Mechanisms of Ageing and Development 138 (2014) 26–44

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Review

The hallmarks of fibroblast ageing

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ARTICLE INFO

Article history:
Received 2 November 2013
Received in revised form 11 March 2014
Accepted 18 March 2014
Available online 29 March 2014

Keywords:
Dermal fibroblasts
Extrinsic skin ageing
Mitochondrial dysfunction
DNA damage
Altered proteostasis
Cellular senescence

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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http://dx.doi.org/10.1016/j.mad.2014.03.004
0047-6374/

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,