providing the SASP are transcribed (Rodier et al., 2011). DNA SCARS are associated with

senescence of fibroblast induced by ionising radiation *in vitro*. Senescence-associated formation of γH2AX-positive DNA damage foci and hetero-chromatinisation are mutually exclusive, since numerous studies have shown that hetero-chromatin is refractory to H2AX phosphorylation (Cann and Dellaire, 2011).

2.7.4. Senescence associated secretory phenotypes

Enhanced secretion of cytokines and other biologically active proteins by senescent cells is termed a senescence-associated secretory phenotype (SASP). SASP enables senescent cells to modulate the tissue microenvironment (Parrinello et al., 2005). It can promote tumour progression and inflammation (Coppe et al., 2010). In hepatocyte and fibroblast cell lines, the onset of replicative senescence is regulated by extracellular growth factors also included in SASP suggesting auto-/paracrine feedback loops (Micutkova et al., 2011; Nelson et al., 2012). Moreover, there is evidence that the AHR could play a role in the induction of SASP through the activation of the EGFR-ligands amphiregulin and epiregulin (Choi et al., 2006; John et al., 2013). Skin fibroblasts subjected to replicative or stress-induced ageing *in vitro* acquire a SASP (Coppe et al., 2010). However, it is questionable whether this holds also true for senescent dermal fibroblasts accumulating in the skin of ageing primates (Herbig et al., 2006) and humans (Dimri et al., 1995). Dermal fibroblasts aged *in vivo* are characterised by enhanced secretion of cysteine-rich, angiogenic inducer protein 61 (CYR61), also known as CCN1 (Quan et al., 2011). CCN1 is a protein that stimulates the secretion of pro-inflammatory cytokines and MMPs and induces cellular senescence. Enhanced CCN1 expression controls fibrosis in wound healing (Jun and Lau, 2010a,b). In aged dermal fibroblasts CCN1 is believed to contribute to the acquisition and maintenance of the senescent cell state and to promote reduced production and increased degradation of collagen (Quan et al., 2011). Holistic secretome analyses demonstrate that already in the non-senescent state dermal fibroblasts secrete more than 1000 individual proteins including transporters, enzymes, peptidases, growth factors and integrines (Boraldi et al., 2003b; Won et al., 2012). Therefore, in fibroblasts, age-related changes in amount and composition of secreted proteins might reflect the switch from one secretory programme to another, rather than acquirement of a secretory phenotype *de novo*. This said, so far there are no systematic studies available regarding alterations of the secreted proteome of dermal fibroblasts during *in situ* ageing.

2.8. Altered intercellular communication

2.8.1. Epidermal growth factor (EGF) signalling

Foreskin fibroblasts subjected to replicative senescence in culture become refractory to EGF receptor (EGFR)-mediated paracrine growth impulses due to down-regulation of the EGFR and up-regulation of tyrosine phosphatases (SHP-1 and PTPN6) (Shiraha et al., 2000; Tran et al., 2003). Acquired EGF unresponsiveness involves down-regulation of caveolin- and clathrin-mediated endocytosis (Park et al., 2002, 2001). EGF-response is attenuated by caveolin overexpression in juvenile fibroblasts and restored by caveolin knock down in senescent fibroblasts (Park et al., 2002). In addition, EGFR-mediated expression of aquaporin-3 is involved in human skin fibroblast migration (Cao et al., 2006).

In keratinocytes, UVA/B irradiation activates EGFR, which plays a dominant role in c-jun-mediated activation of AP-1 leading to enhanced expression of MMPs (Wan et al., 2001). On the other hand, EGFR-dependent activation by oxidative stress may play a protective role in the adaptation of keratinocytes to UV-light, since it is known to enhance cell survival (Wang et al., 2000). Paracrine epidermal/dermal crosstalk of EGF-signalling appears to play a major adaptive role in extrinsic ageing, as estrogen-mediated release of epidermal growth factor from keratinocytes protects the dermal hyaluronan/versican matrix. Thus, EGF-signalling input into dermal fibroblasts acts protective in remodelling processes of the dermal extracellular matrix during extrinsic ageing (Rock et al., 2012) and loss of EGF-responsiveness of the dermal fibroblasts possibly promotes extrinsic skin ageing processes.

2.8.2. AHR-signalling

Skin ageing promoted by polycyclic aromatic hydrocarbons (PAH) is targeted at, and transduced by, the aryl hydrocarbon receptor (AHR) (Morita et al., 2009). All skin cells express the AHR. In keratinocytes, the AHR is activated by UVB irradiation and subsequent signalling events are critically involved in photo carcinogenesis and UVB-induced ageing. Non-genomic AHR signalling in UVB-irradiated keratinocytes induces cyclo-oxigenase 2 and suppresses apoptosis, thereby contributing to the development of skin cancer (Agostinis et al., 2007; Fritsche et al., 2007). In addition, UVB-induced AHR activation in keratinocytes causes increased mRNA and protein expression of MMP1, which probably acts on proteins in the dermal ECM and thus contributes to extrinsic ageing of the dermis in a paracrine manner. Whether AHR – signalling plays a direct role in dermal fibroblasts ageing is currently not known. Dermal fibroblasts express AHR molecules at significant levels (Tigges et al., 2013), but stimulation of these cells with established AHR agonists fails to elicit increased expression of AHR-signature genes such as cytochrome P450, family 1, member A1 (CYP1A1). On the other hand, the AHR in dermal fibroblasts has recently been demonstrated to mediate stimulation of MMP1 expression by tobacco smoke (Ono et al., 2013).

2.9. Alterations of the cytoskeleton

The most obvious morphological changes that are associated with replicative senescence of fibroblasts *in vitro* and recapitulated to some extent in primary dermal fibroblasts isolated from donors of advanced age encompass increases in cell surface and cell volume, as well as changes in cell shape and mobility (reviewed in Hwang et al., 2009). As these properties are to a large part dependent on the function of the cytoskeleton, cytoskeletal modifications are expected to occur during ageing of the dermal fibroblast. Dermal fibroblasts from old donors are less plastic and less mobile than those from young donors (Schulze et al., 2012). It has been suggested that these alterations are due to structural changes in intermediate filaments caused by an increased expression of vimentin. Up-regulation of vimentin was observed in dermal fibroblasts subjected to replicative ageing *in vitro* (Nishio and Inoue, 2005). Conversely, enforced vimentin overexpression induced a senescence-like phenotype (Nishio et al., 2001). Vimentin was also the most prominent among 30 proteins differentially expressed in HGPS fibroblasts as compared to normal fibroblasts (Wang et al., 2012). However a similar increase in vimentin has not been detected in primary dermal fibroblasts isolated form old human donors (Boraldi et al., 2003a). It should also be noted, that vimentin is highly susceptible to modification by lipid peroxidation, glycoxidation and non-enzymatic glycation (Baraibar and Friguet, 2013). Increased glycation of vimentin expressed at normal levels has been observed in primary dermal fibroblasts from donors of advanced age and associated with a loss of contractile capacity of the cells (Kueper et al., 2007). The other relevant cytoskeletal target molecule of ageing processes appears to be actin. Actin content of dermal fibroblasts seems not to change in the course of replicative senescence (Sprenger et al., 2010) or differ between primary cells form old and young donors (Boraldi et al., 2003a), whereas the status of actin filaments has been reported to change during replicative senescence as well as during *in situ*-ageing of human fibroblasts. However, the available studies arrive at contradictory results and it remains unclear whether

during ageing the actin filaments become thicker or thinner or just redistributed within the cell (reviewed in Hwang et al., 2009). More relevant seems an age-related change in actin dynamics leading to an increase in the nuclear content of depolymerised globular (G-)actin and an increased expression or activation of the actin-depolymerising factor cofilin in senescent fibroblasts (Kwak et al., 2004) and primary fibroblast from old humans (Boraldi et al., 2003a; Chen et al., 2006). An altered nuclear content of G-actin possibly plays a causal role in alterations of gene transcription associated with age-associated decreases of stress resistance (Vartiainen, 2008). Incidentally, the actin depolymerising factor cofilin is activated in the skin by UV light, which possibly provides a mechanistic link between age-related changes in nuclear G-actin and extrinsic skin ageing promoted by exposure to sun light (Hensbergen et al., 2005)

2.10. Adaptation/maladaptation to extrinsic stress

Sub-toxic doses of various harmful stressors extend lifespan in *C. elegans* (Cypser et al., 2006; Johnson et al., 2002) and are proposed to have beneficial effects against ageing and diseases in humans (Calabrese et al., 2011; Martins et al., 2011; Wu et al., 2008). Moreover, sub-lethal levels of nuclear or mitochondrial dysfunction modulate ageing and life span of *C. elegans* in a bimodal manner. High levels of transcription-blocking DNA-damage shorten lifespan/accelerate ageing, whereas low levels increase lifespan/attenuate ageing by a mechanisms involving growth retardation and decreased IGF-signalling (Garinis et al., 2009). A similar bimodal adaptive extension/maladaptive abbreviation of lifespan was observed upon reduced expression of various nuclear-encoded mitochondrial proteins, which play a crucial role in the maintenance of the mitochondrial ETC (Rea et al., 2007; Ventura et al., 2005; Ventura and Rea, 2007). These observations have given rise to the concept of mito-hormesis (Tapia, 2006; Ventura et al., 2006), which was later extended (Ristow and Schmeisser, 2011; Ristow and Zarse, 2010) to stipulate that several longevity-promoting interventions including calorie restriction, physical exercise and mild suppression of mitochondrial function converge on an adaptive activation of mitochondrial oxygen consumption thereby promoting ROS formation, which induces downstream effects ultimately inducing endogenous defence mechanisms culminating in increased stress resistance and longevity. In *C. elegans*, the adaptive extension of lifespan in response to cell respiratory dysfunctions depends on the p53 homologue cep-1 (Torgovnick et al., 2010; Ventura et al., 2009). Moreover, it involves genes playing a role in apoptosis and autophagy (beclin and p53) and encompasses reduced lipid storage and increased autophagy (Schiavi et al., 2013). These observations conform to the yeast-derived concept that autophagy counteracts ageing by removing defective mitochondria (Brink et al., 2009). Recently, it has been shown that some of the phenotypic hallmarks of UV-induced skin ageing can be suppressed by preconditioning with mild heat *via* a HSP-70 mediated protective pathway (Matsuda et al., 2013), which indicates that HSP-70 mediated adaptation/maladaptation could play a general and crucial role in extrinsic skin ageing and possibly provides an intervention target (Haarmann-Stemmann et al., 2013). It remains to be investigated, whether these effects are related to the activation of autophagy downstream of mito-hormesis as demonstrated in *C. elegans* (Schiavi et al., 2013).

2.11. Disruption of circadian regulation, decrease of NAD^+ and sirtuin activity

Fibroblasts comprise a molecular circadian oscillator that confers rhythmic diurnal expression to a large number of genes (Menger et al., 2007). The local clockwork in skin and other peripheral organs is under the general control of a light-sensitive master pacemaker in the suprachiasmatic nucleus (Honma et al., 2012) and additionally receives input from a variety of extrinsic factors and cellular response systems including redox state (*e.g. via* SIRT1 and NAD^+/NADH sensing) (Asher et al., 2008, 2010; Chang and Guarente, 2013; Nakahata et al., 2008, 2009; Peek et al., 2013; Ramsey et al., 2009), energy levels (*e.g. via* AMPK) (Lamia et al., 2009), DNA damage (Oklejewicz et al., 2008) and cellular stress response systems (*e.g. via* heat shock factors) (Buhr et al., 2010; Reinke et al., 2008; Saini et al., 2012). Circadian regulation has been demonstrated for various pathways involved in the ageing process of the dermal fibroblast. These encompass NER (Gaddameedhi et al., 2011), autophagy (Ma et al., 2011), expression of proteasome subunits and components of the AHR-pathway (Menger et al., 2007), and anti-oxidative defence systems (Wilking et al., 2013). Genetic or environmental perturbations of circadian regulation induce a variety of pathologies that are also known to increase with age (Kondratova and Kondratov, 2012). Moreover, mouse models defective in specific clock genes have a shortened life span and exhibit features of accelerated organ ageing (Fu et al., 2002; Kondratov and Antoch, 2007; Kondratov et al., 2006; van der Horst et al., 1999). In humans, disruption of circadian rhythm is associated with an earlier onset of metabolic and cardiovascular dysfunction (Maury et al., 2010; Rajaratnam and Arendt, 2001). Of particular interest in this context is the link between circadian regulation and SIRT1, which acts as a conserved, energy-sensitive antiageing protein and mediates the beneficial effects of calorie restriction (Guarente, 2013) by many pathways encompassing the regulation of gene expression through histone deacetylation (Satoh et al., 2013), direct of stimulation of autophagy proteins (Morselli et al., 2010, 2011), adaptation of the retrograde mitobiogenic response to mitochondrial dysfunction (Gomes et al., 2013) and induction of hormetic responses through ROS-generation linked to the methylation of nicotinamide (Schmeisser et al., 2013). In the core clock mechanism SIRT1 directly regulates the stability of the major circadian regulator PER2 (Asher et al., 2008, 2010) and the actelyation status of BMAL1 and chromatin at circadian promoters (Nakahata et al., 2008). Recently, it has been shown that SIRT1 is also involved in the ageing-related decline of clock function in the suprachiasmatic nucleus, which is mediated by reduced levels of SIRT1 and NAD^+ in aged animals (Chang and Guarente, 2013). On the other hand, many of the stresses that promote extrinsic ageing are subjected to circadian dynamics and it seems conceivable that the adequate cellular response systems need to be coordinated with the diurnal incidence of the stressors. Along these lines it has been demonstrated that stress-adaption of mitochondrial function and autophagy is regulated by the clock gene Rev-Erb alpha in skeletal muscle (Woldt et al., 2013) and that autophagy is coordinated with food intake by circadian regulation in the liver through the transcription factor C/EBP beta (Ma et al., 2012, 2011). In summary, these considerations suggest that (i) the susceptibility of the dermal fibroblast to extrinsic ageing is likely to depend on its position in the circadian cycle, (ii) noxae that modulate ageing may act on the dermal fibroblast *via* their input into the molecular clock, *e.g.* through SIRT1, and (iii) this regulation possibly deteriorates with age leading to an age-associated decline in stress resistance. However, so far, there are no data available demonstrating such a connection in dermal fibroblasts.

2.12. Genome wide alterations in gene expression networks

2.12.1. Alterations of the mRNA transcriptome

Age-associated down regulation of genes associated with electron transport chain activity and mitochondrial function is currently considered a common signature of ageing, since it was likewise observed in human skeletal muscle, kidney, skin and brain (Glass et al., 2013; Rodwell et al., 2004; Zahn et al., 2006), as well as

in corresponding organs of mouse (Zahn et al., 2007) and in insects (Zahn et al., 2006). In contrast, genes associated with ECM maintenance, cell progression and pre-mRNA processing exhibit age-related up-regulation only in human muscle and kidney (Zahn et al., 2006), while in human blood lymphocytes age-associated up regulation of genes associated with pre-mRNA processing and mRNA quality control appeared to be the most prominent age-related alteration (Harries et al., 2011). In summary, these observations support a model, in which mitochondrial dysfunction and impaired energy metabolism are common features of aged cells, while most of the other age-related systematic transcriptome changes are consistent with an adaptation to that condition, which may follow different strategies in different organs and cell types. In genetic screens of homogenates of whole human skin biopsies (epidermis + dermis from sun-protected sites/areas) the only gene network that showed age-related changes independently of gender was the wingless/int1 (WNT) pathway (Makrantonaki et al., 2012). In seeming contradiction, another genetic screen of foreskin fibroblasts from young and old human donors revealed a total of 105 genes that changed their expression over 1.7-fold in an ageing-related manner (43 down-regulated, 62 up-regulated), and are involved in diverse cellular processes encompassing cell cycle control, cytoskeletal changes, inflammatory response, signalling and metabolism (Lener et al., 2006). It is puzzling that the gene networks exhibiting ageing-related expression changes in human skin had little to no connection with the common or tissue-specific transcriptome signatures of ageing identified in human human skeletal muscle, kidney, brain (Glass et al., 2013; Rodwell et al., 2004; Zahn et al., 2006) or blood lymphocytes (Harries et al., 2011), nor with the mechanisms known or believed to be involved in bringing about the aged phenotype of the human dermal fibroblast and the dermal stroma controlled by it. For instance, there was no overlap with genes involved in ETC-function, mito-biogenesis, retrograde stress response, AHR-signalling, extracellular matrix, DNA-recombination, DNA-repair or DNA-damage response. This discrepancy could be due to the fact that foreskin fibroblasts analysed in the one of the two available studies (Lener et al., 2006) are subjected to a different ageing process than dermal fibroblasts, whereas differences observed in whole skin biopsies analysed in the other study (Makrantonaki et al., 2012) were possibly related to skin cell types other than dermal fibroblasts, *e.g.* epidermal keratinocytes which in intact skin exceed the dermal fibroblasts by number significantly. Finally, it is also conceivable that ageing of the dermal fibroblast is to a large extent due to post-transcriptional/-translational processes, as is, *e.g.* the case for ageing-related decreases of autophagy in many organs (Morselli et al., 2011).

2.12.2. Alterations of micro-RNA expression

There is growing evidence that miRs are important regulatory molecules deeply involved in the pleiotropic phenomena of ageing. Genome-wide studies in a variety of model organisms show that several miRs are differentially expressed during ageing and regulate age-associated changes in gene expression (Inukai and Slack, 2013). At least four different miR regulated pathways have been found associated with human fibroblast senescence: A family of 15 p53/E2F1-repressed miRs was identified that silence anti-proliferative genes and delay replicative senescence, when over-expressed in human embryonic fibroblast cells (Brosh et al., 2008). A common set of four miRs from the miR 17–92 cluster was identified that suppress p21 and are down regulated in human dermal fibroblasts subjected to replicative senescence *in vitro* as well as in human foreskin fibroblasts established from old donors (Hackl et al., 2010). Two more studies have further addressed potential roles of miRs in fibroblast senescence induced in culture. One study demonstrated that UVB-induced senescence in cultured human diploid fibroblast involving the p53/p21^{WAF1} and p16^{INK4a}/pRb pathways was accompanied by a significant regulation of five miRs. One of these (miR-101) specifically targets the histone-lysine N-methyltransferase EzH2. Overexpression of miR-101 and experimental down-regulation of EzH2 both induced fibroblast senescence, while experimental down-regulation of miR-101 failed to suppress UVB-induced senescence, suggesting redundant mechanisms (Greussing et al., 2013). The other study demonstrated that replicative senescence of human diploid fibroblast in culture was accompanied by up-regulation of miR-152 and miR-181a. Overexpression of these two miRs was sufficient to induce senescence. Interestingly, direct targets regulated by miR-152 and miR-181a include the genes Itga5 and Col16a1, which play a role in cell adhesion and composition of the extracellular matrix (Mancini et al., 2012). In summary, at least four distinct miR-regulated pathways appear to be involved in fibroblast senescence induced by UV-radiation or replicative stress *in vitro*. At least one of these (*i.e.* the miR 17–92 cluster) seems also involved in cellular senescence of human foreskin fibroblasts subjected to ageing *in vivo*. However, it remains to be determined which of these miR-pathways is also regulated during *in vivo* ageing of human dermal fibroblasts.

2.13. Proteome changes

About 22% of the cellular proteome of human foreskin dermal fibroblast from two individuals (4 year old and 9 year old healthy males) subjected to replicative senescence in culture exhibits changes of >1.5 fold, which are mostly due to a moderate down-regulation of proteins associated with transcription and RNA metabolic processes and a pronounced up-regulation of lysosomal proteins (Sprenger et al., 2010). The latter finding conforms to older microscopic studies demonstrating an increase of lysosomes in fibroblasts subjected to replicative ageing *in vitro* (Hwang et al., 2009). A 2D electrophoresis study of the cellular proteome of primary dermal fibroblasts isolated from sun-protected skin areas of a few donors of various ages has identified a total of 38 proteins that are differentially regulated (>3-fold changes) during ageing *in vivo*. The differentially regulated proteome consisted of proteins involved in protein degradation or -repair (mostly components of the ubiquitin proteasome system), proteins involved in stress response and anti-oxidative defence (heat shock proteins, superoxide dismutases, glutathione S-transferase P), and cytoskeletal components and regulators (Boraldi et al., 2003a). Thus, the changes of the cellular proteome of the dermal fibroblast associated with replicative senescence *in vitro* and extrinsic human skin ageing *in vivo* appear to differ notably from each other. It has also been demonstrated that the proteomic changes associated with replicative senescence are significantly different from those associated with premature senescence induced in fibroblasts by subtoxic doses of tert-butyhydroperoxide or ethanol (Dierick et al., 2002). In addition there appear to exist phenotypic differences between fibroblasts from different skin regions (foreskin, abdomen, upper thigh). Moreover, primary human dermal fibroblasts in primary culture are known to secrete a complex protein mix (Boraldi et al., 2003b; Won et al., 2012). Given the accumulation of senescent fibroblasts in skin of aged primates (Herbig et al., 2006) and humans (Ressler et al., 2006) and the acquisition of secretory phenotypes during cellular senescence (Coppe et al., 2010; Quan et al., 2011), it is to be expected that the secreted proteome of dermal fibroblasts will also change during ageing. A screen of the secretome of human new born diploid fibroblasts identified 26 extracellular proteins, the abundance of which was significantly different in media from cells subjected to replicative senescence *in vitro*. Interestingly, one of these was insulin-like growth factor binding protein 6 (IGFBP-6). IGFBP-6 was down regulated upon replicative senescence and may be

involved in supporting the senescent state through a negative autocrine feedback loop, since IGFBP-6 overexpression increased replicative lifespan, whereas experimental down regulation led to premature senescence (Micutkova et al., 2011). However, it has not yet been systematically investigated, whether similar alterations of the proteome are also detectable in dermal fibroblasts retrieved from old individuals.

3. Synopsis, conclusions and open questions

The dermal fibroblast is a long-lived, mostly non-proliferative cell, which is targeted by a number of exogenous influences known or believed to promote extrinsic skin ageing. It is assumed that dermal fibroblasts integrate the chronic effects of such exogenous ageing promoters and thus possibly provide an indicator system for the individual state of extrinsic ageing processes. Table 1 provides in column 1 a catalogue of major age-associated phenotypic changes commonly observed in cells and tissues of humans and other species. It is summarised, which of these changes have so far been also observed in human dermal fibroblasts undergoing spontaneous ageing processes *in vivo* (columns 3 and 4) or subjected to ageing induced in culture by actinic or oxidative stress or continuous replication (column 2). An assessment of these findings with regard to the systematic of the nine ubiquitous hallmarks of ageing (Lopez-Otin et al., 2013) is summarised in Table 2. From these compilations it becomes clear that dermal fibroblasts subjected to ageing in culture exhibit a majority of the ubiquitous hallmarks of ageing (genome instability, telomere attrition, epigenetic alterations, mitochondrial dysfunction, cellular senescence, altered intercellular communication, and loss of proteostasis), while the rest of these hallmarks is not applicable to the culture situation (stem cell exhaustion) or has not yet been investigated (deregulated nutrient sensing/REDOX-imbalance). However, several of the established phenotypic

Table 1
Evidence obtained in dermal fibroblasts of phenotypic changes commonly related to ageing.

Phenotypic changes commonly related to ageing	*Evidence in vitro* senescence induced in culture	*Evidence ex vivo* primary cells from old humans	*Evidence in vivo* dermis of old humans/primates
Persistent DNA damage, nuclear genome instability			
53BP1/γH2AX foci	S[a] (Fumagalli et al., 2012)	HGPS[b] (Richards et al., 2011)	(Herbig et al., 2006; Jeyapalan et al., 2007)
Chromosome instability, aneu-/polyploidy	R[c] (Ohshima, 2012; Suzuki et al., 2002), S (Popescu et al., 1986)	ND[d]	ND
Transposon activation	R (De Cecco et al., 2013)	ND	ND
Decreased DNA repair capacity	R (Seluanov et al., 2004)	(Takahashi et al., 2005)	ND
Oxidative DNA damage	NE[e] (Moller et al., 2010)	NE (Moller et al., 2010)	NE (Moller et al., 2010)
Telomere shortening	R (Harley et al., 1990)	NE (Krunic et al., 2009; Maier et al., 2007)	ND
DNA damage at telomeres	S (Fumagalli et al., 2012)	ND	(Herbig et al., 2006)
Altered pre-mRNA processing			
Misssplicing of Lamin A (Progerin)	(R, S) (Cao et al., 2011; Takeuchi and Runger, 2013)	(Scaffidi and Misteli, 2006)	(McClintock et al., 2007)
Altered splicing of other transcripts	(R, S) (Magnuson et al., 1991)	ND	ND
Altered regulation of splicing genes	R (Hardy et al., 2005)	ND	ND
Cellular senescence			
SAβGal	R (Dimri et al., 1995)	ND	(Dimri et al., 1995)
$p16^{INK4A}$	R (d'Adda di Fagagna et al., 2003), O[f] (Serrano et al., 1997)	ND	(Ressler et al., 2006) (Herbig et al., 2006; Jeyapalan et al., 2007) (Waaijer et al., 2012)
p21	R (Herbig et al., 2004) S (Passos et al., 2010)	ND	ND
SASP	R, S (Coppe et al., 2010)	(Quan et al., 2011)	ND
SAHF	R (Narita et al., 2003)	ND	NE (Kreiling et al., 2011)
HP1β, mH2A (diffuse)	R (Kreiling et al., 2011)	ND	(Kreiling et al., 2011)
53BP1/γH2AX foci	S (Fumagalli et al., 2012)	HGPS (Richards et al., 2011)	(Herbig et al., 2006)
DNA-SCARS	R, S (Rodier et al., 2011)	ND	ND
Epigenetic alterations			
Histone modifications	R (De Cecco et al., 2013)	ND	(Kreiling et al., 2011)
DNA methylation	ND	(Horvath, 2013; Koch et al., 2013)	(Gronniger et al., 2010)
Disturbed Proteostasis			
Chaperone dysfunction	ND	ND	ND
Decline of proteasome activity	R, S (Bulteau et al., 2007; Catalgol et al., 2009)	NE (Koziel et al., 2011)	ND
Decreased Autophagy	ND	ND	ND
Increased matrix metalloproteinase secretion	S (Fisher et al., 1996)	ND	(Brennan et al., 2003)
ECM Proteoglycane Remodelling	ND	ND	(Knott et al., 2009; Koshiishi et al., 1999; Rock et al., 2012)
Altered intercellular communication			
EGF insensitivity	R (Park et al., 2002, 2001; Shiraha et al., 2000; Tran et al., 2003)	ND	ND
Increased AHR-input	S (Ono et al., 2013)	ND	ND

Table 1 (*Continued*)

Phenotypic changes commonly related to ageing	*Evidence in vitro* senescence induced in culture	*Evidence ex vivo* primary cells from old humans	*Evidence in vivo* dermis of old humans/primates
Alterations of cell plascticity and the cytoskeleton			
Decreased cell plasticity	ND	(Schulze et al., 2012)	ND
Upregulation of vimentin	R (Nishio and Inoue, 2005)	NE (Boraldi et al., 2003a)	ND
Enhanced glycation of vimentin	ND	(Kueper et al., 2007)	ND
Increased nuclear content of G-actin and cofilin	R (Kwak et al., 2004)	(Boraldi et al., 2003a; Chen et al., 2006).	ND
Circadian dysregulation	ND	ND	ND
REDOX-Imbalance			
Decreased NAD^+/sirtuins	ND	ND	ND
Increase ROS levels	ND	(Koziel et al., 2011)	ND
Decreased antioxidants	ND	ND	(Rhie et al., 2001a, 2001b)
Mitochondrial dysfunction			
Oxphos-uncoupling	R (Hutter et al., 2004)	(Greco et al., 2003).	
Increased respiration rate		(Greco et al., 2003), NE (Koziel et al., 2011)	
mtDNA mutations	S (Krutmann and Schroeder, 2009)	[g]KS (Krutmann and Schroeder, 2009)	[h]SES (Krutmann and Schroeder, 2009)
Increased mitobiogenesis	R (Goldstein et al., 1984; Hayflick, 1980; Lee et al., 2002b)	ND	ND
Altered fusion/fission equilibirum	ND	ND	ND
Altered gene expression			
mRNA	ND	(Lener et al., 2006)	ND
miR	R (Brosh et al., 2008; Hackl et al., 2010; Mancini et al., 2012), S (Greussing et al., 2013)	(Hackl et al., 2010)	ND

[a] S = stress induced senescence in culture.
[b] HGPs = only observed in Hutchinson Gilford Progeroid Syndrome.
[c] R = replicative senescence in culture.
[d] ND = no data available.
[e] NE = negative evidence.
[f] O = oncogene induced senescence in culture.
[g] KS = observed in Kearns Sayre Syndrome.
[h] SES = observed in sun-exposed skin.

Table 2
Ubiquitous hallmarks of ageing apparent in fibroblast aged in culture or in the skin.

Hallmark[a]	Ageing in culture	Ageing in the skin
Genome instability	Yes	Unclear
Telomere attrition	Yes	Some features
DNA damage at telomeres	Yes	Yes
Telomere shortening	Yes	No
Epigenetic alterations	Yes	Yes
Loss of proteostasis	Some features	Some features
Chaperone dysfunction	Unknown	Unknown
Decline in proteasome activity	Yes	No
Decreased autophagy	Unknown	Unknown
Increased protease secretion and ECM-remodelling	Yes	Yes
Deregulated nutrient sensing (REDOX-imbalance)	Unknown	Some features
Decreased NAD^+ levels	Unknown	Unknown
Decreased sirtuin activity	Unknown	Unknown
Increased ROS levels	Unknown	Yes
Decreased antioxidant activity	Unknown	Yes
Disruption of circadian regulation	Unknown	Unknown
Mitochondrial dysfunction	Yes	Unclear
OXPHOS uncoupling	Yes	Yes
Alteration of cell respiration rate	Yes	Yes/no
mtDNA mutagenesis	Yes	Yes
Altered mitobiogenesis	Yes	Unknown
Altered fusion/fission equilibrium	Unknown	Unknown
Cellular senescence	Yes	Some features
Chronic DNA damage signalling	Yes	Yes
Heterochromatinisation	Yes	Yes
Increased protein secretion (SASP)	Yes	Unknown
Stem cell exhaustion	Not applicable	No
Altered intercellular communication	Yes	Unknown
EGF insensitivity	Yes	Unknown
Increased AHR-input	Yes	Unknown
Altered insulin/IGF-signalling	Unknown	Unknown

[a] According to Lopez-Otin et al. (2013).

features of dermal fibroblasts aged in culture have not yet been investigated in fibroblasts aged *in situ* or were not or only partially detected in the available studies of skin biopsies or primary dermal fibroblasts derived from old donors. Therefore the list of established hallmarks of fibroblast ageing *in situ* is much shorter encompassing just persistent telomere damage, epigenetic alterations, loss of extracellular proteostasis, increased oxidative stress and stress-induced cellular senescence. It is unclear whether mitochondrial dysfunction, genome instability and altered intercellular communication also play a role and it seems unlikely that stem cell exhaustion and telomere shortening are involved in the *in situ* ageing process of the dermal fibroblast. Given these discrepancies, it must be doubted that the fullblown phenotype of cellular senescence of dermal fibroblasts induced in culture is a valid model for the extrinsic ageing process of dermal fibroblasts *in situ*. However, this conclusion should be taken with a grain of salt for the following reasons: (i) Several features clearly associated with replicative or stress-induced senescence *in vitro* have not yet been addressed in the available studies on skin biopsies or primary fibroblasts from old donors (*e.g.* the secretory phenotype, or alterations in mito-biogenesis, or the occurrence of DNA-SCARS). (ii) A number of cellular dysfunctions known to play a role in extrinsic ageing processes of other, comparable cell types and organs, such as a decline in autophagy, a decrease in NAD^+ levels and sirtuin activity, a possible disruption of circadian regulation, or alterations of the mitochondrial fusion/fission equilibrium have not yet been studied at all in aged dermal fibroblasts. (iii) Several features clearly associated with the ageing process of the dermal fibroblast *in vitro* or *in vivo* such as alterations of cell plasticity, cytoskeleton function and pre-mRNA processing have not yet been recognised as ubiquitous hallmarks of ageing. (iv) It is not entirely clear how UV-induced alterations of cell function are related to alterations occurring in the course of extrinsic ageing *in situ*,

because in most *ex vivo* studies of dermal fibroblasts retrieved form old humans, a clear distinction between intrinsic and extrinsic ageing based on the site of cell retrieval is lacking. Along the same lines, primary fibroblasts from tissue compartments other than dermis (*e.g.* foreskin or lung) appear to exhibit an ageing phenotype that markedly differs from that of dermal fibroblasts. Therefore such data should be excluded from synoptic interpretations. Given these numerous restrictions, it must be doubted that the short list of established hallmarks of dermal fibroblasts ageing *in situ* provided in Table 2 is complete. Despite the fact that dermal fibroblasts have been a preferred model of ageing research for more than five decades, there are still large gaps in our knowledge about the ageing process of this cell type within its physiological tissue environment.

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References

Agostinis, P., Garmyn, M., Van Laethem, A., 2007. The aryl hydrocarbon receptor: an illuminating effector of the UVB response. Sci. STKE: Signal Transduct. Knowl. Environ. 2007, pe49.

Ahlqvist, K.J., Hamalainen, R.H., Yatsuga, S., Uutela, M., Terzioglu, M., Gotz, A., Forsstrom, S., Salven, P., Angers-Loustau, A., Kopra, O.H., Tyynismaa, H., Larsson, N.G., Wartiovaara, K., Prolla, T., Trifunovic, A., Suomalainen, A., 2012. Somatic progenitor cell vulnerability to mitochondrial DNA mutagenesis underlies progeroid phenotypes in Polg mutator mice. Cell Metab. 15, 100–109.

Altschmied, J., Haendeler, J., 2009. Thioredoxin-1 and endothelial cell aging: role in cardiovascular diseases. Antioxid. Redox Signal. 11, 1733–1740.

Asher, G., Gatfield, D., Stratmann, M., Reinke, H., Dibner, C., Kreppel, F., Mostoslavsky, R., Alt, F.W., Schibler, U., 2008. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. Cell 134, 317–328.

Asher, G., Reinke, H., Altmeyer, M., Gutierrez-Arcelus, M., Hottiger, M.O., Schibler, U., 2010. Poly(ADP-ribose) polymerase 1 participates in the phase entrainment of circadian clocks to feeding. Cell 142, 943–953.

Avila, J., de Barreda, E.G., Pallas-Bazarra, N., Hernandez, F., 2013. Tau and neuron aging. Aging Dis. 4, 23–28.

Azim, S., Banday, A.R., Sarwar, T., Tabish, M., 2012. Alternatively spliced variants of gamma-subunit of muscle-type acetylcholine receptor in fetal and adult skeletal muscle of mouse. Cell. Mol. Neurobiol. 32, 957–963.

Baker, D.J., Jeganathan, K.B., Cameron, J.D., Thompson, M., Juneja, S., Kopecka, A., Kumar, R., Jenkins, R.B., de Groen, P.C., Roche, P., van Deursen, J.M., 2004. BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. Nat. Genet. 36, 744–749.

Baker, D.J., Wijshake, T., Tchkonia, T., LeBrasseur, N.K., Childs, B.G., van de Sluis, B., Kirkland, J.L., van Deursen, J.M., 2011. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature 479, 232–236.

Balaban, R.S., Nemoto, S., Finkel, T., 2005. Mitochondria, oxidants, and aging. Cell 120, 483–495.

Baraibar, M.A., Friguet, B., 2013. Oxidative proteome modifications target specific cellular pathways during oxidative stress, cellular senescence and aging. Exp. Gerontol. 48, 620–625.

Barja, G., 2007. Mitochondrial oxygen consumption and reactive oxygen species production are independently modulated: implications for aging studies. Rejuven. Res. 10, 215–224.

Bartek, J., Bartkova, J., Lukas, J., 2007. DNA damage signalling guards against activated oncogenes and tumour progression. Oncogene 26, 7773–7779.

Bedard, K., Krause, K.H., 2007. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol. Rev. 87, 245–313.

Bergamini, E., Cavallini, G., Donati, A., Gori, Z., 2007. The role of autophagy in aging: its essential part in the anti-aging mechanism of caloric restriction. Ann. N. Y. Acad. Sci. 1114, 69–78.

Bolognesi, C., Abbondandolo, A., Barale, R., Casalone, R., Dalpra, L., De Ferrari, M., Degrassi, F., Forni, A., Lamberti, L., Lando, C., Migliore, L., Padovani, P., Pasquini, R., Puntoni, R., Sbrana, I., Stella, M., Bonassi, S., 1997. Age-related increase of baseline frequencies of sister chromatid exchanges, chromosome aberrations, and micronuclei in human lymphocytes. Cancer Epidemiol. Biomarkers Prev. 6, 249–256.

Boraldi, F., Bini, L., Liberatori, S., Armini, A., Pallini, V., Tiozzo, R., Pasquali-Ronchetti, I., Quaglino, D., 2003a. Proteome analysis of dermal fibroblasts cultured in vitro from human healthy subjects of different ages. Proteomics 3, 917–929.

Boraldi, F., Bini, L., Liberatori, S., Armini, A., Pallini, V., Tiozzo, R., Ronchetti, I.P., Quaglino, D., 2003b. Normal human dermal fibroblasts: proteomic analysis of cell layer and culture medium. Electrophoresis 24, 1292–1310.

Boukamp, P., 2005. Skin aging: a role for telomerase and telomere dynamics? Curr. Mol. Med. 5, 171–177.

Bracken, A.P., Ciro, M., Cocito, A., Helin, K., 2004. E2F target genes: unraveling the biology. Trends Biochem. Sci. 29, 409–417.

Brennan, M., Bhatti, H., Nerusu, K.C., Bhagavathula, N., Kang, S., Fisher, G.J., Varani, J., Voorhees, J.J., 2003. Matrix metalloproteinase-1 is the major collagenolytic enzyme responsible for collagen damage in UV-irradiated human skin. Photochem. Photobiol. 78, 43–48.

Brink, T.C., Demetrius, L., Lehrach, H., Adjaye, J., 2009. Age-related transcriptional changes in gene expression in different organs of mice support the metabolic stability theory of aging. Biogerontology 10, 549–564.

Brosh, R., Shalgi, R., Liran, A., Landan, G., Korotayev, K., Nguyen, G.H., Enerly, E., Johnsen, H., Buganim, Y., Solomon, H., Goldstein, I., Madar, S., Goldfinger, N., Borresen-Dale, A.L., Ginsberg, D., Harris, C.C., Pilpel, Y., Oren, M., Rotter, V., 2008. p53-Repressed miRNAs are involved with E2F in a feed-forward loop promoting proliferation. Mol. Syst. Biol. 4, 229.

Buhr, E.D., Yoo, S.H., Takahashi, J.S., 2010. Temperature as a universal resetting cue for mammalian circadian oscillators. Science 330, 379–385.

Bulteau, A.L., Moreau, M., Nizard, C., Friguet, B., 2007. Proteasome and photoaging: the effects of UV irradiation. Ann. N. Y. Acad. Sci. 1100, 280–290.

Butow, R.A., Avadhani, N.G., 2004. Mitochondrial signaling: the retrograde response. Mol. Cell 14, 1–15.

Calabrese, V., Cornelius, C., Cuzzocrea, S., Iavicoli, I., Rizzarelli, E., Calabrese, E.J., 2011. Hormesis, cellular stress response and vitagenes as critical determinants in aging and longevity. Mol. Aspects Med. 32, 279–304.

Campisi, J., 2005. Aging, tumor suppression and cancer: high wire-act! Mech. Ageing Dev. 126, 51–58.

Campisi, J., d'Adda di Fagagna, F., 2007. Cellular senescence: when bad things happen to good cells. Nat. Rev. Mol. Cell Biol. 8, 729–740.

Cann, K.L., Dellaire, G., 2011. Heterochromatin and the DNA damage response: the need to relax. Biochem. Cell Biol. (Biochim. Biol. Cell.).89, 45–60.

Cao, C., Sun, Y., Healey, S., Bi, Z., Hu, G., Wan, S., Kouttab, N., Chu, W., Wan, Y., 2006. EGFR-mediated expression of aquaporin-3 is involved in human skin fibroblast migration. Biochem. J. 400, 225–234.

Cao, K., Blair, C.D., Faddah, D.A., Kieckhaefer, J.E., Olive, M., Erdos, M.R., Nabel, E.G., Collins, F.S., 2011. Progerin and telomere dysfunction collaborate to trigger cellular senescence in normal human fibroblasts. J. Clin. Invest. 121, 2833–2844.

Catalgol, B., Ziaja, I., Breusing, N., Jung, T., Hohn, A., Alpertunga, B., Schroeder, P., Chondrogianni, N., Gonos, E.S., Petropoulos, I., Friguet, B., Klotz, L.O., Krutmann, J., Grune, T., 2009. The proteasome is an integral part of solar ultraviolet a radiation-induced gene expression. J. Biol. Chem. 284, 30076–30086.

Chan, D.C., 2012. Fusion and fission: interlinked processes critical for mitochondrial health. Annu. Rev. Genet. 46, 265–287.

Chang, H.C., Guarente, L., 2013. SIRT1 mediates central circadian control in the SCN by a mechanism that decays with aging. Cell 153, 1448–1460.

Chauhan, A.K., Iaconcig, A., Baralle, F.E., Muro, A.F., 2004. Alternative splicing of fibronectin: a mouse model demonstrates the identity of in vitro and in vivo systems and the processing autonomy of regulated exons in adult mice. Gene 324, 55–63.

Chen, H., Chan, D.C., 2009. Mitochondrial dynamics – fusion, fission, movement, and mitophagy – in neurodegenerative diseases. Hum. Mol. Genet. 18, R169–R176.

Chen, I.P., Henning, S., Faust, A., Boukamp, P., Volkmer, B., Greinert, R., 2012. UVA-induced epigenetic regulation of P16(INK4a) in human epidermal keratinocytes and skin tumor derived cells. Photochem. Photobiol. Sci.: Off. J. Eur. Photochem. Assoc. Eur. Soc. Photobiol. 11, 180–190.

Chen, X., Li, Z., Feng, Z., Wang, J., Ouyang, C., Liu, W., Fu, B., Cai, G., Wu, C., Wei, R., Wu, D., Hong, Q., 2006. Integrin-linked kinase induces both senescence-associated alterations and extracellular fibronectin assembly in aging cardiac fibroblasts. J. Gerontol. A: Biol. Sci. Med. Sci. 61, 1232–1245.

Cho, D.H., Nakamura, T., Lipton, S.A., 2010. Mitochondrial dynamics in cell death and neurodegeneration. Cell. Mol. Life Sci. 67, 3435–3447.

Choi, S.S., Miller, M.A., Harper, P.A., 2006. In utero exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin induces amphiregulin gene expression in the developing mouse ureter. Toxicol. Sci.: Off. J. Soc. Toxicol. 94, 163–174.

Chondrogianni, N., Kapeta, S., Chinou, I., Vassilatou, K., Papassideri, I., Gonos, E.S., 2010. Anti-ageing and rejuvenating effects of quercetin. Exp. Gerontol. 45, 763–771.

Chondrogianni, N., Trougakos, I.P., Kletsas, D., Chen, Q.M., Gonos, E.S., 2008. Partial proteasome inhibition in human fibroblasts triggers accelerated M1 senescence or M2 crisis depending on p53 and Rb status. Aging Cell 7, 717–732.

Clegg, A., Young, J., 2011. The frailty syndrome. Clin. Med. 11, 72–75.

Columbaro, M., Capanni, C., Mattioli, E., Novelli, G., Parnaik, V.K., Squarzoni, S., Maraldi, N.M., Lattanzi, G., 2005. Rescue of heterochromatin organization in Hutchinson-Gilford progeria by drug treatment. Cell. Mol. Life Sci. 62, 2669–2678.

Copeland, J.M., Cho, J., Lo Jr., T., Hur, J.H., Bahadorani, S., Arabyan, T., Rabie, J., Soh, J., Walker, D.W., 2009. Extension of Drosophila life span by RNAi of the mitochondrial respiratory chain. Curr. Biol. 19, 1591–1598.

Coppe, J.P., Desprez, P.Y., Krtolica, A., Campisi, J., 2010. The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu. Rev. Pathol. 5, 99–118.

Coppe, J.P., Patil, C.K., Rodier, F., Sun, Y., Munoz, D.P., Goldstein, J., Nelson, P.S., Desprez, P.Y., Campisi, J., 2008. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol. 6, 2853–2868.

Cristofalo, V.J., Allen, R.G., Pignolo, R.J., Martin, B.G., Beck, J.C., 1998. Relationship between donor age and the replicative lifespan of human cells in culture: a reevaluation. Proc. Natl. Acad. Sci. U. S. A. 95, 10614–10619.

Cuervo, A.M., Bergamini, E., Brunk, U.T., Droge, W., Ffrench, M., Terman, A., 2005. Autophagy and aging: the importance of maintaining clean cells. Autophagy 1, 131–140.

Curtis, H., Crowley, C., 1963. Chromosome aberrations in liver cells in relation to the somatic mutation theory of aging. Radiat. Res. 19, 337–344.

Cypser, J.R., Tedesco, P., Johnson, T.E., 2006. Hormesis and aging in *Caenorhabditis elegans*. Exp. Gerontol. 41, 935–939.

d'Adda di Fagagna, F., Reaper, P.M., Clay-Farrace, L., Fiegler, H., Carr, P., Von Zglinicki, T., Saretzki, G., Carter, N.P., Jackson, S.P., 2003. A DNA damage checkpoint response in telomere-initiated senescence. Nature 426, 194–198.

Dai, G., Freudenberger, T., Zipper, P., Melchior, A., Grether-Beck, S., Rabausch, B., de Groot, J., Twarock, S., Hanenberg, H., Homey, B., Krutmann, J., Reifenberger, J., Fischer, J.W., 2007. Chronic ultraviolet B irradiation causes loss of hyaluronic acid from mouse dermis because of down-regulation of hyaluronic acid synthases. Am. J. Pathol. 171, 1451–1461.

Daniell, H.W., 1971. Smoker's wrinkles. A study in the epidemiology of crow's feet. Ann. Intern. Med. 75, 873–880.

De Cecco, M., Criscione, S.W., Peckham, E.J., Hillenmeyer, S., Hamm, E.A., Manivannan, J., Peterson, A.L., Kreiling, J.A., Neretti, N., Sedivy, J.M., 2013. Genomes of replicatively senescent cells undergo global epigenetic changes leading to gene silencing and activation of transposable elements. Aging Cell 12, 247–256.

Dell'agnello, C., Leo, S., Agostino, A., Szabadkai, G., Tiveron, C., Zulian, A., Prelle, A., Roubertoux, P., Rizzuto, R., Zeviani, M., 2007. Increased longevity and refractoriness to Ca(2+)-dependent neurodegeneration in Surf1 knockout mice. Hum. Mol. Genet. 16, 431–444.

Detmer, S.A., Chan, D.C., 2007. Functions and dysfunctions of mitochondrial dynamics. Nat. Rev. Mol. Cell Biol. 8, 870–879.

Diderich, K., Alanazi, M., Hoeijmakers, J.H., 2011. Premature aging and cancer in nucleotide excision repair-disorders. DNA Rep. (Amst.) 10, 772–780.

Dierick, J.F., Eliaers, F., Remacle, J., Raes, M., Fey, S.J., Larsen, P.M., Toussaint, O., 2002. Stress-induced premature senescence and replicative senescence are different phenotypes, proteomic evidence. Biochem. Pharmacol. 64, 1011–1017.

Dillin, A., Hsu, A.L., Arantes-Oliveira, N., Lehrer-Graiwer, J., Hsin, H., Fraser, A.G., Kamath, R.S., Ahringer, J., Kenyon, C., 2002. Rates of behavior and aging specified by mitochondrial function during development. Science 298, 2398–2401.

Dillman, A.A., Hauser, D.N., Gibbs, J.R., Nalls, M.A., McCoy, M.K., Rudenko, I.N., Galter, D., Cookson, M.R., 2013. mRNA expression, splicing and editing in the embryonic and adult mouse cerebral cortex. Nat. Neurosci. 16, 499–506.

Dimri, G.P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E.E., Linskens, M., Rubelj, I., Pereira-Smith, O., et al., 1995. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc. Natl. Acad. Sci. U. S. A. 92, 9363–9367.

Druzhyna, N.M., Wilson, G.L., LeDoux, S.P., 2008. Mitochondrial DNA repair in aging and disease. Mech. Ageing Dev. 129, 383–390.

Durieux, J., Wolff, S., Dillin, A., 2011. The cell-non-autonomous nature of electron transport chain-mediated longevity. Cell 144, 79–91.

Faggioli, F., Vijg, J., Montagna, C., 2011. Chromosomal aneuploidy in the aging brain. Mech. Ageing Dev. 132, 429–436.

Fenech, M., 1998. Chromosomal damage rate, aging, and diet. Ann. N. Y. Acad. Sci. 854, 23–36.

Fenech, M., 2007. Nutrition and genome health. Forum Nutr. 60, 49–65.

Figge, M.T., Osiewacz, H.D., Reichert, A.S., 2013. Quality control of mitochondria during aging: is there a good and a bad side of mitochondrial dynamics? BioEssays: News Rev. Mol. Cell. Dev. Biol. 35, 314–322.

Figge, M.T., Reichert, A.S., Meyer-Hermann, M., Osiewacz, H.D., 2012. Deceleration of fusion-fission cycles improves mitochondrial quality control during aging. PLoS Comput. Biol. 8, e1002576.

Figueroa, R., Lindenmaier, H., Hergenhahn, M., Nielsen, K.V., Boukamp, P., 2000. Telomere erosion varies during in vitro aging of normal human fibroblasts from young and adult donors. Cancer Res. 60, 2770–2774.

Finley, L.W., Haigis, M.C., 2009. The coordination of nuclear and mitochondrial communication during aging and calorie restriction. Ageing Res. Rev. 8, 173–188.

Fischer, H.G., Morawski, M., Bruckner, M.K., Mittag, A., Tarnok, A., Arendt, T., 2012. Changes in neuronal DNA content variation in the human brain during aging. Aging Cell 11, 628–633.

Fisher, G.J., Datta, S.C., Talwar, H.S., Wang, Z.Q., Varani, J., Kang, S., Voorhees, J.J., 1996. Molecular basis of sun-induced premature skin ageing and retinoid antagonism. Nature 379, 335–339.

Fritsche, E., Schafer, C., Calles, C., Bernsmann, T., Bernshausen, T., Wurm, M., Hubenthal, U., Cline, J.E., Hajimiragha, H., Schroeder, P., Klotz, L.O., Rannug, A., Furst, P., Hanenberg, H., Abel, J., Krutmann, J., 2007. Lightening up the UV response by identification of the arylhydrocarbon receptor as a cytoplasmatic target for ultraviolet B radiation. Proc. Natl. Acad. Sci. U. S. A. 104, 8851–8856.

Fu, L., Pelicano, H., Liu, J., Huang, P., Lee, C., 2002. The circadian gene Period2 plays an important role in tumor suppression and DNA damage response in vivo. Cell 111, 41–50.

Fumagalli, M., Rossiello, F., Clerici, M., Barozzi, S., Cittaro, D., Kaplunov, J.M., Bucci, G., Dobreva, M., Matti, V., Beausejour, C.M., Herbig, U., Longhese, M.P., d'Adda di Fagagna, F., 2012. Telomeric DNA damage is irreparable and causes persistent DNA-damage-response activation. Nat. Cell Biol. 14, 355–365.

Gaddameedhi, S., Selby, C.P., Kaufmann, W.K., Smart, R.C., Sancar, A., 2011. Control of skin cancer by the circadian rhythm. Proc. Natl. Acad. Sci. U. S. A. 108, 18790–18795.

Garinis, G.A., Uittenboogaard, L.M., Stachelscheid, H., Fousteri, M., van Ijcken, W., Breit, T.M., van Steeg, H., Mullenders, L.H., van der Horst, G.T., Bruning, J.C., Niessen, C.M., Hoeijmakers, J.H., Schumacher, B., 2009. Persistent transcription-blocking DNA lesions trigger somatic growth attenuation associated with longevity. Nat. Cell Biol. 11, 604–615.

Garm, C., Moreno-Villanueva, M., Burkle, A., Petersen, I., Bohr, V.A., Christensen, K., Stevnsner, T., 2013. Age and gender effects on DNA strand break repair in peripheral blood mononuclear cells. Aging Cell 12, 58–66.

Giangreco, A., Qin, M., Pintar, J.E., Watt, F.M., 2008. Epidermal stem cells are retained in vivo throughout skin aging. Aging Cell 7, 250–259.

Gilchrest, B.A., Eller, M.S., Yaar, M., 2009. Telomere-mediated effects on melanogenesis and skin aging. J. Investig. Dermatol. Sympos. Proc./Soc. Investig. Dermatol. Eur. Soc. Dermatol. Res. 14, 25–31.

Glass, D., Vinuela, A., Davies, M.N., Ramasamy, A., Parts, L., Knowles, D., Brown, A.A., Hedman, A.K., Small, K.S., Buil, A., Grundberg, E., Nica, A.C., Meglio, P., Nestle, F.O., Ryten, M., Durbin, R., McCarthy, M.I., Deloukas, P., Dermitzakis, E.T., Weale, M.E., Bataille, V., Spector, T.D., 2013. Gene expression changes with age in skin, adipose tissue, blood and brain. Genome Biol. 14, R75.

Goldman, R.D., Shumaker, D.K., Erdos, M.R., Eriksson, M., Goldman, A.E., Gordon, L.B., Gruenbaum, Y., Khuon, S., Mendez, M., Varga, R., Collins, F.S., 2004. Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome. Proc. Natl. Acad. Sci. U. S. A. 101, 8963–8968.

Goldstein, S., Moerman, E.J., Porter, K., 1984. High-voltage electron microscopy of human diploid fibroblasts during ageing in vitro. Morphometric analysis of mitochondria. Exp. Cell Res. 154, 101–111.

Gomes, A.P., Price, N.L., Ling, A.J.Y., Moslehi, J.J., Montgomery, M.K., Rajman, L., White, J.P., Teodoro, J.S., Wrann, J.D., Hubbart, B.P., Mercken, E.M., Palmeira, C.M., de Cabo, R., Rolo, A.P., Turner, N., Bell, E.L., Sinclair, D.A., 2013. Declining NAD+ induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. Cell 155, 1624–1638.

Gorbunova, V., Seluanov, A., Mao, Z., Hine, C., 2007. Changes in DNA repair during aging. Nucleic Acids Res. 35, 7466–7474.

Gottlieb, R.A., Mentzer, R.M., 2010. Autophagy during cardiac stress: joys and frustrations of autophagy. Annu. Rev. Physiol. 72, 45–59.

Grady, D., Ernster, V., 1992. Does cigarette smoking make you ugly and old? Am. J. Epidemiol. 135, 839–842.

Greco, M., Villani, G., Mazzucchelli, F., Bresolin, N., Papa, S., Attardi, G., 2003. Marked aging-related decline in efficiency of oxidative phosphorylation in human skin fibroblasts. FASEB J. 17, 1706–1708.

Greussing, R., Hackl, M., Charoentong, P., Pauck, A., Monteforte, R., Cavinato, M., Hofer, E., Scheideler, M., Neuhaus, M., Micutkova, L., Mueck, C., Trajanoski, Z., Grillari, J., Jansen-Durr, P., 2013. Identification of microRNA–mRNA functional interactions in UVB-induced senescence of human diploid fibroblasts. BMC Genomics 14, 224.

Gronniger, E., Weber, B., Heil, O., Peters, N., Stab, F., Wenck, H., Korn, B., Winnefeld, M., Lyko, F., 2010. Aging and chronic sun exposure cause distinct epigenetic changes in human skin. PLoS Genet. 6, e1000971.

Guarente, L., 2013. Calorie restriction and sirtuins revisited. Genes Dev. 27, 2072–2085.

Haarmann-Stemmann, T., Boege, F., Krutmann, J., 2013. Adaptive and maladaptive responses in skin: mild heat exposure protects against UVB-induced photoaging in mice. J. Invest. Dermatol. 133, 868–871.

Hackl, M., Brunner, S., Fortschegger, K., Schreiner, C., Micutkova, L., Muck, C., Laschober, G.T., Lepperdinger, G., Sampson, N., Berger, P., Herndler-Brandstetter, D., Wieser, M., Kuhnel, H., Strasser, A., Rinnerthaler, M., Breitenbach, M., Mildner, M., Eckhart, L., Tschachler, E., Trost, A., Bauer, J.W., Papak, C., Trajanoski, Z., Scheideler, M., Grillari-Voglauer, R., Grubeck-Loebenstein, B., Jansen-Durr, P., Grillari, J., 2010. miR-17, miR-19b, miR-20a, and miR-106a are down-regulated in human aging. Aging Cell 9, 291–296.

Hardy, K., Mansfield, L., Mackay, A., Benvenuti, S., Ismail, S., Arora, P., O'Hare, M.J., Jat, P.S., 2005. Transcriptional networks and cellular senescence in human mammary fibroblasts. Mol. Biol. Cell 16, 943–953.

Harley, C.B., Futcher, A.B., Greider, C.W., 1990. Telomeres shorten during ageing of human fibroblasts. Nature 345, 458–460.

Harman, D., 1956. Aging: a theory based on free radical and radiation chemistry. J. Gerontol. 11, 298–300.

Harries, L.W., Hernandez, D., Henley, W., Wood, A.R., Holly, A.C., Bradley-Smith, R.M., Yaghootkar, H., Dutta, A., Murray, A., Frayling, T.M., Guralnik, J.M., Bandinelli, S., Singleton, A., Ferrucci, L., Melzer, D., 2011. Human aging is characterized by focused changes in gene expression and deregulation of alternative splicing. Aging Cell 10, 868–878.

Hartl, F.U., Bracher, A., Hayer-Hartl, M., 2011. Molecular chaperones in protein folding and proteostasis. Nature 475, 324–332.

Hasty, P., Campisi, J., Hoeijmakers, J., van Steeg, H., Vijg, J., 2003. Aging and genome maintenance: lessons from the mouse? Science 299, 1355–1359.

Hayflick, L., 1980. Recent advances in the cell biology of aging. Mech. Ageing Dev. 14, 59–79.

Hayflick, L., Moorhead, P.S., 1961. The serial cultivation of human diploid cell strains. Exp. Cell Res. 25, 585–621.

Hensbergen, P., Alewijnse, A., Kempenaar, J., van der Schors, R.C., Balog, C.A., Deelder, A., Beumer, G., Ponec, M., Tensen, C.P., 2005. Proteomic profiling identifies an UV-induced activation of cofilin-1 and destrin in human epidermis. J. Invest. Dermatol. 124, 818–824.

Herbig, U., Ferreira, M., Condel, L., Carey, D., Sedivy, J.M., 2006. Cellular senescence in aging primates. Science 311, 1257.

Herbig, U., Jobling, W.A., Chen, B.P., Chen, D.J., Sedivy, J.M., 2004. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). Mol. Cell 14, 501–513.

Hoeijmakers, J.H., 2009. DNA damage, aging, and cancer. N. Engl. J. Med. 361, 1475–1485.

Hoffmann, S., Spitkovsky, D., Radicella, J.P., Epe, B., Wiesner, R.J., 2004. Reactive oxygen species derived from the mitochondrial respiratory chain are not responsible for the basal levels of oxidative base modifications observed in nuclear DNA of Mammalian cells. Free Radic. Biol. Med. 36, 765–773.

Honma, S., Ono, D., Suzuki, Y., Inagaki, N., Yoshikawa, T., Nakamura, W., Honma, K., 2012. Suprachiasmatic nucleus: cellular clocks and networks. Prog. Brain Res. 199, 129–141.

Horvath, S., 2013. DNA methylation age of human tissues and cell types. Genome Biol. 14, R115.

Hsieh, P., Yamane, K., 2008. DNA mismatch repair: molecular mechanism, cancer, and ageing. Mech. Ageing Dev. 129, 391–407.

Hutter, E., Renner, K., Pfister, G., Stockl, P., Jansen-Durr, P., Gnaiger, E., 2004. Senescence-associated changes in respiration and oxidative phosphorylation in primary human fibroblasts. Biochem. J. 380, 919–928.

Hwang, E.S., Yoon, G., Kang, H.T., 2009. A comparative analysis of the cell biology of senescence and aging. Cell. Mol. Life Sci. 66, 2503–2524.

Inukai, S., Slack, F., 2013. MicroRNAs and the genetic network in aging. J. Mol. Biol. 425, 3601–3608.

Jacobs, K.B., Yeager, M., Zhou, W., Wacholder, S., Wang, Z., Rodriguez-Santiago, B., Hutchinson, A., Deng, X., Liu, C., Horner, M.J., Cullen, M., Epstein, C.G., Burdett, L., Dean, M.C., Chatterjee, N., Sampson, J., Chung, C.C., Kovaks, J., Gapstur, S.M., Stevens, V.L., Teras, L.T., Gaudet, M.M., Albanes, D., Weinstein, S.J., Virtamo, J., Taylor, P.R., Freedman, N.D., Abnet, C.C., Goldstein, A.M., Hu, N., Yu, K., Yuan, J.M., Liao, L., Ding, T., Qiao, Y.L., Gao, Y.T., Koh, W.P., Xiang, Y.B., Tang, Z.Z., Fan, J.H., Aldrich, M.C., Amos, C., Blot, W.J., Bock, C.H., Gillanders, E.M., Harris, C.C., Haiman, C.A., Henderson, B.E., Kolonel, L.N., Le Marchand, L., McNeill, L.H., Rybicki, B.A., Schwartz, A.G., Signorello, L.B., Spitz, M.R., Wiencke, J.K., Wrensch, M., Wu, X., Zanetti, K.A., Ziegler, R.G., Figueroa, J.D., Garcia-Closas, M., Malats, N., Marenne, G., Prokunina-Olsson, L., Baris, D., Schwenn, M., Johnson, A., Landi, M.T., Goldin, L., Consonni, D., Bertazzi, P.A., Rotunno, M., Rajaraman, P., Andersson, U., Beane Freeman, L.E., Berg, C.D., Buring, J.E., Butler, M.A., Carreon, T., Feychting, M., Ahlbom, A., Gaziano, J.M., Giles, G.G., Hallmans, G., Hankinson, S.E., Hartge, P., Henriksson, R., Inskip, P.D., Johansen, C., Landgren, A., McKean-Cowdin, R., Michaud, D.S., Melin, B.S., Peters, U., Ruder, A.M., Sesso, H.D., Severi, G., Shu, X.O., Visvanathan, K., White, E., Wolk, A., Zeleniuch-Jacquotte, A., Zheng, W., Silverman, D.T., Kogevinas, M., Gonzalez, J.R., Villa, O., Li, D., Duell, E.J., Risch, H.A., Olson, S.H., Kooperberg, C., Wolpin, B.M., Jiao, L., Hassan, M., Wheeler, W., Arslan, A.A., Bueno-de-Mesquita, H.B., Fuchs, C.S., Gallinger, S., Gross, M.D., Holly, E.A., Klein, A.P., LaCroix, A., Mandelson, M.T., Petersen, G., Boutron-Ruault, M.C., Bracci, P.M., Canzian, F., Chang, K., Cotterchio, M., Giovannucci, E.L., Goggins, M., Hoffman Bolton, J.A., Jenab, M., Khaw, K.T., Krogh, V., Kurtz, R.C., McWilliams, R.R., Mendelsohn, J.B., Rabe, K.G., Riboli, E., Tjonneland, A., Tobias, G.S., Trichopoulos, D., Elena, J.W., Yu, H., Amundadottir, L., Stolzenberg-Solomon, R.Z., Kraft, P., Schumacher, F., Stram, D., Savage, S.A., Mirabello, L., Andrulis, I.L., Wunder, J.S., Patino Garcia, A., Sierrasesumaga, L., Barkauskas, D.A., Gorlick, R.G., Purdue, M., Chow, W.H., Moore, L.E., Schwartz, K.L., Davis, F.G., Hsing, A.W., Berndt, S.I., Black, A., Wentzensen, N., Brinton, L.A., Lissowska, J., Peplonska, B., McGlynn, K.A., Cook, M.B., Graubard, B.I., Kratz, C.P., Greene, M.H., Erickson, R.L., Hunter, D.J., Thomas, G., Hoover, R.N., Real, F.X., Fraumeni Jr., J.F., Caporaso, N.E., Tucker, M., Rothman, N., Perez-Jurado, L.A., Chanock, S.J., 2012. Detectable clonal mosaicism and its relationship to aging and cancer. Nat. Genet. 44, 651–658.

Jendrach, M., Pohl, S., Voth, M., Kowald, A., Hammerstein, P., Bereiter-Hahn, J., 2005. Morpho-dynamic changes of mitochondria during ageing of human endothelial cells. Mech. Ageing Dev. 126, 813–821.

Jeyapalan, J.C., Ferreira, M., Sedivy, J.M., Herbig, U., 2007. Accumulation of senescent cells in mitotic tissue of aging primates. Mech. Ageing Dev. 128, 36–44.

Jiang, M., Liu, K., Luo, J., Dong, Z., 2010. Autophagy is a renoprotective mechanism during in vitro hypoxia and in vivo ischemia-reperfusion injury. Am. J. Pathol. 176, 1181–1192.

John, K., Lahoti, T.S., Wagner, K., Hughes, J.M., Perdew, G.H., 2013. The Ah receptor regulates growth factor expression in head and neck squamous cell carcinoma cell lines. Mol. Carcinog., http://dx.doi.org/10.1002/mc.22032, [Epub ahead of print].

Johnson, T.E., Henderson, S., Murakami, S., de Castro, E., de Castro, S.H., Cypser, J., Rikke, B., Tedesco, P., Link, C., 2002. Longevity genes in the nematode *Caenorhabditis elegans* also mediate increased resistance to stress and prevent disease. J. Inherit. Metab. Dis. 25, 197–206.

Jones, M.R., Ravid, K., 2004. Vascular smooth muscle polyploidization as a biomarker for aging and its impact on differential gene expression. J. Biol. Chem. 279, 5306–5313.

Jun, J.I., Lau, L.F., 2010a. Cellular senescence controls fibrosis in wound healing. Aging 2, 627–631.

Jun, J.I., Lau, L.F., 2010b. The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. Nat. Cell Biol. 12, 676–685.

Jurk, D., Wang, C., Miwa, S., Maddick, M., Korolchuk, V., Tsolou, A., Gonos, E.S., Thrasivoulou, C., Saffrey, M.J., Cameron, K., von Zglinicki, T., 2012. Postmitotic neurons develop a p21-dependent senescence-like phenotype driven by a DNA damage response. Aging Cell 11, 996–1004.

Karran, P., 1996. Microsatellite instability and DNA mismatch repair in human cancer. Semin. Cancer Biol. 7, 15–24.

Katiyar, S.K., Singh, T., Prasad, R., Sun, Q., Vaid, M., 2012. Epigenetic alterations in ultraviolet radiation-induced skin carcinogenesis: interaction of bioactive dietary components on epigenetic targets. Photochem. Photobiol. 88, 1066–1074.

Katsiki, M., Chondrogianni, N., Chinou, I., Rivett, A.J., Gonos, E.S., 2007. The olive constituent oleuropein exhibits proteasome stimulatory properties in vitro and confers life span extension of human embryonic fibroblasts. Rejuven. Res. 10, 157–172.

Khrapko, K., Kraytsberg, Y., de Grey, A.D., Vijg, J., Schon, E.A., 2006. Does premature aging of the mtDNA mutator mouse prove that mtDNA mutations are involved in natural aging? Aging Cell 5, 279–282.

Kipling, D., Davis, T., Ostler, E.L., Faragher, R.G., 2004. What can progeroid syndromes tell us about human aging? Science 305, 1426–1431.

Kirchman, P.A., Kim, S., Lai, C.Y., Jazwinski, S.M., 1999. Interorganelle signaling is a determinant of longevity in *Saccharomyces cerevisiae*. Genetics 152, 179–190.

Kirkwood, T.B., 2005. Understanding the odd science of aging. Cell 120, 437–447.

Kirkwood, T.B., Melov, S., 2011. On the programmed/non-programmed nature of ageing within the life history. Curr. Biol. 21, R701–R707.

Knott, A., Reuschlein, K., Lucius, R., Stab, F., Wenck, H., Gallinat, S., 2009. Deregulation of versican and elastin binding protein in solar elastosis. Biogerontology 10, 181–190.

Koch, C.M., Reck, K., Shao, K., Lin, Q., Joussen, S., Ziegler, P., Walenda, G., Drescher, W., Opalka, B., May, T., Brummendorf, T., Zenke, M., Saric, T., Wagner, W., 2013. Pluripotent stem cells escape from senescence-associated DNA methylation changes. Genome Res. 23, 248–259.

Kondratov, R.V., Antoch, M.P., 2007. The clock proteins, aging, and tumorigenesis. Cold Spring Harb. Symp. Quant. Biol. 72, 477–482.

Kondratov, R.V., Kondratova, A.A., Gorbacheva, V.Y., Vykhovanets, O.V., Antoch, M.P., 2006. Early aging and age-related pathologies in mice deficient in BMAL1, the core componentof the circadian clock. Genes Dev. 20, 1868–1873.

Kondratova, A.A., Kondratov, R.V., 2012. The circadian clock and pathology of the ageing brain. Nat. Rev. Neurosci. 13, 325–335.

Koshiishi, I., Horikoshi, E., Mitani, H., Imanari, T., 1999. Quantitative alterations of hyaluronan and dermatan sulfate in the hairless mouse dorsal skin exposed to chronic UV irradiation. Biochim. Biophys. Acta 1428, 327–333.

Koziel, R., Greussing, R., Maier, A.B., Declercq, L., Jansen-Durr, P., 2011. Functional interplay between mitochondrial and proteasome activity in skin aging. J. Invest. Dermatol. 131, 594–603.

Krause, K.H., 2007. Aging: a revisited theory based on free radicals generated by NOX family NADPH oxidases. Exp. Gerontol. 42, 256–262.

Kreiling, J.A., Tamamori-Adachi, M., Sexton, A.N., Jeyapalan, J.C., Munoz-Najar, U., Peterson, A.L., Manivannan, J., Rogers, E.S., Pchelintsev, N.A., Adams, P.D., Sedivy, J.M., 2011. Age-associated increase in heterochromatic marks in murine and primate tissues. Aging Cell 10, 292–304.

Krichevsky, S., Pawelec, G., Gural, A., Effros, R.B., Globerson, A., Yehuda, D.B., Yehuda, A.B., 2004. Age related microsatellite instability in T cells from healthy individuals. Exp. Gerontol. 39, 507–515.

Krishnan, K.J., Reeve, A.K., Samuels, D.C., Chinnery, P.F., Blackwood, J.K., Taylor, R.W., Wanrooij, S., Spelbrink, J.N., Lightowlers, R.N., Turnbull, D.M., 2008. What causes mitochondrial DNA deletions in human cells? Nat. Genet. 40, 275–279.

Krunic, D., Moshir, S., Greulich-Bode, K.M., Figueroa, R., Cerezo, A., Stammer, H., Stark, H.J., Gray, S.G., Nielsen, K.V., Hartschuh, W., Boukamp, P., 2009. Tissue context-activated telomerase in human epidermis correlates with little age-dependent telomere loss. Biochim. Biophys. Acta 1792, 297–308.

Krutmann, J., Morita, A., Chung, J.H., 2012. Sun exposure: what molecular photodermatology tells us about its good and bad sides. J. Invest. Dermatol. 132, 976–984.

Krutmann, J., Schroeder, P., 2009. Role of mitochondria in photoaging of human skin: the defective powerhouse model. J. Investig. Dermatol. Sympos. Proc./Soc. Investig. Dermatol. Eur. Soc. Dermatol. Res. 14, 44–49.

Kueper, T., Grune, T., Prahl, S., Lenz, H., Welge, V., Biernoth, T., Vogt, Y., Muhr, G.M., Gaemlich, A., Jung, T., Boemke, G., Elsasser, H.P., Wittern, K.P., Wenck, H., Stab, F., Blatt, T., 2007. Vimentin is the specific target in skin glycation. Structural prerequisites, functional consequences, and role in skin aging. J. Biol. Chem. 282, 23427–23436.

Kwak, I.H., Kim, H.S., Choi, O.R., Ryu, M.S., Lim, I.K., 2004. Nuclear accumulation of globular actin as a cellular senescence marker. Cancer Res. 64, 572–580.

Lamia, K.A., Sachdeva, U.M., DiTacchio, L., Williams, E.C., Alvarez, J.G., Egan, D.F., Vasquez, D.S., Juguilon, H., Panda, S., Shaw, R.J., Thompson, C.B., Evans, R.M., 2009. AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. Science 326, 437–440.

Lane, N., 2012. The problem with mixing mitochondria. Cell 151, 246–248.

Lans, H., Hoeijmakers, J.H., 2012. Genome stability, progressive kidney failure and aging. Nat. Genet. 44, 836–838.

Lapointe, J., Stepanyan, Z., Bigras, E., Hekimi, S., 2009. Reversal of the mitochondrial phenotype and slow development of oxidative biomarkers of aging in long-lived Mclk1+/− mice. J. Biol. Chem. 284, 20364–20374.

Lee, C.K., Allison, D.B., Brand, J., Weindruch, R., Prolla, T.A., 2002a. Transcriptional profiles associated with aging and middle age-onset caloric restriction in mouse hearts. Proc. Natl. Acad. Sci. U. S. A. 99, 14988–14993.

Lee, H.C., Yin, P.H., Chi, C.W., Wei, Y.H., 2002b. Increase in mitochondrial mass in human fibroblasts under oxidative stress and during replicative cell senescence. J. Biomed. Sci. 9, 517–526.
Lemasters, J.J., 2005. Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction, and aging. Rejuven. Res. 8, 3–5.
Lener, B., Koziel, R., Pircher, H., Hutter, E., Greussing, R., Herndler-Brandstetter, D., Hermann, M., Unterluggauer, H., Jansen-Durr, P., 2009. The NADPH oxidase Nox4 restricts the replicative lifespan of human endothelial cells. Biochem. J. 423, 363–374.
Lener, T., Moll, P.R., Rinnerthaler, M., Bauer, J., Aberger, F., Richter, K., 2006. Expression profiling of aging in the human skin. Exp. Gerontol. 41, 387–397.
Leufke, C., Leykauf, J., Krunic, D., Jauch, A., Holtgreve-Grez, H., Bohm-Steuer, B., Brocker, E.B., Mauch, C., Utikal, J., Hartschuh, W., Purdie, K.J., Boukamp, P., 2013. The telomere profile distinguishes two classes of genetically distinct cutaneous squamous cell carcinomas. Oncogene, http://dx.doi.org/10.1038/onc.2013.323, [Epub ahead of print].
Lezza, A.M., Pesce, V., Cormio, A., Fracasso, F., Vecchiet, J., Felzani, G., Cantatore, P., Gadaleta, M.N., 2001. Increased expression of mitochondrial transcription factor A and nuclear respiratory factor-1 in skeletal muscle from aged human subjects. FEBS Lett. 501, 74–78.
Li, M., Verdijk, L.B., Sakamoto, K., Ely, B., van Loon, L.J., Musi, N., 2012. Reduced AMPK-ACC and mTOR signaling in muscle from older men, and effect of resistance exercise. Mech. Ageing Dev. 133, 655–664.
Liesa, M., Palacin, M., Zorzano, A., 2009. Mitochondrial dynamics in mammalian health and disease. Physiol. Rev. 89, 799–845.
Lombard, D.B., Chua, K.F., Mostoslavsky, R., Franco, S., Gostissa, M., Alt, F.W., 2005. DNA repair, genome stability, and aging. Cell 120, 497–512.
Lopez-Mejia, I.C., Vautrot, V., De Toledo, M., Behm-Ansmant, I., Bourgeois, C.F., Navarro, C.L., Osorio, F.G., Freije, J.M., Stevenin, J., De Sandre-Giovannoli, A., Lopez-Otin, C., Levy, N., Branlant, C., Tazi, J., 2011. A conserved splicing mechanism of the LMNA gene controls premature aging. Hum. Mol. Genet. 20, 4540–4555.
Lopez-Otin, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G., 2013. The hallmarks of aging. Cell 153, 1194–1217.
Ma, D., Li, S., Molusky, M.M., Lin, J.D., 2012. Circadian autophagy rhythm: a link between clock and metabolism? Trends Endocrinol. Metab.: TEM 23, 319–325.
Ma, D., Panda, S., Lin, J.D., 2011. Temporal orchestration of circadian autophagy rhythm by C/EBPbeta. EMBO J. 30, 4642–4651.
Madeo, F., Tavernarakis, N., Kroemer, G., 2010. Can autophagy promote longevity? Nat. Cell Biol. 12, 842–846.
Magnuson, V.L., Young, M., Schattenberg, D.G., Mancini, M.A., Chen, D.L., Steffensen, B., Klebe, R.J., 1991. The alternative splicing of fibronectin pre-mRNA is altered during aging and in response to growth factors. J. Biol. Chem. 266, 14654–14662.
Maier, A.B., le Cessie, S., de Koning-Treurniet, C., Blom, J., Westendorp, R.G., van Heemst, D., 2007. Persistence of high-replicative capacity in cultured fibroblasts from nonagenarians. Aging Cell 6, 27–33.
Makrantonaki, E., Brink, T.C., Zampeli, V., Elewa, R.M., Mlody, B., Hossini, A.M., Hermes, B., Krause, U., Knolle, J., Abdallah, M., Adjaye, J., Zouboulis, C.C., 2012. Identification of biomarkers of human skin ageing in both genders. Wnt signalling – a label of skin ageing? PLoS ONE 7, e50393.
Makrantonaki, E., Schonknecht, P., Hossini, A.M., Kaiser, E., Katsouli, M.M., Adjaye, J., Schroder, J., Zouboulis, C.C., 2010. Skin and brain age together: the role of hormones in the ageing process. Exp. Gerontol. 45, 801–813.
Mancini, M., Saintigny, G., Mahe, C., Annicchiarico-Petruzzelli, M., Melino, G., Candi, E., 2012. MicroRNA-152 and -181a participate in human dermal fibroblasts senescence acting on cell adhesion and remodeling of the extra-cellular matrix. Aging 4, 843–853.
Mao, Z., Bozzella, M., Seluanov, A., Gorbunova, V., 2008. DNA repair by nonhomologous end joining and homologous recombination during cell cycle in human cells. Cell Cycle 7, 2902–2906.
Martins, I., Galluzzi, L., Kroemer, G., 2011. Hormesis, cell death and aging. Aging 3, 821–828.
Maslov, A.Y., Ganapathi, S., Westerhof, M., Quispe, W., White, R.R., Van Houten, B., Reiling, E., Dolle, M.E., van Steeg, H., Hasty, P., Hoeijmakers, J.H., Vijg, J., 2013. DNA damage in normally and prematurely aged mice. Aging Cell 12, 467–477.
Matsuda, M., Hoshino, T., Yamakawa, N., Tahara, K., Adachi, H., Sobue, G., Maji, D., Ihn, H., Mizushima, T., 2013. Suppression of UV-induced wrinkle formation by induction of HSP70 expression in mice. J. Invest. Dermatol. 133, 919–928.
Maury, E., Ramsey, K.M., Bass, J., 2010. Circadian rhythms and metabolic syndrome: from experimental genetics to human disease. Circ. Res. 106, 447–462.
Mayer, P.J., Lange, C.S., Bradley, M.O., Nichols, W.W., 1989. Age-dependent decline in rejoining of X-ray-induced DNA double-strand breaks in normal human lymphocytes. Mutat. Res. 219, 95–100.
Mazin, P., Xiong, J., Liu, X., Yan, Z., Zhang, X., Li, M., He, L., Somel, M., Yuan, Y., Phoebe Chen, Y.P., Li, N., Hu, Y., Fu, N., Ning, Z., Zeng, R., Yang, H., Chen, W., Gelfand, M., Khaitovich, P., 2013. Widespread splicing changes in human brain development and aging. Mol. Syst. Biol. 9, 633.
McClintock, D., Ratner, D., Lokuge, M., Owens, D.M., Gordon, L.B., Collins, F.S., Djabali, K., 2007. The mutant form of lamin A that causes Hutchinson-Gilford progeria is a biomarker of cellular aging in human skin. PLoS ONE 2, e1269.
McMurray, M.A., Gottschling, D.E., 2003. An age-induced switch to a hyper-recombinational state. Science 301, 1908–1911.
Menger, G.J., Allen, G.C., Neuendorff, N., Nahm, S.S., Thomas, T.L., Cassone, V.M., Earnest, D.J., 2007. Circadian profiling of the transcriptome in NIH/3T3 fibroblasts: comparison with rhythmic gene expression in SCN2.2 cells and the rat SCN. Physiol. Genomics 29, 280–289.
Micutkova, L., Diener, T., Li, C., Rogowska-Wrzesinska, A., Mueck, C., Huetter, E., Weinberger, B., Grubeck-Loebenstein, B., Roepstorff, P., Zeng, R., Jansen-Duerr, P., 2011. Insulin-like growth factor binding protein-6 delays replicative senescence of human fibroblasts. Mech. Ageing Dev. 132, 468–479.
Mizushima, N., Levine, B., Cuervo, A.M., Klionsky, D.J., 2008. Autophagy fights disease through cellular self-digestion. Nature 451, 1069–1075.
Moller, P., Lohr, M., Folkmann, J.K., Mikkelsen, L., Loft, S., 2010. Aging and oxidatively damaged nuclear DNA in animal organs. Free Radic. Biol. Med. 48, 1275–1285.
Morita, A., Torii, K., Maeda, A., Yamaguchi, Y., 2009. Molecular basis of tobacco smoke-induced premature skin aging. J. Investig. Dermatol. Sympos. Proc./Soc. Investig. Dermatol. Eur. Soc. Dermatol. Res. 14, 53–55.
Moriwaki, S., Takahashi, Y., 2008. Photoaging and DNA repair. J. Dermatol. Sci. 50, 169–176.
Morley, J.E., Vellas, B., van Kan, G.A., Anker, S.D., Bauer, J.M., Bernabei, R., Cesari, M., Chumlea, W.C., Doehner, W., Evans, J., Fried, L.P., Guralnik, J.M., Katz, P.R., Malmstrom, T.K., McCarter, R.J., Gutierrez Robledo, L.M., Rockwood, K., von Haehling, S., Vandewoude, M.F., Walston, J., 2013. Frailty consensus: a call to action. J. Am. Med. Directors Assoc. 14, 392–397.
Morselli, E., Galluzzi, L., Kepp, O., Criollo, A., Maiuri, M.C., Tavernarakis, N., Madeo, F., Kroemer, G., 2009. Autophagy mediates pharmacological lifespan extension by spermidine and resveratrol. Aging 1, 961–970.
Morselli, E., Maiuri, M.C., Markaki, M., Megalou, E., Pasparaki, A., Palikaras, K., Criollo, A., Galluzzi, L., Malik, S.A., Vitale, I., Michaud, M., Madeo, F., Tavernarakis, N., Kroemer, G., 2010. Caloric restriction and resveratrol promote longevity through the Sirtuin-1-dependent induction of autophagy. Cell Death Dis. 1, e10.
Morselli, E., Marino, G., Bennetzen, M.V., Eisenberg, T., Megalou, E., Schroeder, S., Cabrera, S., Benit, P., Rustin, P., Criollo, A., Kepp, O., Galluzzi, L., Shen, S., Malik, S.A., Maiuri, M.C., Horio, Y., Lopez-Otin, C., Andersen, J.S., Tavernarakis, N., Madeo, F., Kroemer, G., 2011. Spermidine and resveratrol induce autophagy by distinct pathways converging on the acetylproteome. J. Cell Biol. 192, 615–629.
Muratore, C.R., Hodgson, N.W., Trivedi, M.S., Abdolmaleky, H.M., Persico, A.M., Lintas, C., De la Monte, S., Deth, R.C., 2013. Age-dependent decrease and alternative splicing of methionine synthase mRNA in human cerebral cortex and an accelerated decrease in autism. PLoS ONE 8, e56927.
Nakahata, Y., Kaluzova, M., Grimaldi, B., Sahar, S., Hirayama, J., Chen, D., Guarente, L.P., Sassone-Corsi, P., 2008. The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. Cell 134, 329–340.
Nakahata, Y., Sahar, S., Astarita, G., Kaluzova, M., Sassone-Corsi, P., 2009. Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. Science 324, 654–657.
Narita, M., Nunez, S., Heard, E., Lin, A.W., Hearn, S.A., Spector, D.L., Hannon, G.J., Lowe, S.W., 2003. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell 113, 703–716.
Nelson, G., Wordsworth, J., Wang, C., Jurk, D., Lawless, C., Martin-Ruiz, C., von Zglinicki, T., 2012. A senescent cell bystander effect: senescence-induced senescence. Aging Cell 11, 345–349.
Neri, S., Gardini, A., Facchini, A., Olivieri, F., Franceschi, C., Ravaglia, G., Mariani, E., 2005. Mismatch repair system and aging: microsatellite instability in peripheral blood cells from differently aged participants. J. Gerontol. A: Biol. Sci. Med. Sci. 60, 285–292.
Nishino, K., Toyoda, M., Yamazaki-Inoue, M., Fukawatase, Y., Chikazawa, E., Sakaguchi, H., Akutsu, H., Umezawa, A., 2011. DNA methylation dynamics in human induced pluripotent stem cells over time. PLoS Genet. 7, e1002085.
Nishio, K., Inoue, A., 2005. Senescence-associated alterations of cytoskeleton: extraordinary production of vimentin that anchors cytoplasmic p53 in senescent human fibroblasts. Histochem. Cell Biol. 123, 263–273.
Nishio, K., Inoue, A., Qiao, S., Kondo, H., Mimura, A., 2001. Senescence and cytoskeleton: overproduction of vimentin induces senescent-like morphology in human fibroblasts. Histochem. Cell Biol. 116, 321–327.
Nunnari, J., Suomalainen, A., 2012. Mitochondria: in sickness and in health. Cell 148, 1145–1159.
Oberbauer, A.M., 2013. The regulation of IGF-1 gene transcription and splicing during development and aging. Front. Endocrinol. 4, 39.
Ohshima, S., 2012. Centrosome aberrations associated with cellular senescence and p53 localization at supernumerary centrosomes. Oxid. Med. Cell. Longevity 2012, 217594.
Oklejewicz, M., Destici, E., Tamanini, F., Hut, R.A., Janssens, R., van der Horst, G.T., 2008. Phase resetting of the mammalian circadian clock by DNA damage. Curr. Biol. 18, 286–291.
Ono, Y., Torii, K., Fritsche, E., Shintani, Y., Nishida, E., Nakamura, M., Shirakata, Y., Haarmann-Stemmann, T., Abel, J., Krutmann, J., Morita, A., 2013. Role of the aryl hydrocarbon receptor in tobacco smoke extract-induced matrix metalloproteinase-1 expression. Exp. Dermatol. 22, 349–353.
Osorio, F.G., Navarro, C.L., Cadinanos, J., Lopez-Mejia, I.C., Quiros, P.M., Bartoli, C., Rivera, J., Tazi, J., Guzman, G., Varela, I., Depetris, D., de Carlos, F., Cobo, J., Andres, V., De Sandre-Giovannoli, A., Freije, J.M., Levy, N., Lopez-Otin, C., 2011. Splicing-directed therapy in a new mouse model of human accelerated aging. Sci. Transl. Med. 3, 106ra107.
Pagani, F., Zagato, L., Vergani, C., Casari, G., Sidoli, A., Baralle, F.E., 1991. Tissue-specific splicing pattern of fibronectin messenger RNA precursor during development and aging in rat. J. Cell Biol. 113, 1223–1229.
Park, S.C., Park, J.S., Park, W.Y., Cho, K.A., Ahn, J.S., Jang, I.S., 2002. Down-regulation of receptor-mediated endocytosis is responsible for senescence-associated hyporesponsiveness. Ann. N. Y. Acad. Sci. 959, 45–49.

Park, W.Y., Cho, K.A., Park, J.S., Kim, D.I., Park, S.C., 2001. Attenuation of EGF signaling in senescent cells by caveolin. Ann. N. Y. Acad. Sci. 928, 79–84.

Parrinello, S., Coppe, J.P., Krtolica, A., Campisi, J., 2005. Stromal-epithelial interactions in aging and cancer: senescent fibroblasts alter epithelial cell differentiation. J. Cell Sci. 118, 485–496.

Passos, J.F., Nelson, G., Wang, C., Richter, T., Simillion, C., Proctor, C.J., Miwa, S., Olijslagers, S., Hallinan, J., Wipat, A., Saretzki, G., Rudolph, K.L., Kirkwood, T.B., von Zglinicki, T., 2010. Feedback between p21 and reactive oxygen production is necessary for cell senescence. Mol. Syst. Biol. 6, 347.

Passos, J.F., Saretzki, G., von Zglinicki, T., 2007. DNA damage in telomeres and mitochondria during cellular senescence: is there a connection? Nucleic Acids Res. 35, 7505–7513.

Peek, C.B., Affinati, A.H., Ramsey, K.M., Kuo, H.Y., Yu, W., Sena, L.A., Ilkayeva, O., Marcheva, B., Kobayashi, Y., Omura, C., Levine, D.C., Bacsik, D.J., Gius, D., Newgard, C.B., Goetzman, E., Chandel, N.S., Denu, J.M., Mrksich, M., Bass, J., 2013. Circadian clock NAD+ cycle drives mitochondrial oxidative metabolism in mice. Science 342, 1243417.

Popescu, N.C., Amsbaugh, S.C., Milo, G., DiPaolo, J.A., 1986. Chromosome alterations associated with in vitro exposure of human fibroblasts to chemical or physical carcinogens. Cancer Res. 46, 4720–4725.

Preston, C.R., Flores, C., Engels, W.R., 2006. Age-dependent usage of double-strand-break repair pathways. Curr. Biol. 16, 2009–2015.

Quan, T., Qin, Z., Robichaud, P., Voorhees, J.J., Fisher, G.J., 2011. CCN1 contributes to skin connective tissue aging by inducing age-associated secretory phenotype in human skin dermal fibroblasts. J. Cell Commun. Signal. 5, 201–207.

Rajaratnam, S.M., Arendt, J., 2001. Health in a 24-h society. Lancet 358, 999–1005.

Ramsey, K.M., Yoshino, J., Brace, C.S., Abrassart, D., Kobayashi, Y., Marcheva, B., Hong, H.K., Chong, J.L., Buhr, E.D., Lee, C., Takahashi, J.S., Imai, S., Bass, J., 2009. Circadian clock feedback cycle through NAMPT-mediated NAD+ biosynthesis. Science 324, 651–654.

Ramsey, M.J., Moore 2nd, D.H., Briner, J.F., Lee, D.A., Olsen, L., Senft, J.R., Tucker, J.D., 1995. The effects of age and lifestyle factors on the accumulation of cytogenetic damage as measured by chromosome painting. Mutat. Res. 338, 95–106.

Ramsey, M.R., Sharpless, N.E., 2006. ROS as a tumour suppressor? Nat. Cell Biol. 8, 1213–1215.

Rea, S.L., Ventura, N., Johnson, T.E., 2007. Relationship between mitochondrial electron transport chain dysfunction, development, and life extension in *Caenorhabditis elegans*. PLoS Biol. 5, e259.

Reinke, H., Saini, C., Fleury-Olela, F., Dibner, C., Benjamin, I.J., Schibler, U., 2008. Differential display of DNA-binding proteins reveals heat-shock factor 1 as a circadian transcription factor. Genes Dev. 22, 331–345.

Ressler, S., Bartkova, J., Niederegger, H., Bartek, J., Scharffetter-Kochanek, K., Jansen-Durr, P., Wlaschek, M., 2006. p16INK4A is a robust in vivo biomarker of cellular aging in human skin. Aging Cell 5, 379–389.

Rhie, G., Shin, M.H., Seo, J.Y., Choi, W.W., Cho, K.H., Kim, K.H., Park, K.C., Eun, H.C., Chung, J.H., 2001a. Aging- and photoaging-dependent changes of enzymic and nonenzymic antioxidants in the epidermis and dermis of human skin in vivo. J. Invest. Dermatol. 117, 1212–1217.

Rhie, G.E., Seo, J.Y., Chung, J.H., 2001b. Modulation of catalase in human skin in vivo by acute and chronic UV radiation. Mol. Cells 11, 399–404.

Richards, S.A., Muter, J., Ritchie, P., Lattanzi, G., Hutchison, C.J., 2011. The accumulation of un-repairable DNA damage in laminopathy progeria fibroblasts is caused by ROS generation and is prevented by treatment with N-acetyl cysteine. Hum. Mol. Genet. 20, 3997–4004.

Ricke, R.M., van Deursen, J.M., 2013. Aneuploidy in health, disease, and aging. J. Cell Biol. 201, 11–21.

Ristow, M., Schmeisser, S., 2011. Extending life span by increasing oxidative stress. Free Radic. Biol. Med. 51, 327–336.

Ristow, M., Zarse, K., 2010. How increased oxidative stress promotes longevity and metabolic health: the concept of mitochondrial hormesis (mitohormesis). Exp. Gerontol. 45, 410–418.

Rock, K., Grandoch, M., Majora, M., Krutmann, J., Fischer, J.W., 2011. Collagen fragments inhibit hyaluronan synthesis in skin fibroblasts in response to ultraviolet B (UVB): new insights into mechanisms of matrix remodeling. J. Biol. Chem. 286, 18268–18276.

Rock, K., Meusch, M., Fuchs, N., Tigges, J., Zipper, P., Fritsche, E., Krutmann, J., Homey, B., Reifenberger, J., Fischer, J.W., 2012. Estradiol protects dermal hyaluronan/versican matrix during photoaging by release of epidermal growth factor from keratinocytes. J. Biol. Chem. 287, 20056–20069.

Rodier, F., Munoz, D.P., Teachenor, R., Chu, V., Le, O., Bhaumik, D., Coppe, J.P., Campeau, E., Beausejour, C.M., Kim, S.H., Davalos, A.R., Campisi, J., 2011. DNA-SCARS: distinct nuclear structures that sustain damage-induced senescence growth arrest and inflammatory cytokine secretion. J. Cell Sci. 124, 68–81.

Rodwell, G.E., Sonu, R., Zahn, J.M., Lund, J., Wilhelmy, J., Wang, L., Xiao, W., Mindrinos, M., Crane, E., Segal, E., Myers, B.D., Brooks, J.D., Davis, R.W., Higgins, J., Owen, A.B., Kim, S.K., 2004. A transcriptional profile of aging in the human kidney. PLoS Biol. 2, e427.

Rubinsztein, D.C., Marino, G., Kroemer, G., 2011. Autophagy and aging. Cell 146, 682–695.

Ruiz, M., Cefalu, C., Reske, T., 2012. Frailty syndrome in geriatric medicine. Am. J. Med. Sci. 344, 395–398.

Saini, C., Morf, J., Stratmann, M., Gos, P., Schibler, U., 2012. Simulated body temperature rhythms reveal the phase-shifting behavior and plasticity of mammalian circadian oscillators. Genes Dev. 26, 567–580.

Sander, C.S., Chang, H., Salzmann, S., Muller, C.S., Ekanayake-Mudiyanselage, S., Elsner, P., Thiele, J.J., 2002. Photoaging is associated with protein oxidation in human skin in vivo. J. Invest. Dermatol. 118, 618–625.

Satoh, A., Brace, C.S., Rensing, N., Cliften, P., Wozniak, D.F., Herzog, E.D., Yamada, K.A., Imai, S., 2013. Sirt1 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the DMH and LH. Cell Metab. 18, 416–430.

Scaffidi, P., Misteli, T., 2006. Lamin A-dependent nuclear defects in human aging. Science 312, 1059–1063.

Scheckhuber, C.Q., Hamann, A., Brust, D., Osiewacz, H.D., 2012. Cellular homeostasis in fungi: impact on the aging process. Subcell. Biochem. 57, 233–250.

Scheckhuber, C.Q., Wanger, R.A., Mignat, C.A., Osiewacz, H.D., 2011. Unopposed mitochondrial fission leads to severe lifespan shortening. Cell Cycle 10, 3105–3110.

Schiavi, A., Torgovnick, A., Kell, A., Megalou, E., Castelein, N., Guccini, I., Marzocchella, L., Gelino, S., Hansen, M., Malisan, F., Condo, I., Bei, R., Rea, S.L., Braeckman, B.P., Tavernarakis, N., Testi, R., Ventura, N., 2013. Autophagy induction extends lifespan and reduces lipid content in response to frataxin silencing in *C. elegans*. Exp. Gerontol. 48, 191–201.

Schmeisser, K., Mansfeld, J., Kuhlow, D., Weimer, S., Priebe, S., Heiland, I., Birringer, M., Groth, M., Segref, A., Kanfi, Y., Price, N.L., Schmeisser, S., Schuster, S., Pfeiffer, A.F., Guthke, R., Platzer, M., Hoppe, T., Cohen, H.Y., Zarse, K., Sinclair, D.A., Ristow, M., 2013. Role of sirtuins in lifespan regulation is linked to methylation of nicotinamide. Nat. Chem. Biol. 9, 693–700.

Schroeder, P., Schieke, S., Morita, A., 2006. Premature skin aging by infrared radiation, tobacco smoking and ozone. In: Gilchrest, B., Krutmann, J. (Eds.), Skin Aging. Springer, Heidelberg/New York, pp. 45–54.

Schulze, C., Wetzel, F., Kueper, T., Malsen, A., Muhr, G., Jaspers, S., Blatt, T., Wittern, K.P., Wenck, H., Kas, J.A., 2012. Stiffening of human skin fibroblasts with age. Clin. Plast. Surg. 39, 9–20.

Sedelnikova, O.A., Horikawa, I., Zimonjic, D.B., Popescu, N.C., Bonner, W.M., Barrett, J.C., 2004. Senescing human cells and ageing mice accumulate DNA lesions with unrepairable double-strand breaks. Nat. Cell Biol. 6, 168–170.

Seluanov, A., Mittelman, D., Pereira-Smith, O.M., Wilson, J.H., Gorbunova, V., 2004. DNA end joining becomes less efficient and more error-prone during cellular senescence. Proc. Natl. Acad. Sci. U. S. A. 101, 7624–7629.

Serrano, M., Lin, A.W., McCurrach, M.E., Beach, D., Lowe, S.W., 1997. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell 88, 593–602.

Shin, M.H., Moon, Y.J., Seo, J.E., Lee, Y., Kim, K.H., Chung, J.H., 2008. Reactive oxygen species produced by NADPH oxidase, xanthine oxidase, and mitochondrial electron transport system mediate heat shock-induced MMP-1 and MMP-9 expression. Free Radic. Biol. Med. 44, 635–645.

Shiraha, H., Gupta, K., Drabik, K., Wells, A., 2000. Aging fibroblasts present reduced epidermal growth factor (EGF) responsiveness due to preferential loss of EGF receptors. J. Biol. Chem. 275, 19343–19351.

Shumaker, D.K., Dechat, T., Kohlmaier, A., Adam, S.A., Bozovsky, M.R., Erdos, M.R., Eriksson, M., Goldman, A.E., Khuon, S., Collins, F.S., Jenuwein, T., Goldman, R.D., 2006. Mutant nuclear lamin A leads to progressive alterations of epigenetic control in premature aging. Proc. Natl. Acad. Sci. U. S. A. 103, 8703–8708.

Singh, N.P., Danner, D.B., Tice, R.R., Brant, L., Schneider, E.L., 1990. DNA damage and repair with age in individual human lymphocytes. Mutat. Res. 237, 123–130.

Skinner, A.M., Turker, M.S., 2005. Oxidative mutagenesis, mismatch repair, and aging. Sci. Aging Knowl. Environ.: SAGE KE 2005, re3.

Sprenger, A., Kuttner, V., Biniossek, M.L., Gretzmeier, C., Boerries, M., Mack, C., Has, C., Bruckner-Tuderman, L., Dengjel, J., 2010. Comparative quantitation of proteome alterations induced by aging or immortalization in primary human fibroblasts and keratinocytes for clinical applications. Mol. BioSyst. 6, 1579–1582.

Stern, M.M., Bickenbach, J.R., 2007. Epidermal stem cells are resistant to cellular aging. Aging Cell 6, 439–452.

Stern, R., Maibach, H.I., 2008. Hyaluronan in skin: aspects of aging and its pharmacologic modulation. Clin. Dermatol. 26, 106–122.

Sundberg, J.P., Berndt, A., Sundberg, B.A., Silva, K.A., Kennedy, V., Bronson, R., Yuan, R., Paigen, B., Harrison, D., Schofield, P.N., 2011. The mouse as a model for understanding chronic diseases of aging: the histopathologic basis of aging in inbred mice. Pathobiol. Aging Age Relat. Dis. 1 .

Suzuki, T., Fujii, M., Ayusawa, D., 2002. Demethylation of classical satellite 2 and 3 DNA with chromosomal instability in senescent human fibroblasts. Exp. Gerontol. 37, 1005–1014.

Takahashi, Y., Moriwaki, S., Sugiyama, Y., Endo, Y., Yamazaki, K., Mori, T., Takigawa, M., Inoue, S., 2005. Decreased gene expression responsible for post-ultraviolet DNA repair synthesis in aging: a possible mechanism of age-related reduction in DNA repair capacity. J. Invest. Dermatol. 124, 435–442.

Takeuchi, H., Runger, T.M., 2013. Longwave UV light induces the aging-associated progerin. J. Invest. Dermatol. 133, 1857–1862.

Tandara, A.A., Mustoe, T.A., 2011. MMP- and TIMP-secretion by human cutaneous keratinocytes and fibroblasts – impact of coculture and hydration. J. Plast. Reconstruct. Aesthet. Surg.: JPRAS 64, 108–116.

Tapia, P.C., 2006. Sublethal mitochondrial stress with an attendant stoichiometric augmentation of reactive oxygen species may precipitate many of the beneficial alterations in cellular physiology produced by caloric restriction, intermittent fasting, exercise and dietary phytonutrients: mitohormesis for health and vitality. Med. Hypoth. 66, 832–843.

Thomas, P., Fenech, M., 2008. Chromosome 17 and 21 aneuploidy in buccal cells is increased with ageing and in Alzheimer's disease. Mutagenesis 23, 57–65.

Tigges, J., Weighardt, H., Wolff, S., Gotz, C., Forster, I., Kohne, Z., Huebenthal, U., Merk, H.F., Abel, J., Haarmann-Stemmann, T., Krutmann, J., Fritsche, E., 2013. Aryl hydrocarbon receptor repressor (AhRR) function revisited: repression of CYP1 activity in human skin fibroblasts is not related to AhRR expression. J. Invest. Dermatol. 133, 87–96.

Tollervey, J.R., Wang, Z., Hortobagyi, T., Witten, J.T., Zarnack, K., Kayikci, M., Clark, T.A., Schweitzer, A.C., Rot, G., Curk, T., Zupan, B., Rogelj, B., Shaw, C.E., Ule, J., 2011. Analysis of alternative splicing associated with aging and neurodegeneration in the human brain. Genome Res. 21, 1572–1582.

Tomaru, U., Takahashi, S., Ishizu, A., Miyatake, Y., Gohda, A., Suzuki, S., Ono, A., Ohara, J., Baba, T., Murata, S., Tanaka, K., Kasahara, M., 2012. Decreased proteasomal activity causes age-related phenotypes and promotes the development of metabolic abnormalities. Am. J. Pathol. 180, 963–972.

Torgovnick, A., Schiavi, A., Testi, R., Ventura, N., 2010. A role for p53 in mitochondrial stress response control of longevity in *C. elegans*. Exp. Gerontol. 45, 550–557.

Toussaint, O., Dumont, P., Remacle, J., Dierick, J.F., Pascal, T., Frippiat, C., Magalhaes, J.P., Zdanov, S., Chainiaux, F., 2002. Stress-induced premature senescence or stress-induced senescence-like phenotype: one in vivo reality, two possible definitions? ScientificWorldJournal 2, 230–247.

Toussaint, O., Medrano, E.E., von Zglinicki, T., 2000. Cellular and molecular mechanisms of stress-induced premature senescence (SIPS) of human diploid fibroblasts and melanocytes. Exp. Gerontol. 35, 927–945.

Tran, K.T., Rusu, S.D., Satish, L., Wells, A., 2003. Aging-related attenuation of EGF receptor signaling is mediated in part by increased protein tyrosine phosphatase activity. Exp. Cell Res. 289, 359–367.

Trifunovic, A., Hansson, A., Wredenberg, A., Rovio, A.T., Dufour, E., Khvorostov, I., Spelbrink, J.N., Wibom, R., Jacobs, H.T., Larsson, N.G., 2005. Somatic mtDNA mutations cause aging phenotypes without affecting reactive oxygen species production. Proc. Natl. Acad. Sci. U. S. A. 102, 17993–17998.

Trifunovic, A., Wredenberg, A., Falkenberg, M., Spelbrink, J.N., Rovio, A.T., Bruder, C.E., Bohlooly, Y.M., Gidlof, S., Oldfors, A., Wibom, R., Tornell, J., Jacobs, H.T., Larsson, N.G., 2004. Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature 429, 417–423.

Vallabhaneni, H., O'Callaghan, N., Sidorova, J., Liu, Y., 2013. Defective repair of oxidative base lesions by the DNA glycosylase Nth1 associates with multiple telomere defects. PLoS Genet. 9, e1003639.

van der Horst, G.T., Muijtjens, M., Kobayashi, K., Takano, R., Kanno, S., Takao, M., de Wit, J., Verkerk, A., Eker, A.P., van Leenen, D., Buijs, R., Bootsma, D., Hoeijmakers, J.H., Yasui, A., 1999. Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. Nature 398, 627–630.

van Ham, T.J., Holmberg, M.A., van der Goot, A.T., Teuling, E., Garcia-Arencibia, M., Kim, H.E., Du, D., Thijssen, K.L., Wiersma, M., Burggraaff, R., van Bergeijk, P., van Rheenen, J., Jerre van Veluw, G., Hofstra, R.M., Rubinsztein, D.C., Nollen, E.A., 2010. Identification of MOAG-4/SERF as a regulator of age-related proteotoxicity. Cell 142, 601–612.

Varani, J., Spearman, D., Perone, P., Fligiel, S.E., Datta, S.C., Wang, Z.Q., Shao, Y., Kang, S., Fisher, G.J., Voorhees, J.J., 2001. Inhibition of type I procollagen synthesis by damaged collagen in photoaged skin and by collagenase-degraded collagen in vitro. Am. J. Pathol. 158, 931–942.

Varela, I., Pereira, S., Ugalde, A.P., Navarro, C.L., Suarez, M.F., Cau, P., Cadinanos, J., Osorio, F.G., Foray, N., Cobo, J., de Carlos, F., Levy, N., Freije, J.M., Lopez-Otin, C., 2008. Combined treatment with statins and aminobisphosphonates extends longevity in a mouse model of human premature aging. Nat. Med. 14, 767–772.

Vartiainen, M.K., 2008. Nuclear actin dynamics–from form to function. FEBS Lett. 582, 2033–2040.

Ventura, N., Rea, S., Henderson, S.T., Condo, I., Johnson, T.E., Testi, R., 2005. Reduced expression of frataxin extends the lifespan of Caenorhabditis elegans. Aging Cell 4, 109–112.

Ventura, N., Rea, S.L., 2007. Caenorhabditis elegans mitochondrial mutants as an investigative tool to study human neurodegenerative diseases associated with mitochondrial dysfunction. Biotechnol. J. 2, 584–595.

Ventura, N., Rea, S.L., Schiavi, A., Torgovnick, A., Testi, R., Johnson, T.E., 2009. p53/CEP-1 increases or decreases lifespan, depending on level of mitochondrial bioenergetic stress. Aging Cell 8, 380–393.

Ventura, N., Rea, S.L., Testi, R., 2006. Long-lived C. elegans mitochondrial mutants as a model for human mitochondrial-associated diseases. Exp. Gerontol. 41, 974–991.

Von Zglinicki, T., 2003. Replicative senescence and the art of counting. Exp. Gerontol. 38, 1259–1264.

von Zglinicki, T., Saretzki, G., Ladhoff, J., d'Adda di Fagagna, F., Jackson, S.P., 2005. Human cell senescence as a DNA damage response. Mech. Ageing Dev. 126, 111–117.

Waaijer, M.E., Parish, W.E., Strongitharm, B.H., van Heemst, D., Slagboom, P.E., de Craen, A.J., Sedivy, J.M., Westendorp, R.G., Gunn, D.A., Maier, A.B., 2012. The number of p16INK4a positive cells in human skin reflects biological age. Aging Cell 11, 722–725.

Wagner, W., Bork, S., Horn, P., Krunic, D., Walenda, T., Diehlmann, A., Benes, V., Blake, J., Huber, F.X., Eckstein, V., Boukamp, P., Ho, A.D., 2009. Aging and replicative senescence have related effects on human stem and progenitor cells. PLoS ONE 4, e5846.

Wan, Y.S., Wang, Z.Q., Voorhees, J., Fisher, G., 2001. EGF receptor crosstalks with cytokine receptors leading to the activation of c-Jun kinase in response to UV irradiation in human keratinocytes. Cell. Signal. 13, 139–144.

Wang, L., Yang, W., Ju, W., Wang, P., Zhao, X., Jenkins, E.C., Brown, W.T., Zhong, N., 2012. A proteomic study of Hutchinson-Gilford progeria syndrome: Application of 2D-chromotography in a premature aging disease. Biochem. Biophys. Res. Commun. 417, 1119–1126.

Wang, X., McCullough, K.D., Franke, T.F., Holbrook, N.J., 2000. Epidermal growth factor receptor-dependent Akt activation by oxidative stress enhances cell survival. J. Biol. Chem. 275, 14624–14631.

Welle, S., Brooks, A.I., Delehanty, J.M., Needler, N., Thornton, C.A., 2003. Gene expression profile of aging in human muscle. Physiol. Genomics 14, 149–159.

Westermann, B., 2010. Mitochondrial dynamics in model organisms: what yeasts: worms and flies have taught us about fusion and fission of mitochondria. Semin. Cell Dev. Biol. 21, 542–549.

Weyemi, U., Lagente-Chevallier, O., Boufraqech, M., Prenois, F., Courtin, F., Caillou, B., Talbot, M., Dardalhon, M., Al Ghuzlan, A., Bidart, J.M., Schlumberger, M., Dupuy, C., 2012. ROS-generating NADPH oxidase NOX4 is a critical mediator in oncogenic H-Ras-induced DNA damage and subsequent senescence. Oncogene 31, 1117–1129.

Wiesner, R.J., Zsurka, G., Kunz, W.S., 2006. Mitochondrial DNA damage and the aging process: facts and imaginations. Free Radic. Res. 40, 1284–1294.

Wilking, M., Ndiaye, M., Mukhtar, H., Ahmad, N., 2013. Circadian rhythm connections to oxidative stress: implications for human health. Antioxid. Redox Signal. 19, 192–208.

Woldt, E., Sebti, Y., Solt, L.A., Duhem, C., Lancel, S., Eeckhoute, J., Hesselink, M.K., Paquet, C., Delhaye, S., Shin, Y., Kamenecka, T.M., Schaart, G., Lefebvre, P., Neviere, R., Burris, T.P., Schrauwen, P., Staels, B., Duez, H., 2013. Rev-erb-alpha modulates skeletal muscle oxidative capacity by regulating mitochondrial biogenesis and autophagy. Nat. Med. 19, 1039–1046.

Won, C.H., Kwon, O.S., Kang, Y.J., Yoo, H.G., Lee, D.H., Chung, J.H., Kim, K.H., Park, W.S., Park, N.H., Cho, K., Kwon, S.O., Choi, J.S., Eun, H.C., 2012. Comparative secretome analysis of human follicular dermal papilla cells and fibroblasts using shotgun proteomics. BMB Rep. 45, 253–258.

Wu, D., Cypser, J.R., Yashin, A.I., Johnson, T.E., 2008. The U-shaped response of initial mortality in *Caenorhabditis elegans* to mild heat shock: does it explain recent trends in human mortality? J. Gerontol. A: Biol. Sci. Med. Sci. 63, 660–668.

Yamada, M., Udono, M.U., Hori, M., Hirose, R., Sato, S., Mori, T., Nikaido, O., 2006. Aged human skin removes UVB-induced pyrimidine dimers from the epidermis more slowly than younger adult skin in vivo. Arch. Dermatol. Res. 297, 294–302.

Yang, S.H., Meta, M., Qiao, X., Frost, D., Bauch, J., Coffinier, C., Majumdar, S., Bergo, M.O., Young, S.G., Fong, L.G., 2006. A farnesyltransferase inhibitor improves disease phenotypes in mice with a Hutchinson-Gilford progeria syndrome mutation. J. Clin. Invest. 116, 2115–2121.

Yen, W.L., Klionsky, D.J., 2008. How to live long and prosper: autophagy, mitochondria, and aging. Physiology 23, 248–262.

Zahn, J.M., Poosala, S., Owen, A.B., Ingram, D.K., Lustig, A., Carter, A., Weeraratna, A.T., Taub, D.D., Gorospe, M., Mazan-Mamczarz, K., Lakatta, E.G., Boheler, K.R., Xu, X., Mattson, M.P., Falco, G., Ko, M.S., Schlessinger, D., Firman, J., Kummerfeld, S.K., Wood 3rd, W.H., Zonderman, A.B., Kim, S.K., Becker, K.G., 2007. AGEMAP: a gene expression database for aging in mice. PLoS Genet. 3, e201.

Zahn, J.M., Sonu, R., Vogel, H., Crane, E., Mazan-Mamczarz, K., Rabkin, R., Davis, R.W., Becker, K.G., Owen, A.B., Kim, S.K., 2006. Transcriptional profiling of aging in human muscle reveals a common aging signature. PLoS Genet. 2, e115.

Zhang, J., Bardot, E., Ezhkova, E., 2012. Epigenetic regulation of skin: focus on the Polycomb complex. Cell. Mol. Life Sci. 69, 2161–2172.

Zielonka, J., Kalyanaraman, B., 2010. Hydroethidine- and MitoSOX-derived red fluorescence is not a reliable indicator of intracellular superoxide formation: another inconvenient truth. Free Radic. Biol. Med. 48, 983–1001.