Progeroid Syndromes: Role of Accelerated Fibroblast Senescence and p38 Activation

Terence Davis

Contents

T. Davis (✉)
Institute of Cancer and Genetics, Cardiff University
School of Medicine, Cardiff CF14 4XN, Wales, UK
e-mail: davist2@cardiff.ac.uk

Abstract

Human ageing studies are problematic due to their complex nature so genetic progeroid syndromes that manifest a subset of ageing phenotypes are used as proxies to dissect out specific ageing processes. Many such processes are believed to involve cellular senescence, as senescent cells gradually build-up during life. Two forms of cellular senescence exist; replicative due to telomere dysfunction and stress-induced via activation of p38 MAP kinase. As progeroid syndromes show premature ageing, they are useful for cell ageing studies that may provide support for the linkage between cellular senescence and ageing. For several progeroid syndromes, notably Werner, ATR-Seckel, Hutchinson-Gilford, Ataxia-Telangiectasia, Nijmegen Breakage and Dyskeratosis congenita, there is clear evidence that fibroblasts undergo rapid or premature ageing. For other syndromes such as Cockayne, Rothmund-Thomson and Bloom there is no such clear evidence. In addition, no clear relationship between the severity of ageing features and premature fibroblast senescence is seen. However, as some of these syndromes result in early death from non-age related causes, it may be that insufficient lifespan is available for significant premature ageing to occur. For example in Nijmegen Breakage syndrome, fibroblasts age rapidly but the progeroid features are slight, possibly due to early death from

cancer. In addition, it may be that premature cell senescence occurs in cell types other than fibroblasts. The accelerated cell ageing that does occur results from accelerated telomere dysfunction in some syndromes, activation of p38 in others, or a mix of both mechanisms. In this chapter, I provide a summation of the evidence for accelerated cellular senescence in progeroid syndromes and attempt to relate this to the severity and tissue specificity of the progeroid phenotypes. Finally, I discuss a model that may underlie the accelerated ageing in a subset of these syndromes that may be relevant to normal ageing processes.

Introduction

Human ageing is a process of the gradual build-up of deleterious changes that eventually impinge sufficiently upon normal tissue function to cause failure of that tissue leading to death. An example of this process is the gradual weakening of cardiac function prior to heart failure. As the impairment of normal biological function can result from both the processes of ageing and disease, it would be unsurprising if the basic ageing process itself eventually lead to the onset of those diseases specifically associated with ageing (Burton 2009). Such diseases include, amongst others, type II diabetes, cardiovascular disease (atherosclerosis), osteoporosis, dementia, sarcopenia (muscle wasting) and cancer. If this premise is correct, then an understanding of the basic mechanisms underlying the ageing process should lead to advances in the pathophysiology of much age-related illnesses. This, in turn, should lead to novel therapeutic interventions that are aimed at the basic ageing process itself leading to the amelioration, or even prevention, of age-related disease leading to an improved quality of later life.

To date, basic ageing processes have been considered as a scientific speciality in their own right (biogerontology) that is quite distinct from the process of disease progression in the elderly that has often been the province of the various medical specialities such as geriatrics.

The reasons for this are complex, but may be related to the idea that disease can be treated, but ageing cannot (Bagley et al. 2011). However, it has been increasingly recognised that a significant overlap exits between the fields of biogerontology and disease processes.

Much remains to be discovered regarding the pathophysiology of human ageing (Puzianowska-Kuznicka and Kuznicki 2005), a complex process involving genetic and environmental factors affecting several physiological pathways. Ageing is associated with loss of function of many cells, tissues and organs of the body. Since every tissue and organ is made up of many billions or even trillions of cells, it is necessary to understand how cells age and what contribution old cells make to the tissue in which they lie in order to discover the impact of cell ageing on frailty and disability. As mitotic human tissues consist of cells that have the ability to divide when stimulated, one mechanism commonly postulated as underlying human ageing is cellular senescence, or the observation that many normal human somatic cells are capable of only a finite number of divisions (Burton 2009). How often these cells proliferate is dependent upon how frequently cells become damaged and lost, the so-called 'wear and tear' hypothesis of ageing. As lost cells need to be replaced the remaining stock needs to enter division, eventually leading to a senescent phenotype. Although until recently controversial, it is becoming increasingly accepted that cell ageing (also called *replicative senescence*) is a major player in organismal ageing.

Practical difficulties underlie human ageing studies, most importantly the biological complexity and polygenic nature of the ageing pathologies. An alternative to the study of whole body ageing in normal humans is the study of progeroid syndromes (PSs) whose phenotypes show specific characteristics of ageing (Puzianowska-Kuznicka and Kuznicki 2005). These hereditary disorders are often monogenic and affect only a subset of normal ageing phenotypes, i.e. they are segmental, with different tissues showing accelerated ageing in different PSs. However, the process and pathology of the premature ageing that occurs appears remarkably similar to that seen in

normally aged individuals (Hofer et al. 2005). Thus if these syndromes are reflective of normal ageing processes their study allows distinct ageing pathways to be dissected out from the whole body ageing background leading to the identification of causative genes underlying ageing processes.

Cellular Senescence

Mechanisms of Cellular Senescence

Human mitotically competent cells (e.g. fibroblasts, endothelial cells, lymphocytes) have a limited division capability after which they enter a non-dividing state termed senescence, or mortality stage 1 (M1). Cellular senescence is not death, but a stress response resulting in permanent withdrawal from the cell cycle, with the cells capable of long-term survival. In this regard, senescence resembles terminal differentiation as the cells have distinct morphological and functional changes that impair cellular homeostasis (Kipling et al. 2004). For example, senescent fibroblasts adopt an enlarged and granular morphology with the presence of large arrays of F-actin stress fibres and show high expression of senescence-associated β-galactosidase activity (SAβ-gal). In addition they have an increased expression of inflammatory cytokines such as IL-1α and inflammatory molecules such as ICAM-1, which has been termed the senescence associated secretory phenotype or SASP (Freund et al. 2011).

Cellular senescence is categorised into two distinct types, replicative (or intrinsic) and stress-induced, although this is mostly a definition dependent upon the *mode* of senescence as the resulting cells are remarkably similar in phenotype. Replicative senescence in fibroblasts is largely the consequence of progressive telomere shortening that results from the mechanism of DNA replication being unable to synthesise the 5′ ends of linear chromosomes – the so-called "end-replication problem." Telomeres are capped by a protein complex (the shelterin complex) whose function is to protect the DNA terminus that would otherwise be recognised as a double strand DNA break (DSB) and activate the DNA repair pathway. As fibroblasts divide their telomeres shorten until a critical length is reached, whereupon they lose the shelterin capping function exposing the DSB. This results in a DNA damage response (DDR) that activates the p53/p21^{WAF1} cell cycle arrest pathway inducing senescence (d'Adda di Fagagna et al. 2003).

In addition to replicative senescence, human fibroblasts can undergo stress-induced premature senescence (SIPS) via activation of the MAP kinase p38 (and possibly other mechanisms) that responds to endogenous and exogenous cellular stress (Freund et al. 2011). The p38 MAP kinase is involved in growth arrest in response to the expression of oncogenes such as *ras*, exogenous stress such as arsenite treatment or oxidative stress, ongoing DDR, difficulties in completion of DNA replication (so-called replication stress), and also in telomere-dependent senescence: indeed, p38 defines a common senescence signalling pathway linking both replicative senescence and SIPS (Iwasa et al. 2003). The p38 MAP kinase pathway is important for cell growth arrest and senescence due to its ability to activate both the p53/p21^{WAF1} and the pRb/p16^{INK4A} cell cycle arrest pathways (Fig. 3.1). Activation of p38 leads to the expression of inflammatory molecules that is typical of senescent cells via activation of the kinase MK2 and phosphorylation of the mRNA binding protein TTP, and also the altered morphology of senescent cells via phosphorylation of the small heat shock protein HSP27 and the subsequent formation of F-actin stress fibres. Thus p38 plays a role in both inducing cell senescence and the SASP.

Role of Cellular Senescence in Human Ageing

Many human tissues require extensive and continuous cell turnover throughout life to maintain homeostasis (e.g., small intestine, immune system, skin), and cell division is central to normal function or repair. Tissue function not only requires cells and cell division, but also the
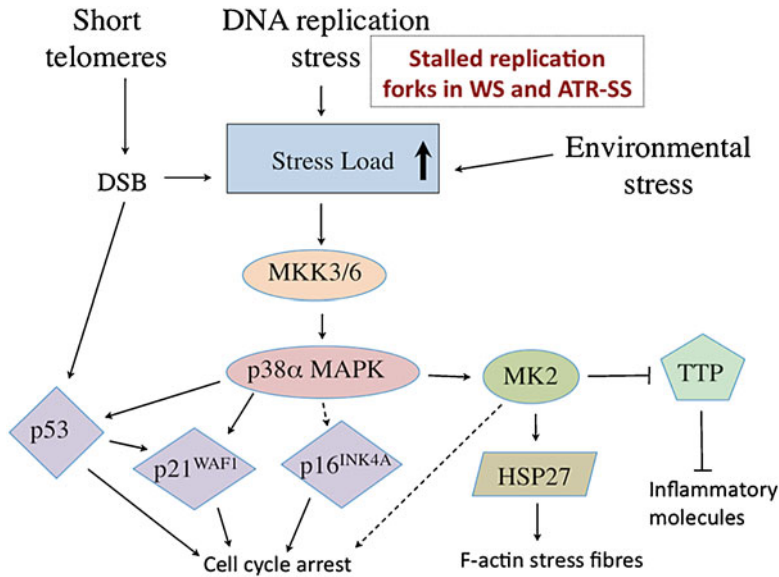
Fig. 3.1 The p38 mitogen-activated protein kinase pathway. This pathway is activated by endogenous and exogenous stress signals including oxidative stress, DNA damage (including short or dysfunctional telomeres), expression of cancer-inducing proteins (oncoproteins), various environmental stresses and possibly psychosocial stress. The actual mechanism whereby the stress is detected as a stress load is currently unknown, but stress results in the activation of MKK3 or MKK6 that activate p38. This leads to inhibition of the cell cycle via activation of the inhibitor proteins p21[WAF1] and/or p16[INK4A], or by activation of p53, leading eventually to stress-induced cellular senescence (SIPS). Additionally, p38 activates MK2 that controls the inflammatory response that typifies senescent cells. In both WS and ATR-SS fibroblasts the stress signal is thought to be due to a decreased ability to restart stalled DNA replication forks

extracellular strata (or matrix) that maintains tissue integrity. When division competent cells reach senescence this will impinge upon tissue integrity in two basic ways: failure to replace cell loss, and the deleterious biochemical features displayed by senescent cells (Kipling et al. 2004). For example, senescent cells secrete inflammatory cytokines such as IL-1α and tumour necrosis factor (TNFα), and express cell surface molecules such as ICAM-1 that are involved in the recruitment of leukocytes during inflammation (Davis and Kipling 2006). In addition, senescent cells up-regulate the expression of matrix degradative enzymes suggesting that, instead of maintaining matrix integrity, they actively degrade it. Moreover, senescent fibroblasts are known to aid the malignant progression of pre-malignant keratinocytes and breast epithelial cells; thus, although cellular senescence has been suggested as a possible tumour suppressive mechanism, the presence of senescent stromal fibroblasts may be

particularly adept at creating a tissue environment that can promote the development of age-related epithelial cancers. By these mechanisms, cellular senescence may contribute to age-related degenerations in division competent tissues and/or the genesis of certain age-related pathologies (Kipling et al. 2004).

Evidence for replicative senescence *in vivo* has been difficult to acquire leading to criticism of this postulate. However, as most human tissues lack detectable telomerase activity the cells in these tissues show progressive telomere shortening with age *in vivo*. Even in tissues with detectable telomerase activity, such as immune cells, this activity is often insufficient to maintain telomere length. Shortened telomeres are correlated with the progression of age-related diseases including immunosenescence, cardiovascular disease, sarcopenia, osteoporosis, osteoarthritis and skin ageing. In addition, there is an association between telomere length and mortality in

people aged 60 years or older [reviewed in (Davis et al. 2009)]. These data provide circumstantial support for a continual build-up of replicatively senescent cells *in vivo* through telomere shortening.

As well as replicative senescence, SIPS may occur in human tissues that are exposed to chronic stress. For example, cells of the vascular system are chronically exposed to a variety of oxidative burdens that would induce apoptosis or premature senescence (i.e. telomere-independent senescence). In addition, endothelium cells in areas of vascular transitions (e.g., blood vessel bifurcations) are under intense haemodynamic stress resulting in low-level (but chronic) injury again leading to premature senescence and/or cell loss through apoptosis. Several lines of evidence indicate that a build up of senescent cells does indeed occur in atherosclerosis-prone areas of the vasculature that may be due to SIPS (Erusalimsky and Kurz 2005). This SIPS then leads to increased proliferation of surrounding cells leading ultimately to their replicative senescence. Similarly, human skin is under mechanical stress and suffers chronic injury due to abrasion that leads to cell loss and/or SIPS. The resulting wound healing process to replace these lost cells may lead to significant cell turnover and ultimately cellular senescence and skin ageing (Wall et al. 2008).

Cellular senescence may have relevance in mental health, incidence of which increases with age, with depression being likened to a state of accelerated ageing (Wolkowitz et al. 2010). Depressed individuals have high plasma levels of inflammatory markers such as TNFα, suggesting elevated inflammation and stress signalling that could lead to enhanced cellular turnover and SIPS. This SIPS may then underlie the increased rates of type II diabetes, osteoporosis, and cardiovascular disease often seen in depressed individuals. However, although psychosocial stress has been proposed to prematurely age cells this has not been formally demonstrated (Wolkowitz et al. 2010).

Overall, data exist suggesting that several ageing phenotypes may be related to the build-up of cells throughout life that have undergone replicative and/or stress-induced senescence. That

senescent cells may contribute to the ageing process in mammals has been shown in mice by eliminating such cells, or preventing their build-up: this slowed or even reversed the acquisition of age-related pathologies in several tissues, including fatty tissue, skeletal muscle, spleen, intestine, the nervous system and the eye (Jaskelioff et al. 2011). Thus, although it may not yet be formally proven, there is strong evidence supporting a link between the build-up of senescent cells and biological ageing processes.

Progeroid Syndromes

Phenotypic Characteristics of Progeroid Syndromes

One of the more intensively studied progeroid syndromes (PSs) is Werner syndrome (WS) due to null mutations in the *WRN* gene encoding the DNA helicase RECQ3 (WRN). Werner syndrome is often first suspected during the teenage years as affected individuals lack the pubertal growth spurt resulting in shortness in height. Major characteristics are bilateral juvenile cataracts, skin atrophy and sclerosis, hair-greying, thymic atrophy and soft tissue calcification, together with age-related diseases such as type II diabetes, atherosclerosis and osteoporosis, all diseases that are inflammatory in nature (Davis et al. 2009). Premature ageing is segmental as no nervous system pathology is noted and there is no obvious immune system dysfunction, i.e. premature ageing affects some tissues but not others. An elevated incidence of cancer is observed, however, not all cancer types are affected, and there is an emphasis on rare non-epithelial cancers such as mesenchymal and soft-tissue sarcomas. Whether this is due to an active mechanism to promote these cancer types, or a suppression of epithelial cancers, is unclear. WS individuals die with a median age of 54 mainly due to cardiovascular disease or cancer.

A second widely studied PS is the extremely rare Hutchinson-Gilford progeria (HGPS) due to splice defects in the *LMNA* gene encoding the nuclear protein lamin A/C. Hutchinson-Gilford

individuals appear normal at birth, but within a year display the effects of premature ageing (Davis et al. 2009). Initial symptoms include severely reduced growth rate and HGPS individuals are short and below average weight. As the condition progresses individuals develop alopecia, skin of wrinkled and aged appearance reminiscent of scleroderma, and inflammatory conditions such as arteriosclerosis, osteoporosis, and atherosclerosis. These individuals show very rapid ageing and appear many decades older than they actually are, having a similar respiratory, cardiovascular and arthritic condition to a senior citizen. Average age at death is 13, with 90% of individuals dying of heart failure and cerebrovascular accident (strokes).

Cockayne syndrome (CS) is of two varieties, CSA and CSB, due to mutations in the proteins ERCC8 and ERCC6 that are involved in DNA repair pathways (Kraemer et al. 2007). However, CS individuals of both types are phenotypically very similar and have characteristic aged facial features, thin hair, cachexia, thin dry skin, retinal degeneration, hearing loss, neurodegeneration (cerebellar ataxia), and cataracts. Their clinical course typifies premature ageing and usually results in early death, with CSA individuals dying in the second or third decade and CSB individuals usually in the first decade of life.

Rothmund-Thomson (RTS) individuals show moderate ageing characteristics, although lifespan is not shortened in the absence of cancer (Davis et al. 2013). Features include poikiloderma, alopecia, grey hair, and short stature, with juvenile cataracts reported in some individuals, and an elevated cancer incidence in others. The syndrome is highly pleiotropic with not all individuals showing all symptoms, probably due to RTS being of two types (I and II) resulting from mutations in (at least) two separate genes. The observed pleiotropy may be due to insufficient historic phenotypic characterisation of the two types as the aetiology is known only recently and only for Type II (due to mutations in the gene encoding the helicase RECQL4), although juvenile cataracts appear to be specific for Type I and osteosarcoma to Type II, whereas poikiloderma is common to both RTS types.

Individuals with ataxia-telangiectasia (AT) show moderate features reminiscent of premature ageing, such as grey hair, wrinkled skin, skin atrophy and sclerosis (scleroderma), and show a reduced lifespan with death usually occurring in the third and fourth decades (Davis et al. 2009). However, AT individuals do not show such inflammatory features as atherosclerosis. Death is usually from recurrent respiratory infection in adolescence or early childhood. It is caused by null mutations of the gene encoding the checkpoint kinase ataxia-telangiectasia, mutated (ATM).

The progeroid features described for Nijmegen Breakage syndrome (NBS), such as sparse grey hair and distinctive 'bird-like' facies, do increase with age, but are relatively mild and there are few inflammatory features (Davis et al. 2009). However, the data on NBS are potentially confounded by many individuals dying at a young age as a result of cancer, so premature ageing has little time to manifest itself. In addition, NBS is complicated by its resemblance to AT, and to other human syndromes, leading to possible misdiagnosis. Those NBS individuals with a known aetiology have hypomorphic mutations in the gene encoding nibrin (*NBN*; sometimes referred to as *NBS1*).

Seckel syndrome (SS) results from mutations in a least six genes that impinge upon centrosome function and affect the activation of the ataxia-telangiectasia and Rad3-related (ATR) checkpoint kinase, with classical SS having hypomorphic mutations in ATR itself (referred to here as ATR-SS, with the term SS referring to non-ATR SS). Seckel syndrome individuals show moderate ageing with few inflammatory features, although accelerated ageing is clearly present in the ATR-SS mouse model (Tivey et al. 2013b). Features include, bird-like facies, short stature, sparse hair, café-au-lait spots, impaired cardiovascular function and type II diabetes, all symptoms that occur in normal ageing.

Dyskeratosis congenita (DC) individuals show many ageing characteristics, such as alopecia, grey hair, wrinkled skin, abnormal skin pigmentation, poikiloderma, osteoporosis and cancer (Davis et al. 2009). The aetiologies of DC

are mutations in members of the telomerase protein complex, with classical X-linked DC mutated for the protein dyskerin. Other mutations occur in the proteins NOP10 and TERT, and the RNA subunit TERC.

Bloom syndrome (BS) individuals have a moderately reduced lifespan (Hofer et al. 2005); however, although classified as a PS, there is little evidence of a premature ageing defect apart from a high incidence of type II diabetes in young BS individuals, and an elevated cancer incidence. Bloom syndrome is due to null mutations in the gene encoding the DNA helicase RECQ2 (BLM).

Are Progeroid Syndromes Associated with Accelerated Fibroblast Senescence?

Associated with premature ageing, accelerated cellular replicative senescence is found in greater than 90% of WS fibroblast strains (Davis et al. 2009). Normal human dermal fibroblasts (NDFs) frequently have replicative capacities greater than 50 population doublings (PDs); in contrast WS fibroblasts usually do fewer than 25 PDs. This accelerated senescence of WS cells *in vitro* has been postulated to correspond to a similar process *in vivo*, and thus contribute to the accelerated ageing of division-competent tissues (Davis et al. 2009). Likewise, many strains of HGPS fibroblasts show reduced replicative capacity, although increased apoptosis is also prevalent, and HGPS individuals undergo rapid ageing and have very short lifespan (Hofer et al. 2005). However, many HGPS fibroblast strains have what appears to be a normal replicative capacity despite the extensive apoptosis that can be present (Davis et al. 2009). These differences may be due to the extensive heterogeneous division capability of cells taken from different individuals at different times of life, and/or due to heterogeneous genetic backgrounds.

Two strains of SS fibroblasts have been examined; the strain with a hypomorphic mutation in *ATR* has a significantly reduced replicative capacity compared to NDFs (Tivey et al. 2013b); the other strain that does not have an *ATR* mutation

appears to have a normal replicative capacity. These differences reflect that SS is a very heterogeneous syndrome resulting from mutations in six different genes.

Nijmegen breakage syndrome fibroblast strains either have no replicative defect (cell life spans greater than 40 PDs), or have a much-reduced replicative lifespan (Ranganathan et al. 2001; Tivey et al. 2013a). The reasons for this large difference are not known, but NBS strongly resembles other PSs (or genomic instability syndromes) in clinical features, notably AT and ataxia-telangiectasia-like disorder (ATLD). It is thus possible that some NBS cases may be misdiagnosed. As the fibroblast strain with a reduced replicative capacity is known to have an *NBN* mutation (Ranganathan et al. 2001), it may be that the fibroblast strains with normal replicative capacities are from an, as yet, unknown syndrome that shares clinical features with NBS. I use the terms NBS and NBSL (NBS-like) respectively, here, for these subtypes solely for ease of clarity.

Cockayne Syndrome has two variants (CSA and CSB), and it has been shown that CSB fibroblasts do not have a replicative capacity defect with the strains used managing greater than 50 PDs (Tivey et al. 2013a). The situation with CSA fibroblasts is unclear, however, but the CSA strains used to date cluster at the low end of the normal range that is suggestive of a reduced division capacity. Those RTS fibroblasts that are mutated for RECQL4 (Type II) do not show an obvious mean replicative defect, although RTS strains cluster at the low end of the normal range for fibroblast lifespan (Davis et al. 2013) that may correlate with the observation that RTS individuals have a normal lifespan (Hofer et al. 2005). The replicative capacity for Type I RTS fibroblasts is unknown. With ataxia-telangiectasia, the replicative capacity of fibroblasts is significantly reduced compared to NDFs almost to the same degree as seen in WS fibroblasts, and most AT fibroblast strains appear to have replicative capacities less than 25 PDs (Davis and Kipling 2009). The only DC fibroblasts that I have studied are from the X-linked variant that has mutations in *DKC1* (dyskerin) and these fibroblast

strains have a very reduced replicative capacity (Davis et al. 2009; Tivey et al. 2013a). Reduced replicative capacity is also seen in fibroblasts from DC cases due to mutations in the other causative genes (Davis et al. 2009). Finally, in the case of BS, there is no apparent replicative defect in any of the fibroblast strains examined to date (Tivey et al. 2013a).

Role of p38 in Accelerated Senescence in Progeroid Fibroblasts

Fibroblasts senesce primarily as a result of telomere erosion that activates p53 and p21^{WAF1}. However, telomere erosion rates in WS fibroblasts have been shown to be similar to that seen in NDFs, although some telomere dysfunction does occur, in particular a low level of sudden telomere truncation (Davis et al. 2005, 2009), suggesting an alternative pathway is involved in the premature WS cell senescence. In addition to a short replicative capacity, WS fibroblasts have very slow growth rates and an enlarged morphology with extensive arrays of F-actin stress fibres that strongly resembles senescent normal cells (Davis et al. 2005). They also have high levels of activated p38, phosphorylated HSP27, and p21^{WAF1}. The stress-associated MAP kinase p38 is involved in cellular senescence processes resulting from both the erosion of telomeres and to endogenous and exogenous stress, and its activation can lead directly to cell cycle arrest by activating either the p53/p21^{WAF1} or the p16^{INK4A} pathways (Iwasa et al. 2003).

Overall, WS fibroblasts resemble cells that have undergone p38 driven stress-induced premature senescence (SIPS). Treatment with the p38 inhibitor SB203580 had a remarkable effect on these cells, more than doubling their replicative capacity to greater than 40 PDs, increasing their growth rate and changing their morphology to that seen in young NDFs. In contrast, p38 inhibition resulted in only a small extension of the replicative lifespan of NDFs (Tivey et al. 2013a). These data suggest that the shortened WS replicative lifespan results from a robust telomere-independent p38-driven SIPS.

A large increase in replicative lifespan for ATR-SS fibroblasts resulted from inhibition of p38 by three different inhibitors, with the increase directly proportional to the extent of p38 inhibition achieved (Tivey et al. 2013b). Untreated ATR-SS fibroblasts show an aged morphology with F-actin stress fibres, activated MK2 (a p38 target and the major HSP27 kinase) and phosphorylated HSP27, and elevated p16^{INK4A}, all features that were corrected by p38 inhibition. These data are consistent with SIPS in ATR-SS cells.

Smaller increases in replicative capacity were found for NBS fibroblasts, although whether p38 is activated in NBS cells is undetermined and the cells may not have an aged morphology (Ranganathan et al. 2001). Increased telomere dysfunction (particularly telomere fusions) is prevalent in NBS cells, but the telomere erosion rates appear not to be significantly elevated (Hou et al. 2012; Ranganathan et al. 2001). These data suggest that the shortened replicative capacity may result from the combined effects of telomere dysfunction and SIPS; however, NBS is not well understood at this time. For the NBSL strains with unknown aetiology, however, the effect of p38 inhibition was similar to that seen in NDFs (Tivey et al. 2013a).

With AT, whilst fibroblast replicative capacity is reduced (Naka et al. 2004; Tchirkov and Lansdorp 2003), there is clear heterogeneity in the response to p38 inhibition, with some AT fibroblast strains showing lifespan extension and increased growth rates greater than seen in NDFs (although much smaller than seen with WS cells), whereas other strains showed a much reduced response (Davis and Kipling 2009). In addition, although some AT fibroblast strains had an altered morphology, they showed no increased level of F-actin stress fibres, and no activated p38. This lack of p38 activation in AT cells agrees with (Naka et al. 2004), but contrasts with the study of (Barascu et al. 2012) who showed activated p38 in AT lymphoblasts and fibroblasts, although the fibroblasts used did not appear to have F-actin stress fibres. The AT fibroblasts in this study did have some features characteristic of senescent cells, such as high SAβ-gal levels, that were corrected by p38 inhi-

bition, or siRNA knockdown (Barascu et al. 2012). These differences suggest that loss of ATM produces a stress signal that activates p38 leading to a low level of SIPS that results in some of the premature senescence seen in AT cells. However, this stress response is much reduced when compared to that seen in WS, ATR-SS and NBS cells, and the major cause of the shortened replicative capacity of AT fibroblasts appears to be the dysfunctional telomeres and accelerated telomere shortening seen in AT cells (Tchirkov and Lansdorp 2003).

Activated p38 and phosphorylated HSP27 are also seen in Type II RTS, CSA and some CSB fibroblasts (Davis et al. 2013; Tivey et al. 2013a). However, these are at a much-reduced level compared to that seen in WS and p38 inhibition produces only a small fibroblast lifespan extension similar to that seen in NDFs. In addition, few enlarged cells with F-actin stress fibres are observed. This suggests only a low level of stress is occurring in these cells and any reduced replicative lifespan for the CSA cells results from a process other than SIPS. The protein mutated in Type II RTS (RECQL4) is believed to play a role in telomere maintenance so the activated p38 may result from a low level of telomere dysfunction (Davis et al. 2013). Alternatively these cells show elevated oxidative stress that would also activate p38. For CSA cells, a defective DNA repair process may lead to cell cycle arrest via p53 activation.

For fibroblasts from HGPS, BS, and SS no p38 activation is seen, the cells have a normal morphology, and p38 inhibition does not extend replicative lifespan beyond that seen in NDFs (Tivey et al. 2013a), suggesting that SIPS is not present in these cells. This is supported by the observation that none of these cells strains show F-actin stress fibres. Thus the premature senescence seen in the HGPS cells used in the study is not due to p38 activation or SIPS. Finally, with DC fibroblasts p38 inhibition had only minimal effects on either replicative lifespan or cellular morphology, but as the cells used were almost at replicative senescence when p38 inhibition began and the activation of p38 was not assessed, no real conclusions could be drawn. However, the premature senescence of DC cells is thought to be due primarily to accelerated telomere erosion (Davis et al. 2009).

Discussion

General Discussion

Human ageing is a gradual process that reduces normal tissue function eventually resulting in tissue failure leading to death; an example being the gradual weakening of cardiac function prior to heart failure. As both ageing and disease impair biological function, it would be unsurprising if basic ageing processes lead eventually to the onset of those diseases specifically associated with ageing. Indeed, biogerontologists have long been aware that an understanding of the basic ageing mechanisms should lead to advances in the pathophysiology of much age-related illnesses, and possibly novel therapeutic interventions. This view is increasingly being accepted amongst medical professionals in addition to biogerontologists (Bagley et al. 2011). Due the polygenic nature and biological complexity of the ageing pathologies, biogerontologists often make use of the group of disorders known as progeroid syndromes (PSs) whose phenotypes show specific characteristics of ageing (Puzianowska-Kuznicka and Kuznicki 2005). As these disorders are often monogenic and affect only a subset of normal ageing phenotypes, these syndromes are useful as their study may allow distinct ageing pathways to be dissected out from the whole body ageing background leading to the identification of causative genes underlying ageing processes.

One possible mechanism that may underlie human ageing is that of cellular senescence as division competent tissues require continuous proliferative capacity throughout life to maintain function due to continuous cell loss. In addition, senescent cells display deleterious biochemical features such as the expression of degradative enzymes and inflammatory molecules (Davis and Kipling 2006; Kipling et al. 2004). Thus instead of their normal function to support tissue function, senescent cells may actively do the opposite

and contribute to the age-related decline in tissue structure and the genesis of age-related pathologies (Kipling et al. 2004). Although evidence for replicative cellular senescence *in vivo* has been difficult to acquire, it has become increasingly evident that a linkage between cellular senescence and the age-related decline of tissue function does indeed exist. Human cells senesce as a result of two basic processes, replicative senescence due to telomere erosion, and SIPS resulting from various stressors throughout life. However, in either case the cells have a strikingly similar phenotype and behaviour. As cellular senescence is proposed as a major causation of human ageing, it is useful to look possible roles for cellular senescence in the various PSs where specific ageing processes may be dissected out from the background of whole human ageing. In addition, as many of the age-related pathologies are inflammatory in nature and senescent cells express high levels of the stress-related kinase p38, a role for p38 in the premature ageing seen in PSs is predicated.

Although fibroblasts from several of the PSs show premature fibroblast senescence, there appears to be no clear relationship between the replicative cellular capacity of the cell strains used and the presence of premature ageing features of PSs. It may be that the presence of accelerated cellular senescence correlates with the severity of the ageing phenotype, e.g. in WS, HGPS and DC the ageing features are marked and the lifespan of individuals is reduced, and the replicative capacity of fibroblasts is much reduced compared to NDFs. In comparison, the ageing characteristics of individuals with RTS and BS are few and the replicative lifespan of fibroblasts is not significantly reduced. However, there are PSs with relatively mild ageing features such as NBS, AT and ATR-SS, where the replicative capacity of fibroblasts is much reduced. However, there is a complicating issue in that individuals with these syndromes have very short lives that do not result from premature ageing, thus it may be that they do not live long enough to manifest significant accelerated ageing phenotypes. This contrasts with other short-lived PSs such as HGPS, where the short lifespan of

individuals results directly from the premature ageing. Finally, in CS significant accelerated ageing is not related to significant accelerated fibroblast senescence in CSB, but may be related to premature cellular ageing in CSA. It should be noted that there appear to be no genes that specifically cause ageing; the processes that affect ageing involve gene products that have diverse additional functions in the body, so mutations in such genes will have broad-ranging phenotypic consequences.

Alternatively, tissue specificity may be important, since dermal fibroblasts from WS, HGPS, DKC, AT and possibly CSA show premature senescence and a notable feature of these PSs is skin ageing, in particular the inflammatory scleroderma seen in WS, HGPS and AT. However, the other PSs with premature fibroblast ageing (NBS and ATS-SS) do not show accelerated skin ageing, although they do manifest the skin conditions of telangiectasias and café-au-lait spots. Moreover, BS also manifests telangiectasias and café-au-lait spots and no accelerated fibroblast ageing. However, a second aged phenotype, that of premature grey hair, is found in all the PSs that have premature fibroblast senescence, but in none of the others.

It should be noted that the only cell type that has been extensively studied are fibroblasts and the premature cellular phenotypes may affect other cell types such as endothelial cells or lymphocytes, although lymphocytes from WS have a normal replicative capacity (Davis et al. 2009). That other cell types may play a role in accelerated ageing phenotypes comes from the observation that large arteries in HGPS individuals are severely depleted in endothelial and vascular smooth muscle cells, with many others thought to be senescent (Stehbens et al. 2001). Senescent endothelial cells have been found extensively in, and are thought to be causative of, atherosclerotic plaques that frequently occur in HGPS individuals. It is possible that features of ageing seen in other PSs may result from the premature ageing of other cell types. Thus, the observed lack of accelerated ageing of fibroblasts in some PSs does not suggest that the observed ageing features are not due to replicative cellular senescence, or the presence of senescent cells of other cell types.
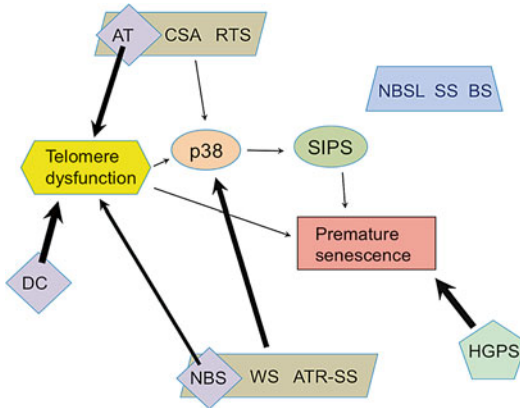
Fig. 3.2 Integrated model for induction of premature senescence in fibroblasts from PSs. Progeroid syndromes induce premature senescence via multiple processes; some via telomere dysfunction (indicated by *purple diamonds*), others directly activate p38 and SIPS (indicated by *brown parallelograms*) and some do both (AT, and possibly NBS) or neither (HGPS). Other PSs do not show premature senescence (*blue trapezium*). The intensity of the inducing signal and thus the degree of premature senescence induced by this mechanism is indicated by the *thickness of the arrow* from the relevant PS

As for a role for p38 in cellular senescence and ageing in PSs, it is clear that no obvious relationship exists between the replicative capacity of fibroblasts and the activation of p38 and SIPS; although it may be a matter of the degree of p38 activation (Fig. 3.2). This is illustrated for WS and ATR-SS cells that have high levels of activated p38 resulting in an altered cellular morphology with F-actin stress fibres: in these cells the loss of replicative capacity appears to be mostly (if not entirely) due to p38 and SIPS. With NBS the situation is more complicated with the cells having increased telomere dysfunction (Ranganathan et al. 2001) and, possibly, SIPS, although which effect predominates is unknown (Fig. 3.2).

For CSA, CSB and RTS, however, the levels of activated p38 seen suggest that only a low level of SIPS is occurring. This SIPS may have little or no effect upon replicative capacity, although any small effects would be hard to detect due to the large variation in replicative lifespan seen in human NDFs (Davis et al. 2013; Tivey et al. 2013a), and is insufficient to have major effects on cell morphology. Moderate p38

activation is also seen in AT and the cells have a much-reduced replicative capacity. In this case a low level of SIPS synergises with a strong telomere dysfunction signal (Tchirkov and Lansdorp 2003) to produce the shortened lifespan (Fig. 3.2). The much-reduced replicative lifespan seen with HGPS cells is not due to activated p38 and SIPS, and it may be independent of dysfunctional telomeres (Davis et al. 2009). In DC cells premature senescence results from accelerated telomere erosion. This leaves NBSL, SS and BS that do not appear to have premature cell senescence, do not activate the p38 pathway and have not been reported to show accelerated telomere dysfunction.

A possible complication in the scenario shown in Fig. 3.2 is that telomere dysfunction can also activate p38 (Iwasa et al. 2003), although it predominantly signals through p53 (d'Adda di Fagagna et al. 2003). Thus some of the p38 signal seen in various PSs (e.g. RTS and WS) may originate from a low level of telomere dysfunction.

As well as a poor relationship between p38 activity and fibroblast premature senescence, there appears to be no clear relationship between p38 activity and the presence of inflammatory disorders in the various PSs. For example the disorder scleroderma has been linked with p38 activity (Ihn et al. 2005), and this condition is seen in the PSs WS, AT and HGPS that show variable p38 activity. Likewise PSs that show high p38 activity such as ATR-SS do not show scleroderma. One possible caveat here is that not all cases of scleroderma show high p38 activity (Ihn et al. 2005), suggesting that others factors are at play that may interact with p38. A similar situation exist with the severity of cardiovascular disease that is noted in HGPS that shows no p38 activation in fibroblasts, compared to WS that does, although as has been stated previously this may be cell type dependent.

Overall it is clear from this work that, whilst there is no overall correlation between premature fibroblast senescence and p38 activity, and the ageing characteristics seen in various PSs, there may be a clear role for these activities in a distinct subset of these disorders. This subset of PSs has defects in a certain type of DNA repair

processes, namely that involving the replication of the so-called common fragile sites: these syndromes are WS, ATR-SS (and possibly NBS) that will be discussed in detail below. For some syndromes (AT, RTS, CSA and some CSB strains), it is possible that the low levels of p38 activity and fibroblast senescence may play a role in the milder ageing characteristics seen in these syndromes. Finally for the other PSs a larger role for these activities may occur in other cell types that have not been studied, although that is speculation. Alternatively, it is possible that SS and NBSL may not actually show premature ageing, as these have not been sufficiently characterised.

Proposed Model for Accelerated Cell Ageing in WS and ATR-SS

Common DNA fragile sites (CFSs) are observed as non-staining gaps or breaks in metaphase chromosomes of cells cultured under conditions of replicative stress. These sites are difficult to replicate and frequently cause replication fork stalling during normal replication (Tivey et al. 2013b). An important function of the kinase ATR is the co-ordination of checkpoint control responses to replication fork stalling (Ozeri-Galai et al. 2008) (see Fig. 3.3). It appears that ATR responds to stalled replication forks in at least two ways: (1) by activating the BLM DNA helicase and MUS81 endonuclease that create transient DNA DSBs (Shimura et al. 2008); (2) by recruiting the WRN DNA helicase to process the DNA DSBs and prevent fork collapse (Ammazzalorso et al. 2010; Pirzio et al. 2008). By keeping these pathways in balance, ATR leads to replication fork stability and coordinates an error-free repair and replication fork restart in a manner that does not involve DNA recombination (Franchitto et al. 2008), although the exact mechanism is not known. Cellular proliferation can then continue normally with the cells eventually reaching senescence that, incidentally, contributes to tissue and whole body ageing (Fig. 3.3, left panel). It is important to note that the function of this pathway is one of tumour suppression as CFS expression is common to many tumour

types, and not ageing *per se* (Arlt et al. 2006; Tivey et al. 2013b).

During this repair process p38 is activated to a low level through an, as yet unknown, pathway leading to a low degree of SIPS. That this SIPS does occur is supported by the observation that normal fibroblasts do show an extension (albeit small) of replicative capacity when treated with p38 inhibitors (Tivey et al. 2013a). It may be that p38 activation results from the formation of the transient DSBs, as both transient DSBs and activated p38 are absent in BS that lacks the BLM protein (Shimura et al. 2008). These DSBs activate ATM; however ATM is not the upstream kinase for p38 in this scheme as ATM inhibition does not prevent p38 activation in ATR-SS cells (my unpublished observations). Thus the actual mechanism whereby p38 is activated is not fully understood.

In WS, lack of the WRN helicase leads to an inability to process the transient DSBs and stabilise the replication fork, and the creation of large numbers of stable DSBs due to ATR activation of BLM and MUS81 (Fig. 3.3, middle panel). Subsequently the fork collapses leading to an error-prone fork repair and restart via ATR activation of CHK1 and RAD51-induced recombination that leads to genomic instability and CFS expression. The increased DSB level results in a much elevated p38 activation and a significant level of SIPS. Likewise, with ATR-SS, the lack of ATR results in the failure to recruit WRN and subsequently large DSB creation (Ammazzalorso et al. 2010) and replication fork collapse (Fig. 3.3, right panel). However, with the lack of ATR, the checkpoint kinase ATM is used in the error-prone fork restart by activating CHK1 (the usual ATM target being CHK2) and CFS expression (Ozeri-Galai et al. 2008). As with WS, the level of p38 activity is elevated leading to extensive SIPS. This idea is supported by the observation that ATR deficiency does not synergise with WRN deficiency in the elevated frequency of CFS expression, which is suggestive of a common pathway (Pirzio et al. 2008). Further support is provided by the extensive similarity between fibroblasts from both ATR-SS and WS, in that they grow slowly, have slow cycling time, increased chromosomal instability, and show increased replication fork
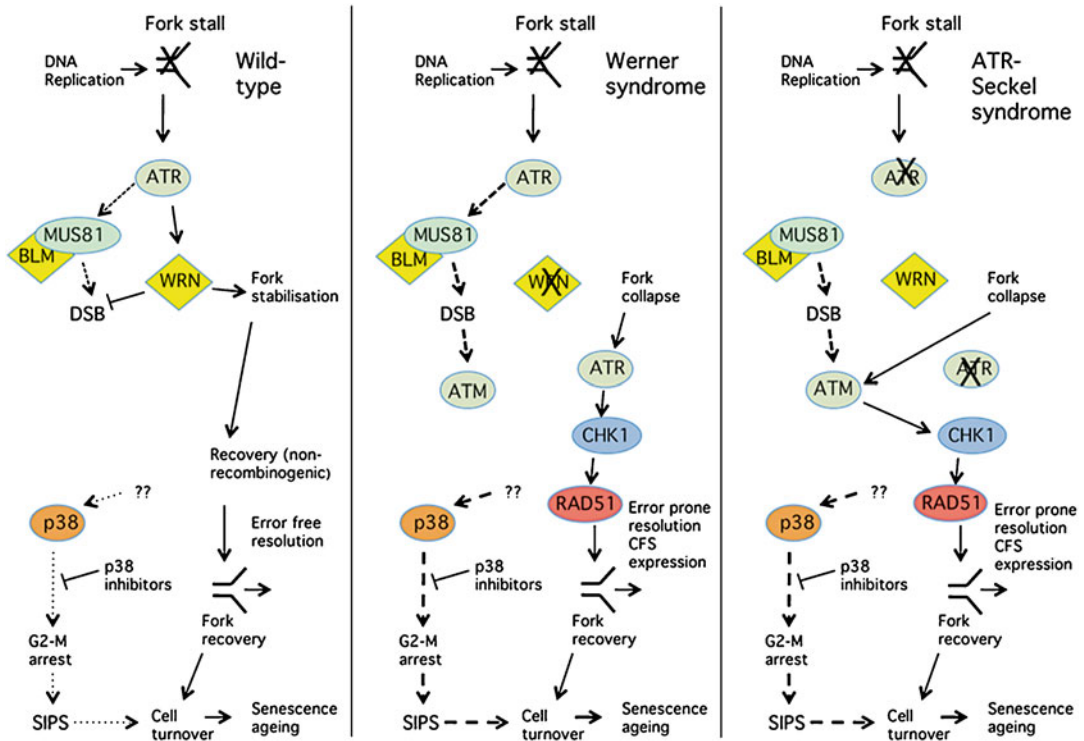
Fig. 3.3 Model for the premature cell senescence and ageing in WS and ATR-SS. Under normal conditions (*left panel*) the DNA replication fork has difficulties processing CFS regions of DNA. This results in a temporary fork stall and the activation of ATR. ATR recruits the WRN helicase resulting in fork stabilisation and an ATR dependent error-free fork recovery, and the restart of DNA synthesis. This allows cellular turnover that leads eventually to senescence and, incidentally, tissue ageing. During this process, p38 is activated leading to a low level of SIPS increasing cellular turnover, although the mechanism by which it is activated is unknown. Loss of either WRN or ATR (*middle* and *right panels*) negatively impinges upon this process resulting in fork collapse, an error-prone fork recovery and CFS expression, and an increased activation of p38. Thus, although fork restart is enabled, the elevated p38 activation results in a significant level of SIPS, increased cell turnover and accelerated ageing. Note that many details of this pathway are still poorly understood

stalling especially at CFSs (Davis et al. 2005; Mokrani-Benhelli et al. 2012; Pirzio et al. 2008).

Overall there is a strong overlap in the cellular phenotype of WS and ATR-SS cells as related to SIPS. This SIPS may lead to aspects of the whole body phenotypes of both ATR-SS and WS such as growth retardation and premature aging due in part to a reduction in cellular division capacity and an accelerated rate of build up of senescent cells. The chronic activation of p38 may also contribute to accelerated aging and the disease predisposition spectrum of these patients – so-called "inflamm-aging" (Franceschi et al. 2000).

Although ultimately ATR-SS and WS are "private" mechanisms of aging (insofar as they are driven by mutations not found in normal individuals), both pathways rapidly converge on a core signalling pathway (p38 MAP kinase) that is subject to substantial regulation by cell intrinsic and extrinsic factors. This in turn raises the possibility that normal human ageing might be affected, even if temporarily, by differential activation of the p38 pathway as a result of other activating circumstances, for example increased oxidative stress. Indeed, the observations of low levels of p38 activation and elevated oxidative stress in other human progerias strengthen the potential relevance of the p38 pathway to human ageing, even though the p38 activity doesn't result in obvious premature cellular senescence

in these syndromes (Davis et al. 2005; Tivey et al. 2013a). It is possible that the gene defects in other PSs can impinge on this pathway at a low level but in a chronic fashion leading to a degree of premature ageing. One such PS is NBS that has *NBN* mutations, as nibrin forms part of the MRN complex that first recognises the DNA replication fork stall and subsequently recruits ATR (Lee and Dunphy 2013). It is not known if fibroblasts from NBS do show p38 activation, but they may undergo a level of SIPS, suggesting a failure in the early part of the pathway shown in Fig. 3.3. It may be that increased telomere dysfunction plays a greater role in NBS premature cellular senescence than in WS or ATR-SS (see Fig. 3.2). It is interesting to note the putative role for BLM in this pathway to create the DSBs that might be responsible for increased p38 activity, as neither SIPS nor p38 activation is seen in BS and premature ageing is minimal.

References[1]

Ammazzalorso F, Pirzio LM, Bignami M, Franchitto A, Pichierri P (2010) ATR and ATM differently regulate WRN to prevent DSBs at stalled replication forks and promote replication fork recovery. EMBO J 29:3156–3169

Arlt MF, Durkin SG, Ragland RL, Glover TW (2006) Common fragile sites as targets for chromosome rearrangements. DNA Repair (Amst) 5:1126–1135

Bagley MC, Davis T, Latimer J, Kipling D (2011) The contribution of biogerontology to quality ageing. Qual Ageing Older Adults 12:26–32

Barascu A, Le Chalony C, Pennarun G, Genet D, Imam N, Lopez B, Bertrand P (2012) Oxidative stress induces an ATM-independent senescence pathway through p38 MAPK-mediated lamin B1 accumulation. EMBO J 31:1080–1094

Burton DG (2009) Cellular senescence, ageing and disease. Age (Dordr) 31:1–9

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

Davis T, Kipling D (2006) Werner syndrome as an example of inflamm-aging: possible therapeutic opportunities for a progeroid syndrome? Rejuvenation Res 9:402–407

Davis T, Kipling D (2009) Assessing the role of stress signalling via p38 MAP kinase in the premature senescence of Ataxia Telangiectasia and Werner syndrome fibroblasts. Biogerontology 10:253–266

Davis T, Baird DM, Haughton MF, Jones CJ, Kipling D (2005) Prevention of accelerated cell aging in Werner syndrome using a p38 mitogen-activated protein kinase inhibitor. J Gerontol A Biol Sci Med Sci 60:1386–1393

Davis T, Tivey HS, Kipling D (2009) Telomere dynamics and biology in human progeroid syndromes. In: Mancini L (ed) Telomeres: function, shortening and lengthening. Novascience, New York, pp 1–75

Davis T, Tivey HS, Brook AJ, Grimstead JW, Rokicki MJ, Kipling D (2013) Activation of p38 MAP kinase and stress signalling in fibroblasts from the progeroid Rothmund-Thomson syndrome. Age (Dordr) 35:1767–1783

Erusalimsky JD, Kurz DJ (2005) Cellular senescence in vivo: its relevance in ageing and cardiovascular disease. Exp Gerontol 40:634–642

Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G (2000) Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci 908:244–254

Franchitto A, Pirzio LM, Prosperi E, Sapora O, Bignami M, Pichierri P (2008) Replication fork stalling in WRN-deficient cells is overcome by prompt activation

---

[1]Note: due to the reference limitations imposed in this chapter (a maximum of 35 permitted), an exhaustive review of the literature is not possible. Therefore I have predominantly cited papers that review the information quoted (many of which are my own works) rather than the primary source. This does not denigrate the primary sources in any way whatsoever (these are listed in the cited references), nor does it imply any form of priority or enhanced importance of the cited references, and sincere apologies are herein expressed for all the authors of the primary literature that I have been unable to cite due to these limitations. If readers wish further information on any of the topics discussed I recommend that they read the original sources that can be found in the cited references.

of a MUS81-dependent pathway. J Cell Biol 183(2):241–252

Freund A, Patil CK, Campisi J (2011) p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. EMBO J 30:1536–1548

Hofer AC, Tran RT, Aziz OZ, Wright W, Novelli G, Shay J, Lewis M (2005) Shared phenotypes among segmental progeroid syndromes suggest underlying pathways of aging. J Gerontol A Biol Sci Med Sci 60:10–20

Hou YY, Toh MT, Wang X (2012) NBS1 deficiency promotes genome instability by affecting DNA damage signaling pathway and impairing telomere integrity. Cell Biochem Funct 30:233–242

Ihn H, Yamane K, Tamaki K (2005) Increased phosphorylation and activation of mitogen-activated protein kinase p38 in scleroderma fibroblasts. J Invest Dermatol 125:247–255

Iwasa H, Han J, Ishikawa F (2003) Mitogen-activated protein kinase p38 defines the common senescence-signalling pathway. Genes Cells 8:131–144

Jaskelioff M, Muller FL, Paik JH, Thomas E, Jiang S, Adams AC, Sahin E, Kost-Alimova M, Protopopov A, Cadinanos J, Horner JW, Maratos-Flier E, DePinho RA (2011) Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. Nature 469:102–106

Kipling D, Davis T, Ostler EL, Faragher RG (2004) What can progeroid syndromes tell us about human aging? Science 305:1426–1431

Kraemer KH, Patronas NJ, Schiffmann R, Brooks BP, Tamura D, DiGiovanna JJ (2007) Xeroderma pigmentosum, trichothiodystrophy and Cockayne syndrome: a complex genotype-phenotype relationship. Neuroscience 145:1388–1396

Lee J, Dunphy WG (2013) The Mre11-Rad50-Nbs1 (MRN) complex has a specific role in the activation of Chk1 in response to stalled replication forks. Mol Cell Biol 24:1343–1353

Mokrani-Benhelli H, Gaillard L, Biasutto P, Le Guen T, Touzot F, Vasquez N, Komatsu J, Conseiller E, Picard C, Gluckman E, Francannet C, Fischer A, Durandy A, Soulier J, de Villartay JP, Cavazzana-Calvo M, Revy P (2012) Primary microcephaly, impaired DNA replication, and genomic instability caused by compound heterozygous ATR mutations. Hum Mutat 34:374–384

Naka K, Tachibana A, Ikeda K, Motoyama N (2004) Stress-induced premature senescence in hTERT-expressing ataxia telangiectasia fibroblasts. J Biol Chem 279:2030–2037

Ozeri-Galai E, Schwartz M, Rahat A, Kerem B (2008) Interplay between ATM and ATR in the regulation of common fragile site stability. Oncogene 27:2109–2117

Pirzio LM, Pichierri P, Bignami M, Franchitto A (2008) Werner syndrome helicase activity is essential in maintaining fragile site stability. J Cell Biol 180:305–314

Puzianowska-Kuznicka M, Kuznicki J (2005) Genetic alterations in accelerated ageing syndromes. Do they play a role in natural ageing? Int J Biochem Cell Biol 37:947–960

Ranganathan V, Heine WF, Ciccone DN, Rudolph KL, Wu X, Chang S, Hai H, Ahearn IM, Livingston DM, Resnick I, Rosen F, Seemanova E, Jarolim P, DePinho RA, Weaver DT (2001) Rescue of a telomere length defect of Nijmegen breakage syndrome cells requires NBS and telomerase catalytic subunit. Curr Biol 11:962–966

Shimura T, Torres MJ, Martin MM, Rao VA, Pommier Y, Katsura M, Miyagawa K, Aladjem MI (2008) Bloom's syndrome helicase and Mus81 are required to induce transient double-strand DNA breaks in response to DNA replication stress. J Mol Biol 375:1152–1164

Stehbens WE, Delahunt B, Shozawa T, Gilbert-Barness E (2001) Smooth muscle cell depletion and collagen types in progeric arteries. Cardiovasc Pathol 10:133–136

Tchirkov A, Lansdorp PM (2003) Role of oxidative stress in telomere shortening in cultured fibroblasts from normal individuals and patients with ataxia-telangiectasia. Hum Mol Genet 12:227–232

Tivey HS, Brook AJ, Rokicki MJ, Kipling D, Davis T (2013a) p38 (MAPK) stress signalling in replicative senescence in fibroblasts from progeroid and genomic instability syndromes. Biogerontology 14:47–62

Tivey HS, Rokicki MJ, Barnacle JR, Rogers MJ, Bagley MC, Kipling D, Davis T (2013b) Small molecule inhibition of p38 MAP kinase extends the replicative lifespan of human ATR-Seckel fibroblasts. J Gerontol A Biol Sci Med Sci 68:1001–1009

Wall IB, Moseley R, Baird DM, Kipling D, Giles P, Laffafian I, Price PE, Thomas DW, Stephens P (2008) Fibroblast dysfunction is a key factor in the non-healing of chronic venous leg ulcers. J Invest Dermatol 128:2526–2540

Wolkowitz OM, Epel ES, Reus VI, Mellon SH (2010) Depression gets old fast: do stress and depression accelerate cell aging? Depress Anxiety 27:327–338