General introduction and outline of the thesis

General introduction

The skin is a most crucial organ, but often under-valued in its importance for human health. It is the main organ through which we interact with the outside world, functioning as a sensor of external stimuli and as a barrier to protect our internal milieu from damaging external exposures. Its appearance is not only of cosmetic interest, but reflects the internal physiological state of the body. The skin is connected to the entire body, and many biological processes in the skin span across the body, such as vitamin D metabolism [1], immune responses [2] and thermoregulation [3]. Hence, the process of skin aging is likely to also reflect aging processes occurring in other tissues and is therefore a good model in which we can study the aging process and its impact on human health.

The degree skin has aged is reflected by an individual's perceived age in facial images[4], i.e. how old an individual looks irrespective of their actual calendar age. This measure of facial aging has been strongly linked to systemic aging: already in younger subjects perceived age has been linked to a sharper decline in a composite of biomarkers reflecting aging of several organ systems [11]. In middle-aged to elderly individuals a higher perceived age is associated with indicators of poorer health such as high glucose levels, high blood pressure and carotid atherosclerosis [5-7]. In the elderly a higher perceived age has also been linked to lower survival [8;9]. Other phenotypes indicative of poorer health such as lower cognitive performance and low handgrip strength are also associated with a higher perceived age in elderly persons [9;10].

The appearance of skin is closely related its structural properties- i.e. its histologic and morphological characteristics. The most exterior layer of the skin, the epidermis typically shows atrophy with advanced age. The interface of the epidermis with the layer underneath, the dermis, also flattens with age [12-15]. The dermis itself consists of various cell types, collagen, elastic fibers and extracellular matrix proteins, plus various appendages such as vasculature, glands and hair follicles. At a higher age collagen is less synthesized by fibroblasts, and collagen networks are disorganised and thickened compared to at younger ages [16]. Elastic fibres are found in higher amounts in aged skin and have a larger and less structured appearance [16-18]. These features are found at sun-protected sites but are also evoked to a greater extent by external stressors such as UV damage and smoking [19;20].

Another age related phenomenon that occurs in the skin is cellular senescence – i.e. stable cell cycle arrest. This phenomenon was first described by Hayflick and Moorhead in the form of so-called replicative senescence, observing the limited replicative capacity of cultured fibroblasts. They theorized that this in vitro phenomenon could occur in vivo as well and contributes to the aging process [21;22]. Cellular senescence can be triggered by insults such

as genomic damage, oncogenic signals and telomere attrition [23;24]. Next to the occurrence of cellular senescence in vitro, an increasing number of studies report a higher prevalence of senescence in aged tissues of several mammals [25-30]. In human skin higher amounts of senescence-associated markers have been found in older persons compared to young [31-33]. In addition to this presumed age-dependency of cellular senescence, links with age-related disease have been described. For example, senescent cells have been linked to glomerular disease, lung emphysema, Alzheimer's disease and diabetic nephropathy [30;34-36].

Detrimental effects of cellular senescence could be the result of loss of tissue homeostasis with reduced numbers of cells with replicative potential, but could also be the result of factors secreted by senescent cells, i.e. the senescence-associated secretory phenotype (SASP). Amongst these SASP factors are cytokines, proteases and growth factors [37], and there is some evidence that in vitro the SASP adds to inflammation [23] and can have tumorigenic properties in neighbouring cells [38;39]. These clues that cellular senescence might be implicated in the aging process have led to studies investigating its use of as a potential marker for the aging process and its potential for slowing the aging process [40-45].

In this thesis we focus on the skin as a model to study aging, using several methodologies: the appearance of facial skin, histological and morphological characteristics, the presence of cellular senescence in skin biopsies and characteristics of cultured skin fibroblasts. All these skin phenotypes were measured in middle-aged to old participants of the Leiden Longevity Study. The Leiden Longevity Study aimed to determine factors contributing to familial longevity [46]. Families were defined as long-lived if at least two siblings were alive and aged 89 (male) or 91 (female) or older. From these families their middle-aged to old (63 years on average) offspring were asked to participate, as it was hypothesized that a familial propensity for longevity would be (partially) conveyed to these offspring. Their partners were included as age and environmentally matched controls. The offspring of these nonagenarian sibling indeed appear to age at a slower pace when compared to their partners, as indicated by a lower standardized mortality rate [46], a lower prevalence of cardiovascular and metabolic diseases [47], enhanced insulin sensitivity [48] and better cognitive performance [49]. In addition, fibroblasts derived from skin biopsies of these offspring display less cellular senescence upon stress in vitro compared to their partners [50].

From both the offspring and their partners we obtained their perceived ages from facial photographs, and skin biopsies were obtained from the sun-protected upper inner arm to assess histologic morphological characteristics and cellular senescence. The cultured fibroblasts from these biopsies were assessed for different senescence-associated features in vitro. In order to study larger age differences we also made use of fibroblasts strains obtained

from 90 year old participants from the Leiden 85-plus Study, a prospective population-based study [51], and young controls (22 years on average) [52].

Aim of the thesis

We aim to study the manner and extent to which the skin reflects the aging process. We will study the appearance of facial skin, histologic morphological characteristics, cellular senescence in skin biopsies and in cultured skin fibroblasts, and their respective associations with age, membership of a long-lived family and health status.

Outline of the thesis

In **Part I** we question whether skin fibroblasts in vitro mirror the aging process. We study the association between several senescence-associated features in cultured fibroblasts (microRNA-663 expression, DNA damage markers) with (1) the age of the donor from who the cultured fibroblasts were derived, (2) membership of a long-lived family (propensity for longevity) and (3) health status (presence of cardiovascular or metabolic diseases). We further study whether different senescence markers are associated in vitro, and whether these markers are associated intra-individually with the senescence-associated protein p16INK4a in situ. **Part II** focusses on phenotypes of the skin biopsies from the Leiden Longevity Study participants, to study whether skin tissue mirrors the aging process. Differences in the exterior appearance of the skin, histologic morphological characteristics and amount of cellular senescence in situ were studied dependent on age, membership of a long-lived family and health status. In addition the interrelations between these skin phenotypes were studied. In **Part III** we aim to further place the phenomenon of cellular senescence in context, firstly recapitulating published work on cellular senescence dependent on age in various human tissues in a systematic review. Secondly we compared its value as potential marker of the aging process to currently used measures of functional capacity.

References

(1) Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357:266-281.

(2) Di MP, Perera GK, Nestle FO. The multitasking organ: recent insights into skin immune function. *Immunity* 2011;35:857-869.

(3) Charkoudian N. Skin blood flow in adult human thermoregulation: how it works, when it does not, and why. *Mayo Clin Proc* 2003;78:603-612.

(4) Gunn DA, Rexbye H, Griffiths CE et al. Why some women look young for their age. *PLoS One* 2009;4:e8021.

(5) Noordam R, Gunn DA, Tomlin CC et al. High serum glucose levels are associated with a higher perceived age. *Age (Dordr)* 2013;35:189-195.

(6) Gunn DA, de Craen AJ, Dick JL et al. Facial appearance reflects human familial longevity and cardiovascular disease risk in healthy individuals. *J Gerontol A Biol Sci Med Sci* 2013;68:145-152.

(7) Kido M, Kohara K, Miyawaki S, Tabara Y, Igase M, Miki T. Perceived age of facial features is a significant diagnosis criterion for age-related carotid atherosclerosis in Japanese subjects: J-SHIPP study. *Geriatr Gerontol Int* 2012;12:733-740.

(8) Dykiert D, Bates TC, Gow AJ, Penke L, Starr JM, Deary IJ. Predicting mortality from human faces. *Psychosom Med* 2012;74:560-566.

(9) Christensen K, Thinggaard M, McGue M et al. Perceived age as clinically useful biomarker of ageing: cohort study. *BMJ* 2009;339:b5262.

(10) Gunn DA, Larsen LA, Lall JS, Rexbye H, Christensen K. Mortality is Written on the Face. *J Gerontol A Biol Sci Med Sci* 2016;71:72-77.

(11) Belsky DW, Caspi A, Houts R et al. Quantification of biological aging in young adults. *Proc Natl Acad Sci U S A* 2015;112:E4104-E4110.

(12) Fenske NA, Conard CB. Aging skin. *Am Fam Physician* 1988;37:219-230.

(13) Fenske NA, Lober CW. Structural and functional changes of normal aging skin. *J Am Acad Dermatol* 1986;15:571-585.

(14) Kurban RS, Bhawan J. Histologic changes in skin associated with aging. *J Dermatol Surg Oncol* 1990;16:908-914.

(15) Lavker RM, Zheng PS, Dong G. Morphology of aged skin. *Dermatol Clin* 1986;4:379-389.

(16) Baumann L. Skin ageing and its treatment. *J Pathol* 2007;211:241-251.

(17) Robert C, Lesty C, Robert AM. Ageing of the skin: study of elastic fiber network modifications by computerized image analysis. *Gerontology* 1988;34:291-296.

(18) Frances C, Branchet MC, Boisnic S, Lesty CL, Robert L. Elastic fibers in normal human skin. Variations with age: a morphometric analysis. *Arch Gerontol Geriatr* 1990;10:57-67.

(19) Warren R, Gartstein V, Kligman AM, Montagna W, Allendorf RA, Ridder GM. Age, sunlight, and facial skin: a histologic and quantitative study. *J Am Acad Dermatol* 1991;25:751-760.

(20) Just M, Ribera M, Monso E, Lorenzo JC, Ferrandiz C. Effect of smoking on skin elastic fibres: morphometric and immunohistochemical analysis. *Br J Dermatol* 2007;156:85-91.

(21) Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. *Exp Cell Res* 1961;25:585-621.

(22) Hayflick L. The limited in vitro lifetime of human diploid cell strains. *Exp Cell Res* 1965;37:614-636.

(23) Campisi J. Aging, cellular senescence, and cancer. *Annu Rev Physiol* 2013;75:685-705.

(24) Campisi J, d'Adda di FF. Cellular senescence: when bad things happen to good cells. *Nat Rev Mol Cell Biol* 2007;8:729-740.

(25) Krishnamurthy J, Torrice C, Ramsey MR et al. Ink4a/Arf expression is a biomarker of aging. *J Clin Invest* 2004;114:1299-1307.

(26) Herbig U, Ferreira M, Condel L, Carey D, Sedivy JM. Cellular senescence in aging primates. *Science* 2006;311:1257.

(27) Jeyapalan JC, Ferreira M, Sedivy JM, Herbig U. Accumulation of senescent cells in mitotic tissue of aging primates. *Mech Ageing Dev* 2007;128:36-44.

(28) Melk A, Schmidt BM, Takeuchi O, Sawitzki B, Rayner DC, Halloran PF. Expression of p16INK4a and other cell cycle regulator and senescence associated genes in aging human kidney. *Kidney Int* 2004;65:510-520.

(29) Liu Y, Sanoff HK, Cho H et al. Expression of p16(INK4a) in peripheral blood T-cells

is a biomarker of human aging. *Aging Cell* 2009;8:439-448.

(30) Bhat R, Crowe EP, Bitto A et al. Astrocyte senescence as a component of Alzheimer's disease. *PLoS One* 2012;7:e45069.

(31) Dimri GP, Lee X, Basile G et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci U S A* 1995;92:9363-9367.

(32) Ressler S, Bartkova J, Niederegger H et al. p16INK4A is a robust in vivo biomarker of cellular aging in human skin. *Aging Cell* 2006;5:379-389.

(33) Kalfalah F, Seggewiss S, Walter R et al. Structural chromosome abnormalities, increased DNA strand breaks and DNA strand break repair deficiency in dermal fibroblasts from old female human donors. *Aging (Albany NY)* 2015;7:110-122.

(34) Sis B, Tasanarong A, Khoshjou F, Dadras F, Solez K, Halloran PF. Accelerated expression of senescence associated cell cycle inhibitor p16INK4A in kidneys with glomerular disease. *Kidney Int* 2007;71:218-226.

(35) Tsuji T, Aoshiba K, Nagai A. Alveolar cell senescence in patients with pulmonary emphysema. *Am J Respir Crit Care Med* 2006;174:886-893.

(36) Verzola D, Gandolfo MT, Gaetani G et al. Accelerated senescence in the kidneys of patients with type 2 diabetic nephropathy. *Am J Physiol Renal Physiol* 2008;295:F1563-F1573.

(37) Coppe JP, Patil CK, Rodier F et al. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol* 2008;6:2853-2868.

(38) Laberge RM, Awad P, Campisi J, Desprez PY. Epithelial-mesenchymal transition induced by senescent fibroblasts. *Cancer Microenviron* 2012;5:39-44.

(39) Parrinello S, Coppe JP, Krtolica A, Campisi J. Stromal-epithelial interactions in aging and cancer: senescent fibroblasts alter epithelial cell differentiation. *J Cell Sci* 2005;118:485-496.

(40) Gingell-Littlejohn M, McGuinness D, McGlynn LM et al. Pre-transplant CDKN2A expression in kidney biopsies predicts renal function and is a future component of donor scoring criteria. *PLoS One* 2013;8:e68133.

(41) Koppelstaetter C, Schratzberger G, Perco P et al. Markers of cellular senescence in zero hour biopsies predict outcome in renal transplantation. *Aging Cell* 2008;7:491-497.

(42) Pustavoitau A, Barodka V, Sharpless NE et al. Role of senescence marker p16INK4a measured in peripheral blood T-lymphocytes in predicting length of hospital stay after coronary artery bypass surgery in older adults. *Experimental Gerontology*. In press.

(43) Baker DJ, Wijshake T, Tchkonia T et al. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature* 2011;479:232-236.

(44) Zhu Y, Tchkonia T, Pirtskhalava T et al. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. *Aging Cell* 2015;14:644-658.

(45) Baker DJ, Childs BG, Durik M et al. Naturally occurring p16-positive cells shorten healthy lifespan. *Nature* 2016.

(46) Schoenmaker M, de Craen AJ, de Meijer PH et al. Evidence of genetic enrichment for exceptional survival using a family approach: the Leiden Longevity Study. *Eur J Hum Genet* 2006;14:79-84.

(47) Westendorp RG, van Heemst D, Rozing MP et al. Nonagenarian siblings and their offspring display lower risk of mortality and morbidity than sporadic nonagenarians: The Leiden Longevity Study. *J Am Geriatr Soc* 2009;57:1634-1637.

(48) Wijsman CA, Rozing MP, Streefland TC et al. Familial longevity is marked by enhanced insulin sensitivity. *Aging Cell* 2011;10:114-121.

(49) Stijntjes M, de Craen AJ, van HD et al. Familial longevity is marked by better cognitive performance at middle age: the Leiden Longevity Study. *PLoS One* 2013;8:e57962.

(50) Dekker P, Maier AB, van HD et al. Stress-induced responses of human skin fibroblasts in vitro reflect human longevity. *Aging Cell* 2009;8:595-603.

(51) Bootsma-van der Wiel A, Gussekloo J, de Craen AJ, Van EE, Bloem BR, Westendorp RG. Common chronic diseases and general impairments as determinants of walking disability in the oldest-old population. *J Am Geriatr Soc* 2002;50:1405-1410.

(52) Maier AB, le CS, de Koning-Treurniet C, Blom J, Westendorp RG, van HD. Persistence of high-replicative capacity in cultured fibroblasts from nonagenarians. *Aging Cell* 2007;6:27-33.