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Author: Waaijer, Mariëtte
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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, immune responses and thermoregulation. 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, 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. 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. In the elderly a higher perceived age has also been linked to lower survival. 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.

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. 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. Elastic fibres are found in higher amounts in aged skin and have a larger and less structured appearance. 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.

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. Cellular senescence can be triggered by insults such...
as genomic damage, oncogenic signals and telomere attrition. 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. In human skin higher amounts of senescence-associated markers have been found in older persons compared to young. 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.

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, and there is some evidence that in vitro the SASP adds to inflammation and can have tumorigenic properties in neighbouring cells. These clues that cellular senescence might be implicated in the aging process have led to studies investigating its use as a potential marker for the aging process and its potential for slowing the aging process.

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. 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, a lower prevalence of cardiovascular and metabolic diseases, enhanced insulin sensitivity and better cognitive performance. In addition, fibroblasts derived from skin biopsies of these offspring display less cellular senescence upon stress in vitro compared to their partners.

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.
General introduction and outline of the thesis

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