Cellular senescence in vitro and organismal ageing

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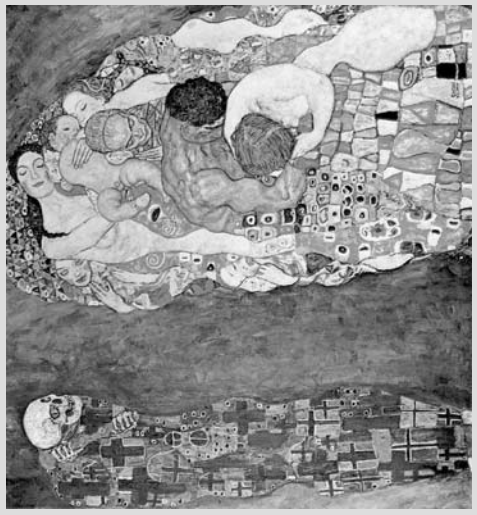


Chapter 1

General introduction

Introduction

Cells are the building blocks of life; the human body is composed of approximately 100 trillion of them. It is not an unreasonable assumption that properties of the species should be reflected in properties of their cells. Cell turnover during life history, which is essential for tissue maintenance, might be a primary mechanism of organismal ageing (Hayflick, 1985). Very recently conducted studies support a model in which ageing may results of adult stem cell dysfunction and progressive deterioration of tissue functions (Scaffidi and Misteli, 2006, 2008).

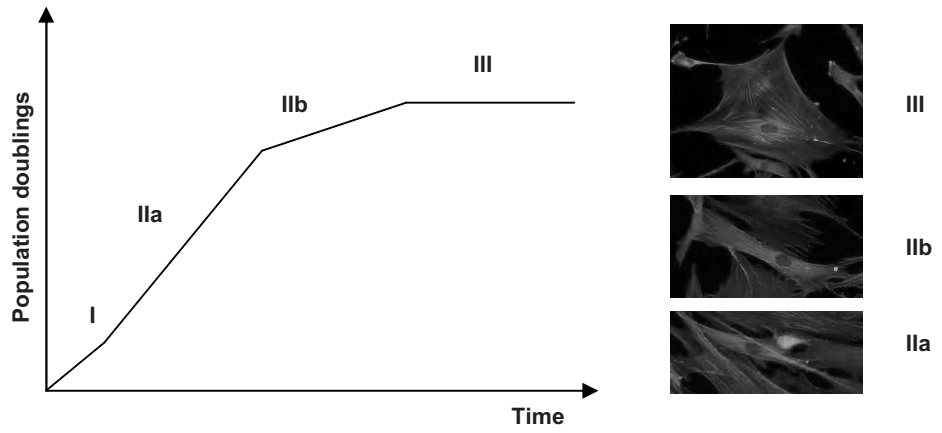
The most widely accepted interpretation for the biological function of cellular senescence is that it serves as a mechanism for restricting cancer progression (Sager, 1991; Cosme-Blanco *et al*., 2007; Ventura *et al*., 2007). The suppression of cancer acts as a beneficial trait, selected in reproductively active individuals, however, cellular senescence may have harmful effects later in life by altering tissue structures and functions (Kirkwood and Austad, 2000).

Two broad categories of cellular senescence can be distinguished. On the one hand, replicative capacity of many human cells is limited by telomere attrition that causes cells to undergo **replicative senescence** with short telomeres (Harley *et al*., 1990); on the other hand, sustained effects after exposure to subcytotoxic stress induces irreversible growth arrest, known as **stress-induced premature senescence** (Toussaint *et al*., 2002).

Replicative senescence

Growth kinetics have been studied extensively using human diploid fibroblasts, which undergo irreversible cellular arrest after a finite number of divisions owing to the process called replicative senescence (Campisi, 1996), as was first described by Hayflick and Moorhead in 1961. As shown in Figure 1, the course of a fibroblast culture *ex vivo* can be distinguished into three phases (Swim and Parker, 1957; Hayflick and Moorhead, 1961). Phase I consists of taking a skin biopsy and transferring the cells from the *in vivo* environment to *in vitro* conditions to initiate

Figure 1. Typical growth phases and phenotypes during the course of fibroblast *in vitro* ageing.



the culture. Following the establishment of the culture, cells undergo a long period of steady proliferation (phase IIa). Hereafter, a period follows in which the growth speed of the culture, measured by the time required to reach confluence, significantly decreases (phase IIb). Cultures then degenerate and cell division ceases, resulting in growth arrest (phase III or senescence).

Senescent fibroblasts have a characteristic phenotype; cells increase in overall size and lose their spindle shape appearance (Matsumura *et al*., 1979; Hayflick and Moorhead, 1961; Cristofalo and Kritchevsky, 1969). Senescent cells fail to respond to mitogenic stimuli, but maintain metabolic activity and can remain viable in culture essentially indefinitely (Matsumura *et al*, 1979; Pignolo *et al*., 1994) owing to resistance to apoptosis (Marcotte *et al*., 2004, Hampel *et al*., 2005). On a molecular level, changes occur in gene expression and protein processing during the course of cellular senescence (Cristofalo and Sharf, 1973; Matsumura *et al*., 1979; Gonos *et al*., 1998; Trougakos *et al*., 2006; Cong *et al*., 2006) including an increased activity of the enzyme β-galactosidase (Dimri *et al*., 1995).

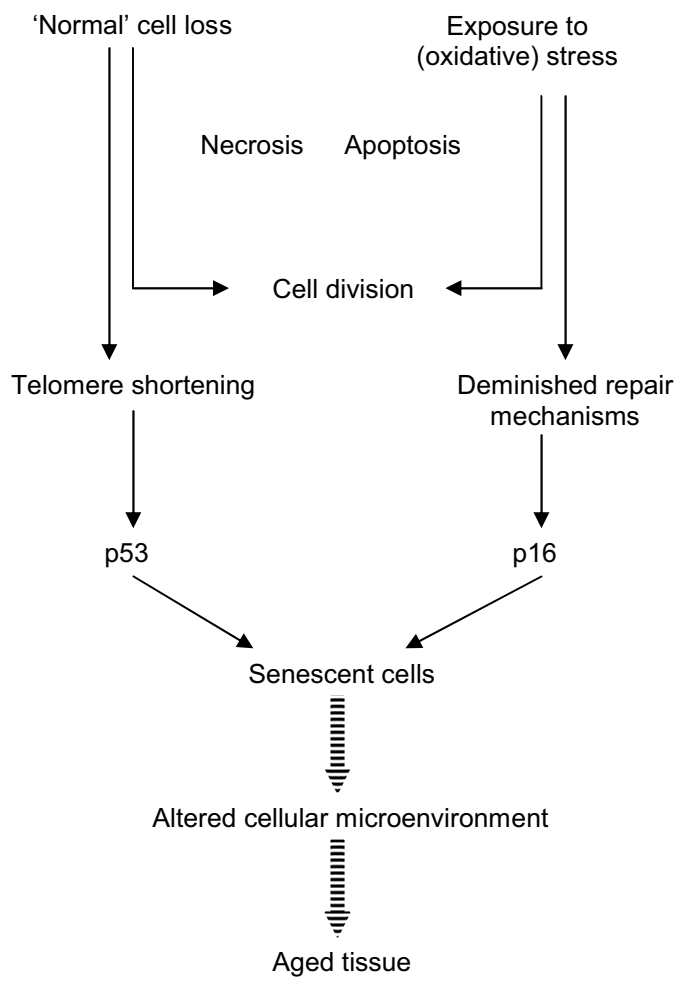
It is generally accepted that fibroblasts senesce because of one or more short and dysfunctional telomeres (Harley *et al*., 1990; de Lange, 2001; Karlseder *et al*., 2002; Levy *et al*., 1992). Telomeres, the ends of vertebrate chromosomes build up out of TTAGGG sequences, protect the ends of chromosomes from being recognized as broken DNA and provide a source of expendable DNA. Telomere shortening is the consequence of cell turn over by DNA replication in the absence of telomerase expression (Wright *et al*., 1996). Telomeres have been shown to shorten in tissues as a function of donor age (Lindsey *et al*., 1991) and in cultures as a function of the number of cell divisions (Harley *et al*., 1990). Furthermore, disrupted or dysfunctional telomeres trigger permanent cell cycle arrest, the hallmark of cellular senescence; p53, a pleiotropic tumor suppressor, plays a major role in senescence induced by telomere erosion (Chin *et al*., 1999; Saretzki *et al*., 1999; Herbig *et al*., 2004).

Stress induced premature senescence

Various human proliferative cell types undergo stress induced premature senescence after exposure to many types of subcytotoxic stressors under *in vitro* conditions. The senescence arrest depends on the p16 tumor suppressor, a cyclin-dependent kinase inhibitor that keeps the pRB (retino blastoma) tumor suppressor cell cycle regulator in its unphosphorylated form (Ohtani *et al*., 2004). p16 has been shown to be upregulated *in vivo* with age and in response to cellular stress (Zindy *et al*., 1997; Schmitt *et al*., 2002; Krishnamurthy *et al*., 2004; Ressler *et al*., 2006). However, premature senescence can also be achieved in a telomere dependent manner by exposure to mild oxidative stress (Duan *et al*., 2005; von Zglinicki *et al*., 1995) because of single strand breaks in telomere regions by oxidative stress which consequently cause accelerated telomere shortening (von Zglinicki *et al*., 2000, 2002). In fibroblasts, the phenotype of premature senescence often exhibits many features shared with replicative senescence, including distinct morphology and β-galactosidase activity.

It has been suggested that the pathways of replicative senescence and stress induced premature senescence can intersect (Figure 2). Thus, while one pathway might predominate in the induction of cellular senescence under given circumstances, the pathways can also cooperate to prevent indefinite cell proliferation (Lin *et al*., 1998; Shapiro *et al*., 1998; Rheinwald *et al*., 2002; Schmitt *et al*., 2002; Itahana *et al*., 2003).

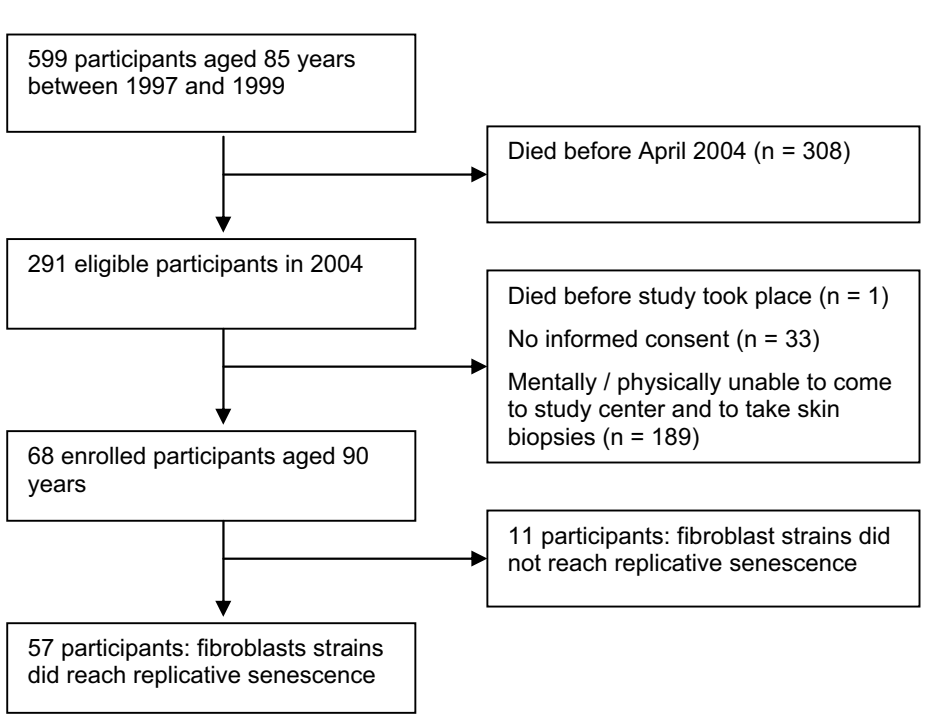
Figure 2. Mechanism of cellular senescence.



The Leiden 85-plus Study

All studies presented in this thesis, except for the study described in chapter 7, were performed within the Leiden 85-plus Study. The Leiden 85-plus Study is a prospective population based follow-up study, in which all inhabitants of the city Leiden, the Netherlands, in the month after their 85th birthday were ask to participate. Of the 705 eligible subjects, 599 subjects (85%) were enrolled in the initial cohort (der Wiel et al., 2002) as outlined in Figure 3. There were no selection criteria related to health or demographic characteristics at baseline. After five years of follow-up, 68 well functioning relatively healthy community-dwelling nonagenarians were invited for sampling of skin biopsies (11.4% of baseline cohort).

Figure 3. Flow chart of participants, Leiden 85-plus Study.



Aim and outline of the thesis

The aim of the work described in this thesis was to study *in vitro* senescence of human cells, in particular the relation of cellular *in vitro* ageing and its relation to chronological ageing. Each chapter focuses on a different aspect of cellular *in vitro* senescence.

Chapter 2 reports on our first study describing the interindividual variation in replicative capacity of human fibroblast strains obtained from 68 relatively healthy community-dwelling nonagenarians. In **chapter 3**, we studied the transitions between the different growth phases in fibroblasts cultured up to the onset of senescence by using the biomarker β-galactosidase. The onset of replicative senescence *in vitro* may last up to several years; therefore, in **chapter 4**, we tested the colony formation assay as surrogate indicator for the onset of replicative senescence. In **chapter 5**, we tested the influence of p53 genotypes on cellular stress induced by X-irradiation. As body mass has been shown to be a better correlate for the replicative capacity across species than average longevity, in **chapter 6** we studied the relation between individual's body size and replicative capacity within humans. **Chapter 7** addresses the difference between cellular mixed cultures and clonal cultures in myoblasts. Finally, in **chapter 8** we discuss the use of replicative capacity of human fibroblasts as a model for *in vitro* ageing and its relation to chronological ageing.

Framework

The research presented in this thesis was carried out within the framework of the "Innovative Oriented Research" (IOP) project entitled "Genetic determinants of longevity and disease in old age", subsidized by the Dutch Ministry of Economic Affairs (grant number IGE0100114). This project brought together physicians, biologists and geneticists with the aim to identify mechanisms that determine longevity and disease in old age.

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