Diplomarbeit

The role of p53 in the process of aging

eingereicht von

Sandra Toferer

zur Erlangung des akademischen Grades

Doktor(in) der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt am

Gottfried Schatz Forschungszentrum (für zelluläre Signaltransduktion, Stoffwechsel und Altern)

Lehrstuhl für Zellbiologie, Histologie und Embryologie

unter der Anleitung von

Assoz.-Prof. Priv.-Doz. Dr.techn. Andreas Prokesch

Graz, 13.08.2019

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am 13.08.2019 Sandra Katharina Toferer eh.

Danksagungen

Hier möchte ich die Gelegenheit nutzen, um jenen, dich mich während meines Studiums und bei der Diplomarbeit unterstützt haben meinen Dank auszusprechen.
Erwähnen möchte ich meinen Mann, dessen Lernmotivation und vorbildliches Leben mich oft inspiriert haben weiterzumachen.
Ein besonderer Dank gilt auch meinem Diplomarbeitsbetreuer, Prof. Prokesch, für seine außerordentliche Geduld bei der Vorbereitung und während des Verfassens dieser Arbeit.

Abstract

The protein p53 is very well known as a tumor suppressor and recent evidence suggests that it is also involved in the process of aging and longevity. As transcription factor, p53 regulates cellular stress responses, mainly through target gene activation. This thesis focuses on p53 in relation to the hallmarks of cellular aging. Interestingly, p53 is involved in all nine identified cellular aging hallmarks: genomic instability, mitochondrial dysfunction, telomere shortening, cellular senescence, stem cell exhaustion, epigenetic alterations, loss of proteostasis, deregulated nutrient-sensing and altered intercellular communication. The interplay of p53, caloric restriction, and autophagy as an anti-aging tool spanning all species is also discussed. Furthermore, the dispute if p53 is a pro-aging or anti-aging factor will be highlighted, mainly with evidence coming from various mouse models. In conclusion, p53 can be pro-aging or anti-aging, depending on the context. It seems to act as pro-aging molecule when constitutively active or deregulated, while it might have anti-aging functions when aiding down-regulation of nutrient sensing pathways.

Key words: **p53, aging, autophagy, senescence, caloric restriction**

Zusammenfassung

Das Protein p53 ist in seiner Funktion als Tumorsuppressor bereits gut bekannt und vielfach erforscht. Doch es spielt nicht nur in der Krebsentstehung eine Rolle, sondern auch in vielen anderen Bereichen wie zum Beispiel dem Alterungsprozess. In dieser Arbeit wird auch auf das Thema Fasten als ein wirksamer Mechanismus gegen das Altern und wie p53 damit zusammenhängt eingegangen. p53 ist in Zellen dafür zuständig, dass die Zelle richtig auf Angriffe unterschiedlicher Art reagieren kann, indem Zielgene aktiviert werden. Diese Arbeit möchte mit Hilfe der neun Kennzeichen der Zellalterung und dem Prozess Autophagie die Stellung von p53 im Alterungsprozess beschreiben. p53 ist am Autophagieprozess und an den folgenden neun von neun Kennzeichen der Zellalterung beteiligt: Genetische Instabilität, Telomerverkürzung, Funktionsstörungen der Mitochondrien, zelluläre Seneszenz, Erschöpfung des Stammenzellpools, epigenetische Veränderungen und Störungen der Proteinhomeostase, der Signalwege von Wachstum und Nährstoffen sowie der zellulären Kommunikation. Dem Protein p53 werden im Alterungsprozess entgegengesetzte Rollen zugeschrieben. Diese Arbeit möchte mit Hilfe verschiedener Modell, vor allem von Mäusen, die Frage klären, ob p53 das Altern stoppen kann oder ob es dazu beiträgt. Es bestätigt sich die Dualität von p53: je nach Kontext kann p53 Altern beschleunigen oder verlangsamen. Das Protein kann das Altern beschleunigen, wenn es über lange Zeit überaktiviert ist oder die normale Regulation nicht funktioniert. p53 könnte dem Altern aber auch entgegenwirken indem es Signalwege von Wachstumshormonen und Nährstoffen herunterreguliert.

Inhaltsverzeichnis

Aging and disease

The median age of Austria's population in 1950 was 35.7 years (1). Currently, in the European Union it reached 42.6 years (2,3). Life expectancy at birth is growing around 0.22% per year and in 2018 it was 81.9 years with a gap of 4.6 years between males (79.6 years) and females (84.2 years) (4). An aging population will also lead to a rise in age-related diseases. Thereby it is essential that healthspan is increasing at the same rate as lifespan. Healthspan can be defined as the years without a serious disease, i.e. a disease that leads to death (5). So it is more important than ever to find strategies that prolong healthspan. On the non-clinical front, various lifestyle modifications can be useful tools to extend healthspan. It has been shown in experimental models including primates that caloric restriction or fasting could be such strategies prolonging lifespan and healthspan.

Living twice as long as normal mice, a male dwarf mouse was reported with the longest lifespan of four years (6). Dwarf mice have a deficiency or resistance of growth hormone (7). In the above mentioned case the mouse lacked the anabolic IGF-1 (insulin-like-growth-factor 1) which is activated by growth hormone (GH) (8). These dwarf mice not only live longer but also have an extended healthspan (7).

Human aging is characterized by greying of hair, development of wrinkles, loss of fertility, and pathological features such as hearing loss, presbyopia, reduced cognitive ability, osteoporosis and loss of muscle mass resulting in decreased mobility. Apart from these common characteristics the risk of cancer, heart diseases and neurocognitive diseases increases during the process of aging (9).

At first sight, aging and cancer seem to be two separate fields but in fact they have a common underlying cause: accumulation of molecular damage (10). One plausible connection of aging and cancer is the prominent tumor suppressor p53, which has a disputed function in the context of aging and is the focus of this thesis. To search for literature mainly PubMed has been used with the following keywords: p53 and aging, p53 and longevity.

1 The process of aging

1.1 Aging at the cellular level

About 60 years ago Leonhard Hayflick developed the concept of the Hayflick-limit, stating that the number of cell divisions of normal human embryonic cells in culture is limited to around 50 times before they reach a state of mitotic inactivity, known as replicative senescence (11). This presented one of the first studies, describing the process of aging at the cellular level. More recently, in a review from *Lopez-Otin et al,* (12)*,* the authors defined the following nine key hallmarks of the process of aging: genomic instability, telomere dysfunction, mitochondrial dysfunction, cellular senescence, altered intercellular communication, stem cell exhaustion, epigenetic alterations, loss of proteostasis and deregulated nutrient-sensing (12). To these nine aging hallmarks the molecular mechanism of autophagy was added as it is also of importance for cellular and organismal aging proven by many different papers.

1.1.1 Genomic instability and accelerated aging disorders

The first aging hallmark dealt with in this thesis is genomic instability. Cumulative genetic damage over time contributes to cellular and organismal aging. The DNA can be challenged endogenously and exogenously compromising its stability and integrity. Exogenous threats include biological, chemical and physical components and endogenous threats are DNA replication errors, reactive oxygen species (ROS) and hydrolytic reactions (12). All these stressors can lead to chromosomal gains or losses, translocations, gene disruptions, telomere attrition or point mutations (12). It has been estimated that each cell is affected by ten thousand of the described damages per day (13). The initial notion was that most damages are caused by ROS which has been disproved by more recent studies showing replication errors are major culprits (12,14). It is crucial that cellular responses envolved that ensure diverse repair and control mechanisms to prevent permanent damage. Furthermore, mitochondrial DNA (mDNA) damage has been shown to be involved in the aging process (4), as it has been evidenced in mouse models of mDNA damage showing signs of aging like greying of hair (15). There is also a link between damaged nuclear architecture and the accelerated aging disorder

Hutchinson-Gilford progeria, an autosomal dominant genetic disease caused by mutations in the gene coding for laminin A (16). The wild-type protein is responsible for the architecture of the nuclear lamina and is safeguarding genomic stability (16). Several genetic causes for accelerated aging are known as described below. Progeroid syndromes or accelerated aging disorders are rare genetic disorders ending up in death at a young age, mostly as a result of cardiovascular disease or musculoskeletal decline. They mimic normal ageing in loss of hair, skin tightness, cardiovascular diseases and osteoporosis (9) . Human progeroid syndromes are classified by their molecular pathways underlying the disease: (i) defects in the nuclear envelope or (ii) errors affecting DNA repair mechanisms (9). Hutchinson-Gilford progeria is an example for the first group with a defect in the nuclear lamina. Physiologically this lamina divides the nucleus in eukaryotic cells from the cytoplasm and also contains proteins related to gene regulation and chromatin organization (17). The responsible gene defect is a mutation in the LMNA gene resulting in a Lamin protein, named progerin, missing 50 amino acids with a fatal outcome in nuclear architecture (18). The estimated incidence in the USA is 1 per 8 million births. In most cases a sporadic mutation is causing Hutchinson-Gilford progeria. Only three reported cases of familial occurrence exist in the literature (19). At birth patients appear normal but at the age of around two years growth retardation is noticed (19). Patients' clinical appearance include micrognathia, midface-hypoplasia, alopecia and the absence of subcutaneous fat tissue. Problematic pathologies are osteodysplasia resulting in pathological fractures and atherosclerosis causing strokes and coronary heart disease leading to death in their early teens (20). Interestingly, patients do not suffer from a higher risk of cancer nor they are affected by cognitive decline (21), compared to old individuals aged physiologically. Werner's syndrome belongs to the second group of progeroid syndromes, i.e. harbouring defects in DNA repair mechanisms. Humans have five helicases in order to maintain genomic stability (9). They repair double-strand breaks occurring during DNA-replication and one of these helicases is defect in Werner's syndrome (9,22). This syndrome is autosomal-recessive and begins in early adulthood with patients showing signs of premature aging (23). Retrospectively the first sign noticed is retardation in growth and quite a small stature in adulthood (24). Later, in their early thirties, they

present with skin thinning and with grey hair and hair loss (24). With progressing age, patients' are suffering from age-related diseases such as bilateral cataracts, hypogonadism, diabetes mellitus type 2, osteoporosis as well as atherosclerosis and malignancies leading to death around the age of 54 (24,25).
Genomic damage contributes to the aging process and so does defective mDNA. In Hutchinson-Gilford progeria and Werner's syndrome two examples of rare genetic diseases leading to accelerated aging are listed.

1.1.2 Telomere dysfunction and telomerase

Telomeres are DNA structures at the ends of chromosomes. They protect the chromosomes from degradation and consist of tandem repeats, which are often rich of the base guanine (26). Chromosomes need the protection because they are minimized whenever they are replicated, because enzymes are incapable of a full DNA strand replication (27). Shortening telomeres without any protein coding information is better than damaging other genes (28). Telomeres also preserve the chromosomes from breaking and thereby they build a barrier against end-to-end fusion or degradation (27). They are bound by a protecting protein complex in order to not be recognized by the DNA damage repair machinery (29). After many cell divisions cultured human fibroblasts show short telomeres and enter replicative senescence (for senescence see 1.1.4 cellular senescence). At this point the Hayflick-limit is reached and mitosis is halted. However, other cell types may enter apoptosis when their telomeres are too short (27).
Telomerase is a reverse transcriptase that extends telomeres, but is absent in most cells except germline cells, lymphocytes during the immune answer, basal ceratinocytes, intestine cryptocytes, some blood stem cells and unsurprisingly most cancer cells (30). In 1998, induction of telomerase in human diploid cells was discovered to enable prolongation of cellular lifespan and empower more cell divisions before the cell enters replicative senescence (31).
Short telomeres are a hallmark of cellular aging as the cell stops mitosis. The enzyme telomerase is able to extend telomeres and prolong lifespan, but telomerase is absent in most cells.

1.1.3 Mitochondrial dysfunction

As another hallmark of aging mitochondrial dysfunction has long been considered to have multiple impacts on the ageing process. Besides their main cellular function of energy production as ATP (adenosine triphosphate), they produce ROS (reactive oxygen species) and participate in the process of apoptosis and autophagy (32). There are several theories on how mitochondria are involved in ageing, as described below.

The mitochondrial free radical theory, is based on the permise that old mitochondria produce more ROS leading to further mitochondrial detoriation (12). However, in the last years, it has been shown that increased ROS may lengthen lifespan in yeast countered this theory (33–35). Contributing to that, genetically manipulated mice that produce more mitochondrial ROS did not show signs of premature aging, although they had a higher incidence of pathology (36). For a long time ROS was seen as the evil by-product of a limited system, but they also partake in intracellular signaling, since they trigger proliferation and survival as the answer to physiological processes and cellular stressors (37).

Another concept, although contrary to the ROS theory of aging, is mitohormesis. Mitohormesis describes the process of a system gaining more fitness after chronic exposition to mildly elevated levels of ROS by improving defense mechanisms due to an adaptive response (38). This concept can be compared to the mechanisms underlying vaccination, where stimulation of system resulting in more fitness afterwards (38).

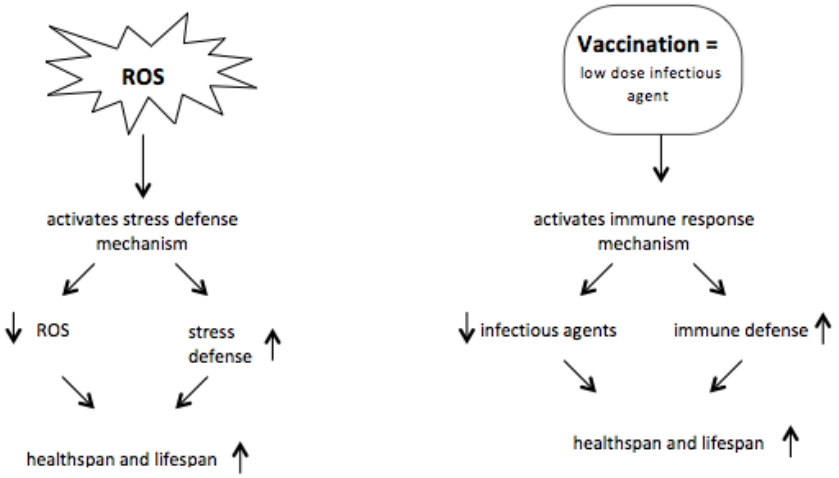

Figure 1: Mitohormesis compared with vaccination (38)

Apart from ROS levels mitochondria impact cellular ageing via other pathways as it has been shown in mice deficient for mitochondrial DNA polymerase y, some underlying mechanisms discussed include inflammation, altered apoptotic signaling or a direct impact on intercellular signaling (12).

Further telomere length also affects mitochondria. That is the case in telomerase deficient mice, where a decline of mitochondrial biogenesis was considered to be the consequence of telomere shortening (39). This process has been found in wild-type mice during aging and was partly reversible through telomerase initiation (40).

To provide enough healthy mitochondria to the cells the balance between mitochondrial biogenesis and degradation is essential. Mitochondrial biogenesis is modulated through Sirtuin1 (a histone deacetylase; see 1.1.7 epigenetic alterations) with an effect on PGC-1α (peroxisome proliferator-activated receptor gamma, coactivator 1 alpha), a transcriptional co-activator (12). The PGC proteins are important for mitochondrial function and energy metabolism, since there repression results in a decline of mitochondrial biogenesis and elevated ROS levels (41). PGC-1α is master regulator of mitochondrial biogenesis. PGC1-β is important in mitochondria for beta-oxidation of fatty acids and oxidative phosphorylation (42,43). Aged and defect mitochondria are degraded by the cell itself through the process of autophagy, which is named mitophagy in mitochondrial elimination (for autophagy see 1.1.10 autophagy) (12). The causes of defective mitochondria include mutations and deletions in mDNA, changes of the lipids in the membrane, oxidation of mitochondrial proteins and defective control mechanisms (44).

So a combination of decreased mitochondrial biogenesis and increased mitochondrial damage may function as causative driver of aging. The outlined facts serve as evidence for mitochondria playing a central role in the process of aging. Different theories on how ROS are involved in aging exist in literature. Further, mitochondrial biogenesis is telomere dependent and they are degraded via mitophagy. All these show the connection between the cellular aging hallmarks.

1.1.4 Cellular senescence

During a human's life time cells are exposed to many stressful endogenous and exogenous events. Cells respond with evolutionary conserved mechanisms that prevent accumulation of damaged cells by evoking cell death (e.g. apoptosis), maintain cellular function (e.g. DNA damage repair), or forstall proliferation of aged or damaged cells. To unsure the latter a cell can undergo senescence, a permanent cell-cycle arrest (45). Senescent cells do not react with growth and proliferation upon growth and mitotic factors anymore (46). Although recent findings provide evidence, that this cell state is not permanent (41). The state of senescence caused by genomic damage is called premature senescence whereas it is called replicative senescence denominates shortened telomeres as reason for halted proliferation. Senescent cells appear to be more flattened and respond worse to stress (10). ROS also contribute to senescence by generating oxidative damage (41). Important pathways regulating cellular senescence are the p53/p21 pathway and the p16/Retinoblasoma protein pathway (47). In mammals, including humans, cells with senescent aspects increase with age (48). These hyporeplicative cells aquire a so-called senescence-associated secretory phenotype (SASP) by producing a chronic inflammatory environment through secretion of pro-inflammatory chemokines like interleukin-6, tumor necrosis factor, growth factors, matrix metalloproteases, and others (10). In general, chronic inflammation is part of nearly every age-related degenerative or hyperplastic disease (49). Clearance of senescent cells is associated with delayed aging, whereas their accumulation is linked to accelerated aging and the development of the SASP. Senescent cells secrete pro-inflammatory chemokines, on one hand this attracts further immune cells to clear senescent cells, but on the other hand it could also lead to immune evasion and less clearance of senescent cells. This would lead to further aging (10).

Cells with senescent aspects increase with age, wheather this is a permanent state or not, and they can lead to the SASP if they accumulate and accelerate aging.

1.1.5 Altered intercellular communication

Aging is accompanied with altered systemic hormone levels and tissues show a higher level of inflammation, immunosurveillance against malignant cells decreases, and extracellular matrix changes (12). All these affect the communication between cells. The term ‘inflammaging’ is described to be a chronic non-infectious and low-grade inflammation occurring in different tissues undergoing the aging process (50). A connection to the hallmark cellular senescence and the SASP can be made, since senescent cells secrete an increased amount of inflammatory cytokines, chemokines and proteases (51). Inflammation is strongly associated with age-related diseases such as atherosclerosis and diabetes mellitus type 2 (12).

1.1.6 Stem cell exhaustion

Stem cells divide symmetrically in order to reproduce themselves and asymmetrically in order to generate tissue-specific cells as a replacement in adult tissue as soon as cells are damaged, injured or unable to function properly (52). During the aging process stem cells’ functions decline (53). Greying of hair as a characteristic feature of aging has been linked to melanocyte stem cell dysfunction (54). Blood is more likely affected by the decline in regenerative potential with age, resulting in pathologies such as moderate anemia, haematological neoplasia, bone marrow failure and decreased immunity (53).

1.1.7 Epigenetic alterations

Epigenetic mechanisms control access to DNA information and its transmission to the following generation, regulates gene expression, and stabilizes genes. This is achieved by using three main mechanisms: DNA methylation in order to block gene expression, the modification of the DNA’s packaging material, called histones, and the regulation of mRNA stability and translation by interference through short RNA sequences (30).

Histone demethylases in Caenorhabditis elegans interact with the IGF-1 and insulin signaling pathway (IIS) which plays a key role in the aging process (12). For more information about IIS see 1.1.9. deregulated nutrient sensing. There is growing interest on sirtuins in the field of aging research. They are a group of

histone-deacetlyases which suppress and enhance transcription. To date, eleven different classes of sirtuins have been discovered in humans (55). Sirt1, Sirt3, Sirt6 have been identified to influence the aging process: Sirt1 overexpression in mammals improves their health during aging, while Sirt6 deficient mice showed signs of accelerated aging. Sirt3 overexpression has been connected to the regenerative potential of hematopoietic stem cells and the pro-longevity aspects of caloric restriction. Interestingly Sirt3 is located in mitochondria placing at the center of an aging hallmark (12).

Further, heterochromatin which is tightly packed DNA material with lots of repetitive sequences and can be found in centromeres and telomeres (56). Cells from individuals affected by Hutchinson-Gilford progeria show less heterochromatin structures compared to cells of individuals undergoing the physiological aging process (57). Telomeres mainly consist of heterochromatin providing another connection between several hallmarks of aging namely epigenetics, genomic stability, and telomere shortening (12).

Epigenetic alterations also influence cellular senescence. For instance, the accumulation of heterochromatic structure in the promotor of E2F-target genes, resulting in their repression, has been shown. E2F is a transcription factor regulating cell cycle. This accumulation of heterochromatic structures in E2F-target promoters is associated with senescence and therefore is referred to as senescence-associated heterochromatic foci (SAHF) (41,46).

The outlined facts serve as evidence for epigenetic mechanisms influencing the aging process and therefor being a promising field to conduct further study.

1.1.8 Loss of proteostasis

The word proteostasis is a combination of protein and homeostasis. Proteostasis is essential for proper cellular function and encompasses protein stabilization, correct folding, maintenance and degradation through proteasome or lysosome (58). The protein homeostasis is altered during the aging process which has been proven by many studies. The connection between loss of proteostasis and age-related diseases such as Alzheimer's and Parkinson's disease is reviewed elsewhere (12). For instance, in Alzheimer's disease accumulation of mis-folded amyloid beta protein or tau proteins are believed to be causative (59).

Every protein is folded very specifically to ensure correct function and the organism's health. Sometimes proteins need assistance to fold correctly, especially under conditions of cellular stress like heat, and this assistance is provided by chaperone proteins (60). Chaperones stabilize proteins and polypeptides and they avoid their aggregation. Prominent chaperones are the so called heat shock proteins (HSPs) (61). HSPs play a role in longevity and aging, since their expression is needed to avoid protein damage and a decline in the heat shock response while aging has been reported (62).

Old or misfolded proteins must be eliminated by the cell. This elimination is ensured by the ubiquitin-proteasome system and the autophagy-lysosomal system. Proteasomes degrade proteins marked for elimination by the small protein ubiquitin through an ATP-demanding process. In contrast, lysosomal elimination does not require ATP although whole cell organelles like mitochondria can be degraded via hydrolytic enzymes (63). For more information about autophagy see 1.10 autophagy. The activity and fidelity of the ubiquitin-proteasome and the autophagy-lysosomal systems are reduced with increasing age (12).

Proteostasis changes during the aging process and it impacts aging. There is a decline in HSPs respone as well as in fidelity of the ubiquitin-proteasome and the autophagy-lysosomal systems with the age. Alzheimer's and Parkinson's disease also show altered proteostasis.

1.1.9 Deregulated nutrient-sensing

If there are enough macronutrients (lipids, glucose and amino acids) and growth factors available, nutrient sensing- and growth factor pathways confer signals for cell growth and division (64). Mutations or polymorphisms resulting in decreased function of the nutrient sensing pathways, such as reduced function of the IGF-1 receptor and the insulin receptor or diminished intracellular signals via AKT (AKT is an important kinase for intracellular signal transduction), mechanistic target of Rapamycin (mTOR) or forkhead box protein O (FOXO) have been linked to longevity in humans and other organisms (12). One nutrient-sensing pathway involved in aging is the mTOR pathway and the other one is the IGF-1 and insulin signaling pathway or short IIS. The latter is called IIS, because the intracellular signaling of IGF-1 and insulin while the presence of glucose is the same (12).

The mTOR pathway recognizes amino acid and glucose levels (12), but it is also involved in sensing low energy levels with decreasing ATP and increasing adenosine monophosphate (AMP), hypoxia and growth factors via the IIS pathway (64). mTOR contains two enzymatic complexes: mechanistic target of rapamycin complex 1 (mTORC1) (contains mTOR, Raptor, and mLST8 proteins) and mTORC2 (contains mTOR, Rictor, Sin1 and mLST1) (65). In response to nutrient levels mTOR regulates cell growth and protein translation. To sense low energy states, with increasing AMP/ATP ratio liver kinase B 1 (LKB1) and AMP-activated protein kinase (AMPK) are activated. Subsequently, AMPK phosphorylates tuberous sclerosis complex 2 (TSC2) (64). The TSC2 protein, also called tuberin, is defect in tuberous sclerosis, where patients affected develop multiple at least non-malign tumours (66). TSC2, on the other hand, regulates the small binding protein Rheb to activate mTORC1. Normally, eIF4E binding protein and a repressor of mRNA translation (4EBP) and the p70 ribosomal protein S6 kinase (S6K) are bound to eukaryotic initiation factor 3 (eIF3) and are dormant until growth hormone binds to the cell and mTORC1 inhibits eIF3 and activates S6K and 4EBP. 4EBP then releases eukaryotic initiation factor 4E (eIF4E) (64). Finally, protein translation and cell growth start.

How does the IIS pathway work? As soon as GH is released by the anterior pituitary gland of mammals and it reaches the cells via the circulation they produce IGF-1, especially hepatocytes (12). When IGF-1 binds to the IGF-1 receptor the enzyme phosphatidylinositol 3-kinase (PI3K) produces phosphatidylinositol-3,4,5-triphosphate (PIP3) that activates lipidkinase PDK1 and mTORC 2 then AKT kinase is activated and moved to the nucleus where it phosphorylates FOXO transcription factors, then they leave the nucleus (67). The nuclear export of FOXO proteins inhibits apoptotic signaling (64). This results in cell growth and division.

Additionally, AKT can also stimulate nuclear factor kappa-light chain-enhancer of activated B-cells (NFκB) (68), which works as a transcription factor and plays a role in proliferation, apoptosis and immune answer (69). There is also a connection between the IIS and mTOR pathway: AKT activates mTORC1 via inactivation of TSC2 (68).

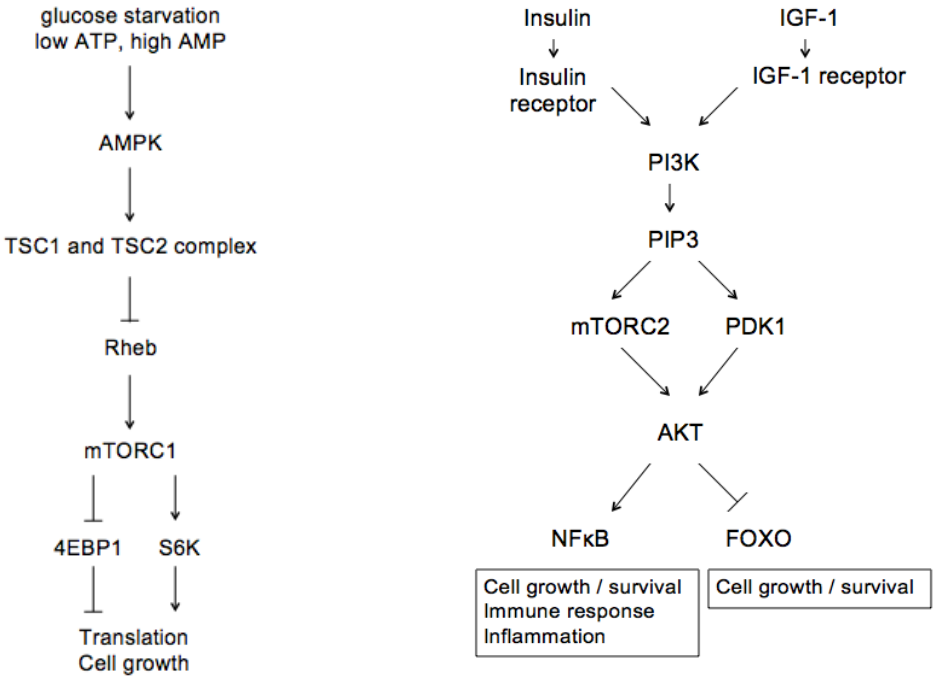

Figure 2:
The process of growth regulated by the mTOR (left) (64) and in the IIS pathway (right) (68)

The downregulation of the nutrient sensing pathways mTOR and IIS through mutations is linked to longevity (12), and a better understanding of these pathways is essential for following chapters.

1.1.10 Autophagy

Autophagy can be seen as a part of the hallmark protein homeostasis. Anyway, as this process might be of importance it is discussed separately. There are three different types of autophagy: macroautophagy, chaperone-mediated autophagy and microautophagy (70). In the following the word autophagy is used to refer to macroautophagy. Autophagy is essential during states of nutrient deprivation. The process ensures recycling and it provides nutrients and energy (70).
Autophagy starts with a membrane wrapping around the cytoplasmatic element, which has to be eliminated, for example old mitochondria. Then the edges of this membrane melt together and forming the autophagosome. The autophagosome's outer membrane fuses with the lysosome leading to the autophagolysosome. Next, enzymes (lysosomal hydrolases) degrade the cytoplasmatic material inside the autophagolysosome including the inner membrane. The recycled products can

be used again for a broad range of cellular processes. Hence, such as ribonucleotide synthesis and protein translation (68). The process of autophagy is important to adapt to the cells need in times of diminished resources and to prevent accumulation of non-functional proteins (69). In many organisms autophagy plays a key role in regulating lifespan: As soon as autophagy is reduced the aging process accelerates. Thus, the autophagy process is generally considered an anti-aging process (70). Although reports of the beneficial effects of autophagy are prevalent, this notion is challenged by recent studies such as the one by *Zhou et al*. (72). They show that autophagy induction can have detrimental effects on organismal health in the context of increased mitochondrial permeability.

On the cellular level, autophagy is primarily a process to protect the cell (70). Autophagy allows the cell to adapt to stressful conditions as it provides nutrients (proteins, lipids and carbohydrates) in times of starvation from the breakdown of cellular components. The inactivation of the mTOR pathway can activate autophagy. With that mTOR switches between cell growth in the presence of nutrients and catabolic degradation in times of starvation (64). Also, mitochondrial homeostasis is regulated through mitophagy (12). Furthermore, autophagy is essential for proteostasis and therefore has an impact on the aging process.

In summary, autophagy is mainly considered as an anti-aging process and evidence showing pro-aging effects are so far strictly context-dependent.

1.2 Longevity and healthspan

1.2.1 Genetic basics

Many genetic variations connected to longevity have been discovered yet and some of them interact with the IIS pathway (73). A significant proportion of genetic variations linked to longevity exist, a few of which are discussed in the following: At the beginning the dwarf mouse lacking IGF-1 and living twice as long as her litter mates, was already mentioned. Another prominent variation is that of the FOXO3A gene, encoding a transcription factor and key downstream effctor in the IIS pathway. It gained special interest when three singe nucleotide polymorphisms (SNPs) in this gene were connected with prolonged survival in humans (74). Data from studies across several species suggest a strong link between FOXO transcription factors and autophagy. Specifically, FOXO3A can stimulate autophagy in mammals as in response to Sirt1 activation or starvation (70). Another one of genetic variations prolonging lifespan is the p53 polymorphism Arg72Pro. Individuals with this polymorphism live longer (10). Paradoxically this p53 variant shows reduced apoptotic potential (74). Further discussion about this p53 polymorphism can be found in chapter 3.12 p53 SNP increases lifespan. Sexual dimorphism also influences lifespan. Usually females and males of a species differ in their characteristic behavior. Interestingly females and males are also different concerning lifespan and cancer development. In general, females of a species live longer and develop cancer later than males (67).
In conclusion, many genetic variations determining lifespan exist and gender also seems to influence longevity.

1.2.2 Lifestyle interventions

Caloric restriction (CR) extends lifespan and slows the aging process in many species including primates. (76) Until now CR has been the most successful tool to extend lifespan and reduce age-related diseases in animal models (77). The first CR experiments were performed in 1935. Rats fed with 30% less food compared to a group of ad libitum fed rats. The finding was an increase in mean and maximum lifespan by more than 30%. Data has shown that CR may reverse

the molecular aging hallmarks: genomic instability, mitochondrial dysfunction, telomere shortening, cellular senescence, stem cell exhaustion, loss of proteostasis, deregulated nutrient sensing and altered cellular communication (78). In humans, a healthy diet and normal body weight are believed to have positive influence in preventing many diseases related to age. Diabetes mellitus type 2, colorectal cancer, atherosclerosis and cardiac disease are connected to inadequate nutrition. High blood glucose and elevated cholesterol are risk factors for many other diseases, and can be partly regulated through nutrition.

CALERIE is a study of human CR published in 2015, where 218 non-obese humans between 21 years and 25 years have been examined. They were randomized into two groups, caloric restriction (CR) or ad libitum (AL) diet, for over two years. CR was feasible to use in non-obese humans. Metabolic slowing, reduced core temperature and reduced triiodthyronine (T3) have been found in animal models of CR and might correlate with the positive effects on lifespan. The resting metabolic rate within the CR group droped significantly more compared to the AL group. Also, T3 droped significantly more in CR than in AL while tumor necrosis factor α (TNFα) decreased significantly in the CR group. The hormone T3 from the thyroid gland assists the body in adapting to environmental conditions and is therefore of great significance for human metabolism (79). Inflammation is part of the aging process therefore it is interesting, that TNFα which is a pro-inflammatory cytokine droped in the CR group. This study points out that CR had positive effects on cardiometabolic risk factors without showing any negative findings in quality of life (80). However, chronic CR could lead to loss of bone density with the possibility of pathologic fractures and therefore intermittent fasting may be the better alternative to reduce aging biomarkers in humans since both CR and IF show similar lifespan extensions in rodents without a major decrease on bodymass (70). The longevity extension upon CR is most likely regulated via the following nutrient-sensing pathways: mTOR, AMPK, sirtuins and IIS (81). On the molecular basis mTOR seems to play an important role concerning the beneficial effects of CR, because CR fails to prolong lifespan without mTOR signaling (41). AMPK and Sirt1 act as energy sensors, as soon as there is a lack of energy they regulate many cellular processes of energy homeostasis, such as autophagy (70). Further, the decrease of the metabolic rate in times of CR also descends the

production of toxic components like ROS, but it is not clear if this corresponds to the longevity effect (67).

Exercise in general is a modifiable risk factor for cardiovascular disease which is among the most frequent causes of death. Prevention of mitochondrial degeneration is one suggested mechanism through which endurance training may improve healthspan (12). It has been shown in enducance-trained persons, that no age-related decline in mitochondria's oxidative potential occured, although mtDNA and mitochondrial transcription factors showed signs of age-related damage. Further, it has been seen a decline of Sirt3 in inactive persons with age while it was equally elevated in endurance-trained persons through all included ages (82). Hence, besides genetics, also lifestyle decisions such as CR and exercise impact longevity and healthspan.

1.2.3 CR and fasting mimetics

Taking a pill to achieve longevity and healthspan would be the more comfortable method compared to caloric restriction and physical exercise. A pharmaceutical approach to utilize the positive effects of CR, are caloric restriction mimetics, which mimic CR without restricted food intake. Resveratrol and its relatives, metformin, rapamycin and other mTOR inhibitors have been proposed as such (83).

Grapes, and with that red wine, contain the polyphenol resveratrol to a varying degree. Red wine was suggested to have various beneficial effects on health and longevity with special interest on the substance resveratrol. Resveratrol was shown to possess cardioprotective, anti-inflammatory, anti-cancer, and neuroprotective, cumulating in an anti-aging effect (84). Anti-aging effects of Resveratrol have been found in in yeast, flies and worms via indirect activation of Sirt2, which is ortholog to Sirt1, and the induction of autophagy in yeast and C. elegans has been reported (70). No effect on longevity has been found in rodents, but an improvement in health parameters in the resveratrol-fed animals has been recognized in animals fed with a high fat diet (84). No beneficial effects of resveratrol in healthy individuals could be found, whereas in exercise studies the effect was unfavorable. Only in small patient studies some improvements have been found (85). Other pro-autophagic substances include spermidin and curcumin (70).

Metformin is a drug widely used in the treatment of diabetes mellitus type 2. Metformin has been proposed to have positive effects on lifespan as it might interfere with the IIS and mTOR pathway via the activation of AMPK, which makes it a caloric restriction mimetic (85).

In 2009 rapamycin has been discovered to extend the lifespan in mice (86). Today it is approved as an anti-cancer drug and immunosuppressant, the latter of which is at the same time indicated as adverse effect in the use of rapamycin supplementation in healthy adults. Rapamycin functions as a selective inhibitor of mTORC1, a major nutrient sensing pathway as described above (87). The drug rapamycin inhibits mTORC1 while mTORC2 is insensitive to its inhibition (64). In human fibroblasts rapamycin has been able to delay senescence (65). Hence, through modulation of the mTOR pathway rapamycin can mimick effects of CR or IF (70). Besides immunosuppression rapamycin has various harmful effects including disregulation of the glucose and insulin homeostasis and cataracts, although there is evidence that these side effects could be avoided by regulation of dose and timing (85). mTOR also interacts with PGC-1 in the regulation of mitochondrial genes, but it is not clear yet if this also pertains to the longevity effects of rapamycin (85).

In sum, CR and fasting mimetics have at least some of the positive effects of CR but are often plagued with harmful side effects.

1.2.4 Parabiosis – young blood

Connecting the circulatory systems of old and young mice was done in heterochronic parabiosis experiments (10). This procedure leads to old mice being exposed to factors in the young mouse's blood. Strikingly, it has been shown that factors in the circulatory system can modulate ageing by rejuvenating the brain and numerous other organs (88,89). One such factor was shown to be growth differentiation factor 11 (GDF11) that can reverse age-related decline in some cells, while other studies show controversial outcomes (10).

2 p53: the guardian of the genome

The TP53 gene encodes for the correspondent protein p53 with a molecular weight of 53kD, giving the protein its name. In 1992 it was declared The Guardian of the Genome and awarded as Molecule of the Year in 1993. As a transcription factor it regulates numerous genes functioning as tumor suppressor in most contexts, as it prevents a genomic damaged cell from proliferation. p53 has a wide spectrum of functions. The connection between p53 and cancer is well established already, in contrast to its role in ageing and longevity. Normally the concentration of p53 in the cell is kept on a low level and the protein has a half-life of 20 minutes (28). Initially, the protein was thought to be localized in the nucleus, but some functions are also mediated by protein-protein intarctions in the cytoplasma and at the outer mitochondrial membrane (90). In general, p53 acts as a sensor of extrinsic and intrinsic stress signals like DNA damage, activation of oncogenes, hypoxia, extreme temperatures and diminished nutrient levels in times of starvation (64). For a long time the protein was ascribed two main functions due to its responses to stress signals: cell cycle arrest and apoptosis. p53-mediated cell cycle arrest blocks the proliferation of damaged cells until they are repaired. Major DNA defects, that are beyond repair, are detected and apoptosis is initiated by p53's transcriptional activity as well as through interaction with the apoptosis regulator BCL2 (B-cell lymphoma 2) at the mitochondrial membrane (28). As a response to stress it controls transcription through sequence-specific DNA binding to its target genes. (91) These target genes provide genomic integrity to protect the whole organism against a defective dividing cell through either cell cycle arrest and repair or senescence or apoptosis. (64) Besides these transcriptional functions, p53 also has non-transcriptional functions such as DNA-repair and mitochondrial protein survival (92,93).

However, research on p53 continues to deliver surprising results as it seems to be essential for molecular processes way beyond its canonical functions (94).

2.1 The p53 protein domain structure

Like all transcription factors, p53 has a distinct and defined domain structure. Most mutations associated with cancer are localized in the protein-binding domain (94).

At the N-terminus p53 contains two transcriptional activation domains, TAD1 and TAD2, with the range of amino acid residues 1-40 and 40-60. Further, TAD is important for the regulation of p53's transcriptional activity as an transcriptional enhancer of p53 target genes (94). Interestingly, the TAD1 region is the binding site for the negative p53 regulators MDM2 and MDMX (95).

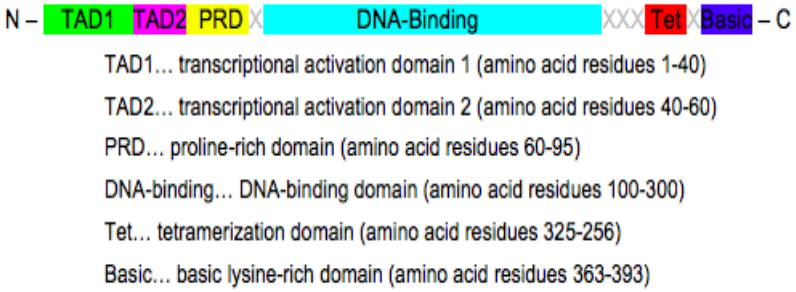

Figure 3: The p53 protein domain structure (94)

The next part of the protein domain structure is a proline-rich region (PRD) which might play a role in protein-protein interactions (94). C-terminal to the PRD, the DNA-binding domain ranges from residue 100 to 300. This region is responsible for the exact binding of the protein to the corresponding recognition sites in the DNA. p53 binds to DNA sites as a homotetramer. Tetramerization is ensured by the tetramerization domain (Tet) stretching from 325 to 356 amino acid residue (94,95). At the C-terminus p53 has a basic lysine-rich domain, at residues 363-393. This domain supports DNA binding of the p53 tetramer. It also harbours several residues prone to posttranslational modifications that can affect p53 stabilization (94).

2.2 Regulation of the protein p53

The level of the p53 protein in a proliferating, non-stressed cell is kept very low due to constant degradation by the ubiquitin-proteasome system. As soon as the cell has to respond to a stress signal the p53 protein concentration rises through stabilization. Inappropriate regulation could result in a damaged cell proliferating and passing the defect on to further cells which would affect the whole organism.
p53 is regulated on the transcriptional level and also post-transcriptionally through

diverse modifications on the protein. Ubiquitilation, acetylation and phosphorylation might be the most prominent (93). Ubiquitilation marks p53 for degradation, for phosphorylation p53 protein has Ser/Thr amino acid residues serving as phosphorylation sites for enzymes and many lysine residues act as acetylation sites as acetylation prevents the ubiquitilation of these sites resulting in p53 stabilization (93).

One of p53's well-known regulators is the MDM2 protein. It is the most important of the E3 ubiquitin ligases to negatively regulate p53 activity. (94) MDM2 can decrease p53 protein levels via at least three mechanisms: First, poly-ubiquitylation marks p53 for degradation mediated by proteasomes; Secondly, mono-ubiquitilation by MDM2 translocates p53 out of the nucleus; Thirdly, MDM2 can directly binding to the transactivation domain of p53, hampering its ability to engage with recognition sites and activate target genes (10). As response to stress p53 is released via several mechanisms. One is the post-translational modification of MDM2 and p53 to decrease their interaction. The aminoacid residues Ser15 (Ser18 in mice) and Ser20 (Ser23 in mice) become phosphorylated by ATM (ataxia-teleangectasia mutated) and other kinases. This results in disturbed MDM2-p53 interaction and p53 stabilization (93,94). Another mechanism is the inhibtion of p53's ubiquitilation. And last MDM2 sequestration by the tumor suppressor alternate reading frame (ARF) when oncogenes are activated (94). ARF accumulates when oncogenic or aberrant hyperproliferative signals prevail (96). Synonyms are p14ARF in humans or p19ARF in mice. Acting through p53, it can induce cell cycle arrest or apoptosis. The encoding gene is INK4a and is located on the short arm of chromosome 9. This gene also encodes p16INK4a, a major senescence regulator (97).

On the flip side, the enzyme ubiquitin-specific-processing protease 7 (USP7), or also called herpesvirus-associated ubiquitin-specific protease (HAUSP) since it has first been identified as a reaction partner with a Herpes-simplex-virus 1 protein. (98) USP7 is a de-ubiquitylation enzyme that divides ubiquitin from p53 and protects it from degradation, so it works as an antagonist of MDM2 (99).

Overall, p53 is exceedingly regulated which highlights its importance in cellular processes.

2.3 Cellular processes regulated by p53

It is not completely understood yet, why p53 makes cell cycle arrest in some types of cells and apoptosis in others (91). The cell fate is dependent not only on the cell type but also on the nature and extent of stress and the cellular environment (94). p53 works as a transcriptional regulator of target genes. While some p53 target genes are regulated by high levels of p53, others require basal levels (10).

One p53 function is cell cycle arrest and repair. If stress is occurring cellular p53 concentrations rise within a few minutes through phosphorylation via upstream kinases, outcompeting MDM2 in binding p53. Interruption of the cell cycle in damaged cells happens at the checkpoint in the G1-phase (Gap1-phase) and G2-phase (Gap2-phase) of the cell cycle. This point between G1- and S-phase (synthesis) is also called restriction-point, where a cell decides whether DNA is replicated or not. The second checkpoint is between G2- and the M-phase (mitosis) and at this point the replicated DNA is checked again (28).

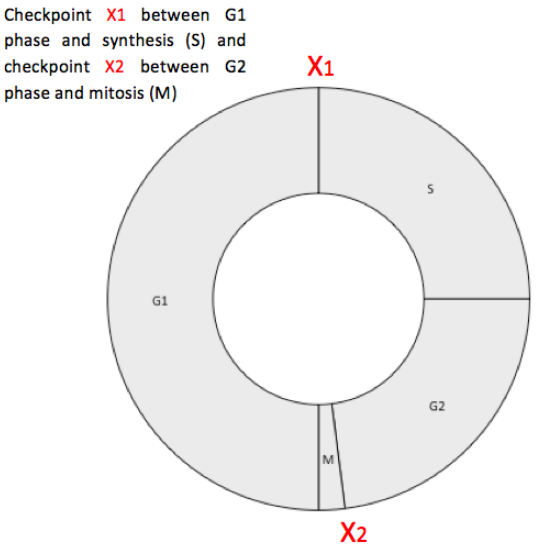

Figure 4: Simplified cell cycle with p53 dependent checkpoints (28)

Irreparably damaged cells have to be eliminated via apoptosis to protect the whole organism against neoplasia (94). p53 starts apoptosis via downregulation of BCL2, first found in a b-cell-lymphoma, that releases cytochrom c from the mitochondrial membrane into the cytoplasm where it activates caspase 9 that itself activates

caspase 3 to start the apoptotic process. Another protein belonging to the BCL2 family is BAX. This protein helps to release more cytochrome c from the mitochondria, rendering it pro-apoptotic. Transactive nuclear p53 enhances BAX transcription (28), as well as that of other factors of the apoptotic pathway: FAS, NOXA and PUMA (94).

In some contexts, p53 reacts to damage with the induction of senescence, a cell state widely known as a permanent cell cycle arrest, although there is now evidence for it to be reversible under certain conditions. Premature senescence denotes a cell cycle arrest before the cell would naturally stop dividing. The natural end of mitosis in aged cells is called replicative senescence. In contrast to replicative senescence is premature senescence independent from telomere attrition (100). Senescence also works as a tumor protection as it prevents a benign tumor cell from malignant transformation (100). The p53 / p21 and p16INK4A / Retinoblastoma protein pathways are the two main pathways underlying the mechanism of senescence, p53 is able to activate p21 and plasminogen activator inhibitor-1 (PAI-1) to lead the cell into senescence (41). It has been shown in mouse embryonic fibroblasts, that the senescence function of p53 also relies on p21 working properly, as the cells failed to undergo cell cycle arrest after DNA damage when p21 was deleted (101). Additionally, E2F7 has been described as another partner of p53 in senescence (41). E2F7, which is a p53 target, belongs to a family of transcriptional regulators involved in cell cycle repression (102). When inducing senescence in the laboratory through oncogenes and proliferative signals, E2F7 is upregulated depending on p53 (41).

Thus, cell cycle arrest, apoptosis and senescence are three distinct cellular processes all regulated by p53.

2.4 p53 tumorsuppressor and beyond

About 50% of human cancer cells have a loss or mutation in the p53 gene locus (94). A severe example for the protein not working correctly is the Li-Fraumeni-Syndrome. Humans showing a mutant p53 allele are predisposed to suffer from cancer in early ages, such as renal carcinomas, breast carcinomas, sarcomas and brain tumors (103). Before the age of routine genetic testing the Li-Fraumeni-Syndrome was defined as follows: a person with a sarcoma under the age of 45

years who has got a first-degree relative with any kind of cancer under the age of 45 years plus another relative (first- or second-degree) with cancer under the age of 45 years or a sarcoma at any age. Suggesting an autosomal dominant transmission of the gene (104). The importance of p53 in tumor suppression has also been evident in p53-null mice, which developed tumors in nearly 100% (105). Most cancer mutations are located In the DNA-binding domain and most of those mutations are missense mutations, which prevent efficient DNA-binding, underlying the importance of p53's transactivation function for its role as a tumor suppressor (94,95).

Besides tumor suppression p53 plays a role in the physiological processes of aging, development, cell differentiation, stem cell function, fertility and tissue homeostasis as well as in many other pathologies (94). Pathologies relying on defective ribosomal biogenesis, like Diamond-Blackfan anemia, Trecher Collins syndrome, 5q-syndrome macrocytic anemia have been linked to aberrant p53 activation in mouse models (94). Further, p53 was also suggested to play a role in the origin of diabetes concerning the senescence response in fat cells (94). Ongoing research has been establishing roles of p53 in the fields of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and Huntington's disease (94).

As listed above, the prominent tumor suppressor impacts numerous other physiological and pathological fields of study.

3 Pro-aging and anti-aging roles of p53

p53 assures longevity also due to its cancer protecting functions as it has been shown in a mouse model, where both allels of p53 were deleted, resulting in early death caused by cancer (106). Evidence for p53 regulating longevity and aging beside its function as a tumor suppressor has been growing in the last two decades. Mice aged naturally showed a decline in p53's function (10). Further, the decline of effective p53 stress response has been associated with the increasing incidence of cancer with the age (67). The global average age of cancer incidence in men and woman was 65.7 years in 2018 (107). Cancer is most commonly in the last part of an animal's lifespan, although animals have very different life spans (67). In an experiment to test if the effectiveness of p53's stress response changes with age, mice at different ages were "treated" with gamma rays (5 Gray) and the transcription of six p53 target genes (p21, MDM2, Cycin G, Puma, Noxa, Fas) was examined. Strikingly, the induction in response to irradiation of all six genes decreased with age (108). Interestingly, their lifespan correlated with the onset of p53's functional decline (67). This supports a positive association between p53 and its anti-aging function. So does a study from the worm C. elegans: mutations increasing longevity also lifted p53 activity (96,109). On the other hand, elevated levels of the protein p53 have been shown in astrocytes, fibroblasts and retinal pigment epithelial cells from aged individuals (10).

As can be glanced from these reports, there is evidence for both p53 as pro-aging and anti-aging as further discussed in the following chapters. Importantly, p53 impacts nine out of nine cellular aging hallmarks as well as the process of autophagy.

3.1 p53 in genomic instability

Over time cells accumulate genomic damage and p53 has functions of cell cycle arrest, repair and apoptosis to conserve genomic integrity. Progeroid syndromes with defects in DNA repair mechanisms, like Werner's Syndrome, show genomic instability with high levels of p53 (10). One stress response pathway in Hutchinson-Gilford Progeria Syndrome to the defect nuclear lamina is the activation of p53 (12). Similarly, it has been evidenced in a mouse model

(Zmpste24-/-) mimicking Hutchinson-Gilford progeria by lacking a metalloprotease and resulting in destroyed nuclear architecture. Here, p53 target gene expression was upregulated, whereas basal p53 protein levels were unchanged. When crossing these Zmpste24-/- mice into p53-null mice their aging phenotype could be partly rescued (110). Further, in another mouse model of accelerated aging due to defective breast cancer 1 (Brca1), which is important for double-strand break repair, p53 deletion reduced cellular senescence and the aging phenotype (111). Additionally, generated Ku80(-/-) mice had a defect in double-strand brake repair and showed skin atrophy, osteopenia, and reduced longevity (92). In this model, fibroblasts showed an elevated level of p53 and entered replicative senescence early. When these fibroblasts were made deficient for p53, they did not present with early replicative senescence but died earlier due to neoplasia (112). In the same way, SnoN(m/m) mice were not able to antagonize transforming growth factor beta (TFB beta) signalling anymore and showed accelerated aging, unless made deficient of p53 their aging phenotype could be rescued partially (113). These studies support the idea that constitutive p53 activation as a response to genomic damage causes accelerated aging and it indicates p53 to be a key driver of this accelerated aging phenotype.

On the flip side, a mouse model (ATRs/s) of the ATR-Seckel syndrome showed an aggravated aging phenotype when loosing p53 (114). This syndrome is a rare genetic disease caused by mutations in ataxia telangiectasia and Rad3 related protein (ATR), which is a component of DNA damage signaling. Patients affected have dramatic microcephaly, dwarfism and developmental delay (115). This diverse outcome in p53 loss could be seen as the failure of an already damaged system due to additional stress when loosing p53 (10).

In summary, most mentioned studies support the idea, that constitutively active p53 in response to genomic damage accelerates aging. On the flip side are mice mimicking ATR-Seckel syndrome with an aggravated aging phenotype when loosing p53.

3.2 p53 in telomere dysfunction and its results

Dysfunctional telomeres limit the proliferative potential in human cells and induce the organismal aging process by p53 and p21 activation (116). So p53

also interferes with short telomeres. The erosion of telomeres while cell proliferation also results in replicative senescence. Short telomeres are recognized as DNA-damage (more specifically as double-strand breaks) which activates DNA damage response. In the DNA damage response the pathway around ATM kinase is activated, which activates p53 to induce cell-cycle arrest and senescence (41). In another mouse model (terc -/-), telomerase has been eliminated resulting in mice with decreased lifespan, short telomeres, increased senescence as well as infertility in the sixth generation (117). When p53 has been eliminated in this model their phenotype could be saved regardless of telomere dysfunction (10). This supports the pro-aging role of p53.

3.3 p53 and mitochondria

p53 is able to inhibit mitochondrial biogenesis and function through inhibition of PGC-1α and PGC1-β in response to short telomeres. This itself leads to further ROS production activating p53 in a forward-feedback loop (10). p53 in general is also partly responsible for the expression of cytochrome c oxidase 2 which is part of complex IV of the electron transport chain, but it is not entirely clear yet if this impacts the aging process (41). In a study on animals lacking telomerase a link between mitochondria and p53 in aging has been found. When telomeres become too short they are recognized as DNA-damage and this activates p53 resulting in age-related mitochondrial decline. In these telomerase-deficient animals a reduction of p53's function diminished this age-related mitochondrial decline, partly because of reduced p53-mediated cell death (118). Further, p53 is able to interact with mTOR and to regulate autophagy, that suggests that p53 also plays a role in mitophagy, but more research is needed to clarify the axis (41). Depending on the type and level of stress p53 can induce opposing outcomes in mitochondrial fidelity. Basal p53 activity optimizes mitochondrial function and reduces ROS through its target genes SCO2, TIGAR and sestrins. Upon mild stress, p53 can induce the transcription of anti-oxidant genes whereas upon acute stress p53 activates pro-oxidant mechanisms leading to apoptosis and senescence (10).

To sum up, p53 has various functions from inhibition of mitochondrial biogenesis to an improvement of mitochondrial function, which are dependent on type and level of stress.

3.4 p53 in cellular senescence

Senescent cells show increased expression of p53, p21, p16 and other inhibitors of the cell cycle. p53 is essential for senescence as it has been proven by p53 deletion when the induction of senescence through oncogenes was not possible anymore (41). A p53 dependent senescence response has been shown in Studies of diabetes in fat cells from obese mice. Interestingly, that response generated insulin resistance (92). Additionally, it has been proposed, that excessive calorie intake led to a type 2 diabetes like disease and to the following senescent changes: increased p53 expression, increased proinflammatory cytokine production and increased activity of senescence-associated beta-galactosidase. Whereas p53 inhibition in mice's adipose tissue improved insulin resistance, decreased pro-inflammatory cytokines and ameliorated senescent changes. p53 upregualtion caused an inflammatory response resulting in insulin resistance (119). Populations of ku80(-/-) cells have defects in double strand brake repair leading to replicative senescence. The reduction of p53 levels could rescue these cells from replicative senescence and enabled immortalization (112). In the above cases, p53 acts pro-senescent. On the other hand, it has been shown that p53 can allure macrophages due to CC-chemokine ligand 2 (CCL2) and Death domain 1α (DD1α) expression for clearing senescent cells as the clearance of senescent cells is associated with delayed aging (10). Also, cellular damage mediated through ROS takes part in inducing cellular senescence. By regulating ROS levels p53 can prevent or promote senescence. There is evidence for p53 to regulate antioxidant genes (mitochondrial superoxide dismutase 2, glutathione peroxidase 1, mammalian sestrin homologs 1 and 2), suggesting the suppression of senescence. DNA-damage activates p53 and produces an intracellular ROS peak resulting in apoptosis or senescence. This dual role of p53 and ROS might also contribute to senescence in a dual way (41).

In the two above described studies p53 has been labeled pro-senescent, but there is also evidence for a dual role as it has been described in cell clearance and ROS.

3.5 p53 in intercellular communication

As discussed above, p53 plays an important role in senescence. But does it also play a role in the SASP? Cells with wild-type p53 were compared to cells lacking p53 function: Those lacking p53 secreted higher levels of cytokines known as SASP components (90). Further, p53 has a role in the regulation of the immune system (120). Toll-like receptors, natural killer cell ligands (ULBP1 and ULBP2) and cytokines are also p53 targets as it is able to enhance their transcription (121). Through these mechanisms p53 modulates innate immune answer, apoptosis and inflammation (10). Additionally, p53 loss accelerates aging of the immune system: memory T-cells accumulate, more cytokines are produced and T-cell proliferation is stopped earlier. p53 can upregulate Toll-like receptors, cytokines and natural killer cell ligands. It can downregulate the expression of programmed cell death ligand 1 (PDL1) (10).

Overall, p53 is involved in intercellular communication, like in the immune system, where is shows protection against aging. Concerning the SASP phenomenon p53 also has an anti-aging function in cells.

3.6 p53 in stemcell function

p53 is involved in the restriction of the self-renewal in some stem cells, like in neural stem cells and hematopoietic stem cells (94). In the absence of p53 enhanced efficiency in reprogramming somatic cells to induced pluripotent stem cells has been reported, leading to the conclusion that p53 maintains a differentiated cell state (94,122). It has also been shown in aged mice with reduced p53 activity (p53+/-), that they had a higher number of proliferating hematopoietic stem cells compared to mice with normal p53 activity (p53+/+). Indicating that an alteration in p53 activity in aged organisms can influence the number of stem cells, their proliferative capacity and hematopoiesis (123). This also corresponds to the pro-aging activity of p53. Similarly, p53 +/m mice were generated carrying one copy wild type p53 and one copy with truncation at the N-terminus, where MDM2 interaction happens. p53+/m mice showed accelerated aging, diminished self-renewal and differentiation potential of their stem cell population (123). Contributing to this, the p44+/+ mice with the short isoform of

p53 lacking the transactivation domain at the N-terminus displayed accelerated aging phenotypes and they also showed defective regeneration potential of neural progenitor cells (68,124).

These data suggest that downregulation of p53 activity could enhance the regenerative capacity in stem cells, while p53 deficiency could contribute positively to the proliferative capacity of hematopoietic and neuronal progenitor cells (68). p53 has pro-aging roles in stem cell populations, especially when it is deregulated like in p44+/+ and p53+/m mice.

3.7 p53 in epigenetics

The sirtuins and their role in aging have already been discussed in chapter 1.1.7 epigenetic alterations. p53 interacts with several sirtuins. Sirt1 not only deacetylates histone complexes but also p53, so it prevents interaction with some of the p53 target genes involved in proliferation, ROS production, senescence and apoptosis (41). Through acetylation p53 shows enhanced stability (125). Cells with mutations in the last seven lysine residues of p53 mimic acetylation. These cells entered the state of senescence accelerated. Further, cells with mutation preventing acetylation fail to enter replicative and oncogene induced senescence. These data suggest, that p53 deacetylation by Sirt1 inhibits senescence (41). However, further studies are necessary to analyze how this is connected to the process of aging.

Sirt6 also acts as deacetylase and regulates p53 stability and activity negatively. In mice loss of Sirt6 lead to accelerated aging and premature death. This aging phenotype could be rescued upon heterozygous deletion of p53 (126). In this study p53 has pro-aging functions.

Summing up, p53 interacts with epigenetics exemplified by the cases of Sirt1 and Sirt6. In Sirt6 deficient mice a pro-aging role of p53 could be demonstrated.

3.8 p53 in dysregulated proteostasis in Parkinson's disease and Alzheimer's disease

A connection between dysregulated p53 and neurodegenerative disorders has been shown in many studies (10). The apoptotic potential of p53 causes pathologies associated with neurodegenerative disorders, such as Parkinson's

disease and Alzheimer's disease (94). In Parkinson's disease the main pathology is the degeneration of dopaminergic neurons. DJ-1 is a mutated gene in human Parkinson's disease. A connection between DJ-1 and p53 was made in animal models (127). In experimental parkinsonism p53 inhibitors were tested and the finding was that p53 inhibitors preserve dopamine neurons from degeneration (128). In animal models it has been shown that a loss of DJ-1 expression activates p53 and leads dopaminergic neurons into degeneration (127). Here, p53 was involved in disease progression. Further, Parkin (encoded by PARK2), a Parkinson disease-associated gene, has been identified as a p53 target. p53 increased the transcription of Parkin in mice and humans. Parkin contributed to p53's role in glucose metabolism as Parkin deficiency activated glycolysis and lead to a reduction in mitochondrial respiration, the so-called Warburg effect. The Warburg effect could be reversed through restoration of Parkin expression (129). In Alzheimer's disease the amyloid cascade hypothesis says that amyloid precursor protein is converted into amyloid β, which accumulates in neurons and forms plaques. This has a toxic effect and cause cell death and dysfunction (59). It has been suggested that pathologic amyloid β peptides change p53's conformation (127). Additionally, it has been shown in Alzheimer's disease that p53 is upregulated (10), and it was implicated as a reason for neuronal cell death (127).

These results indicate that p53 plays a role in modification of these age-related neurodegenerative disorders.

3.9 p53 in nutrient sensing pathways

Reduced signaling in the mTOR and the IIS pathways is linked to longevity (12). mTOR and IIS pathways together with p53 partly explain the longevity effect of caloric restriction (67). As a response to nutrient stress p53 is able to regulate mTOR and ISS pathway negatively to restrict cell growth and division to avoid replicative errors (64). p53 interacts with these pathways through the expression of the following target genes: The insulin like growth factor binding protein (IGF-BP3) and phosphatase and tensin homolog (pTEN) to diminish IIS and it can reduce mTOR signaling via tuberous sclerosis complex 2 (TSC-2), AMPK-β an isoform of AMPK, sestrins and regulated in development and DNA damage responses 1

(REDD1). So the cell is able to inhibit growth, division, nutrient sensing and metabolic regulation under conditions of nutrient scarcity (67). IGF-BP3 binds free IGF-1. Bound IGF-1 cannot interact with the IGF-1 receptor to activate IIS signaling (130). pTEN is a tumor suppressor and phosphatase which stops cell growth in different tissues (131). It functions as a phosphatidylinositol-3,4,5-triphosphate (PIP3) phosphatase as it makes PIP3 to phosphatidylinositol-4,5-bisphosphonate (PIP2) and this leads to decreased AKT activation via diminished PDK1 and mTORC2 signaling. It also affects the mTORC1 pathway because of the connection between AKT and TSC2. AKT decreases in function and as an effect TSC1 and TSC2 activity increases and blocks mTORC1 activity (64). As mentioned, p53 can also induce TSC2 expression (64) and then together with TSC1 shut down the mTORC1 pathway (66). AMPK-β regulates AMPK formation and activity in cells (132). Increased expression of AMPK-β can stimulate AMPK activity and the TSC complex positively and this leads to inhibition of the mTOR pathway (64). The sestrins (Sestrin 1 and Sestrin 2) are target genes of p53, and they activate AMPK, that itself interacts with TSC2 and inhibits mTOR. It has been shown that mice lacking Sestrin 2 are not able to inhibit mTOR during genotoxic stress (133). REDD1 is also a p53 target gene (134), that can be induced via hypoxia and has an important role in inhibiting mTOR during hypoxic stress (135). Further, IIS signaling can activate p53's endogenous inhibitor MDM2. The activation of the insulin receptor substrate 1 (IRS1) and PI3K/AKT can activate MDM2 leading to enhanced ubiquitin ligase activity and p53 can be degraded faster (136). Additionally, MDM2 can bind IRS-1 and IGF-1 receptor to target them for degradation (10). In summary, p53 interacts in a complex way with many players of nutrient sensing pathways.

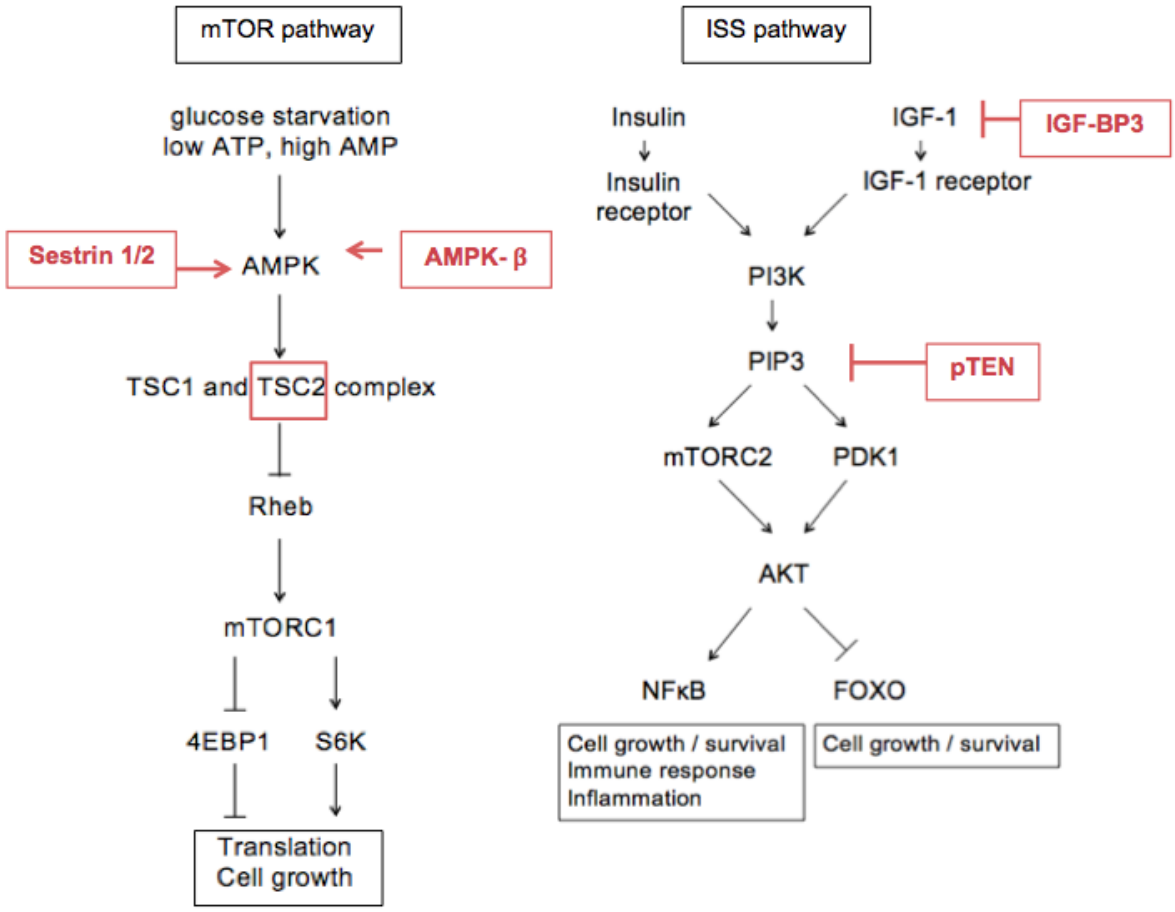

Figure 5: p53 interacts with the IIS and mTOR pathways through the expression of target genes (IGF-BP3, pTEN, TSC2, AMPK- β and Sestrin 1/2) (64,68)

p44 is a short isoform of p53 without the transactivation domain at the N-terminus. Deregulation of the IIS pathway has been seen in p44 mice, contributing to accelerated aging in these mice. In their cells p53 targets such as p21, MDM2 and IGF-BP3 were upregulated and others such as IGF-1-R were downregulated (137). In a short-lived (137) and a long-lived mouse model showing higher p53 activity, elevated levels of IGF-1 and IIS have been found (90). This hints to p53's pro-aging function in nutrient sensing pathways. On the other hand, in a short-lived hyper-p53 mouse model reduced IIS has been seen (90). Further, the p53 protein might also have the function of mTOR downregulation due to its ability to protect cells against ROS and the activation of antioxidant genes. High ROS levels are a consequence of mTOR activity (136).

Therefore, p53 is able to act anti-aging through downregulation of nutrient sensing pathways, although recent findings do not suggest p53 to be a key player in life span extension by CR (138). On the other hand, elevated p53 activity could also elevate IIS in some mouse models.

3.10 p53 in autophagy

It has been shown, that p53 can activate or inactivate autophagy (68), therefore it has been suggested that p53 in the nucleus promotes autophagy, whereas it inhibits autophagy in cytoplasm (41). It could also positively affect autophagy via inhibition of the mTOR and the IIS pathway under conditions of stress (67). Additionally, through AMPK, PTEN and sestrins, p53 acts positively on autophagy, mostly due to mTOR inhibition (41). Additionally, it has been shown that p53 can stimulate autophagy as mouse embryo fibroblasts with normal p53 produce more autophagosomes during stress than the same cells lacking p53 (64). Similarily, p53 regulates autophagy directly through the transcription of its target gene damage-regulated autophagy modulator (DRAM) encoding for a lysosomal protein. It has been found out, that DRAM overexpression can lead to accumulation of autophagosomes in cells. In contrast to that, DRAM knockdown could avoid this p53-driven autophagosome accumulation (64).

On the other hand, p53 in cytoplasm represses autophagy. The underlying mechanism is unknown. Guido Kroemer's group could show, that cytoplasmatic p53 was responsible for the inhibition of autophagy and that p53 loss resulted positively on autophagy. The autophagy inducers starvation and rapamycin degraded p53 so autophagy could be activated (41).

These studies indicate that p53 has a dual role in autophagy. However, autophagy itself is generally considered an anti-aging process, but challenged by recent findings (see above).

3.11 Arf and p53

The ARF/p53 pathway is relevant in tumor suppression protecting cells against many types of damage. It has been described in mammals that this pathway also protects an organism against the aging process (139). It has to be mentioned, that the ARF allele includes the Ink4a gene which could act pro-aging itself and the

anti-aging effect of ARF could be underestimated (96). Mice expressing 'super-Arf/super-p53' due to the induction of multiple transgenes of p53 and Arf have been generated and displayed a significant increase in median longevity due to delayed-onset aging and not due to cancer protection (140). This group created transgenic mice carrying four Arf alleles in total and these mice also displayed delayed onset aging, longevity and cancer protection. So it is still not clear if the longevity effect is dependent on p53 or Arf, because it is known that Arf has functions independent from p53 (96). However, 'super-Arf/super-p53' mice showed protection against oxidative stress and efficient cell elimination, what could explain part of its longevity effect. Also DNA damage accumulated more slowly compared to wild-type mice (96,140).

These findings also lead to p53 as an anti-aging component in the ARF/p53 pathway.

3.12 p53 polymorphism increases lifespan

p53-Pro72 is a single nucleotide polymorphism (SNP) affecting codon 72 of p53 in humans and is linked to longevity. p53-Arg72 is the most common variant. Individuals carrying the p53-Pro72 variation can show extended longevity, unless they die early because this polymorphism also has higher risk of developing cancer (96). The p53-Arg72 has higher apoptotic potential compared to p53-Pro72 (141). In contrast, cells with the longevity polymorphism p53-Pro72 are arrested into G1 phase of the cell cycle more frequently (142) and show a higher level of senescence (96). Indicating, that p53's apoptotic function may work pro-aging while cell-cycle-arrest and senescence function are anti-aging. These findings have been underlined by a mouse model carrying the p53 longevity SNP single nucleotide polymorphism with proline (P72) compared to the more common variant with arginine (R72) (75). Thus, this p53 SNP may act as anti-aging factor due to its reduced apoptotic potential. Indicating, that p53's apoptotic potential might act pro-aging and so p53 itself.

3.13 p53 levels and regulation contribute to the aging process

'super-p53' mice were created via induction of multiple transgenes of wild- type p53 and did not display signs of accelerated aging, but these mice were very

resistant to neoplasia (143). It becomes evident in the following mouse models that aberrant p53 regulation leads to an accelerated aging phenotype (10). Heterozygote mice (p53+/m) with one copy of wild-type p53 and one copy of p53 with truncation on the N-terminus have been created and showed an accelerated aging phenotype with organ atrophy and skeletal defects (144). In this region the MDM2 interaction domain is located, indicating that dysregulated p53 is connected to aging (10). These p53+/m mice showed hyperactive p53 through deletion of the first six exons. The finding was, that p53 levels did not change significantly to the cells without this alteration, but the p53 response was augmented. Another mouse model (p44 TG) with an overexpression of p53's short isoform p44 lacking the transactivation domain at the N-terminus also showed accelerated aging (137). Mouse embryonic fibroblasts from the p44 TG and the p53+/m mice showed normal p53 levels but p53 was constitutively active (10). The accelerated aging phenotype of these MDM2-insensitive mice has been explained by massive apoptotic cell loss caused by the aggravated and constitutive p53 activation. At least these mice were very resistant to neoplasia (96).

Further, mice deficient of REGγ (REG: 11S regulatory particles, 28-kDa proteasome activator) were created and showed accelerated aging phenotypes. REGγ deficient mice accumulate an enzyme degrading MDM2 and thereby elevating p53 levels. Crossing these mice into a p53+/- background could rescue the aging phenotype (138). Moreover, mice with modest elevation of p53 levels but under normal regulation did not show an accelerated aging phenotype but an increased cancer resistance (96). Mice with elevated p53 wild-type gene ('super p53') (143), with large genomic segments of the ARF gene (145) or with reduced MDM2 activity are included here (146).

In a case report, a patient with a special Progeria has been described (147). This patient had the putative diagnosis of a *Werner syndrome–like segmental progeroid disorder.* When he was 19 years old he presented with gray hair, short stature, pinched facial features, high-pitched voice, scleroderma-like skin, few pubic hair, hypogonadism, small kidneys and kidney failure resulting in severe arterial hypertension. The patient's parents were of Saudi-Arabian origin and showed consanguinity, they were not affected by progeria. Two of his sisters showed similar symptoms and died at the ages of 31 and 23. Conventional sequencing for

progeria did not offer results. Special investigations lead to a homozygous antiterminating mutation in MDM2 at the C-terminus which is essential for its E3 ubiquitin ligase activity. It has been shown that this MDM2 is not able to degrade p53. In patient's primary dermal fibroblasts higher levels of p53 protein and higher p53 induction was found. Their data indicate that this mutated MDM2 was not able to repress p53 at basal activity whereas upon stress it lead to hyperactivation of p53. Cells from the patient also showed increased protection against ionizing radiation with increased p53 stabilization, at the expense of decreased regenerative capacity. Consequently, the cells entered replicative senescence earlier than control cells.

Contributing to previous studies, this case report also pictures deregulated p53 to act pro-aging.

3.14 Apoptosis, senescence and ferroptosis drive aging

Mice lacking the ATM phosphorylation site (due to a mutation in serine 18) which is necessary for apoptosis through PUMA had a high rate of neoplasia. While the non-tumor-bearing mice showed accelerated aging and their cells were lead into premature senescence (148). Analog to the previous mice, p53-S18A/S23A mice were also resistant to apoptosis (10,149). Xrcc4 (-/-) mice, lacking the protein Xrcc4 important in DNA repair mechanisms, were embryonic lethal unless crossed into p53-S18A/S23A background. The resulting mice (Xrcc4-/-; p53S18A/S23A) showed accelerated aging. Moreover, the Xrcc4 (-/-) mice's early lethality could be rescued by p53 knockout (10,150). Thus, the apoptotic function is not necessary for the accelerated aging phenotype. In another study, the Xrcc4(-/-) mice with the defect DNA repair mechanism were crossed with mice (p53-K3R/K3R) exhibiting loss of p53-dependend apoptosis, senescence and cell cycle arrest via the loss of p53 acetylation. These mice (Xrcc4−/−; p53-K3R/K3R) showed signs of accelerated aging including testicular atrophy. In testicular cells an increase in ferroptosis could be found. So it has been speculated, that the process of ferroptosis could also contribute to the aging process (151). Ferroptosis is a relatively recent discovered form of cell death dependent on iron and resulting in lipid peroxide accumulation (152).

These results indicate, that besides initiating apoptosis, p53 also contribute to the aging process by regulating senescence, cell cycle arrest and the process of ferroptosis.

3.15 p53 and the antagonistic pleiotrophy

The antagonistic pleiotropy is a theory hypothesizing that organisms have genes that contribute positively to fitness at a young age but have a negative influence on fitness late in life (153). Early in life high p53 levels are essential for the embryonic implantation in the uterus and also for fertility (154). Further, high p53 activity during lifespan protected mice from cancer development (96). Higher levels or higher activity of p53 were the reason for these mice do die at younger ages, due to higher rates of apoptosis leading to stem cell exhaustion (155). p53 is a good example for antagonistic pleiotropy.

3.16 p53 and sexual dimorphism

After triggering p53 activation and analyzing p53 functions in C57BL/6 mice, a sexual dimorphism was recognized in p53 loss during the aging process. Female mice showed a decline in p53 activity about three months earlier than the males. Males lived about three month longer than their female litter mates. In humans, females live longer than man. Usually sexual dimorphism can be explained through hormonal signaling, factors linked to the X or Y chromosome or epigenetic imprinting, but it is still unknown which of these aspects are relevant for p53 levels and signaling. Notably, MDM2 and leukemia inhibitory factor (LIF), which is important during embryonic implantation, are regulated via p53 and estrogen (67).

4 Conclusion

The protein p53 is regulatory involved in nine out of nine aging hallmarks as well as the process of autophagy. From the literature review conducted in this thesis it can be concluded that p53 has a very important but dual and highly context-dependend role in the process of aging.

If p53 is constitutively active it can enhance the aging process and reduce lifespan but it offers tumor protection. The literature analysis of this thesis also yielded, that mild elevation of p53 under normal regulation does not accelerate the aging process whereas deregulated p53 results in accelerated aging phenotypes.

In contrast, p53 could act as anti-aging factor through its negative regulation in growth and growth-related stress or in nutrient sensing pathways. p53 is able to down-regulate the nutrient sensing pathways IIS and mTOR. Reduced signaling in these pathways is linked to longevity (10). It can repress the IIS pathway via the expression of IGF-BP3 and PTEN and the mTOR pathway via TSC-2, AMPK-beta, sestrins, and REDD1. p53's ability to inhibit the mTOR pathway on one hand and induce cell-cycle arrest on the other hand could offer an explanation for why modest elevation of p53 activity both prolongs lifespan and protects against cancer (41).

p53 has wide-ranged functions from flies to mammals including humans concerning cancer-protection, disease-modification and aging. In general, p53 seems to have a plethora of functions and interactions in cells and will continue to surprise scientist over many more years. Especially in the field aging, much more research is needed to exactly define pre-conditions, contexts, tissues and cells, and stimuli that tilt p53 between a pro-aging and an anti-aging function.

5 References

1. • Austria - average age of the population 1950-2050 | Statistic [Internet]. [cited 2019 Apr 11]. Available from: https://www.statista.com/statistics/385777/average-age-of-the-population-in-austria/
2. England K, Azzopardi-Muscat N. Demographic trends and public health in Europe. Eur J Public Health [Internet]. 2017 Oct 1 [cited 2019 Apr 11];27(suppl_4):9–13. Available from: http://academic.oup.com/eurpub/article/27/suppl_4/9/4430516/Demographic-trends-and-public-health-in-Europe
3. Median age ranges from 18 to 54 across EU regions - Product - Eurostat [Internet]. [cited 2019 Apr 11]. Available from: https://ec.europa.eu/eurostat/web/products-eurostat-news/-/DDN-20170215-1?inheritRedirect=true
4. Austria Life expectancy, 1950-2018 - knoema.com [Internet]. [cited 2019 Apr 11]. Available from: https://knoema.com/atlas/Austria/topics/Demographics/Population-forecast/Life-expectancy
5. Healthspan Is More Important Than Lifespan, So Why Don't More People Know About It? | Institute for Public Health | Washington University in St. Louis [Internet]. [cited 2019 Apr 11]. Available from: https://publichealth.wustl.edu/heatlhspan-is-more-important-than-lifespan-so-why-dont-more-people-know-about-it/
6. World's oldest mouse reaches milestone birthday [Internet]. [cited 2019 Apr 15]. Available from: http://www.ur.umich.edu/0304/Apr19_04/26.shtml
7. Masternak MM, Darcy J, Victoria B, Bartke A. Dwarf Mice and Aging. In: Progress in molecular biology and translational science [Internet]. 2018 [cited 2019 Apr 15]. p. 69–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29653683
8. Dean L. Yoda and the fountain of youth? 2014 Jul 21 [cited 2019 Apr 15]; Available from: https://www.ncbi.nlm.nih.gov/sites/books/NBK222181/
9. Carrero D, Soria-Valles C, López-Otín C. Hallmarks of progeroid syndromes:

lessons from mice and reprogrammed cells. Dis Model Mech [Internet]. 2016 [cited 2018 Dec 5];9(7):719–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27482812

10. Wu D, Prives C. Relevance of the p53-MDM2 axis to aging. Cell Death Differ [Internet]. 2018;25(1):169–79. Available from: http://dx.doi.org/10.1038/cdd.2017.187
11. Watts G. Leonard Hayflick and the limits of ageing [Internet]. Vol. 377, The Lancet. 2011 [cited 2019 Jan 11]. Available from: www.thelancet.com
12. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell [Internet]. 2013 Jun 6 [cited 2018 Dec 3];153(6):1194–217. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23746838
13. Ou H-L, Schumacher B. DNA damage responses and p53 in the aging process. Blood [Internet]. 2018;131(5):488–95. Available from: http://www.bloodjournal.org/lookup/doi/10.1182/blood-2017-07-746396
14. Linnane A, Ozawa T, Marzuki S, Tanaka M. MITOCHONDRIAL DNA MUTATIONS AS AN IMPORTANT CONTRIBUTOR TO AGEING AND DEGENERATIVE DISEASES. Lancet [Internet]. 1989 Mar 25 [cited 2018 Dec 12];333(8639):642–5. Available from: https://www.sciencedirect.com/science/article/pii/S0140673689921454
15. Kujoth GC, Bradshaw PC, Haroon S, Prolla TA. The role of mitochondrial DNA mutations in mammalian aging. PLoS Genet [Internet]. 2007 Feb 23 [cited 2018 Dec 12];3(2):e24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17319745
16. Dechat T, Pfleghaar K, Sengupta K, Shimi T, Shumaker DK, Solimando L, et al. Nuclear lamins: major factors in the structural organization and function of the nucleus and chromatin. Genes Dev [Internet]. 2008 Apr 1 [cited 2018 Dec 12];22(7):832–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18381888
17. Gonzalo S, Kreienkamp R, Askjaer P. Hutchinson-Gilford Progeria Syndrome: A premature aging disease caused by LMNA gene mutations. Ageing Res Rev [Internet]. 2017 Jan [cited 2018 Dec 5];33:18–29. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27374873

18. Scaffidi P, Gordon L, Misteli T. The Cell Nucleus and Aging: Tantalizing Clues and Hopeful Promises. PLoS Biol [Internet]. 2005 Nov 15 [cited 2018 Dec 5];3(11):e395. Available from: https://dx.plos.org/10.1371/journal.pbio.0030395
19. Sarkar PK, Shinton RA. Hutchinson-Guilford progeria syndrome Hutchinson-Guilford progeria syndrome. Postgrad Med J [Internet]. 2001 [cited 2018 Dec 5];312–7. Available from: www.postgradmedj.com
20. De Sandre-Giovannoli A, Bernard R, Cau P, Navarro C, Amiel J, Boccaccio I, et al. Lamin a truncation in Hutchinson-Gilford progeria. Science [Internet]. 2003 Jun 27 [cited 2018 Dec 11];300(5628):2055. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12702809
21. Progeroid Syndromes - an overview | ScienceDirect Topics [Internet]. [cited 2018 Dec 11]. Available from: https://www.sciencedirect.com/topics/medicine-and-dentistry/progeroid-syndromes
22. Bernstein KA, Gangloff S, Rothstein R. The RecQ DNA helicases in DNA repair. Annu Rev Genet [Internet]. 2010 [cited 2018 Dec 11];44:393–417. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21047263
23. Maierhofer A, Flunkert J, Oshima J, Martin GM, Haaf T, Horvath S. Accelerated epigenetic aging in Werner syndrome. Aging (Albany NY) [Internet]. 2017 [cited 2018 Dec 11];9(4):1143–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28377537
24. Oshima J, Sidorova JM, Monnat RJ, Jr. Werner syndrome: Clinical features, pathogenesis and potential therapeutic interventions. Ageing Res Rev [Internet]. 2017 Jan [cited 2018 Dec 11];33:105–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26993153
25. Huang S, Lee L, Hanson NB, Lenaerts C, Hoehn H, Poot M, et al. The spectrum of WRN mutations in Werner syndrome patients. Hum Mutat [Internet]. 2006 Jun [cited 2018 Dec 11];27(6):558–67. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16673358
26. Chan SRWL, Blackburn EH. Telomeres and telomerase. [cited 2018 Dec 18]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1693310/pdf/15065663.pdf

27. Wong JM, Collins K. Telomere maintenance and disease. Lancet [Internet]. 2003 Sep 20 [cited 2019 Jan 11];362(9388):983–8. Available from: https://www.sciencedirect.com/science/article/pii/S0140673603143693#cesec10
28. Horn Fl. Biochemie des Menschen: Das Lehrbuch für das Medizinstudium. 5th ed. Thieme. Stuttgard; 2009. 255-267; 298-299; p.
29. Schmidt JC, Cech TR. Human telomerase: biogenesis, trafficking, recruitment, and activation. Genes Dev [Internet]. 2015 Jun 1 [cited 2018 Dec 18];29(11):1095–105. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26063571
30. Höhn H, Holinski-Feder E, Meitinger T, Poeggel G, Zerres K. Grundlagen der Genetik. In: Murken JD, Grimm T, Holinski-Feder E, Zerres K, editors. 9. teilakt. Georg Thieme Verlag; 2017. Available from: https://eref.thieme.de/ebooks/1879364#/ebook_1879364_SL73445214 BT - Taschenlehrbuch Humangenetik
31. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, et al. Extension of life-span by introduction of telomerase into normal human cells. Science [Internet]. 1998 Jan 16 [cited 2019 Jan 11];279(5349):349–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9454332
32. Boengler K, Kosiol M, Mayr M, Schulz R, Rohrbach S. Mitochondria and ageing: role in heart, skeletal muscle and adipose tissue. J Cachexia Sarcopenia Muscle [Internet]. 2017 Jun [cited 2018 Dec 12];8(3):349–69. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28432755
33. Doonan R, McElwee JJ, Matthijssens F, Walker GA, Houthoofd K, Back P, et al. Against the oxidative damage theory of aging: superoxide dismutases protect against oxidative stress but have little or no effect on life span in Caenorhabditis elegans. Genes Dev [Internet]. 2008 Dec 1 [cited 2018 Dec 12];22(23):3236–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19056880
34. Mesquita A, Weinberger M, Silva A, Sampaio-Marques B, Almeida B, Leão C, et al. Caloric restriction or catalase inactivation extends yeast chronological lifespan by inducing H2O2 and superoxide dismutase activity. Proc Natl Acad Sci U S A [Internet]. 2010 Aug 24 [cited 2018 Dec

12];107(34):15123–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20696905

35. Van Raamsdonk JM, Hekimi S. Deletion of the mitochondrial superoxide dismutase sod-2 extends lifespan in Caenorhabditis elegans. PLoS Genet [Internet]. 2009 Feb [cited 2018 Dec 12];5(2):e1000361. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19197346
36. Zhang Y, Ikeno Y, Qi W, Chaudhuri A, Li Y, Bokov A, et al. Mice deficient in both Mn superoxide dismutase and glutathione peroxidase-1 have increased oxidative damage and a greater incidence of pathology but no reduction in longevity. J Gerontol A Biol Sci Med Sci [Internet]. 2009 Dec [cited 2018 Dec 12];64(12):1212–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19776219
37. Sena LA, Chandel NS. Physiological roles of mitochondrial reactive oxygen species. Molecular Cell. 2012.
38. Ristow M, Schmeisser K. Mitohormesis: Promoting Health and Lifespan by Increased Levels of Reactive Oxygen Species (ROS). Dose Response [Internet]. 2014 May [cited 2019 Jan 5];12(2):288–341. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24910588
39. Sahin E, DePinho RA. Axis of ageing: telomeres, p53 and mitochondria. Nat Rev Mol Cell Biol [Internet]. 2012 Jun 16 [cited 2018 Dec 17];13(6):397–404. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22588366
40. Bernardes de Jesus B, Vera E, Schneeberger K, Tejera AM, Ayuso E, Bosch F, et al. Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer. EMBO Mol Med [Internet]. 2012 Aug [cited 2018 Dec 17];4(8):691–704. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22585399
41. Rufini A, Tucci P, Celardo I, Melino G. Senescence and aging: The critical roles of p53. Oncogene [Internet]. 2013;32(43):5129–43. Available from: http://dx.doi.org/10.1038/onc.2012.640
42. Shao D, Liu Y, Liu X, Zhu L, Cui Y, Cui A, et al. PGC-1β-Regulated mitochondrial biogenesis and function in myotubes is mediated by NRF-1 and ERRα. Mitochondrion [Internet]. 2010 Aug [cited 2019 Jan 22];10(5):516–27. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/20561910

43. Cheng A, Wan R, Yang J-L, Kamimura N, Son TG, Ouyang X, et al. Involvement of PGC-1α in the formation and maintenance of neuronal dendritic spines. Nat Commun [Internet]. 2012 Jan 4 [cited 2019 Jan 22];3(1):1250. Available from: http://www.nature.com/articles/ncomms2238
44. Wang K, Klionsky DJ. Mitochondria removal by autophagy. Autophagy [Internet]. 2011 Mar [cited 2018 Dec 18];7(3):297–300. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21252623
45. Campisi J, d'Adda di Fagagna F. Cellular senescence: when bad things happen to good cells. Nat Rev Mol Cell Biol [Internet]. 2007 Sep 1 [cited 2019 Jan 7];8(9):729–40. Available from: http://www.nature.com/articles/nrm2233
46. Kuilman T, Michaloglou C, Mooi WJ, Peeper DS. The essence of senescence. Genes Dev [Internet]. 2010 Nov 15 [cited 2019 Apr 29];24(22):2463–79. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21078816
47. Tominaga K. The emerging role of senescent cells in tissue homeostasis and pathophysiology. Pathobiol Aging Age-related Dis [Internet]. 2015 Jan 19 [cited 2019 Jan 7];5(1):27743. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25994420
48. He S, Sharpless NE. Senescence in Health and Disease. Cell [Internet]. 2017 Jun 1 [cited 2019 Jan 7];169(6):1000–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28575665
49. Regulski MJ. Cellular Senescence: What, Why, and How. Wounds a Compend Clin Res Pract [Internet]. 2017 Jun [cited 2019 Jan 7];29(6):168–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28682291
50. Franceschi C, Garagnani P, Vitale G, Capri M, Salvioli S. Inflammaging and 'Garb-aging.' Trends Endocrinol Metab [Internet]. 2017 Mar 1 [cited 2019 Jan 14];28(3):199–212. Available from: https://www.sciencedirect.com/science/article/pii/S1043276016301254?via%3Dihub
51. Watanabe S, Kawamoto S, Ohtani N, Hara E. Impact of senescence-associated secretory phenotype and its potential as a therapeutic target for

senescence-associated diseases. Cancer Sci [Internet]. 2017 Apr [cited 2019 Jan 14];108(4):563–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28165648

52. Keyes BE, Fuchs E. Stem cells: Aging and transcriptional fingerprints. J Cell Biol [Internet]. 2018 Jan 2 [cited 2019 Jan 8];217(1):79–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29070608
53. Sharpless NE, DePinho RA. How stem cells age and why this makes us grow old. Nat Rev Mol Cell Biol. 2007;8(9):703–13.
54. Nishimura EK, Granter SR, Fisher DE. Mechanisms of hair graying: incomplete melanocyte stem cell maintenance in the niche. Science [Internet]. 2005 Feb 4 [cited 2019 Jan 8];307(5710):720–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15618488
55. Gregoretti I, Lee Y-M, Goodson H V. Molecular Evolution of the Histone Deacetylase Family: Functional Implications of Phylogenetic Analysis. J Mol Biol [Internet]. 2004 Apr 16 [cited 2019 Jan 10];338(1):17–31. Available from: https://www.sciencedirect.com/science/article/pii/S0022283604001408?via%3Dihub
56. Heterochromatin - Verpackungskünstler am Werk | Max-Planck-Gesellschaft [Internet]. [cited 2019 Jan 10]. Available from: https://www.mpg.de/6705389/MPIIB_JB_2013
57. Sidler C, Kovalchuk O, Kovalchuk I. Epigenetic Regulation of Cellular Senescence and Aging. Front Genet [Internet]. 2017 [cited 2019 Jan 10];8:138. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29018479
58. Klaips CL, Jayaraj GG, Hartl FU. Pathways of cellular proteostasis in aging and disease. J Cell Biol [Internet]. 2018 Jan 2 [cited 2019 Jan 11];217(1):51–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29127110
59. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer's disease. Lancet [Internet]. 2011 Mar 19 [cited 2019 Jan 11];377(9770):1019–31. Available from: https://www.sciencedirect.com/science/article/pii/S0140673610613499?via%3Dihub#cesec20

60. Bradbury J. Chaperones: keeping a close eye on protein folding. Lancet (London, England) [Internet]. 2003 Apr 5 [cited 2019 Jan 12];361(9364):1194–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12686051
61. Creagh EM, Sheehan D, Cotter TG. Heat shock proteins - Modulators of apoptosis in tumour cells. Leukemia. 2000;14(7):1161–73.
62. Calderwood SK, Murshid A, Prince T. The shock of aging: Molecular chaperones and the heat shock response in longevity and aging - A mini-review. Gerontology. 2009.
63. Horn F. Der Proteinabbau. In: Horn F, editor. 7., korrig. Georg Thieme Verlag; 2018. Available from: https://eref-1thieme-1de-1lkwv1kii09ed.han.medunigraz.at/ebooks/2373922#/ebook_2373922_SL89065481 BT - Biochemie des Menschen
64. Feng Z. p53 regulation of the IGF-1/AKT/mTOR pathways and the endosomal compartment. Cold Spring Harb Perspect Biol [Internet]. 2010 Feb [cited 2019 Jan 30];2(2):a001057. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20182617
65. Ghosh K, Capell BC. The Senescence-Associated Secretory Phenotype: Critical Effector in Skin Cancer and Aging. J Invest Dermatol [Internet]. 2016 Nov 1 [cited 2019 Jan 14];136(11):2133–9. Available from: https://www.sciencedirect.com/science/article/pii/S0022202X1632098X?via%3Dihub
66. Henske EP, Jóźwiak S, Kingswood JC, Sampson JR, Thiele EA. Tuberous sclerosis complex. Nat Rev Dis Prim [Internet]. 2016 May 26 [cited 2019 Jan 25];2:16035. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27226234
67. Feng Z, Hu W, Rajagopal G, Levine AJ. The tumor suppressor p53: Cancer and aging. Cell Cycle. 2008;7(7):842–7.
68. Feng Z, Lin M, Wu R. The Regulation of Aging and Longevity: A New and Complex Role of p53. Genes Cancer [Internet]. 2011;2(4):443–52. Available from: http://gan.sagepub.com/lookup/doi/10.1177/1947601911410223
69. Karin M, Ben-Neriah Y. Phosphorylation Meets Ubiquitination: The Control of NF-κB Activity. Annu Rev Immunol [Internet]. 2000 Apr 28 [cited 2019 Feb 1];18(1):621–63. Available from:

http://www.annualreviews.org/doi/10.1146/annurev.immunol.18.1.621

70. Rubinsztein DC, Mariño G, Kroemer G. Autophagy and Aging. Cell [Internet]. 2011 Sep 2 [cited 2019 Jan 12];146(5):682–95. Available from: https://www.sciencedirect.com/science/article/pii/S0092867411008282?via%3Dihub

71. Mizushima N, Ohsumi Y, Yoshimori T. Autophagosome Formation in Mammalian Cells [Internet]. Vol. 27, CELL STRUCTURE AND FUNCTION. 2002 [cited 2019 Jan 18]. Available from: https://www.jstage.jst.go.jp/article/csf/27/6/27_6_421/_pdf/-char/en

72. Savini M, Wang MC. Does Autophagy Promote Longevity? It Depends. Cell [Internet]. 2019 Apr [cited 2019 Jul 16];177(2):221–2. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0092867419302831

73. Brooks-Wilson AR. Genetics of healthy aging and longevity. Hum Genet [Internet]. 2013 Dec 8 [cited 2019 Apr 18];132(12):1323–38. Available from: http://link.springer.com/10.1007/s00439-013-1342-z

74. Flachsbart F, Caliebe A, Kleindorp R, Blanché H, von Eller-Eberstein H, Nikolaus S, et al. Association of FOXO3A variation with human longevity confirmed in German centenarians. Proc Natl Acad Sci [Internet]. 2009 Feb 24 [cited 2019 Jan 21];106(8):2700–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19196970

75. Zhao Y, Wu L, Yue X, Zhang C, Wang J, Li J, et al. A polymorphism in the tumor suppressor p53 affects aging and longevity in mouse models. Elife. 2018;7:1–18.

76. Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM, et al. Caloric restriction delays disease onset and mortality in rhesus monkeys. Science [Internet]. 2009 Jul 10 [cited 2019 Jan 14];325(5937):201–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19590001

77. López-Lluch G, Navas P. Calorie restriction as an intervention in ageing. J Physiol [Internet]. 2016 Apr 15 [cited 2019 Jan 14];594(8):2043–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26607973

78. Carmona JJ, Michan S. Biology of Healthy Aging and Longevity. Rev Invest Clin [Internet]. [cited 2018 Nov 28];68(1):7–16. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/27028172

79. Deutzmann R. Zelluläre Wirkungen. In: Behrends J, Bischofberger J, Deutzmann R, Ehmke H, Frings S, Grissmer S, et al., editors. 3., vollst. Georg Thieme Verlag; 2016. Available from: https://eref.thieme.de/ebooks/1502150#/ebook_1502150_SL62138788 BT - Duale Reihe Physiologie
80. Ravussin E, Redman LM, Rochon J, Das SK, Fontana L, Kraus WE, et al. A 2-Year Randomized Controlled Trial of Human Caloric Restriction: Feasibility and Effects on Predictors of Health Span and Longevity. J Gerontol A Biol Sci Med Sci [Internet]. 2015 Sep [cited 2019 Jan 16];70(9):1097–104. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26187233
81. Kenyon CJ. The genetics of ageing. Nature [Internet]. 2010 Mar [cited 2019 Jan 21];464(7288):504–12. Available from: http://www.nature.com/articles/nature08980
82. Lanza IR, Short DK, Short KR, Raghavakaimal S, Basu R, Joyner MJ, et al. Endurance exercise as a countermeasure for aging. Diabetes [Internet]. 2008 Nov [cited 2019 Jan 17];57(11):2933–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18716044
83. Roth GS, Ingram DK. Manipulation of health span and function by dietary caloric restriction mimetics. Ann N Y Acad Sci [Internet]. 2016 Jan [cited 2019 Apr 18];1363(1):5–10. Available from: http://doi.wiley.com/10.1111/nyas.12834
84. Pearson KJ, Baur JA, Lewis KN, Peshkin L, Price NL, Labinskyy N, et al. Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. Cell Metab [Internet]. 2008 Aug [cited 2019 Apr 18];8(2):157–68. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18599363
85. Handschin C. Caloric restriction and exercise “‘mimetics’”: Ready for prime time? Pharmacol Res [Internet]. 2016 Jan [cited 2019 Apr 18];103:158–66. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26658171
86. Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous

mice. Nature [Internet]. 2009 Jul 8 [cited 2019 Jan 16];460(7253):392–5. Available from: http://www.nature.com/articles/nature08221

87. Lamming DW. Inhibition of the Mechanistic Target of Rapamycin (mTOR)-Rapamycin and Beyond. Cold Spring Harb Perspect Med [Internet]. 2016 [cited 2019 Jan 12];6(5). Available from: http://www.ncbi.nlm.nih.gov/pubmed/27048303
88. Wyss-Coray T. Ageing, neurodegeneration and brain rejuvenation. Nature [Internet]. 2016 Nov 9 [cited 2019 Apr 4];539(7628):180–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27830812
89. Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature [Internet]. 2005 Feb [cited 2019 Apr 4];433(7027):760–4. Available from: http://www.nature.com/articles/nature03260
90. de Keizer PLJ, Laberge RM, Campisi J. P53: Pro-aging or pro-longevity? Aging (Albany NY). 2010;2(7):377–9.
91. Kastenhuber ER, Lowe SW. Putting p53 in Context. Cell [Internet]. 2017 [cited 2019 Jan 21];170:1062–78. Available from: http://dx.doi.org/10.1016/j.cell.2017.08.028
92. Donehower LA. Using mice to examine p53 functions in cancer, aging, and longevity. Cold Spring Harb Perspect Biol. 2009;1(6):1–18.
93. Hasty P, Christy BA. P53 As an Intervention Target for Cancer and Aging. Pathobiol Aging Age-related Dis [Internet]. 2013;3(1):22702. Available from: https://www.tandfonline.com/doi/full/10.3402/pba.v3i0.22702
94. Brady CA, Attardi LD. p53 at a glance. J Cell Sci. 2010;123(15):2527–32.
95. Joerger AC, Fersht AR. The tumor suppressor p53: from structures to drug discovery. Cold Spring Harb Perspect Biol. 2010;2(6):a000919.
96. Matheu A, Maraver A, Serrano M. The Arf/p53 pathway in cancer and aging. Cancer Res. 2008;68(15):6031–4.
97. Schwab M, editor. ARF. In: Encyclopedia of Cancer [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 2011. p. 265. Available from: https://doi.org/10.1007/978-3-642-16483-5_383
98. Faesen AC, Sixma TK, Everett RD. Ubiquitin-Specific Protease USP7 [Internet]. Handbook of Proteolytic Enzymes. Academic Press; 2013 [cited

2018 Nov 27]. 2057-2062 p. Available from: https://www.sciencedirect.com/science/article/pii/B9780123822192004634

99. Li M, Chen D, Shiloh A, Luo J, Nikolaev AY, Qin J, et al. Deubiquitination of p53 by HAUSP is an important pathway for p53 stabilization. Nature [Internet]. 2002 Apr [cited 2018 Nov 27];416(6881):648–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11923872
100. Bolden JE, Lowe SW. Cellular Senescence. Mol Basis Cancer [Internet]. 2015 Jan 1 [cited 2019 Jan 22];229–238.e2. Available from: https://www.sciencedirect.com/science/article/pii/B9781455740666000159
101. Brugarolas J, Chandrasekaran C, Gordon JI, Beach D, Jacks T, Hannon GJ. Radiation-induced cell cycle arrest compromised by p21 deficiency. Nat 1995 3776549 [Internet]. 1995 [cited 2019 Apr 29];377(6549):552. Available from: https://www.nature.com/articles/377552a0
102. Carvajal LA, Hamard P-J, Tonnessen C, Manfredi JJ. E2F7, a novel target, is up-regulated by p53 and mediates DNA damage-dependent transcriptional repression. Genes Dev [Internet]. 2012 Jul 15 [cited 2019 Apr 29];26(14):1533–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22802528
103. Olivier M, Hollstein M, Hainaut P. TP53 mutations in human cancers: origins, consequences, and clinical use. Cold Spring Harb Perspect Biol. 2010;2(1):a001008.
104. Malkin D. Li-fraumeni syndrome. Genes Cancer. 2011;2(4):475–84.
105. Kenzelmann Broz D, Attardi LD. In vivo analysis of p53 tumor suppressor function using genetically engineered mouse models. Carcinogenesis. 2010;31(8):1311–8.
106. Venkatachalam S, Tyner S, Pickering C, Boley S, Recio L, French J, et al. Is p53 Haploinsufficient for Tumor Suppression? Implications for the p53 +/- Mouse Model in Carcinogenicity Testing. Toxicol Pathol [Internet]. 2001 Sep 1 [cited 2019 May 1];29(5):147–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11695551
107. Lin HN, Gu XY, Zhang SW, Zeng HM, Wei WW, Zheng RS. [Analysis on incidence and mean age at diagnosis for Global Cancer]. Zhonghua Zhong Liu Za Zhi. 2018 Jul;40(7):543–9.

108. Feng Z, Hu W, Teresky AK, Hernando E, Cordon-Cardo C, Levine AJ. Declining p53 function in the aging process: A possible mechanism for the increased tumor incidence in older populations. Proc Natl Acad Sci [Internet]. 2007 Oct 16 [cited 2019 Apr 25];104(42):16633–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17921246
109. Pinkston JM, Garigan D, Hansen M, Kenyon C. Mutations that increase the life span of C. elegans inhibit tumor growth. Science [Internet]. 2006 Aug 18 [cited 2019 Apr 25];313(5789):971–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16917064
110. Varela I, Cadiñanos J, Pendás AM, Gutiérrez-Fernández A, Folgueras AR, Sánchez LM, et al. Accelerated ageing in mice deficient in Zmpste24 protease is linked to p53 signalling activation. Nature [Internet]. 2005 Sep 3 [cited 2019 Apr 19];437(7058):564–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16079796
111. Cao L, Li W, Kim S, Brodie SG, Deng C-X. Senescence, aging, and malignant transformation mediated by p53 in mice lacking the Brca1 full-length isoform. Genes Dev [Internet]. 2003 Jan 15 [cited 2019 Apr 19];17(2):201–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12533509
112. Lim DS, Vogel H, Willerford DM, Sands AT, Platt KA, Hasty P. Analysis of ku80-mutant mice and cells with deficient levels of p53. Mol Cell Biol [Internet]. 2000 Jun [cited 2019 Apr 19];20(11):3772–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10805721
113. Pan D, Zhu Q, Conboy MJ, Conboy IM, Luo K. SnoN activates p53 directly to regulate aging and tumorigenesis. Aging Cell [Internet]. 2012 Oct [cited 2019 Apr 19];11(5):902–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22805162
114. Murga M, Bunting S, Montaña MF, Soria R, Mulero F, Cañamero M, et al. A mouse model of ATR-Seckel shows embryonic replicative stress and accelerated aging. Nat Genet [Internet]. 2009 Aug 20 [cited 2019 Apr 19];41(8):891–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19620979
115. O'Driscoll M, Gennery AR, Seidel J, Concannon P, Jeggo PA. An overview

of three new disorders associated with genetic instability: LIG4 syndrome, RS-SCID and ATR-Seckel syndrome. DNA Repair (Amst) [Internet]. 2004 Aug 1 [cited 2019 Apr 19];3(8–9):1227–35. Available from: https://www.sciencedirect.com/science/article/pii/S1568786404000849?via%3Dihub

116. Begus-Nahrmann Y, Lechel A, Obenauf AC, Nalapareddy K, Peit E, Hoffmann E, et al. p53 deletion impairs clearance of chromosomal-instable stem cells in aging telomere-dysfunctional mice. Nat Genet [Internet]. 2009 Oct 30 [cited 2019 Apr 19];41(10):1138–43. Available from: http://www.nature.com/articles/ng.426
117. Rudolph KL, Chang S, Lee HW, Blasco M, Gottlieb GJ, Greider C, et al. Longevity, stress response, and cancer in aging telomerase-deficient mice. Cell [Internet]. 1999 Mar 5 [cited 2019 Apr 19];96(5):701–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10089885
118. Chin L, Artandi SE, Shen Q, Tam A, Lee SL, Gottlieb GJ, et al. p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. Cell [Internet]. 1999 May 14 [cited 2019 Apr 30];97(4):527–38. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10338216
119. Minamino T, Orimo M, Shimizu I, Kunieda T, Yokoyama M, Ito T, et al. A crucial role for adipose tissue p53 in the regulation of insulin resistance. Nat Med [Internet]. 2009 Sep 30 [cited 2018 Nov 14];15(9):1082–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19718037
120. Cooks T, Harris CC, Oren M. Caught in the cross fire: p53 in inflammation. Carcinogenesis [Internet]. 2014 Aug [cited 2019 Jan 25];35(8):1680–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24942866
121. Muñoz-Fontela C, Mandinova A, Aaronson SA, Lee SW. Emerging roles of p53 and other tumour-suppressor genes in immune regulation. Nat Rev Immunol [Internet]. 2016 [cited 2019 Jan 25];16(12):741–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27667712
122. Krizhanovsky V, Lowe SW. NEWS & VIEWS The promises and perils of p53. Nature. 2009;1085–6.
123. Dumble M, Moore L, Chambers SM, Geiger H, Van Zant G, Goodell MA, et

al. The impact of altered p53 dosage on hematopoietic stem cell dynamics during aging. Blood [Internet]. 2007 Feb 15 [cited 2019 Apr 24];109(4):1736–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17032926

124. Medrano S, Burns-Cusato M, Atienza MB, Rahimi D, Scrable H. Regenerative capacity of neural precursors in the adult mammalian brain is under the control of p53. Neurobiol Aging [Internet]. 2009 Mar [cited 2019 May 1];30(3):483–97. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17850928
125. Brooks CL, Gu W. The impact of acetylation and deacetylation on the p53 pathway. Protein Cell [Internet]. 2011 Jun 12 [cited 2019 Apr 30];2(6):456–62. Available from: http://link.springer.com/10.1007/s13238-011-1063-9
126. Ghosh S, Wong SK, Jiang Z, Liu B, Wang Y, Hao Q, et al. Haploinsufficiency ofTrp53dramatically extends the lifespan of Sirt6-deficient mice. Elife [Internet]. 2018;7:1–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29474172%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5825207
127. Vousden KH, Prives C. Blinded by the Light: The Growing Complexity of p53. Cell. 2009;137(3):413–31.
128. Duan W, Zhu X, Ladenheim B, Yu Q-S, Guo Z, Oyler J, et al. p53 inhibitors preserve dopamine neurons and motor function in experimental parkinsonism. Ann Neurol [Internet]. 2002 Nov [cited 2018 Nov 14];52(5):597–606. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12402257
129. Zhang C, Lin M, Wu R, Wang X, Yang B, Levine AJ, et al. Parkin, a p53 target gene, mediates the role of p53 in glucose metabolism and the Warburg effect. Proc Natl Acad Sci U S A [Internet]. 2011 Sep 27 [cited 2019 Jul 23];108(39):16259–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21930938
130. Bentov I, Werner H. Insulin-like Growth Factor-1. Handb Biol Act Pept [Internet]. 2013 Jan 1 [cited 2019 Jan 25];1627–32. Available from: https://www.sciencedirect.com/science/article/pii/B9780123850959002220?via%3Dihub
131. Leslie NR, Kriplani N, Hermida MA, Alvarez-Garcia V, Wise HM. The PTEN

protein: cellular localization and post-translational regulation. Biochem Soc Trans [Internet]. 2016 Feb 15 [cited 2019 Jan 25];44(1):273–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26862215

132. Houde VP, Donzelli S, Sacconi A, Galic S, Hammill JA, Bramson JL, et al. AMPK β1 reduces tumor progression and improves survival in p53 null mice. Mol Oncol [Internet]. 2017 [cited 2019 Jan 30];11(9):1143–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28544264
133. Budanov A V, Karin M. p53 target genes sestrin1 and sestrin2 connect genotoxic stress and mTOR signaling. Cell [Internet]. 2008 Aug 8 [cited 2019 Jan 30];134(3):451–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18692468
134. Ellisen LW, Ramsayer KD, Johannessen CM, Yang A, Beppu H, Minda K, et al. REDD1, a Developmentally Regulated Transcriptional Target of p63 and p53, Links p63 to Regulation of Reactive Oxygen Species. Mol Cell [Internet]. 2002 Nov 1 [cited 2019 Jan 30];10(5):995–1005. Available from: https://www.sciencedirect.com/science/article/pii/S1097276502007062?via%3Dihub
135. Katiyar S, Liu E, Knutzen CA, Lang ES, Lombardo CR, Sankar S, et al. REDD1, an inhibitor of mTOR signalling, is regulated by the CUL4A-DDB1 ubiquitin ligase. EMBO Rep [Internet]. 2009 Aug [cited 2019 Jan 30];10(8):866–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19557001
136. Poyurovsky M V., Prives C. P53 and aging: A fresh look at an old paradigm. Aging (Albany NY). 2010;2(7):380–2.
137. Maier B, Gluba W, Bernier B, Turner T, Mohammad K, Guise T, et al. Modulation of mammalian life span by the short isoform of p53. Genes Dev [Internet]. 2004 Feb 1 [cited 2019 Apr 23];18(3):306–19. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14871929
138. Hasty P, Campisi J, Sharp ZD. Do p53 stress responses impact organismal aging? Transl Cancer Res [Internet]. 2016 Dec [cited 2019 Apr 29];5(6):685–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30984573
139. Matheu A, Maraver A, Klatt P, Flores I, Garcia-Cao I, Borras C, et al. Delayed ageing through damage protection by the Arf/p53 pathway. Nature.

2007;448(7151):375–9.

140. Matheu A, Maraver A, Klatt P, Flores I, Garcia-Cao I, Borras C, et al. Delayed ageing through damage protection by the Arf/p53 pathway. Nature [Internet]. 2007 Jul 19 [cited 2019 Apr 22];448(7151):375–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17637672
141. Dumont P, Leu JI-J, Della Pietra AC, George DL, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat Genet [Internet]. 2003 Mar 3 [cited 2019 Apr 25];33(3):357–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12567188
142. Pim D, Banks L. p53 polymorphic variants at codon 72 exert different effects on cell cycle progression. Int J Cancer [Internet]. 2004 Jan 10 [cited 2019 Apr 25];108(2):196–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14639602
143. García-Cao I, García-Cao M, Martín-Caballero J, Criado LM, Klatt P, Flores JM, et al. 'Super p53' mice exhibit enhanced DNA damage response, are tumor resistant and age normally. EMBO J [Internet]. 2002 Nov 15 [cited 2019 Apr 22];21(22):6225–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12426394
144. Tyner SD, Venkatachalam S, Choi J, Jones S, Ghebranious N, Igelmann H, et al. P53 mutant mice that display early ageing-associated phenotypes. Nature. 2002;415(6867):45–53.
145. Matheu A, Pantoja C, Efeyan A, Criado LM, Martín-Caballero J, Flores JM, et al. Increased gene dosage of Ink4a/Arf results in cancer resistance and normal aging. Genes Dev [Internet]. 2004 Nov 15 [cited 2019 Apr 24];18(22):2736–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15520276
146. Mendrysa SM, O'Leary KA, McElwee MK, Michalowski J, Eisenman RN, Powell DA, et al. Tumor suppression and normal aging in mice with constitutively high p53 activity. Genes Dev [Internet]. 2006 Jan 1 [cited 2019 Apr 22];20(1):16–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16391230
147. Lessel D, Wu D, Trujillo C, Ramezani T, Lessel I, Alwasiyah MK, et al. Dysfunction of the MDM2/p53 axis is linked to premature aging. J Clin

Invest. 2017;127(10):3598–608.

148. Armata HL, Garlick DS, Sluss HK. The Ataxia Telangiectasia Mutated Target Site Ser18 Is Required for p53-Mediated Tumor Suppression. Cancer Res [Internet]. 2007 Dec 15 [cited 2019 Apr 23];67(24):11696–703. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18089799
149. Chao C, Herr D, Chun J, Xu Y. Ser18 and 23 phosphorylation is required for p53-dependent apoptosis and tumor suppression. EMBO J [Internet]. 2006 [cited 2019 Apr 23];25(11):2615. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1478190/
150. Gao Y, Ferguson DO, Xie W, Manis JP, Sekiguchi J, Frank KM, et al. Interplay of p53 and DNA-repair protein XRCC4 in tumorigenesis, genomic stability and development. Nature [Internet]. 2000 Apr [cited 2019 Apr 23];404(6780):897–900. Available from: http://www.nature.com/articles/35009138
151. Li T, Liu X, Jiang L, Manfredi J, Zha S, Gu W. Loss of p53-mediated cell-cycle arrest, senescence and apoptosis promotes genomic instability and premature aging. Oncotarget [Internet]. 2016 Mar 15 [cited 2019 Apr 23];7(11):11838–49. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26943586
152. Yu H, Guo P, Xie X, Wang Y, Chen G. Ferroptosis, a new form of cell death, and its relationships with tumourous diseases. J Cell Mol Med [Internet]. 2017 Apr [cited 2019 Apr 23];21(4):648–57. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27860262
153. Austad SN, Hoffman JM. Is antagonistic pleiotropy ubiquitous in aging biology? Evol Med Public Heal [Internet]. 2018 Jan 1 [cited 2019 Apr 25];2018(1):287–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30524730
154. Hu W, Feng Z, Teresky AK, Levine AJ. p53 regulates maternal reproduction through LIF. Nature. 2007;450(7170):721–4.
155. Dumble M, Moore L, Chambers SM, Geiger H, Van Zant G, Goodell MA, et al. The impact of altered p53 dosage on hematopoietic stem cell dynamics during aging. Blood [Internet]. 2007 Feb 15 [cited 2019 Apr 25];109(4):1736–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17032926