[Frontiers in Bioscience 13, 6966-6990, May 1, 2008]

# DNA repair, insulin signaling and sirtuins: at the crossroads between cancer and aging

**Raul Mostoslavsky**[1]

[1]*Department of Medicine, Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA*

**TABLE OF CONTENTS**



## 1. ABSTRACT

For many years organismal aging and cancer were viewed as separate entities. Recent studies however have suggested that these two seemingly disparate biological processes may in fact share common biochemical pathways. One area of emerging convergence involves the intersection of pathways known to mediate DNA repair with pathways previously implicated in insulin signaling. Recent evidence suggests that the sirtuin family of proteins act as central mediators of this molecular crosstalk. The coordination of DNA repair with overall energy balance may be essential for reducing the risk of developing cancer as well as for determining the rate at which we age. This review will summarize our current knowledge on how the maintenance of genomic integrity and insulin signaling intersect, the potential regulation of sirtuins in this crosstalk, and how this coordinated regulation may have important implication for both tumor-free and overall survival.

## 2. INTRODUCTION

In recent years, definitive experimental evidence from a variety of organisms has taught us that the natural process of aging, or increased mortality with time, has its roots in specific molecular pathways. In particular, DNA damage repair and the insulin/Igf1-like signaling (IIS) pathway represent two evolutionary conserved pathways influencing lifespan across species. Interestingly, it is becoming clearer that those same pathways seem to play critical roles in the development of cancer. Indeed, aging and cancer seem to be two divergent outcomes following defects in conserved pathways, suggesting an underlying common biology (reviewed in (1)). Whether organisms end up with impaired homeostasis or increased susceptibility to cancer seems to depend on the severity of the malfunction and the integrity of the downstream checkpoints, as discussed in detail below.

A recent development in defining the relationship between aging and cancer is the discovery of the sirtuins, homologs of the yeast Sir2 protein. These proteins regulate lifespan in many species and have been implicated in cancer pathogenesis (2, 3). Functionally, sirtuins—which encode NAD-dependent deacetylases and ADP-ribosyl transferases— appear to regulate either DNA repair or metabolism through the IIS pathway, while another—Sirt6— appears to integrate both of these processes (2, 4). Therefore, sirtuins represent a group of proteins at the crossroad between DNA repair and IIS, and thus might influence lifespan and/or cancer development through regulation of those two pathways. This review will summarize the DNA repair and IIS pathways, and how they relate to aging and cancer. It will focus then on the sirtuin proteins, with a particular emphasis on the role of SIRT6 in these pathways.

### 2.1. DNA REPAIR MECHANISMS

The eukaryotic cell is faced with more than $1x10^5$ DNA lesions/day that need to be repaired, in order to avoid chromosomal instability, mutations and ultimately, cell death (5). Therefore, cells have developed five major repair pathways, each one responsible for repairing different types of lesion. Namely, single-strand lesions (SSBs) are repaired through the so-called Excision repair pathways: base excision repair, nucleotide excision repair and mismatch repair, while double strand breaks (DSBs) are repaired through homologous recombination and non-homologous end joining. These pathways have been extensively described elsewhere (6-8), thus they will be briefly introduced here, to focus later on their relationship to aging and the development of malignancies.

#### 2.1.1. Double Strand Breaks (DSBs)

DSBs arise from ionizing radiation, chemical mutagens, replication of SSBs and free radical lesions. Following detection of a DSB, a complex cascade of events is triggered, in what is known as the DNA damage response (DDR). This coordinated response includes activation of transcription, cell cycle control, apoptosis, senescence and DNA repair processes (see (9), for a detailed review). As mentioned above, two mechanisms are responsible for the repair of DSBs: homologous recombination and non-homologous end joining.

##### 2.1.1.1. Homologous recombination (HR)

HR represents the preferred repair mechanism when a second copy of the damaged DNA is available as a template, namely during late S- and G2-phases of the cell cycle. In this case, the repair is error-free. The cellular response to a DSB is initiated by the activity of phosphatidylinositol-3-OH-kinase-like kinases (PIKKs), principally ATM (Ataxia-Telangiectasia-Mutated), ATR (ATM and Rad3 related) and DNA-PK (DNA-dependent protein kinase) (reviewed in (10)). ATM is likely loaded onto DSBs through an interaction with the DSB sensor complex MRN, which consists of MRE11 (Meiotic recombination 11 homologue), RAD50 and NBS1 (Nijmegen breakage syndrome 1). Upon recruitment, ATM is activated, likely through the catalytic activity of the MRN complex, to initiate the DDR (10) (Figure1). Another early event is the phosphorylation of the histone H2A variant H2AX, which is mostly dependent on ATM, and in certain types of damage, on ATR and DNA-PK (11) (Figure 1). H2AX phosphorylation seems to be required for the recruitment of repair factors. Following activation, ATM recruits and activates additional MRN complexes, which promote strand invasion of homologous sequences through the 5'-3' exonuclease activity of MRE11 (12). The repair reaction requires the concerted action of additional proteins, such as Replication Protein A (RPA), the strand-exchange factor RAD51, the DNA-dependent ATPase RAD54, and the BRCA1-BRCA2 proteins (Figure 1). BRCA1 is a multi-domain protein that possesses E3 ubiquitin ligase activity. BRCA1 forms a complex with RAD51 and BRCA2, and is present in damage-induced foci together with RAD51 (13). BRCA1 is also present in a complex with the ubiquitin-binding protein RAP80, an association required for targeting BRCA1 to sites of DNA damage (14-16).

##### 2.1.1.2. Non-homologous end joining (NHEJ)

During G1 and early S-phase, when a sister chromatid is not available as a template for HR, DSBs are repaired through direct end-ligation, a reaction that usually involves gain or loss of a few nucleotides (17). NHEJ represents the main repair mechanism for V (D)J and class switch recombination events that occur as part of the immune response in the antigen receptor genes. As in HR, ATM and H2AX are activated during NHEJ, and this is followed by recruitment of the end-binding proteins Ku70/Ku80, together with the catalytic subunit of DNA-PK (DNA-PKcs). Ligation is then performed by the LigaseIV/XRCC4 complex (Figure 1). In some cases, additional factors are required, like the end-processing protein Artemis and the factor Cernunnos, which seems to participate in the ligation step, although its' specific function remains unclear (18-20) (Figure 1). In addition, in cases where NHEJ is not able to participate in the DSB repair reaction, an alternative micro-homology mediated end-joining reaction exists as a salvage pathway, although the factors involved in this particular reaction remain unknown (21, 22).

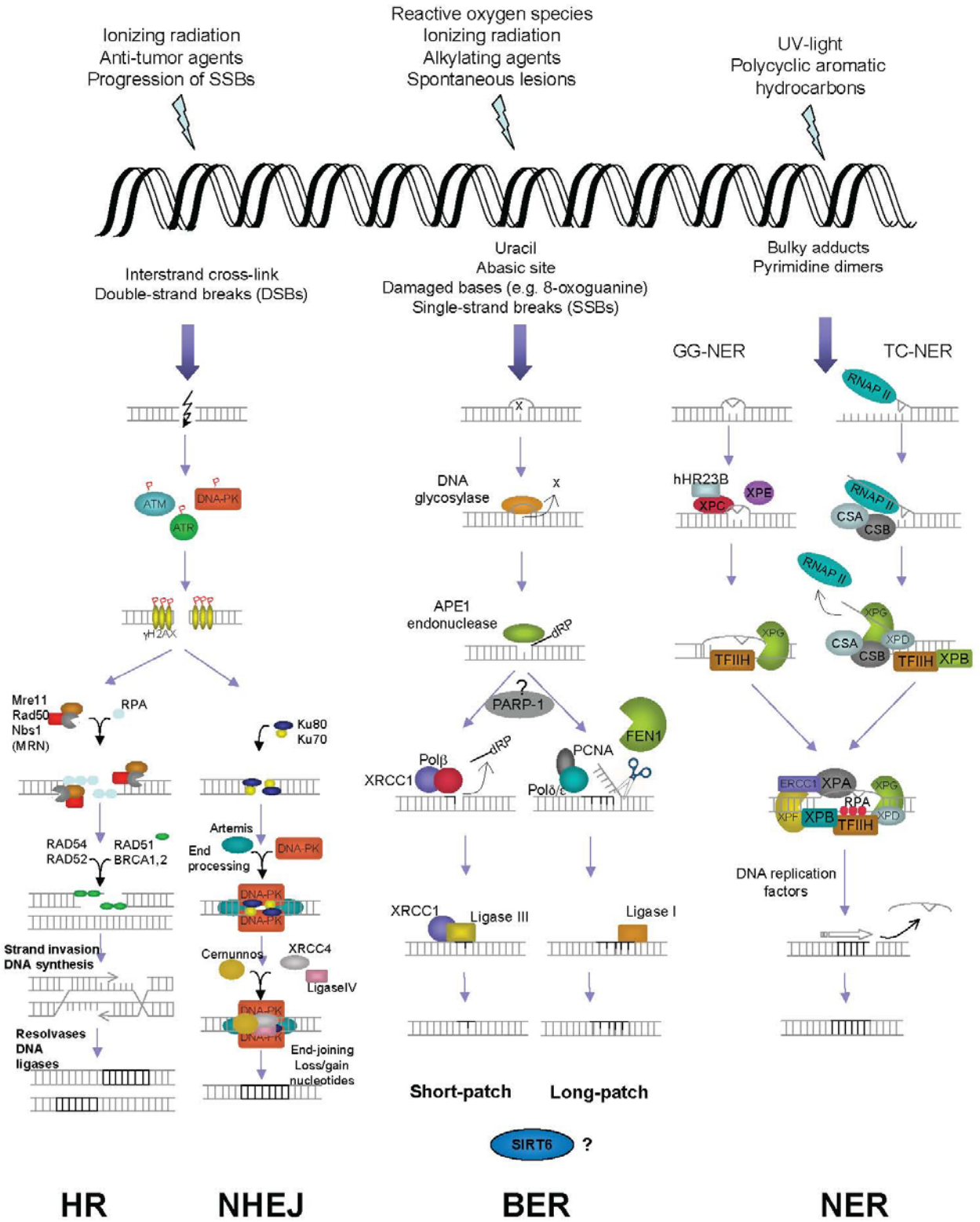


**Figure 1.** DNA repair pathways.The four main repair pathways in eukaryotes are depicted: homologous recombination (HR), non-homologous end joining (NHEJ), base-excision repair (BER) and nucleotide-excision repair (NER). HR: following a double-strand break (DSB) in G2, ATM phosphorylates the RAD50/MRE11/NBS1 (MRN) complex, which 5'-3' exonuclease activity exposes both 3' ends. The replication-protein A (RPA) protein binds the single-strand exposed DNA to facilitate assembly of the RAD51 nucleoprotein (likely including associated factors XRCC2, XRCC3, RAD51B, C and D. RAD51 exchanges the single strand with the same sequence from the homologous double-stranded DNA molecule. Nuclear translocation of RAD51 depends

on BRCA2. Cohesins probably facilitate the identification and synapsis of the sequences. RAD54 is a member of the SWI/SNF family of ATP-dependent remodeling complexes, likely involved in chromatin accessibility. DNA is synthesized using the intact DNA sequence as a template, and the Holliday-junctions are resolved by resolvases. It is unclear what is the ligase involved in the reaction. Additional proteins, like BRCA1, are also involved in the reaction. NHEJ: in G1, when a second copy is not available for repair, cells simply links the ends of a DSB without any template, using the end-binding proteins Ku70-Ku80, which facilitate DNA end-alignment and end-protection together with DNA-PKcs. Ligation occurs through the XRCC4-LigaseIV complex. End-joining is sometimes associated with gain or loss of few nucleotides if processing of the ends occurs, a step that requires Artemis. The role of the recently discovered Cernunnos protein is unclear. BER: repair of single-strand lesions starts with the excision of the damaged base by one of several specific DNA glycosylases, resulting in an abasic site (which can also occurs spontaneously by hydrolysis. The APE1 endonuclease incises the DNA, resulting in a nick. In the short-patch repair pathway DNA pol-β performs a one-nucleotide gap filling reaction and removes the 5'deoxy-ribose via its lyase activity. Sealing of the nick is then performed by the XRCC1-LigaseIII complex. The long-patch repair process involves DNA polδ/ε and proliferating cell nuclear antigen (PCNA) for synthesis (2-10 bases) as well as FEN1 to remove the displaced DNA flap and LigaseI for sealing. NER: in the GG-NER reaction, the XPC-hHR23B complex first screen for disrupted base-pairing. In TC-NER, the stalled RNA polymerase is recognized and displaced by the CSA and CSB proteins. The subsequent steps of GG-NER and TC-NER are identical. The XPB/XPD helicases –subunits of the TFIIH transcription factor-- opens ~30 base pairs of DNA surrounding the damage, while XPA screens for abnormal structures. RPA then stabilizes the "bubble" intermediate. The XPG and ERCC1/XPF endonucleases subsequently cleave the 3' and 5' ends in the damaged strand, respectively. This generates a 24-32 gap that is filled by the DNA replication machinery.

### 2.1.2. Single Strand lesions

#### 2.1.2.1. Base Excision Repair (BER)

BER is responsible for the repair of small chemical alterations. Even though these lesions may not impede transcription, they frequently miscode, therefore this pathway is particularly relevant for preventing mutagenesis. BER is the main sentinel against damage due to cellular metabolism, including those resulting from reactive oxygen species, methylation and alkylation, and thus it is particularly important with regard to aging, as discussed below. In its core reaction (named short-patch repair), a set of DNA glycosylases, each recognizing a specific lesion, cleaves the damaged base, generating an abasic site, which is processed by the APE1 endonuclease to generate a nick. DNA polymerase β (polβ) then performs a one-nucleotide gap-filling reaction and removes the 5' baseless sugar residue via its lyase activity. The XRCC1-Ligase3 complex finishes the reaction (Figure 1). Additional factors, such as the poly (ADP-ribose) polymerases PARP-1 and PARP-2 have been proposed to participate in the reaction, although their specific roles remain unclear. In the alternative pathway (long-patch repair), 2-10 nucleotides are removed and synthetized *de novo*, in a reaction that involves additional proteins, such as Polδ/ε, the co-factor PCNA, the FEN1 endonuclease and Ligase1 (Figure 1).

#### 2.1.2.2. Nucleotide Excision Repair (NER)

This pathway deals with a wide class of helix distorting lesions that interfere with base pairing and obstruct transcription and replication. Most NE lesions arise from exogenous sources, such as UV light. This pathway is the most versatile in terms of lesion recognition. It can be subdivided into global genome NER (GG-NER) which surveys the entire genome for lesions, and transcription-coupled repair (TCR) that focuses on damage that blocks transcription by the RNA polymerases (Figure 1). The major difference between the two pathways resides basically at the recognition of the lesion. For GG-NER, a specific complex (XPC-hHR23B) recognizes disrupted base-pairing. In the case of TCR, it is the ability of the lesion to block the RNA Polymerase what signals the pathway, and two specific proteins are involved in this process, CSA and CSB. The subsequent stages are identical and involve helicases (XPB-XPD), endonucleases (XPG-ERCC1/XPF), auxiliary factors (such as the single-strand binding protein RPA) and the DNA replication machinery to fill the gap (Figure 1).

#### 2.1.2.3. Mismatch Repair (MMR)

MMR removes mispaired nucleotides that result from replication errors and defective homologous recombination, such as base-base mismatches and insertion/deletion loops (8). It is as well involved in the repair of oxidative damage and damage generated by alkylating agents, and it has been suggested to act as a back-up repair mechanism to BER (23).

Several genes including MSH2, MSH3, MSH6, MLH1, MLH3, PMS1 and PMS2 are involved in MMR. Initially, the nucleotide mismatch is recognized by the MSH complex, followed by interaction with the MLH/PMS complex, in an ordered reaction that led to nucleotide excision and resynthesis. Cells deficient in MMR display microsatellite instability, a genomic biomarker of MMR defects. Even though defects in MMR have shown causative roles in several malignancies such as colorectal cancer (8), little is known about the relationship of MMR with aging, and thus this pathway will not be further discussed.

## 3. GENOMIC INSTABILITY, AGING-LIKE SYNDROMES AND TUMORIGENESIS

Extensive literature for both human syndromes and mouse models has shown that mutations in several components of the above described DNA repair mechanisms can lead to two seemingly opposite outcomes: either segmental progeria or an increased in cancer incidence, thus suggesting a common biology underlying these two processes (1). The different human syndromes and mouse models exhibit great variation with respect to their phenotypic manifestations, and they will be summarized below. In general, it seems that in those cases where the DNA damage signaling proteins or gatekeepers

(checkpoint factors such as the tumor suppressors ATM, p53, p19Arf and p16INK4a) are affected, DNA damage accumulates because of a failure to activate a repair or an arrest/apoptosis response, thus leading to increased genomic instability and cancer predisposition. In contrast, when the defects occur in the DNA repair factors *per se*, the so-called caretakers, massive accumulation of DNA damage triggers the activation of the above-mentioned gatekeepers, leading to accelerated cellular senescence/apoptosis and to premature aging phenotypes (1). In this case, protection against tumor development is achieved at the expense of longevity (the so-called "antagonistic pleiotropy" model) (24). However, this tentative model does not provide a mechanistic framework for fully defining the distinction between the two outcomes, nor does it explain all manifestations of progeria. In many cases, as described below, both progeroid manifestations and tumors appear together, illustrating the complexity of these processes; how they evolved remains far from being completely understood.

### 3.1. DSB defects in aging and cancer

#### 3.1.1. Gatekeepers in aging and cancer

Defects in upstream factors in the DNA damage response (gatekeepers) can lead to aging-like and/or tumor-prone phenotypes. In humans, loss of ATM function causes Ataxia-Telangiectasia (A-T), a syndrome characterized by immunodeficiency, lymphoma, cerebellar degeneration, sterility, and an overall progeroid appearance (25). Mutations in MRE11 can produce a clinically similar condition called ATLD (Ataxia-Telangiectasia Like Disorder) (26). A-T mouse models recapitulate many of these phenotypes although with less prominent progeroid features, and neurological dysfunction rather than frank neurodegeneration (4). ATM-deficient mice do however demonstrate age-associated hematopoietic stem cell failure and early onset of lymphomagenesis (27, 28). These defects, as well as neuronal dysfunction, can be ameliorated by antioxidant treatment, suggesting that dysregulation of reactive oxygen species (ROS) levels constitute a functionally important element of ATM-deficiency (27, 29-33). Indeed, ATM itself appears to have a direct role in regulating ROS levels in addition to its function in regulating the DNA Damage Response (DDR) (34). Thus, ROS seem to play an important role in the phenotypic manifestations of these biological processes, as it will be detailed below. The relationship between ATM, cancer and aging is very complex; in addition to its functions in the DDR and ROS regulation, ATM also plays important roles in telomere maintenance and, as we shall see below, in insulin signaling (35).

The p53 gene encodes a protein that plays a crucial role in the cell-cycle checkpoint response, promoting either cell-cycle arrest (senescence) or apoptosis in response to genotoxic damage. It is well known that defects in this tumor suppressor cause genomic instability, secondary to lack of cell cycle arrest following damage. The role of p53 in tumorigenesis has been documented extensively, and thus it is not surprising that p53 is found mutated or deleted in a high percentage of human cancers (36). Nevertheless, recent studies have uncovered a role for p53 in aging as well. A short isoform of p53, termed ΔN-p53 or p44, has been isolated; in the absence of full-length p53, the truncated form is tumorigenic (37). However, in the presence of wild-type p53, p44 leads to constitutive p53 activity, promoting growth arrest (38). In this regard, two groups have independently found that mice expressing an N-terminally truncated form of p53 exhibit signs of premature aging (39, 40). This progeroid syndrome includes osteopenia, lordokyphosis, reduced subcutaneous fat, defective wound healing, and a shortened lifespan. Thus, unregulated constitutive activation of p53 seems to accelerate aging, likely through its role in cell-cycle arrest and senescence. However, these results should be taken with care, given the artificial nature of the unregulated expression of p53 in this model. Indeed, overexpression of a p53 transgene carrying its endogenous promoter ("super-p53") protected against tumors without an effect in lifespan (41). Furthermore, combination of super-p53 with super-Arf-p19, the tumor-supressor protein encoded by the CDKN2a locus (an inhibitor of MDM2-mediated proteolysis of p53) causes a synergistic effect on tumor resistance and delayed aging (42). In this case, a marked reduction in ROS levels was detected, once again highlighting the role of ROS downstream of p53 in these processes, as it will be further discussed below.

The other component of the CDKN2a locus, the tumor-suppressor p16INK4a that encodes a cyclin-dependent kinase-4 inhibitor, has also been clearly linked with aging in mice and humans (43). p16Ink4a protein levels rise dramatically with age (44), and mice lacking INK4a are tumor-prone but show increased regenerative potential in diverse stem-cell niches. Furthermore, a number of aging associated disorders in humans map to the CDKN2a locus, presumably affecting Ink4a expression (although Arf or other genes may be involved) (45-47). Thus, it seems that p16INK4a might serve as a checkpoint to preclude proliferation of cancer cells, although at the expense of limiting the long-term renewal of stem cells. This "stem-cell theory of aging and cancer", which could easily apply to most of the gatekeepers discussed before, it is beyond the scope of this review, and therefore the reader is referred to other recent reviews that discuss this theory in detail (1, 48).

#### 3.1.2. Caretakers in aging and cancer

Two core HR factors, BRCA1 and RAD50, are linked to aging-like and cancer-prone phenotypes in mouse models. Homozygous inactivation of BRCA1 or RAD50 results in embryonic and cellular lethality, respectively (12, 49). BRCA1 deficiency leads to impaired HR-mediated DSB repair, hypersensitivity to DNA damaging agents and genomic instability (50). These defects could potentially explain the embryonic lethality. However, mice homozygous for hypomorphic alleles of these genes exhibit several features of accelerated aging and increased tumor incidence: wasting, skin atrophy, osteopenia and various forms of malignancies (mainly mammary tumors) in the case of BRCA1 (51), and shortened lifespan, cancer predisposition (thymic lymphomas) and hematopoietic stem cell failure in RAD50 mutant mice (52). In the latter case, the phenotype is partially p53-dependent, suggesting

that it occurs in response to unrepaired DNA damage as part of the DDR.

Segmental progeroid phenotypes have been observed in several NHEJ mutant mice as well. Similar to the HR deficiencies, the progeroid syndromes are accompanied with an increased tendency to develop tumors. Specifically, although mutations in the core NHEJ factors Ligase IV or XRCC4 cause embryonic lethality, in the context of p53 deficiency, compound mutant mice are viable but they develop lymphomas (53, 54). In addition, XRCC4/p53-deficient mice also show degenerative phenotypes --spinal curvature, thinning of the skin, and testicular atrophy (55)-- and a hypomorphic allele of Ligase IV is associated with progressive loss of hematopoietic stem cell function (56). Both Ku70 and Ku80 deficient mice show increased incidence of lymphomas, a phenotype greatly enhanced in the context of p53 deficiency (57, 58). In addition, a line of Ku80-deficient mice exhibits osteopenia, atrophic skin, liver lesions and shortened lifespan (59). Although Ku70-deficiency had previously been linked to malignancy but not to degenerative phenotypes, recent results suggest that both Ku70- and Ku80-deficient animals develop similar degenerative syndromes when raised under similar housing conditions (60). This finding highlights the importance of the environment in modulating progeria phenotypes. Similarly, a strain of DNA-PKcs-deficient mice shows osteopenia, intestinal atrophy, thymic lymphoma and reduced longevity (61). However, other lines of DNA-PKcs-deficient mice, and mice deficient in Artemis, do not demonstrate these phenotypes (4, 62). In this regard, both the Ku80 and DNA-PKcs-deficient mice show signs of ongoing inflammation, thus some of the observed phenotypes might well reflect degenerative changes caused by chronic infection rather than accelerated aging due to the DNA repair defect (4). Similarly, Ku and DNA-PKcs function in other pathways distinct from NHEJ such as telomere maintenance (4), raising the possibility that aging-like effects observed in these mutant mice may also occur through mechanisms unrelated to loss of NHEJ function.

### 3.2. Single-Strand lesions in lifespan and tumorigenesis

#### 3.2.1. BER in aging and cancer

Even though BER represents the main pathway to repair oxidative lesions, defects in known BER factors do not produce progeroid manifestations, likely due to redundancy of the glycosylases, on one hand, and the requirement for viability of the core BER factors on the other (63, 64). However, deficiencies in certain BER factors have been associated with the prevention of aging-associated neurodegeneration (64). Clearly, the link between BER and aging remains as an open question. In this context, recent studies in our lab have shown that cells lacking the mammalian sirtuin SIRT6 exhibit evidence of defective BER accompanied by an accelerated aging phenotype, and will be discussed in detail below.

On the other hand, defects in several BER factors have been linked to the development of malignancies. Specific polymorphisms in the OGG1 glycosylase and the XRCC1 genes have been related to increased risk of lung cancer (65, 66) and altered expression/activity of Ape1, Polβ and OGG1 has been linked to development of different types of cancers (67-69). Overall, BER defects seem to manifest as predisposition to malignancies rather than aging-like syndromes. This is probably due to the fact that null mutations are lethal; tumors will arise when changes in the expression of these genes are subtle enough to be compatible with life, although at the cost of increased genomic instability and concomitant risk of developing cancer.

#### 3.2.2. NER in aging and cancer

Several human patients and mouse strains with defects in NER factors have phenotypes that either resemble premature aging or else exhibit increased tendency to develop tumors (70). Defects in the TC-NER factors CSA or CSB –encoding proteins that block RNA polymerase II activity-- lead to Cockayne syndrome (CS) in humans, a complex disorder with developmental delay, neurodegeneration and progeroid features. In addition, patients with defects in the XPD helicase suffer from trichothiodystrophy (TTD), a disease characterized by brittle hair, skin defects and shortened lifespan (71). Notably, mice with a mutation in XPD that mimics the one observed in TTD develop similar aging associated changes, including wasting, osteoporosis and melanocyte loss (72). More recently, a human patient with a dramatic progeroid syndrome was found to possess defects in the XPF gene. Mice deficient for the XPF exonuclease, its binding partner ERCC1 or mice deficient in CSB/XPA or XPD/XPA also exhibit severe acute degeneration, dying prior to one month of age with osteopenia, skin and bone marrow abnormalities, kyphosis, and, at the cellular level, sensitivity to oxidative stress (73, 74, 75-77). Remarkably, in all these mouse mutants a dramatic inhibition of the somatotrophic axis was observed (74, 77, 78), leading the authors to suggest that unrepaired DNA damage triggers a metabolic response mediated via the insulin-signaling pathway. The potential modulation of insulin signaling by genotoxic stress might be relevant for other progeroid models, including SIRT6, as discuss in more detail below.

NER defects are associated with cancer as well, and the best example is xeroderma pigmentosum (XP), a rare autosomal-recessive inherited disease characterized by a 1000-fold increased risk of sun-induced skin cancer (79) accompanied in some cases with neurologic abnormalities. Mutations in several NER genes, such as XPD or XPF, can lead to XP. Interestingly, XP-CS and XP-TTD patients exhibit a combination of progeroid and cancer prone conditions, as do mouse models of these diseases (70).

Overall, it is clear that defects in the different DNA repair pathways can lead to an aging-like syndrome, cancer, or a combination of both. The ultimate outcome depends on the specific function affected and the severity of the defect: a progeroid syndrome will develop in those mutants in which checkpoints and senescence programs can be activated early on, while tumors will arise in cases where the increased genomic instability is unable to activate the gatekeepers, leading to chronic accumulation of genomic instability and ultimately cancer. The underlying basis for

these distinct outcomes remains unclear. The combined progeroid/tumor prone syndromes --such as A-T, XP-CS and XP-TTD—present a notable paradox. In these diseases, the activation of gatekeepers resulting in arrest or apoptosis, and consequent progeroid symptoms would be predicted to protect against, rather than promote tumorigenesis. Molecular analysis of mouse models mimicking those specific mutants will likely shed light into this particular puzzle.

### 3.3. Reactive oxygen species as a source of DNA damage

Reactive oxygen species (ROS), the product of endogenous metabolism, likely produce much of the DNA damage that is repaired through one of the pathways discussed above (80). Thus, changes in ROS metabolism could lead to aging-like or tumor prone phenotypes even when the DNA repair pathways are fully functional. For instance, overexpression of catalase targeted to the mitochondria extends murine lifespan along with lower levels of oxidative damage (81). Mutation of p66sch –a mitochondrial redox enzyme— or the adenylyl cyclase AC5 –a key enzyme that catalyzes the synthesis of cAMP from ATP— is also associated with decreased oxidative stress and extended lifespan (82-84). Interestingly, increasing the levels of oxidative stress can cause both aging-like and cancer-prone phenotypes, depending on the severity of the oxidative damage. For instance, mice heterozygous for mitochondrial superoxide dismutase (SOD2), a critical detoxifier of ROS, exhibit increased oxidative damage and elevated incidence of lymphomas (85), but normal lifespan. In contrast, mice lacking Hif-2a, a regulator of antioxidant genes, develop metabolic defects including hypoglycemia, systemic degeneration of mitochondria-rich organs, and short lifespan (86). The relatively short lifespan of these mice precluded analysis on tumor phenotypes. As discussed below, intra-cellular ROS appear to regulate insulin signaling as well (reviewed in Leslie, 2006), and thus it could affect lifespan and tumor development independent of its effect on DNA damage. On the other hand, chronic accumulation of DNA mutations is a hallmark of aging in mammals (87), and thus although DNA mutations were not directly measured in these animal models, the critical role of ROS levels in their phenotypes suggest that accumulation of DNA damage likely plays an important role. Overall, although unanswered questions remain on the complex relationship between ROS, aging and cancer, ROS represent an important player in these biological processes, and a subject that will certainly benefits from future studies.

### 3.4. Werner and Hutchinson-Gilford Progeria syndromes: DNA Repair Deficiencies with Striking Progeroid Features

Besides the syndromes described above, two particular human diseases bearing progeroid features with incompletely-defined DNA repair defects deserve mention (88, 89). Werner syndrome (WS) is an autosomal recessive disorder in humans characterized by the early onset of many manifestations of aging: premature graying, bilateral cataracts, thinning of the skin and loss of subcutaneous adipose tissue, type II diabetes, arteriosclerosis and myocardial infarctions, and neoplasia. However, WS is a not a perfect simulacrum of normal aging (88) (89). The gene defective in WS, WRN, encodes a large DNA helicase that also possesses an exonuclease domain. The exact function of WRN is unclear; however it has been implicated in several DNA repair pathways and in DNA replication, perhaps in the resolution of unusual DNA structures (64, 89). Of note, WRN-deficient mice only develop progeroid features in the setting of artificially shortened telomeres –due to combined mutations of the telomerase gene— implying that telomere maintenance is an important role of WRN; loss of this function is evident otherwise in humans but not mice owing to the very long murine telomeres (90-92). The role of telomere function in these processes are also highlighted by the Dyskeratosis Congenita progeria and cancer syndrome, which is associated with defects in processing of the telomerase RNA component resulting in loss of telomerase activity (93). Even though the role of telomere dysfunction in aging and cancer has been extensively documented, it is beyond the scope of this review, and the reader is referred to any of several excellent studies on this matter (1, 93).

In humans, different mutations in the LMNA gene, encoding the nuclear lamins A and C, are associated with a spectrum of disorders termed the laminopathies, affecting many different tissues (89). Three of these diseases possess progeroid features; the best characterized, Hutchinson-Guilford Progeria syndrome (HGPS), is a severe condition with onset in childhood, characterized by atherosclerosis and myocardial infarction, skin changes and hair loss, loss of subcutaneous fat, and skeletal abnormalities. Most LMNA mutations associated with HGPS interfere with proteolytic processing of prelamin A to mature lamin A; these phenotypes can be reproduced in various LMNA mouse mutants or mice lacking the protease that processes LMNA, Zmpste24 (89). Even though it remains unclear how lamin A deficiency causes HGPS, recent studies indicate that LMNA defects lead to abnormalities in nuclear structure and DNA repair: genomic instability, genotoxin sensitivity, and impaired recruitment of repair factors (94-96). The survival of Zmpste24-deficient mice is improved in a p53-deficient background, implying that checkpoint responses to unrepaired DNA damage likely play an important role in the pathogenesis of HGPS (97), analogous to the situation in RAD50 mutant mice (52). Overall, although the specific DNA repair defects in WS and HGPS have not been fully characterized, there is ample evidence that these disorders may be caused at least in part by impaired DNA repair.

### 3.5. The Fanconi Anemia pathway

This section will be incomplete without a mention on the Fanconi Anemia (FA) disorder, a rare recessive disease characterized by bone marrow failure due to increased apoptosis of hematopoietic cells, congenital defects and cancer predisposition. In addition, signs of accelerated aging are also observed, likely due to increased cell death in the proliferating compartments of the organism (98, 99). At the cellular level, FA patients exhibit increased chromosomal aberrations and extreme sensitivity to DNA interstrand-cross linking (ICL) agents. Until recently, little was known about the molecular defects

behind this disease. Recent studies indicate that it is caused by mutations in any of several genes of the FA pathway, a specific pathway in charge of repairing DNA ICLs. The FA multi-protein complex includes helicases and endonucleases, capable of recruiting DNA repair proteins such as BRCA1, RPA, Topoisomerase III, DNA polymerases and the MRN complex to sites of DNA crosslinks (see (98) for review).

## 4. THE INSULIN/IGF1-LIKE SIGNALING (IIS) PATHWAY

IIS represents a conserved pathway, regulating metabolism in many organisms. Similar to the DNA repair pathways described before, extensive literature has linked the IIS pathway to both lifespan regulation and cancer.

IIS is initiated when insulin or its related peptides bind to their cognate transmembrane receptors leading to receptor autophosphorylation (100) (Figure 2). In turn, this receptor activation causes phosphorylation of insulin receptor substrate (IRS) proteins, allowing recruitment of several proteins, initiating multiple signaling cascades. One important cascade involves the activation of phosphatidylinositol-3-OH kinase (PI3K), which leads to the activation of PDK1 kinase. This, in turn, activates the AKT/PKB kinase, a critical mediator of multiple downstream processes, such as metabolic adaptation, stress resistance, cell cycle, survival and growth (101). The FoxO family of transcription factors represents one of the main effector arms of the PI3K-AKT signaling pathway. AKT activation leads to FOXO phosphorylation and sequestration of the proteins in the cytoplasm. Reduced IIS allows nuclear translocation of FoxO proteins, where they activate or repress transcription, promoting stress resistance, cell death, cell cycle arrest and metabolic changes in a cell-type specific and context-dependent manner (102) (Figure 2). The specific response will likely depend on the type and intensity of the original stimulus. For instance, DNA damage and the DDR activates a strong IIS response to allow cellular adaptation, as we learned recently (103), and such links between these conserved pathways will be further discussed below.

### 4.1. Insulin signaling in aging and cancer

#### 4.1.1. IIS in lifespan regulation

Reductions in IIS are associated with longevity in many model organisms. Deletion of the AKT homolog *sch9* promotes stress resistance and increased lifespan in yeast (see below) (104, 105). In *C. elegans*, mutations in the Insulin/IGF1 receptor homolog *daf-2*, the PI3K homolog *age-1*, or *pdk-1* confer extended longevity dependent on the FOXO protein DAF-16 (106). Interestingly, knock-down of the insulin receptor in *C. elegans* and knock-down of several downstream targets of DAF-16, affected both tumor growth (in a worm model of cancer) and lifespan of tumor-free animals, indicating that in lower organisms, cancer and aging are processes mechanistically connected (107, 108). In this case, reducing insulin signaling had a beneficial effect on both cancer and lifespan, and thus they offer a distinct view from the "antagonistic pleiotropy" model, where cancer protection is exerted at the expense of longevity (24). Therefore, it will be interesting to test whether mammalian homologs of these FOXO-target genes protect against tumor and aging as well. In *Drosophila* mutations in the insulin receptor *Inr* (109) the IRS homolog *chico (*110), or overexpression of FOXO proteins in the fat body (111) also lead to extended lifespan. Suppressing IIS in *Drosophila* heart reduces age-related cardiac decline that occurs in this organism (112). Finally, perturbations in the TOR pathway, another Akt target impacting on nutrient sensing and IIS (Figure 2), increase lifespan in lower organisms (105, 113-115).

Mammals possess distinct hormones that provoke IIS to signal different outcomes: insulin, secreted by the beta cells of the pancreas, directs anabolic metabolism, whereas IGF1, secreted mostly by the liver in response to pituitary growth hormone (GH), predominantly directs somatic growth and differentiation (116). Evidence suggests that IGF1 is most relevant for lifespan determination in mammals; reduced IGF1 signaling is associated with extended longevity in mice (116). Also, deletion of the Irs2 gene specifically in brain leads to increased longevity (117). In contrast, reduction in insulin sensitivity or insulin secretion produces diabetes and shortens lifespan in mice and humans. Even though deletion of the insulin receptor specifically in adipose tissue does extend longevity, this effect may stem from reduced adiposity in these animals rather than altered IIS per se (118). In humans, reduced IIS has not been clearly linked with longevity; indeed low levels of IGF1 have been linked to cardiovascular disease and diabetes, whereas high IGF1 levels may confer susceptibility to cancer (116). Overall, while we lack convincing data supporting a connection between IIS and longevity in humans, compelling evidence links reduction of IIS to increased lifespan in diverse organisms, including mammals.

#### 4.1.2. IIS and cancer

Similar to DNA repair mutants, defects in the insulin-signaling pathway have not only been correlated with lifespan regulation, as described above, but defects in several of its components also correlate with increased appearance of tumors in humans (119). For instance, the phosphatase PTEN that inactivates the PI3K/Akt pathway is mutated in a wide variety of tumors (120, 121). In addition, germline mutations in the PTEN gene were found in tumor-prone syndromes such as Bannayan-Zonana and Cowden syndromes (121, 122). Consistent with this effect in humans, mice deficient in PTEN develop a broad range of tumors, such as skin, prostate, colon and breast carcinomas, as well as thymic lymphomas (123-127). PTEN encodes a major lipid phosphatase that functions in the PI3K signaling cascade, and loss of PTEN leads to the activation of the insulin-signaling pathway, stimulating cell growth and proliferation (128, 129). Furthermore, PTEN-deficient cells exhibit genomic instability due to hyperactivity of AKT in these mice leading to phosphorylation and degradation of Chk1 and defective checkpoint responses (130, 131). Therefore, mutations in PTEN were thought to influence tumor development solely through its effect in cell proliferation and cell cycle modulation. However, in a recent study, PTEN was shown to move to the nucleus and interact with both the centromeric protein CENP-C and the DNA repair factor

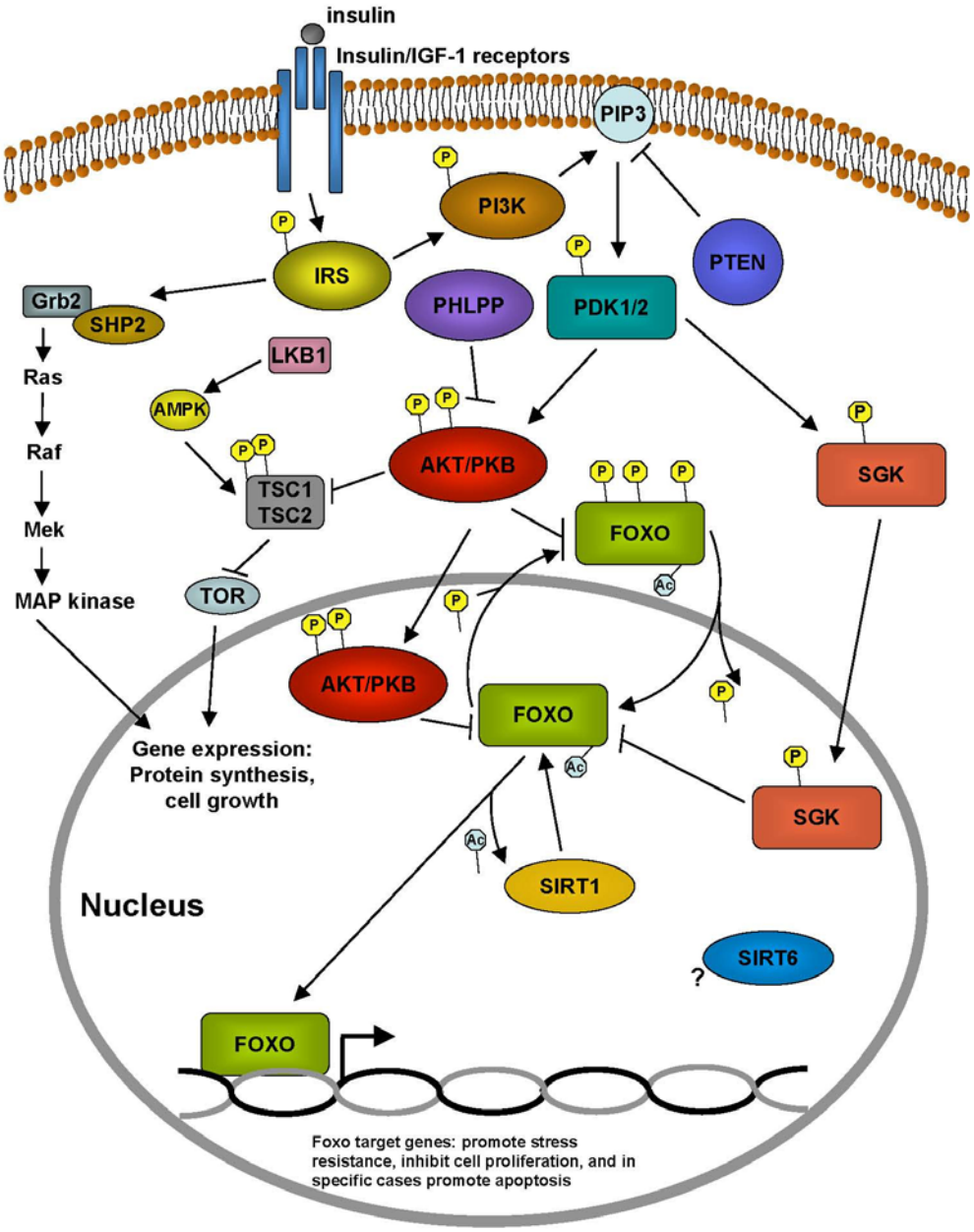


**Figure 2.** The insulin-signaling pathway. Insulin activates phosphatidylinositol 3-kinase (PI3K) through phosphorylation of the insulin receptor susbtrate 1-4 (IRS-1/IRS-4) proteins. PI3K generates phosphatidylinositol-3,4,5-triphosphate (PIP3) that triggers the phosphorylation of PDK1 and subsequent phosphorylation and activation of the AKT/PKB and SGK kinases. Recent studies identified the phosphatase PHLPP as a direct inhibitor of AKT. This first step of the pathway can also be regulated by the phosphatase PTEN, which reverts the formation of PIP3. Activated AKT then phosphorylates FOXO, sequestering it in the cytoplasm. SGK can also phosphorylate FOXO independently of AKT. When insulin signaling is inhibited, FOXO is dephosphorylated (through an unknown mechanism) and translocates to the nucleus, impinging on several downstream target genes to promote stress resistance (a process promoted by FOXO deacetylation through SIRT1), and --in specific contexts— inhibition of cell proliferation or apoptosis. In parallel, AKT activation also leads to phosphorylation and inactivation of the tuberous sclerosis-2 (TSC2) protein, which promotes activation of target of rapamycin (TOR). TOR, in turn, promotes protein synthesis and cell growth. TOR signaling can be inhibited under conditions of stress, which increases AMP/ATP ratio, activating AMPK through a mechanism involving the LKB1 tumor suppressor protein. AMPK subsequently phosphorylates and activates TSC2 – at different sites from AKT—causing inhibition of TOR signaling. Cell growth and protein synthesis can also be regulated through the MAP-kinase pathway, a third parallel sub-pathway activated by the IRS proteins. The precise role of SIRT6 in the insulin-signaling pathway remains unclear.

RAD51. Indeed, PTEN-deficient cells exhibited a striking increase in chromosomal instability (132). Hence, the chromosomal instability in PTEN mutant cells is likely the result from both direct engagement of PTEN with the repair machinery as well as to aberrant Akt activation. It is then conceivable that the tumorigenic potential of insulin-signaling defects might also be secondary to the effect of some of these factors in genomic stability, rather than to its sole effect on cell proliferation.

As mentioned above, the TOR pathway has recently been shown to influence lifespan in lower organisms. However, activating mutations in several TOR regulators have been linked to tumors in humans as well (133), and the usage of the TOR inhibitor rapamycin is currently tested in clinical trials for cancer treatment. In this same context, deficiency in the Foxo family of transcription factors were long predicted to influence cancer, given the prominent role of Foxo proteins as downstream negatively-regulated effectors in the PI3K/Akt signaling pathway. Indeed, somatic deletion of all three Foxo members (Foxo1, Foxo3 and Foxo4) in mice leads to the development of aggressive lymphomas as well as benign hamartomas (134). It is not known whether these tumors exhibit genomic instability. However, overexpression of Foxo3a increased UV-damaged DNA repair (135). In addition, a recent study indicates that inactivation of FOXO causes decreased expression of catalase, with the concomitant increase in ROS levels and genomic instability (136). Thus, as in PTEN mutants, the neoplastic phenotype observed in FOXO-deficient animals might involve effects on genomic stability. As noted above, targets of the FOXO homolog in C. elegans, DAF-16, also influence both lifespan and tumor growth (108), thus indicating that the effect of FOXO in cancer and aging seems to be evolutionary conserved.

Other factors in the insulin-signaling pathway have also been shown to influence tumorigenesis. For instance, overexpression of the adaptor signaling molecules IRS-1 and IRS-2 caused increased tumor incidence and metastasis in a mouse model of breast cancer (137). In this same context, increased activity of the PI3K Akt1 pathway is found in numerous human cancers, and overexpression in mice caused tumor resistance against chemoterapy (138, 139), while Akt deficiency in mice suppresses oncogenesis (140). The impact of PI3K activation status on the degree of genomic instability of human tumors has not been determined, thus it remains unclear whether DNA damage directly provoked by this pathway plays a role in these cases.

Overall, it appears that unregulated IIS activation leads to tumor development, while suppression of IIS promotes longevity. As shown recently for PTEN, some of these phenotypes are not only due to the effect of these factors on cell proliferation but rather on their role in regulating genomic stability as well. It will be important to demonstrate whether this applies for other insulin-signaling factors as well. In this regard, it appears that communication between these two pathways (IIS and DNA repair) would lead to a better coordinated response, which certainly will be beneficial for the cells. This cross talk appears more general than previously suspected, and it will be further discussed below.

### 4.2. ROS and IIS

Apart from the effect of ROS on DNA damage, recent studies indicate that ROS levels can influence insulin signaling, affecting organismal metabolism as well. In this context, increased ROS levels cause oxidation and inactivation of several inhibitors in the insulin-signaling pathway, such as PTEN (reviewed in (141) and increased overall IIS. However, these results conflict other studies indicating that cancer cells with increased glycolysis (and therefore reduced oxidative-phosphorylation and ROS), exhibit increased NADH levels that cause inactivation of PTEN (142). In this case, reduced levels of ROS were accompanied by increased Akt phosphorylation, and therefore increased insulin signaling. This study provides an explanation for the "Warburg" effect observed in cancer cells, where these cells experience growth-stimulatory signals under hypoxic conditions that favor glycolysis. In general, the relationship between ROS and insulin signaling is a complex one, and the discrepancies between these studies suggest that the net effect of ROS on IS will probably be cell- and context-dependent.

## 5. THE SIRTUIN FAMILY OF PROTEINS

Sirtuins are homologs of the yeast Sir2 protein, the founding member of this family, originally discovered in a screen for proteins that were able to silence the mating type locus (*Sir*: *S*ilent *I*nformation *R*egulator) (143, 144). The sirtuins represent another category of genes with conserved functions in lifespan and likely in cancer as well. Overexpression or hyperactivity of sirtuins in many species, including yeast, worms, flies, fish and potentially mammals, promotes longevity (145). In mammals, seven homologs, termed SIRT1-SIRT7, have been described (145, 146). Sirtuins exert their effects via NAD-dependent enzymatic activity; some of them possess robust deacetylase activity, like SIRT1, while others, like SIRT4 and SIRT6, appear to function primarily as ADP-ribosyl transferases, with little or no deacetylase activity, at least in *in-vitro* assays (147, 148). Although yeast Sir2 apparently only modifies histones, other sirtuins have evolved to modify a large array of proteins. Among mammalian sirtuins, SIRT1 is the best-characterized; SIRT1 binds to and/or deacetylates a wide variety of factors impacting on numerous cellular processes, including stress resistance (p53, FOXO, NF-κB, E2F1, Ku70) and metabolism (FOXO, PPARγ, PGC1α), as well as many others (145, 149, 150).

### 5.1. Sirtuins and DNA repair

Given the conserved role of DNA repair pathways and IIS in lifespan, it is remarkably that both pathways seem to be affected by sirtuins. In budding yeast, Sir2 suppresses recombination at the rDNA array, and thus the formation and accumulation of extrachromosomal ribosomal circles (ERCs). ERCs represents a major cause of yeast aging in a replicative lifespan assay – that is, when lifespan is measured in terms of number of divisions undergone by a mother cell (151). Modest overexpression

of Sir2 increases yeast lifespan, whereas loss of Sir2 reduces lifespan (152, 153); in both cases lifespan modulation was directly proportional to the stability of the rDNA array. Thus, at least in yeast, genomic integrity seems to play a critical role in the ability of sirtuins to influence lifespan. In addition to the role of Sir2 in rDNA integrity, some sirtuins are involved in the DDR; yeast lacking Sir2 are sensitive to a variety of DNA-damaging agents (154-156). Furthermore, a protein complex containing Sir2 relocalizes to sites of DNA damage in response to DSBs in a manner dependent upon Mec1, the yeast ATM homolog (155-157). However, the role of Sir2 in DNA repair is largely indirect and occurs predominantly via repression of the silent mating type loci; mating type itself regulates DNA repair in *S. cerevisiae*. (158, 159). In addition, the yeast sirtuins Hst3 and Hst4 deacetylate K56 of histone H3; lack of this activity results in DNA damage sensitivity, genomic instability, temperature sensitivity, and silencing defects (160-162). Hst3 and Hst4 are downregulated during S-phase and following DNA damage, conditions under which acetylation of H3-K56 is detectable, implying that precise temporal regulation of this modification is required to ensure proper genome maintenance. How this modification promotes genomic stability is unclear. In *Trypanosoma brucei*, the sirtuin TbSIR2RP1 deacetylates and ADP-ribosylates histones and promotes survival in reponse to DNA damage. TbSIR2RP1 increases chromatin accessibility to micrococcal nuclease following genotoxin treatment; thus one plausible model of TbSIR2RP1 function is that this sirtuin modifies chromatin following genomic insult to allow greater access to DNA repair enzymes (163). This activity of TbSIR2RP1 may serve as a paradigm for understanding how SIRT6 functions to facilitate DNA repair (see below). Although little is known about the role of mammalian sirtuins in DNA repair, a recent study indicates that SIRT1 can deacetylate the DDR protein NBS1, an activity required for proper activation of NBS1 and regulation of the intra-S phase checkpoint (164). In addition, SIRT6 can also affect genomic integrity, through an effect in BER, as described further below (148).

### 5.2. Sirtuins and IIS

In *C. elegans* and mammals, sirtuins can also modulate IIS, suggesting a second mechanism by which these proteins could affect lifespan in higher organisms. Lifespan extension provided by increased dosage of Sir2.1 in *C. elegans* depends upon the FOXO protein DAF-16 and on 14-3-3 chaperone proteins that bind both FOXOs and Sir2.1 (165-167). However, extended lifespan mediated by defects in IIS are not dependent upon Sir2.1 (168). These data suggest that Sir2.1 acts in parallel with IIS, both pathways converging to regulate DAF16 (166). In mammals, the capacity of SIRT1 to deacetylate FOXO proteins promoting their transactivation of stress resistance genes, could potentially contribute to lifespan extension (102). Recently, SIRT2 has been shown to deacetylate FOXO3A, causing decreased ROS levels under conditions of mild stress but promoting cell death under severe stress (169).

Sirtuins can also act upstream of intracellular IIS to influence insulin secretion itself. Mice overexpressing SIRT1 specifically in the beta cells of the pancreas produce higher levels of insulin (170), whereas SIRT1-deficient mice secrete lower levels of insulin (171), effects occurring via regulation of uncoupling protein 2 (UCP2) transcription by SIRT1. SIRT1-deficient animals also possess elevated levels of IGF-1 binding protein (IGFBP1), resulting in suppression of IGF-1 signaling; it has been suggested that many of the effects of SIRT1 deficiency – in particular, small size, failure to thrive, and sterility -- stem from reduced IGF-1 signaling (172). However these results must be interpreted with some caution, because these phenotypes are somewhat non-specific. Moreover, given that most SIRT1-deficient mice on a pure strain background die perinatally, analysis of adult SIRT1-deficient mice necessarily occurs using a highly-selected subset of animals (173, 174). With respect to other mammalian sirtuins, SIRT4 ADP-ribosylates and inactivates the metabolic enzyme glutamate dehydrogenase (GDH) resulting in reduced amino acid-induced insulin secretion (147). During periods of prolonged fasting, inhibition of GDH by SIRT4 is relieved via an unknown mechanism, allowing regulation of insulin secretion by amino acids rather than glucose (147).

#### 5.2.1 Calorie restriction: the intersection of IIS and sirtuins?

Calorie restriction (CR) is an intervention capable of extending lifespan in a wide variety of eukaryotic organisms (175). Both IIS and sirtuins have been implicated in the organismal response to CR. In yeast replicative aging, the CR response depends on integrity of AKT/SCH9 (105). In metazoans however, the CR and IIS pathways are separable. In *C. elegans*, lifespan extension by CR is dependent on the PHA4 forkhead family transcription factor rather than DAF-16 (176). Moreover, worms and flies with impaired IIS still respond to CR (177, 178). In mammals, CR is associated with lower levels of ROS, and concomitantly, a lower frequency of ROS-induced mutations (179, 180). Additionally CR leads to multiple endocrine changes, including alterations in IIS: reduction in IGF1, insulin, and blood glucose levels, as well as increased insulin sensitivity (116, 181). However, the data regarding the relationship between IIS and CR in mammals is somewhat contradictory. Long-lived dwarf mice -- carrying a mutation that negatively influences IGF-1 production but affects many other aspects of physiology as well -- live even longer when exposed to CR, indicating that CR can activate an IIS-independent pathway (182). However, mice lacking the growth hormone receptor, with low levels of IGF-1, do not show further extension of lifespan under CR (183), suggesting that the CR response and IIS occur via overlapping mechanisms.

Based on work in yeast and other invertebrates, studies using the sirtuin activator resveratrol, and indirect data in mammals, sirtuins have been proposed to mediate the effects of CR (147, 184-186); however this connection is currently controversial (187). Although in *Drosophila* the CR response is Sir2-dependent (188), in *C. elegans*, different CR models reveal a partial dependence (168) or

an independence of Sir2.1 (189). It should be noted that recent studies showed a minor sirtuin-independent effect for resveratrol in lifespan extension in *C. elegans* and *Drosophila*, questioning the role of sirtuins in lifespan extension in these organisms (190); future studies will be necessary to fully clarify the relevance of these proteins in lifespan extension in these organisms. In mammals, levels of SIRT1, SIRT2, and SIRT3 proteins rise in tissues of animals subject to CR (169, 191-193). Moreover, in response to nutrient deprivation, SIRT1 deacetylates and activates the central metabolic transcriptional regulator PGC-1α (194, 195). Unlike wild-type mice, SIRT1-deficient animals do not raise their overall activity in response to CR, suggesting that SIRT1 might mediate behavioral aspects of the CR response; however, changes in several serum components in response to CR were intact in SIRT1 animals, demonstrating, at the very least, SIRT1 is not the sole mediator of the CR response (185). Overall, sirtuins, IIS, and CR represent overlapping but distinct paradigms of lifespan extension.

### 5.3. Mammalian Sirtuins in aging and cancer

Overall, there is clear evidence to support a role for sirtuins in lifespan extension in lower organisms, as described before. Furthermore, some sirtuins seem to influence lifespan through a role in DNA repair while others regulate IIS. In the context of mammalian sirtuins, their functions in stress resistance, metabolic homeostasis and CR are consistent with a potential beneficial effect in lifespan. Indeed, both SIRT1 and SIRT2 exhibit protective role in models of neurodegeneration (196, 197, 198), and recent studies in mice showed that resveratrol promotes longevity and improves glucose homeostasis in a Sirt1-dependent manner (199, 200). A specific allele of SIRT3 that maintains an enhancer shows higher frequency in elderly people, suggesting a link between SIRT3 expression and longevity (201, 202). In addition, SIRT1 may also inhibit replicative senescence by deacetylating histone H1 and inhibiting the formation of heterochromatin (203). Recent studies demonstrated that loss of H1 occurs during formation of senescent associated heterochromatic foci (SAHFs) (204), and thus we could hypothesize that increased levels of SIRT1 would be beneficial to avoid cellular senescence via deacetylation of H1, in this way promoting cellular and organismal lifespan (3). However, other results are potentially inconsistent with a beneficial effect for sirtuins in lifespan. While modest overexpression of SIRT1 protects cardiac muscle from age-related deterioration, higher levels actually promote degenerative changes in this tissue (205). As mentioned before, SIRT1 appears to promote insulin secretion and IGF-1 signaling, activities predicted to reduce longevity (172). Furthermore, we found that SIRT1 promotes fibroblast senescence under conditions of low oxidative stress, via regulation of the tumor suppressor Arf (206). In this context, a potential role for SIRT1 in senescence of critical cell populations –like stem cells- would promote tissue decline via stem cell depletion.

Less is known about the effect of sirtuins in cancer. Recent studies suggest that SIRT1 promotes tumorigenesis via its deacetylation and down-regulation of p53 activity (207, 208). In these tumor models, SIRT1 expression was up regulated due to lack of HIC1, a tumor suppressor frequently inactivated in human cancer (209), and a negative regulator of SIRT1 expression (207). In addition, SIRT1 deacetylates E2F1 and Foxo proteins, decreasing DNA damage- and stress-induced apoptosis (150, 210). These roles of SIRT1 in down-regulating p53, Foxo and E2F1 could have a beneficial effect in lifespan extension by promoting survival under conditions of stress. However, some of these same pro-survival properties of SIRT1 could be pro-tumorigenic, and thus impacting negatively on lifespan. Along these lines, SIRT1 is overexpressed in a number of tumor types (94, 211-213). On the other hand, forced overexpression of SIRT1 inhibited the *in vitro* growth and proliferation of prostate cancer cells through deacetylation of the androgen receptor (214). Other sirtuins have potential roles in cancer as well. SIRT2 is down-regulated in human gliomas; since SIRT2 delays mitotic exit, lack of this protein might cause defective mitotic checkpoint and genomic instability (215-217). However, a direct effect of SIRT2 on genomic integrity and cancer remains as yet to be described. SIRT7 is overexpressed in human thyroid carcinoma and breast cancers (218-219), but the significance of these results remains unclear. As described below, the effect of SIRT6 on both DNA repair and IS suggests a role for this protein in both lifespan regulation and tumorigenesis, and it will be discussed in detail below. Altogether, these results highlight the complexity of sirtuin roles in cancer development, in particular for SIRT1. Future studies will help to pinpoint the precise function for these proteins in influencing the development of malignancies.

## 6. SIRT6 LINKS DNA REPAIR AND IS

Thus far the roles of these two conserved mechanisms –IS and DNA repair- were described in the context of lifespan regulation and tumorigenesis, and how certain sirtuins appears to regulate them. Recent studies have shown that these pathways appear to communicate with each other, in order to establish a coordinated and appropriate response in the face of stress. In this context, the sirtuin SIRT6 likely exemplifies one of those regulators capable of modulating metabolic responses in order to handle stress and DNA damage.

### 6.1. SIRT6 influences DNA repair

Mouse embryonic fibroblasts (MEFs) and embryonic stem (ES) cells homozygous for SIRT6 deletion exhibited slow growth, and increased sensitivity to genotoxic insults, such as ionizing radiation. In addition, analysis of metaphase spreads indicated increased levels of spontaneous genomic instability, characterized by numerous non-clonal chromosomal aberrations (148). These phenotypes suggested a defect in the ability of the cells to cope with DNA damage. Indeed, SIRT6-deficient cells exhibited increased sensitivity to alkylating and oxidizing agents, such as methyl methane sulfonate (MMS) and hydrogen peroxide (H2O2), but normal response to UV damage (repaired through NER) and normal repair of DNA DSBs. These spectrums of sensitivities suggest a role for SIRT6 in BER; in support of this notion, these sensitivities

were rescued by expression of the dRP-lyase domain of DNA polymerase β (polβ) (148). How might SIRT6 influence BER? Given its tight association with chromatin, one possibility is that SIRT6 influence the chromatin environment to allow access of the BER factors to the site of damage. Notably, several BER factors performance is severely reduced on chromatinized templates (220, 221). In this regard, extracts from SIRT6-deficient cells exhibited a normal response in an *in vitro* BER assay with naked non-chromatinized DNA templates (148). On the other hand, it is still possible that SIRT6 plays no direct role in the BER process, but rather influences DNA damage accumulation via modulation of ROS, a possibility discussed further below.

### 6.2. Organismal degeneration: SIRT6 function in metabolic homeostasis

From the aging research standpoint, SIRT6 deficiency causes the most dramatic phenotype among sirtuins. As mentioned above, at the cellular level SIRT6 deficiency leads to genomic instability. In the whole organism, metabolic defects dominate the manifestations of SIRT6 deficiency. SIRT6 knockout animals are born at a Mendelian ratio, and appear relatively normal until approximately two weeks of age. At this point, mice degenerate in an acute manner, exhibiting extremely low levels of the hormone IGF-1, osteopenia (likely due to low IGF-1 levels), lymphopenia and a severe hypoglycemia, which causes the demise at around a month of age (148). Overall, SIRT6 deficiency is associated with a complex phenotype resembling some aspects of models of "premature aging" (4). We have recently found that these mice exhibit low insulin levels as well (unpublished results), suggesting that the animals are experiencing increased insulin sensitivity, and indicating that SIRT6 might directly down-regulate this pathway to promote normoglycemia. In this regard, recent studies in several cell types demonstrate that SIRT6 down-regulates Akt phosphorylation, allowing nuclear relocalization of Foxo proteins (R.M., David Lombard, Bjoern Schwer, and Frederick Alt, unpublished results). Furthermore, SIRT6-deficient mice exhibit increased Akt phosphorylation despite low levels of circulating insulin, suggesting that, indeed, these mice are experiencing increased insulin sensitivity and further reinforcing the possibility that SIRT6 functions to down-modulate insulin signaling.

### 6.3. Crosstalk between DNA repair and insulin signaling: the SIRT6 connection

It is remarkable that SIRT6 impacts on these two conserved pathways. What is the connection between these roles? One possibility is that SIRT6 influences both DNA repair and IIS independently of one another, influencing numerous substrates, as shown for SIRT1 and SIRT3. However, another possibility is that SIRT6 function could link both the DNA damage response and the metabolic adaptation to that response (Figure 3). As discussed below, the DNA damage response can be accompanied by changes in the IIS pathway, and conversely, metabolism can affect DNA damage repair. In this context, SIRT6 deficiency could lead to a primary DNA repair defect that triggers a metabolic response (model 1 in Figure 3) in order to cope with the damage. In this case, the effect of SIRT6 in IS will be indirect. In contrast, SIRT6 could directly down-modulate IS (model 2 in Figure 3), causing decreased overall levels of ROS due to reduced metabolic activity. Decreased ROS levels could in turn protect against DNA lesions that arise following chronic exposure to ROS. Finally, SIRT6 could directly regulate ROS levels, which in turn can influence both DNA repair and insulin-signaling response (Model 3 in Figure 3), as discussed in Sections 2.2.3 and 3.2. Undoubtedly, future studies will be critical in order to determine the precise molecular role for SIRT6 in these pathways.

## 7. A GENERAL LINK BETWEEN METABOLISM AND DNA DAMAGE: OVERLAP BETWEEN CONSERVED PATHWAYS OF AGING AND CANCER

### 7.1. DDR can modulate IIS pathways

We have described the possibility that SIRT6 could link these two pathways, therefore suggesting that their effects on cancer and lifespan might be connected. In this regard, SIRT6 is probably only one example of concerted regulation of DNA repair and IIS pathways (Figure 4). For instance, recent studies have uncovered a general link between IIS and the DNA damage response (DDR), suggesting means by which cellular metabolism might be regulated in the face of DNA damage (222). During the DDR, proteins implicated in IIS are phosphorylated, potentially modulating IIS (103). The ATM kinase, central to the DDR, has also been linked to metabolic regulation. ATM positively regulates expression of the IGF-1 receptor (IGF-1R), and enforced expression of the IGF-1R in ATM-deficient cells rescues their radiation sensitivity (223) In addition, ATM positively regulates insulin sensitivity and negatively regulates the development of atherosclerosis in a mouse model of dyslipidemia (224).

More generally, several mouse models of defective DNA repair, like the NER-deficient mice, show profound metabolic abnormalities. We have already discussed the appearance of both genomic instability and metabolic defects in the absence of SIRT6 (148). Furthermore, some of the changes in the insulin-signaling pathway seems to depend on the level of genotoxic stress, thus suggesting that SIRT6 is involved in regulating metabolic changes in response to DNA damage (R.M., David Lombard and Frederick.W.Alt, unpublished results). Thus, in the case of SIRT6, both responses appear to be linked. This also seems to be the case with regards to defects in the NER pathway. As described above, in several mouse models of NER deficiency, such as ERCC1, XPF, CSA/XPD and CSA/XPA-deficient mice, the progeroid phenotype, secondary to increased DNA damage, was accompanied by an inhibition in the somatotrophic axis (e.g., low insulin and low IGF-1 levels) (74). In these cases, the mice experienced increased insulin sensitivity and hypoglycemia as well, thus the overall phenotype strikingly resembled the one observed in SIRT6-deficient mice. In the case of the NER-deficient mice, Hoeijmakers and colleagues proposed that the inhibition of the somatotrophic axis represents a response (albeit a non-successful one in this case) aiming to

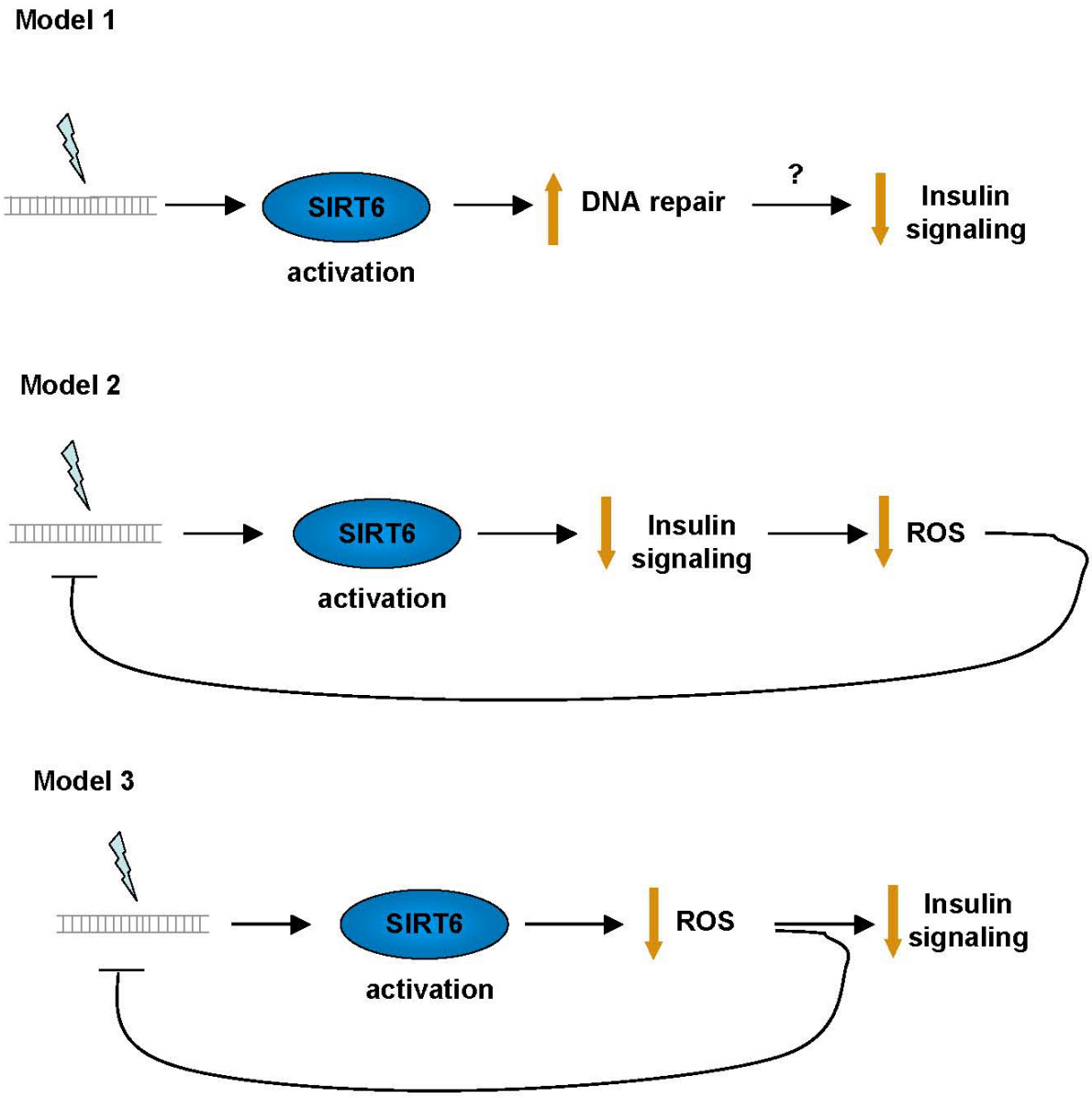


**Figure 3.** Three models for SIRT6 function. 1-SIRT6 is activated by DNA damage, which causes increased SIRT6-dependent DNA repair. In turn, this could lead to down-regulation of insulin signaling as a mechanism to avoid further damage. In this case, SIRT6 effect on insulin signaling will be indirect. 2- In this model, DNA damage activates SIRT6, causing SIRT6 to directly down-regulate insulin signaling, with subsequent less production of ROS and less DNA damage. In the absence of SIRT6, increased ROS production secondary to increased insulin signaling would lead to chronic accumulation of DNA damage which could explain the genomic instability observed in SIRT6-deficient cells. 3- Following DNA damage, activated SIRT6 down-regulates ROS production, which in turn can impinge on both insulin signaling (142) and DNA damage.

diminish the generation of ROS, in order to better handle the damage. However, the mice are experiencing increased insulin sensitivity and hypoglycemia, thus, as in the case of the SIRT6 deficiency, another possibility could as well be that the low levels of insulin and IGF-1 are secondary to the hypoglycemia. In this case, the increased insulin sensitivity, with the concomitant increase in metabolism and ROS production, might directly contribute to the observed DNA damage, which in conjunction with the primary NER defect could lead to the irreversible phenotype observed. However, it remains unclear why these mice experience increased insulin sensitivity, and determining whether insulin sensitivity represents a primary response to the increased DNA damage in these mice will be critical in order to understand the phenotypes observed.

### 7.2. IIS can influence DNA repair

In a reciprocal way to the effect of DDR in insulin signaling, factors implicated in IIS can impact upon the

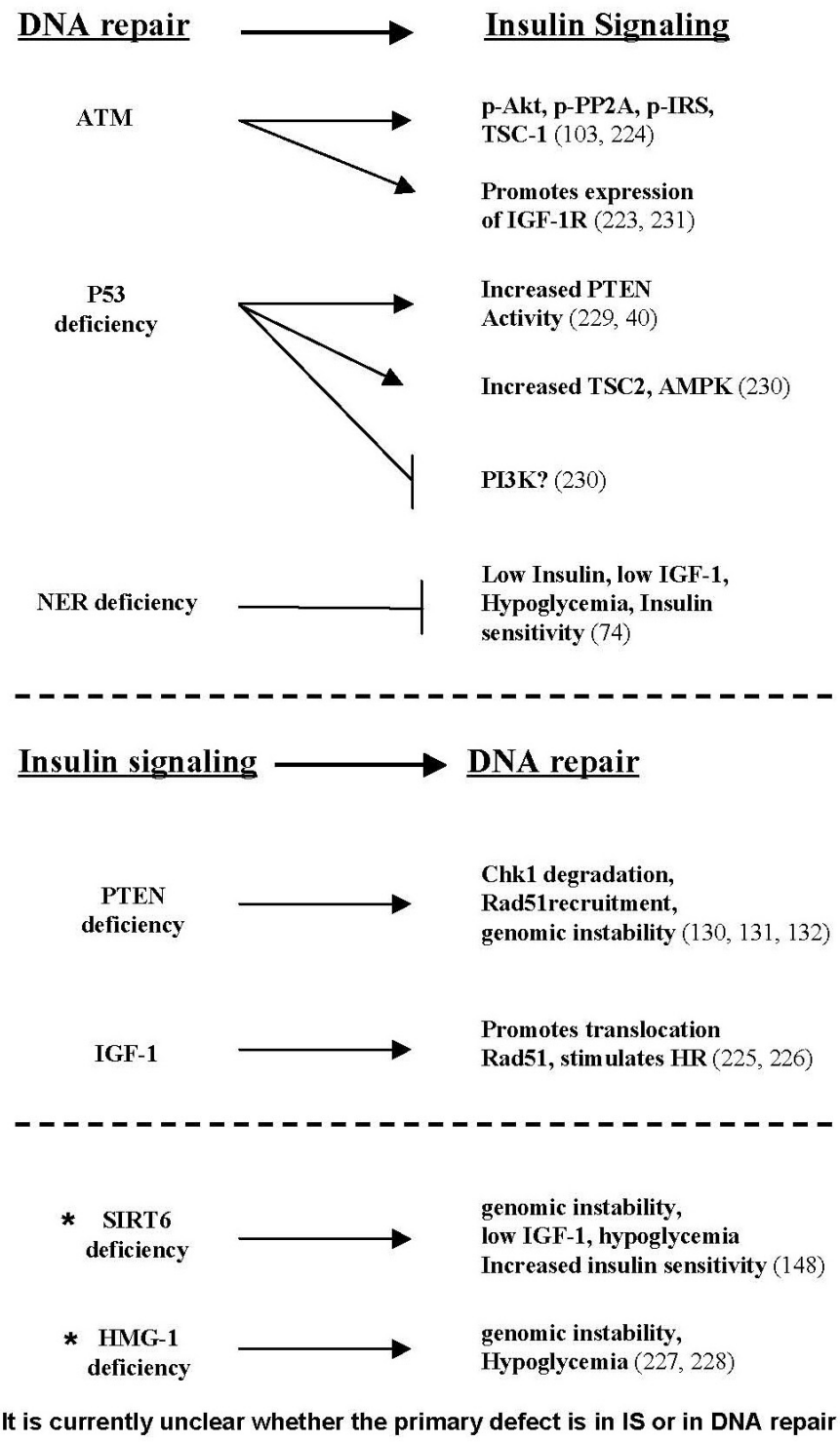


**Figure 4.** Crosstalk between DNA repair and insulin-signaling pathways. See main text for details.

DDR and/or DNA repair (Figure 4). We mentioned before that cells deficient in PTEN, a negative regulator of IIS, show genomic instability as a consequence of hyperactivity of AKT, leading to phosphorylation and degradation of Chk1 and defective checkpoint responses (130, 131). PTEN also functions to maintain genomic stability via regulation of Rad51 and centromeric stability (132). IGF-1 itself stimulates HR by promoting translocation of Rad51, a critical protein involved in HR, to sites of DNA damage (225, 226). This overlap between the

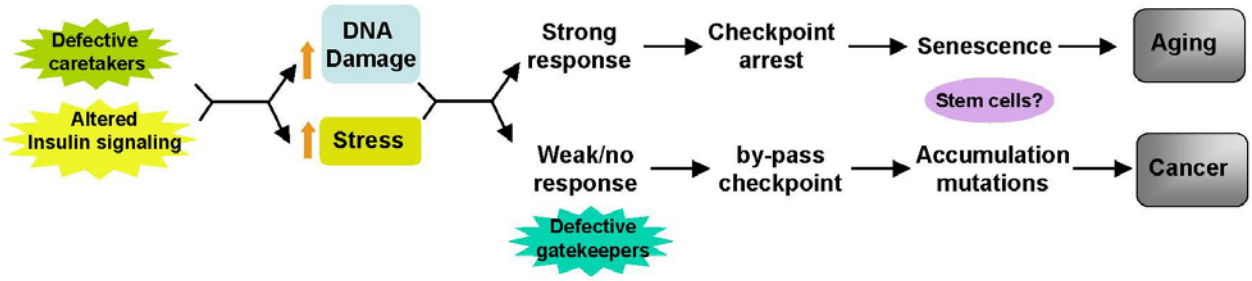


**Figure 5.** Aging and cancer: similar defects-two outcomes. Defects in insulin signaling and caretakers (DNA repair factors) lead to increase DNA damage and stress. In the case where these defects trigger a checkpoint response, cells will arrest, leading to a senescence phenotype. Chronic lost of these cells, or depletion of the stem-cell pool in critical tissues will end up in tissue degeneration and organismal aging. In those cases where a checkpoint response fails to mount, as observed when gatekeepers (checkpoint proteins) are affected, mutations in the DNA accumulate beyond control. Mutations in tumor-suppressor genes and oncogenes will lead to the development of malignancies.

DDR and IIS suggests that cells may modulate IIS in the face of DNA damage, perhaps to reduce overall cellular metabolism, lowering ROS levels and averting further DNA damage, similar to what we proposed for SIRT6 (Figure 3).

Notably, there are two other cases where defects in DNA repair and metabolic responses appear to be linked (Figure 4). Mice deficient in the chromatin adaptor HMG1 exhibited severe hypoglycemia (227), and, similar to SIRT6, cells deficient in HMG1 display pronounced genomic instability (228). Given the strong binding of SIRT6 to chromatin, it will be interesting to test whether SIRT6 acts in concert with HMG1 in order to promote genomic stability and metabolic homeostasis. The second case involves the tumor suppressor p53. It has been reported that p53 directly activates PTEN and the TOR regulator TSC2 (229, 230). As mentioned above, transgenic mice over-expressing the p44 isoform in the context of WT p53 exhibited signs of premature aging (40). Interestingly, tissues and MEFs from the p44 transgenic mice display a striking increase in insulin-signaling activity, including increased Akt phosphorylation, Foxo phosphorylation and reduced PTEN levels. Similar to SIRT6-deficient MEFs, p44 transgenic MEFs show signs of reduced proliferation capacity (40). In addition, these authors found increased phosphorylation of the MAP kinase Erk, which was followed by activation of the p53-target p21. p21 mediates a cell-cycle response, thus explaining the reduced proliferation capacity in these cells. It is still unclear whether this side of the pathway is affected in the SIRT6 and HMG1 mutants, a possibility that certainly deserves future attention.

## 8. PERSPECTIVE

DNA repair, insulin signaling and sirtuins represent common regulatory mechanisms at the crossroads between cancer and aging. The same defects in these pathways that distinguish normal cells from their cancer counterparts, appear to influence how we age as well. It is clear that both the energetic status and the genomic integrity of our cells play a primordial role in cell-fate decisions such as proliferation vs. arrest and repair vs. apoptosis. Given the role of the insulin-signaling and the DNA repair pathways in those decisions, it comes as no surprise that de-regulation of these pathways could lead to these two seemingly distinctive outcomes. However, the fact that IIS and DNA repair seem to act in a concerted fashion indicates that communication between different pathways is crucial to maintain a balance homeostasis. In this regard, sirtuins appear to influence those pathways, therefore representing *bona fide* regulators of cellular homeostasis. Whether cells take the road of cancer or the road of aging will probably depends on the intensity of the signals: likely, a checkpoint-arrest response following excessive DNA damage and/or deregulated IIS will lead to depletion of critical cell populations –like stem cells- promoting tissue decline, degenerative diseases and eventually aging. In contrast, in those situations where these same defects are not able to signal the checkpoint machinery (the so-called gatekeepers), or the defects impinge directly on those gatekeepers, uncontrolled proliferation and chronic accumulation of further DNA damage will eventually result in the appearance of tumorigenic cells (Figure 5).

Clearly, the relationship between the DDR and IIS is complex and reciprocal. Overall, in the short term, increased IIS improves survival in the face of DNA damage. However, chronic reduction of IIS promotes resistance to a variety of stresses, including DNA damage, via FOXO and potentially other factors. Conversely, IIS can be activated as part of the DDR, perhaps to promote cell survival acutely. Chronically, DNA damage may lead to downregulation of IIS in an attempt to lower ROS levels and forestall further DNA damage (74). Alternatively, metabolic defects could conceivably lead to higher ROS production, thus increasing DNA damage. Sirtuins can potentially modulate both the DDR and IIS and thus are likely to interact with these responses in complex ways. In this regard, SIRT6 might play a direct role in reducing IIS in response to genotoxic stress, thereby decreasing ROS levels and retarding accumulation of DNA damage (Figure 3). In this way, SIRT6 could function as a metabolic "rheostat", contributing to critical cell-fate decisions. Overall, elucidation of the mechanistic interplay between these longevity assurance pathways will no doubt yield important new insights into our understanding of aging and

cancer, that may in the long term result in new therapeutic approaches to treat and prevent age-related ailments and the development of malignancies.

## 9. ACKNOWLEDGMENTS

I would like to thank David Lombard for all the insightful comments and major role he played in putting this review together. Also Bjoern Schwer for helpful discussions and Nabeel Bardeesy for critically reading the manuscript. R.M is the recipient of a V Foundation Scholar Award.

## 10. REFERENCES

1. Finkel, T., M. Serrano & M. A. Blasco: The common biology of cancer and ageing. *Nature*, 448, 767-74 (2007)
2. Dali-Youcef, N., M. Lagouge, S. Froelich, C. Koehl, K. Schoonjans & J. Auwerx: Sirtuins: the 'magnificent seven', function, metabolism and longevity. *Ann Med*, 39, 335-45 (2007)
3. Saunders, L. R. & E. Verdin: Sirtuins: critical regulators at the crossroads between cancer and aging. *Oncogene*, 26, 5489-504 (2007)
4. Lombard, D. B., K. F. Chua, R. Mostoslavsky, S. Franco, M. Gostissa & F. W. Alt: DNA repair, genome stability, and aging. *Cell*, 120, 497-512 (2005)
5. Lindahl, T. & D. E. Barnes: Repair of endogenous DNA damage. *Cold Spring Harb Symp Quant Biol*, 65, 127-33 (2000)
6. Ataian, Y. & J. E. Krebs: Five repair pathways in one context: chromatin modification during DNA repair. *Biochem Cell Biol*, 84, 490-504 (2006)
7. Hoeijmakers, J. H.: Genome maintenance mechanisms for preventing cancer. *Nature*, 411, 366-74 (2001)
8. Kennedy, R. D. & A. D. D'Andrea: DNA repair pathways in clinical practice: lessons from pediatric cancer susceptibility syndromes. *J Clin Oncol*, 24, 3799-808 (2006)
9. Zhou, B. B. & S. J. Elledge: The DNA damage response: putting checkpoints in perspective. *Nature*, 408, 433-9 (2000)
10. Downs, J. A., M. C. Nussenzweig & A. Nussenzweig: Chromatin dynamics and the preservation of genetic information. *Nature*, 447, 951-8 (2007)
11. Bassing, C. H. & F. W. Alt: The cellular response to general and programmed DNA double strand breaks. *DNA Repair (Amst)*, 3, 781-96 (2004)
12. Stracker, T. H., J. W. Theunissen, M. Morales & J. H. Petrini: The Mre11 complex and the metabolism of chromosome breaks: the importance of communicating and holding things together. *DNA Repair (Amst)*, 3, 845-54 (2004)
13. Dong, Y., M. A. Hakimi, X. Chen, E. Kumaraswamy, N. S. Cooch, A. K. Godwin & R. Shiekhattar: Regulation of BRCC, a holoenzyme complex containing BRCA1 and BRCA2, by a signalosome-like subunit and its role in DNA repair. *Mol Cell*, 12, 1087-99 (2003)
14. Kim, H., J. Chen & X. Yu: Ubiquitin-binding protein RAP80 mediates BRCA1-dependent DNA damage response. *Science*, 316, 1202-5 (2007)
15. Sobhian, B., G. Shao, D. R. Lilli, A. C. Culhane, L. A. Moreau, B. Xia, D. M. Livingston & R. A. Greenberg: RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. *Science*, 316, 1198-202 (2007)
16. Wang, B., S. Matsuoka, B. A. Ballif, D. Zhang, A. Smogorzewska, S. P. Gygi & S. J. Elledge: Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response. *Science*, 316, 1194-8 (2007)
17. Bassing, C. H., W. Swat & F. W. Alt: The mechanism and regulation of chromosomal V (D)J recombination. *Cell*, 109 Suppl, S45-55 (2002)
18. Hentges, P., P. Ahnesorg, R. S. Pitcher, C. K. Bruce, B. Kysela, A. J. Green, J. Bianchi, T. E. Wilson, S. P. Jackson & A. J. Doherty: Evolutionary and functional conservation of the DNA non-homologous end-joining protein, XLF/Cernunnos. *J Biol Chem*, 281, 37517-26 (2006)
19. Lu, H., U. Pannicke, K. Schwarz & M. R. Lieber: Length-dependent binding of human XLF to DNA and stimulation of XRCC4.DNA ligase IV activity. *J Biol Chem*, 282, 11155-62 (2007)
20. Ma, Y., U. Pannicke, K. Schwarz & M. R. Lieber: Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V (D)J recombination. *Cell*, 108, 781-94 (2002)
21. Corneo, B., R. L. Wendland, L. Deriano, X. Cui, I. A. Klein, S. Y. Wong, S. Arnal, A. J. Holub, G. R. Weller, B. A. Pancake, S. Shah, V. L. Brandt, K. Meek & D. B. Roth: Rag mutations reveal robust alternative end joining. *Nature*, 449, 483-6 (2007)
22. Yan, C. T., C. Boboila, E. K. Souza, S. Franco, T. R. Hickernell, M. Murphy, S. Gumaste, M. Geyer, A. A. Zarrin, J. P. Manis, K. Rajewsky & F. W. Alt: IgH class switching and translocations use a robust non-classical end-joining pathway. *Nature*, 449, 478-82 (2007)
23. Dzierzbicki, P., P. Koprowski, M. U. Fikus, E. Malc & Z. Ciesla: Repair of oxidative damage in mitochondrial DNA of Saccharomyces cerevisiae: involvement of the MSH1-dependent pathway. *DNA Repair (Amst)*, 3, 403-11 (2004)
24. Campisi, J.: Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. *Cell*, 120, 513-22 (2005)
25. Shiloh, Y. & M. B. Kastan: ATM: genome stability, neuronal development, and cancer cross paths. *Adv Cancer Res*, 83, 209-54 (2001)
26. Stewart, G. S., R. S. Maser, T. Stankovic, D. A. Bressan, M. I. Kaplan, N. G. Jaspers, A. Raams, P. J. Byrd, J. H. Petrini & A. M. Taylor: The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder. *Cell*, 99, 577-87 (1999)
27. Ito, K., A. Hirao, F. Arai, S. Matsuoka, K. Takubo, I. Hamaguchi, K. Nomiyama, K. Hosokawa, K. Sakurada, N. Nakagata, Y. Ikeda, T. W. Mak & T. Suda: Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. *Nature*, 431, 997-1002 (2004)
28. Xu, Y. & D. Baltimore: Dual roles of ATM in the cellular response to radiation and in cell growth control. *Genes Dev*, 10, 2401-10 (1996)
29. Barlow, C., S. Hirotsune, R. Paylor, M. Liyanage, M. Eckhaus, F. Collins, Y. Shiloh, J. N. Crawley, T. Ried, D.

Tagle & A. Wynshaw-Boris: Atm-deficient mice: a paradigm of ataxia telangiectasia. *Cell*, 86, 159-71 (1996)
30. Browne, S. E., L. J. Roberts, 2nd, P. A. Dennery, S. R. Doctrow, M. F. Beal, C. Barlow & R. L. Levine: Treatment with a catalytic antioxidant corrects the neurobehavioral defect in ataxia-telangiectasia mice. *Free Radic Biol Med*, 36, 938-42 (2004)
31. Ito, K., A. Hirao, F. Arai, K. Takubo, S. Matsuoka, K. Miyamoto, M. Ohmura, K. Naka, K. Hosokawa, Y. Ikeda & T. Suda: Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. *Nat Med*, 12, 446-51 (2006)
32. Ito, K., K. Takubo, F. Arai, H. Satoh, S. Matsuoka, M. Ohmura, K. Naka, M. Azuma, K. Miyamoto, K. Hosokawa, Y. Ikeda, T. W. Mak, T. Suda & A. Hirao: Regulation of reactive oxygen species by Atm is essential for proper response to DNA double-strand breaks in lymphocytes. *J Immunol*, 178, 103-10 (2007)
33. Schubert, R., L. Erker, C. Barlow, H. Yakushiji, D. Larson, A. Russo, J. B. Mitchell & A. Wynshaw-Boris: Cancer chemoprevention by the antioxidant tempol in Atm-deficient mice. *Hum Mol Genet*, 13, 1793-802 (2004)
34. Barzilai, A., G. Rotman & Y. Shiloh: ATM deficiency and oxidative stress: a new dimension of defective response to DNA damage. *DNA Repair (Amst)*, 1, 3-25 (2002)
35. Wong, K. K., R. S. Maser, R. M. Bachoo, J. Menon, D. R. Carrasco, Y. Gu, F. W. Alt & R. A. DePinho: Telomere dysfunction and Atm deficiency compromises organ homeostasis and accelerates ageing. *Nature*, 421, 643-8 (2003)
36. Harris, S. L. & A. J. Levine: The p53 pathway: positive and negative feedback loops. *Oncogene*, 24, 2899-908 (2005)
37. Mowat, M., A. Cheng, N. Kimura, A. Bernstein & S. Benchimol: Rearrangements of the cellular p53 gene in erythroleukaemic cells transformed by Friend virus. *Nature*, 314, 633-6 (1985)
38. Rovinski, B. & S. Benchimol: Immortalization of rat embryo fibroblasts by the cellular p53 oncogene. *Oncogene*, 2, 445-52 (1988)
39. Tyner, S. D., S. Venkatachalam, J. Choi, S. Jones, N. Ghebranious, H. Igelmann, X. Lu, G. Soron, B. Cooper, C. Brayton, S. Hee Park, T. Thompson, G. Karsenty, A. Bradley & L. A. Donehower: p53 mutant mice that display early ageing-associated phenotypes. *Nature*, 415, 45-53 (2002)
40. Maier, B., W. Gluba, B. Bernier, T. Turner, K. Mohammad, T. Guise, A. Sutherland, M. Thorner & H. Scrable: Modulation of mammalian life span by the short isoform of p53. *Genes Dev*, 18, 306-19 (2004)
41. Garcia-Cao, I., M. Garcia-Cao, J. Martin-Caballero, L. M. Criado, P. Klatt, J. M. Flores, J. C. Weill, M. A. Blasco & M. Serrano: "Super p53" mice exhibit enhanced DNA damage response, are tumor resistant and age normally. *Embo J*, 21, 6225-35 (2002)
42. Matheu, A., A. Maraver, P. Klatt, I. Flores, I. Garcia-Cao, C. Borras, J. M. Flores, J. Vina, M. A. Blasco & M. Serrano: Delayed ageing through damage protection by the Arf/p53 pathway. *Nature*, 448, 375-9 (2007)
43. Kim, W. Y. & N. E. Sharpless: The regulation of INK4/ARF in cancer and aging. *Cell*, 127, 265-75 (2006)
44. Krishnamurthy, J., C. Torrice, M. R. Ramsey, G. I. Kovalev, K. Al-Regaiey, L. Su & N. E. Sharpless: Ink4a/Arf expression is a biomarker of aging. *J Clin Invest*, 114, 1299-307 (2004)
45. Helgadottir, A., G. Thorleifsson, A. Manolescu, S. Gretarsdottir, T. Blondal, A. Jonasdottir, A. Jonasdottir, A. Sigurdsson, A. Baker, A. Palsson, G. Masson, D. F. Gudbjartsson, K. P. Magnusson, K. Andersen, A. I. Levey, V. M. Backman, S. Matthiasdottir, T. Jonsdottir, S. Palsson, H. Einarsdottir, S. Gunnarsdottir, A. Gylfason, V. Vaccarino, W. C. Hooper, M. P. Reilly, C. B. Granger, H. Austin, D. J. Rader, S. H. Shah, A. A. Quyyumi, J. R. Gulcher, G. Thorgeirsson, U. Thorsteinsdottir, A. Kong & K. Stefansson: A common variant on chromosome 9p21 affects the risk of myocardial infarction. *Science*, 316, 1491-3 (2007)
46. Melzer, D., T. M. Frayling, A. Murray, A. J. Hurst, L. W. Harries, H. Song, K. Khaw, R. Luben, P. G. Surtees, S. S. Bandinelli, A. M. Corsi, L. Ferrucci, J. M. Guralnik, R. B. Wallace, A. T. Hattersley & P. D. Pharoah: A common variant of the p16 (INK4a) genetic region is associated with physical function in older people. *Mech Ageing Dev*, 128, 370-7 (2007)
47. Saxena, R., B. F. Voight, V. Lyssenko, N. P. Burtt, P. I. de Bakker, H. Chen, J. J. Roix, S. Kathiresan, J. N. Hirschhorn, M. J. Daly, T. E. Hughes, L. Groop, D. Altshuler, P. Almgren, J. C. Florez, J. Meyer, K. Ardlie, K. Bengtsson Bostrom, B. Isomaa, G. Lettre, U. Lindblad, H. N. Lyon, O. Melander, C. Newton-Cheh, P. Nilsson, M. Orho-Melander, L. Rastam, E. K. Speliotes, M. R. Taskinen, T. Tuomi, C. Guiducci, A. Berglund, J. Carlson, L. Gianniny, R. Hackett, L. Hall, J. Holmkvist, E. Laurila, M. Sjogren, M. Sterner, A. Surti, M. Svensson, M. Svensson, R. Tewhey, B. Blumenstiel, M. Parkin, M. Defelice, R. Barry, W. Brodeur, J. Camarata, N. Chia, M. Fava, J. Gibbons, B. Handsaker, C. Healy, K. Nguyen, C. Gates, C. Sougnez, D. Gage, M. Nizzari, S. B. Gabriel, G. W. Chirn, Q. Ma, H. Parikh, D. Richardson, D. Ricke & S. Purcell: Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science*, 316, 1331-6 (2007)
48. Sharpless, N. E. & R. A. DePinho: How stem cells age and why this makes us grow old. *Nat Rev Mol Cell Biol*, 8, 703-13 (2007)
49. Valerie, K. & L. F. Povirk: Regulation and mechanisms of mammalian double-strand break repair. *Oncogene*, 22, 5792-812 (2003)
50. Scully, R., A. Xie & G. Nagaraju: Molecular Functions of BRCA1 in the DNA Damage Response. *Cancer Biol Ther*, 3, 521-7 (2004)
51. Cao, L., W. Li, S. Kim, S. G. Brodie & C. X. Deng: Senescence, aging, and malignant transformation mediated by p53 in mice lacking the Brca1 full-length isoform. *Genes Dev*, 17, 201-13 (2003)
52. Bender, C. F., M. L. Sikes, R. Sullivan, L. E. Huye, M. M. Le Beau, D. B. Roth, O. K. Mirzoeva, E. M. Oltz & J. H. Petrini: Cancer predisposition and hematopoietic failure in Rad50 (S/S) mice. *Genes Dev*, 16, 2237-51 (2002)
53. Frank, K. M., N. E. Sharpless, Y. Gao, J. M. Sekiguchi, D. O. Ferguson, C. Zhu, J. P. Manis, J. Horner, R. A. DePinho & F. W. Alt: DNA ligase IV deficiency in mice

leads to defective neurogenesis and embryonic lethality via the p53 pathway. *Mol Cell*, 5, 993-1002 (2000)
54. Gao, Y., D. O. Ferguson, W. Xie, J. P. Manis, J. Sekiguchi, K. M. Frank, J. Chaudhuri, J. Horner, R. A. DePinho & F. W. Alt: Interplay of p53 and DNA-repair protein XRCC4 in tumorigenesis, genomic stability and development. *Nature*, 404, 897-900 (2000)
55. Chao, C., D. Herr, J. Chun & Y. Xu: Ser18 and 23 phosphorylation is required for p53-dependent apoptosis and tumor suppression. *Embo J*, 25, 2615-22 (2006)
56. Nijnik, A., L. Woodbine, C. Marchetti, S. Dawson, T. Lambe, C. Liu, N. P. Rodrigues, T. L. Crockford, E. Cabuy, A. Vindigni, T. Enver, J. I. Bell, P. Slijepcevic, C. C. Goodnow, P. A. Jeggo & R. J. Cornall: DNA repair is limiting for haematopoietic stem cells during ageing. *Nature*, 447, 686-90 (2007)
57. Difilippantonio, M. J., J. Zhu, H. T. Chen, E. Meffre, M. C. Nussenzweig, E. E. Max, T. Ried & A. Nussenzweig: DNA repair protein Ku80 suppresses chromosomal aberrations and malignant transformation. *Nature*, 404, 510-4 (2000)
58. Gu, Y., K. J. Seidl, G. A. Rathbun, C. Zhu, J. P. Manis, N. van der Stoep, L. Davidson, H. L. Cheng, J. M. Sekiguchi, K. Frank, P. Stanhope-Baker, M. S. Schlissel, D. B. Roth & F. W. Alt: Growth retardation and leaky SCID phenotype of Ku70-deficient mice. *Immunity*, 7, 653-65 (1997)
59. Vogel, H., D. S. Lim, G. Karsenty, M. Finegold & P. Hasty: Deletion of Ku86 causes early onset of senescence in mice. *Proc Natl Acad Sci U S A*, 96, 10770-5 (1999)
60. Li, H., H. Vogel, V. B. Holcomb, Y. Gu & P. Hasty: Deletion of Either Ku70, Ku80 or Both Causes Early Aging Without Substantially Increased Cancer. *Mol Cell Biol*, 23, 8205-8214 (2007)
61. Espejel, S., M. Martin, P. Klatt, J. Martin-Caballero, J. M. Flores & M. A. Blasco: Shorter telomeres, accelerated ageing and increased lymphoma in DNA-PKcs-deficient mice. *EMBO Rep*, 5, 503-9 (2004)
62. Karanjawala, Z. E. & M. R. Lieber: DNA damage and aging. *Mech Ageing Dev*, 125, 405-16 (2004)
63. Hasty, P., J. Campisi, J. Hoeijmakers, H. van Steeg & J. Vijg: Aging and genome maintenance: lessons from the mouse? *Science*, 299, 1355-9 (2003)
64. Wilson, D. M., 3rd & V. A. Bohr: The mechanics of base excision repair, and its relationship to aging and disease. *DNA Repair (Amst)*, 6, 544-59 (2007)
65. Hung, R. J., J. Hall, P. Brennan & P. Boffetta: Genetic polymorphisms in the base excision repair pathway and cancer risk: a HuGE review. *Am J Epidemiol*, 162, 925-42 (2005)
66. Zhang, X., X. Miao, G. Liang, B. Hao, Y. Wang, W. Tan, Y. Li, Y. Guo, F. He, Q. Wei & D. Lin: Polymorphisms in DNA base excision repair genes ADPRT and XRCC1 and risk of lung cancer. *Cancer Res*, 65, 722-6 (2005)
67. Albertella, M. R., A. Lau & M. J. O'Connor: The overexpression of specialized DNA polymerases in cancer. *DNA Repair (Amst)*, 4, 583-93 (2005)
68. Paz-Elizur, T., M. Krupsky, S. Blumenstein, D. Elinger, E. Schechtman & Z. Livneh: DNA repair activity for oxidative damage and risk of lung cancer. *J Natl Cancer Inst*, 95, 1312-9 (2003)
69. Robertson, K. A., H. A. Bullock, Y. Xu, R. Tritt, E. Zimmerman, T. M. Ulbright, R. S. Foster, L. H. Einhorn & M. R. Kelley: Altered expression of Ape1/ref-1 in germ cell tumors and overexpression in NT2 cells confers resistance to bleomycin and radiation. *Cancer Res*, 61, 2220-5 (2001)
70. Andressoo, J. O., J. H. Hoeijmakers & J. R. Mitchell: Nucleotide excision repair disorders and the balance between cancer and aging. *Cell Cycle*, 5, 2886-8 (2006)
71. Bergmann, E. & J. M. Egly: Trichothiodystrophy, a transcription syndrome. *Trends Genet*, 17, 279-86 (2001)
72. de Boer, J., J. O. Andressoo, J. de Wit, J. Huijmans, R. B. Beems, H. van Steeg, G. Weeda, G. T. van der Horst, W. van Leeuwen, A. P. Themmen, M. Meradji & J. H. Hoeijmakers: Premature aging in mice deficient in DNA repair and transcription. *Science*, 296, 1276-9 (2002)
73. Tian, M., R. Shinkura, N. Shinkura & F. W. Alt: Growth retardation, early death, and DNA repair defects in mice deficient for the nucleotide excision repair enzyme XPF. *Mol Cell Biol*, 24, 1200-5 (2004)
74. Niedernhofer, L. J., G. A. Garinis, A. Raams, A. S. Lalai, A. R. Robinson, E. Appeldoorn, H. Odijk, R. Oostendorp, A. Ahmad, W. van Leeuwen, A. F. Theil, W. Vermeulen, G. T. van der Horst, P. Meinecke, W. J. Kleijer, J. Vijg, N. G. Jaspers & J. H. Hoeijmakers: A new progeroid syndrome reveals that genotoxic stress suppresses the somatotroph axis. *Nature*, 444, 1038-43 (2006)
75. McWhir, J., J. Selfridge, D. J. Harrison, S. Squires & D. W. Melton: Mice with DNA repair gene (ERCC-1) deficiency have elevated levels of p53, liver nuclear abnormalities and die before weaning. *Nat Genet*, 5, 217-24 (1993)
76. Murai, M., Y. Enokido, N. Inamura, M. Yoshino, Y. Nakatsu, G. T. van der Horst, J. H. Hoeijmakers, K. Tanaka & H. Hatanaka: Early postnatal ataxia and abnormal cerebellar development in mice lacking Xeroderma pigmentosum Group A and Cockayne syndrome Group B DNA repair genes. *Proc Natl Acad Sci U S A*, 98, 13379-84 (2001)
77. van de Ven, M., J. O. Andressoo, V. B. Holcomb, M. von Lindern, W. M. Jong, C. I. Zeeuw, Y. Suh, P. Hasty, J. H. Hoeijmakers, G. T. van der Horst & J. R. Mitchell: Adaptive Stress Response in Segmental Progeria Resembles Long-Lived Dwarfism and Calorie Restriction in Mice. *PLoS Genet*, 2, e192 (2006)
78. van der Pluijm, I., G. A. Garinis, R. M. Brandt, T. G. Gorgels, S. W. Wijnhoven, K. E. Diderich, J. de Wit, J. R. Mitchell, C. van Oostrom, R. Beems, L. J. Niedernhofer, S. Velasco, E. C. Friedberg, K. Tanaka, H. van Steeg, J. H. Hoeijmakers & G. T. van der Horst: Impaired genome maintenance suppresses the growth hormone--insulin-like growth factor 1 axis in mice with Cockayne syndrome. *PLoS Biol*, 5, e2 (2006)
79. de Boer, J. & J. H. Hoeijmakers: Nucleotide excision repair and human syndromes. *Carcinogenesis*, 21, 453-60 (2000)
80. Balaban, R. S., S. Nemoto & T. Finkel: Mitochondria, oxidants, and aging. *Cell*, 120, 483-95 (2005)
81. Schriner, S. E., N. J. Linford, G. M. Martin, P. Treuting, C. E. Ogburn, M. Emond, P. E. Coskun, W. Ladiges, N. Wolf, H. Van Remmen, D. C. Wallace & P. S.

Rabinovitch: Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science*, 308, 1909-11 (2005)
82. Migliaccio, E., M. Giorgio, S. Mele, G. Pelicci, P. Reboldi, P. P. Pandolfi, L. Lanfrancone & P. G. Pelicci: The p66shc adaptor protein controls oxidative stress response and life span in mammals. *Nature*, 402, 309-13 (1999)
83. Giorgio, M., E. Migliaccio, F. Orsini, D. Paolucci, M. Moroni, C. Contursi, G. Pelliccia, L. Luzi, S. Minucci, M. Marcaccio, P. Pinton, R. Rizzuto, P. Bernardi, F. Paolucci & P. G. Pelicci: Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. *Cell*, 122, 221-33 (2005)
84. Yan, L., D. E. Vatner, P. O'Connor J, A. Ivessa, H. Ge, W. Chen, S. Hirotani, Y. Ishikawa, J. Sadoshima & S. F. Vatner: Type 5 adenylyl cyclase disruption increases longevity and protects against stress. *Cell*, 130, 247-58 (2007)
85. Van Remmen, H., Y. Ikeno, M. Hamilton, M. Pahlavani, N. Wolf, S. R. Thorpe, N. L. Alderson, J. W. Baynes, C. J. Epstein, T. T. Huang, J. Nelson, R. Strong & A. Richardson: Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Physiol Genomics*, 16, 29-37 (2003)
86. Scortegagna, M., K. Ding, Y. Oktay, A. Gaur, F. Thurmond, L. J. Yan, B. T. Marck, A. M. Matsumoto, J. M. Shelton, J. A. Richardson, M. J. Bennett & J. A. Garcia: Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in Epas1-/- mice. *Nat Genet*, 35, 331-40 (2003)
87. Vijg, J.: Somatic mutations and aging: a re-evaluation. *Mutat Res*, 447, 117-35 (2000)
88. Martin, G. M.: Genetic modulation of senescent phenotypes in Homo sapiens. *Cell*, 120, 523-32 (2005)
89. Kudlow, B. A., B. K. Kennedy & R. J. Monnat, Jr.: Werner and Hutchinson-Gilford progeria syndromes: mechanistic basis of human progeroid diseases. *Nat Rev Mol Cell Biol*, 8, 394-404 (2007)
90. Du, X., J. Shen, N. Kugan, E. E. Furth, D. B. Lombard, C. Cheung, S. Pak, G. Luo, R. J. Pignolo, R. A. DePinho, L. Guarente & F. B. Johnson: Telomere shortening exposes functions for the mouse werner and bloom syndrome genes. *Mol Cell Biol*, 24, 8437-46 (2004)
91. Chang, S., A. S. Multani, N. G. Cabrera, M. L. Naylor, P. Laud, D. Lombard, S. Pathak, L. Guarente & R. A. DePinho: Essential role of limiting telomeres in the pathogenesis of Werner syndrome. *Nat Genet*, 36, 877-82 (2004)
92. Lombard, D. B., C. Beard, B. Johnson, R. A. Marciniak, J. Dausman, R. Bronson, J. E. Buhlmann, R. Lipman, R. Curry, A. Sharpe, R. Jaenisch & L. Guarente: Mutations in the WRN gene in mice accelerate mortality in a p53-null background. *Mol Cell Biol*, 20, 3286-91 (2000)
93. Blasco, M. A.: Telomere length, stem cells and aging. *Nat Chem Biol*, 3, 640-9 (2007)
94. Liu, G., X. Yuan, Z. Zeng, P. Tunici, H. Ng, I. R. Abdulkadir, L. Lu, D. Irvin, K. L. Black & J. S. Yu: Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. *Mol Cancer*, 5, 67 (2006)
95. Liu, B., J. Wang, K. M. Chan, W. M. Tjia, W. Deng, X. Guan, J. D. Huang, K. M. Li, P. Y. Chau, D. J. Chen, D. Pei, A. M. Pendas, J. Cadinanos, C. Lopez-Otin, H. F. Tse, C. Hutchison, J. Chen, Y. Cao, K. S. Cheah, K. Tryggvason & Z. Zhou: Genomic instability in laminopathy-based premature aging. *Nat Med*, 11, 780-5 (2005)
96. Manju, K., B. Muralikrishna & V. K. Parnaik: Expression of disease-causing lamin A mutants impairs the formation of DNA repair foci. *J Cell Sci*, 119, 2704-14 (2006)
97. Varela, I., J. Cadinanos, A. M. Pendas, A. Gutierrez-Fernandez, A. R. Folgueras, L. M. Sanchez, Z. Zhou, F. J. Rodriguez, C. L. Stewart, J. A. Vega, K. Tryggvason, J. M. Freije & C. Lopez-Otin: Accelerated ageing in mice deficient in Zmpste24 protease is linked to p53 signalling activation. *Nature*, 437, 564-8 (2005)
98. Gurtan, A. M. & A. D. D'Andrea: Dedicated to the core: understanding the Fanconi anemia complex. *DNA Repair (Amst)*, 5, 1119-25 (2006)
99. Niedernhofer, L. J., A. S. Lalai & J. H. Hoeijmakers: Fanconi anemia (cross)linked to DNA repair. *Cell*, 123, 1191-8 (2005)
100. Saltiel, A. R. & C. R. Kahn: Insulin signalling and the regulation of glucose and lipid metabolism. *Nature*, 414, 799-806 (2001)
101. Cantley, L. C.: The phosphoinositide 3-kinase pathway. *Science*, 296, 1655-7 (2002)
102. Greer, E. L. & A. Brunet: FOXO transcription factors at the interface between longevity and tumor suppression. *Oncogene*, 24, 7410-25 (2005)
103. Matsuoka, S., B. A. Ballif, A. Smogorzewska, E. R. McDonald, 3rd, K. E. Hurov, J. Luo, C. E. Bakalarski, Z. Zhao, N. Solimini, Y. Lerenthal, Y. Shiloh, S. P. Gygi & S. J. Elledge: ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science*, 316, 1160-6 (2007)
104. Fabrizio, P., F. Pozza, S. D. Pletcher, C. M. Gendron & V. D. Longo: Regulation of longevity and stress resistance by Sch9 in yeast. *Science*, 292, 288-90 (2001)
105. Kaeberlein, M., R. W. Powers, 3rd, K. K. Steffen, E. A. Westman, D. Hu, N. Dang, E. O. Kerr, K. T. Kirkland, S. Fields & B. K. Kennedy: Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. *Science*, 310, 1193-6 (2005)
106. Guarente, L. & C. Kenyon: Genetic pathways that regulate ageing in model organisms. *Nature*, 408, 255-62. (2000)
107. Pinkston, J. M., D. Garigan, M. Hansen & C. Kenyon: Mutations that increase the life span of C. elegans inhibit tumor growth. *Science*, 313, 971-5 (2006)
108. Pinkston-Gosse, J. & C. Kenyon: DAF-16/FOXO targets genes that regulate tumor growth in Caenorhabditis elegans. *Nat Genet*, 39, 1403-9 (2007)
109. Tatar, M., A. Kopelman, D. Epstein, M. P. Tu, C. M. Yin & R. S. Garofalo: A mutant Drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science*, 292, 107-10 (2001)
110. Clancy, D. J., D. Gems, L. G. Harshman, S. Oldham, H. Stocker, E. Hafen, S. J. Leevers & L. Partridge: Extension of life-span by loss of CHICO, a Drosophila insulin receptor substrate protein. *Science*, 292, 104-6 (2001)

111. Hwangbo, D. S., B. Gershman, M. P. Tu, M. Palmer & M. Tatar: Drosophila dFOXO controls lifespan and regulates insulin signalling in brain and fat body. *Nature*, 429, 562-6 (2004)
112. Wessells, R. J., E. Fitzgerald, J. R. Cypser, M. Tatar & R. Bodmer: Insulin regulation of heart function in aging fruit flies. *Nat Genet*, 36, 1275-81 (2004)
113. Kapahi, P., B. M. Zid, T. Harper, D. Koslover, V. Sapin & S. Benzer: Regulation of lifespan in Drosophila by modulation of genes in the TOR signaling pathway. *Curr Biol*, 14, 885-90 (2004)
114. Sarbassov, D. D., S. M. Ali & D. M. Sabatini: Growing roles for the mTOR pathway. *Curr Opin Cell Biol*, 17, 596-603 (2005)
115. Vellai, T., K. Takacs-Vellai, Y. Zhang, A. L. Kovacs, L. Orosz & F. Muller: Genetics: influence of TOR kinase on lifespan in C. elegans. *Nature*, 426, 620 (2003)
116. Rincon, M., E. Rudin & N. Barzilai: The insulin/IGF-1 signaling in mammals and its relevance to human longevity. *Exp Gerontol*, 40, 873-7 (2005)
117. Taguchi, A., L. M. Wartschow & M. F. White: Brain IRS2 signaling coordinates life span and nutrient homeostasis. *Science*, 317, 369-72 (2007)
118. Bluher, M., B. B. Kahn & C. R. Kahn: Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science*, 299, 572-4 (2003)
119. Cully, M., H. You, A. J. Levine & T. W. Mak: Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. *Nat Rev Cancer*, 6, 184-92 (2006)
120. Di Cristofano, A. & P. P. Pandolfi: The multiple roles of PTEN in tumor suppression. *Cell*, 100, 387-90 (2000)
121. Eng, C.: PTEN: one gene, many syndromes. *Hum Mutat*, 22, 183-98 (2003)
122. Wanner, M., J. T. Celebi & M. Peacocke: Identification of a PTEN mutation in a family with Cowden syndrome and Bannayan-Zonana syndrome. *J Am Acad Dermatol*, 44, 183-7 (2001)
123. Di Cristofano, A., B. Pesce, C. Cordon-Cardo & P. P. Pandolfi: Pten is essential for embryonic development and tumour suppression. *Nat Genet*, 19, 348-55 (1998)
124. Li, G., G. W. Robinson, R. Lesche, H. Martinez-Diaz, Z. Jiang, N. Rozengurt, K. U. Wagner, D. C. Wu, T. F. Lane, X. Liu, L. Hennighausen & H. Wu: Conditional loss of PTEN leads to precocious development and neoplasia in the mammary gland. *Development*, 129, 4159-70 (2002)
125. Podsypanina, K., L. H. Ellenson, A. Nemes, J. Gu, M. Tamura, K. M. Yamada, C. Cordon-Cardo, G. Catoretti, P. E. Fisher & R. Parsons: Mutation of Pten/Mmac1 in mice causes neoplasia in multiple organ systems. *Proc Natl Acad Sci U S A*, 96, 1563-8 (1999)
126. Suzuki, A., M. T. Yamaguchi, T. Ohteki, T. Sasaki, T. Kaisho, Y. Kimura, R. Yoshida, A. Wakeham, T. Higuchi, M. Fukumoto, T. Tsubata, P. S. Ohashi, S. Koyasu, J. M. Penninger, T. Nakano & T. W. Mak: T cell-specific loss of Pten leads to defects in central and peripheral tolerance. *Immunity*, 14, 523-34 (2001)
127. Wang, S., J. Gao, Q. Lei, N. Rozengurt, C. Pritchard, J. Jiao, G. V. Thomas, G. Li, P. Roy-Burman, P. S. Nelson, X. Liu & H. Wu: Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. *Cancer Cell*, 4, 209-21 (2003)
128. Stambolic, V., A. Suzuki, J. L. de la Pompa, G. M. Brothers, C. Mirtsos, T. Sasaki, J. Ruland, J. M. Penninger, D. P. Siderovski & T. W. Mak: Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. *Cell*, 95, 29-39 (1998)
129. Sun, H., R. Lesche, D. M. Li, J. Liliental, H. Zhang, J. Gao, N. Gavrilova, B. Mueller, X. Liu & H. Wu: PTEN modulates cell cycle progression and cell survival by regulating phosphatidylinositol 3,4,5,-trisphosphate and Akt/protein kinase B signaling pathway. *Proc Natl Acad Sci U S A*, 96, 6199-204 (1999)
130. Puc, J., M. Keniry, H. S. Li, T. K. Pandita, A. D. Choudhury, L. Memeo, M. Mansukhani, V. V. Murty, Z. Gaciong, S. E. Meek, H. Piwnica-Worms, H. Hibshoosh & R. Parsons: Lack of PTEN sequesters CHK1 and initiates genetic instability. *Cancer Cell*, 7, 193-204 (2005)
131. Puc, J. & R. Parsons: PTEN loss inhibits CHK1 to cause double stranded-DNA breaks in cells. *Cell Cycle*, 4, 927-9 (2005)
132. Shen, W. H., A. S. Balajee, J. Wang, H. Wu, C. Eng, P. P. Pandolfi & Y. Yin: Essential role for nuclear PTEN in maintaining chromosomal integrity. *Cell*, 128, 157-70 (2007)
133. Sabatini, D. M.: mTOR and cancer: insights into a complex relationship. *Nat Rev Cancer*, 6, 729-34 (2006)
134. Paik, J. H., R. Kollipara, G. Chu, H. Ji, Y. Xiao, Z. Ding, L. Miao, Z. Tothova, J. W. Horner, D. R. Carrasco, S. Jiang, D. G. Gilliland, L. Chin, W. H. Wong, D. H. Castrillon & R. A. DePinho: FoxOs are lineage-restricted redundant tumor suppressors and regulate endothelial cell homeostasis. *Cell*, 128, 309-23 (2007)
135. Tran, H., A. Brunet, J. M. Grenier, S. R. Datta, A. J. Fornace, Jr., P. S. DiStefano, L. W. Chiang & M. E. Greenberg: DNA repair pathway stimulated by the forkhead transcription factor FOXO3a through the Gadd45 protein. *Science*, 296, 530-4 (2002)
136. Venkatesan, B., L. Mahimainathan, F. Das, N. Ghosh-Choudhury & G. Ghosh Choudhury: Downregulation of catalase by reactive oxygen species via PI 3 kinase/Akt signaling in mesangial cells. *J Cell Physiol*, 211, 457-67 (2007)
137. Dearth, R. K., X. Cui, H. J. Kim, I. Kuiatse, N. A. Lawrence, X. Zhang, J. Divisova, O. L. Britton, S. Mohsin, D. C. Allred, D. L. Hadsell & A. V. Lee: Mammary tumorigenesis and metastasis caused by overexpression of insulin receptor substrate 1 (IRS-1) or IRS-2. *Mol Cell Biol*, 26, 9302-14 (2006)
138. Altomare, D. A. & J. R. Testa: Perturbations of the AKT signaling pathway in human cancer. *Oncogene*, 24, 7455-64 (2005)
139.Cheng, J. Q., C. W. Lindsley, G. Z. Cheng, H. Yang & S. V. Nicosia: The Akt/PKB pathway: molecular target for cancer drug discovery. *Oncogene*, 24, 7482-92 (2005)
140. Skeen, J. E., P. T. Bhaskar, C. C. Chen, W. S. Chen, X. D. Peng, V. Nogueira, A. Hahn-Windgassen, H. Kiyokawa & N. Hay: Akt deficiency impairs normal cell proliferation and suppresses oncogenesis in a p53-independent and mTORC1-dependent manner. *Cancer Cell*, 10, 269-80 (2006)
141. Leslie, N. R.: The redox regulation of PI 3-kinase-dependent signaling. *Antioxid Redox Signal*, 8, 1765-74 (2006)

142. Pelicano, H., R. H. Xu, M. Du, L. Feng, R. Sasaki, J. S. Carew, Y. Hu, L. Ramdas, L. Hu, M. J. Keating, W. Zhang, W. Plunkett & P. Huang: Mitochondrial respiration defects in cancer cells cause activation of Akt survival pathway through a redox-mediated mechanism. *J Cell Biol*, 175, 913-23 (2006)
143. Klar, A. J., J. N. Strathern, J. R. Broach & J. B. Hicks: Regulation of transcription in expressed and unexpressed mating type cassettes of yeast. *Nature*, 289, 239-44 (1981)
144. Rine, J. & I. Herskowitz: Four genes responsible for a position effect on expression from HML and HMR in Saccharomyces cerevisiae. *Genetics*, 116, 9-22. (1987)
145. Blander, G. & L. Guarente: The sir2 family of protein deacetylases. *Annu Rev Biochem*, 73, 417-35 (2004)
146. Frye, R. A.: Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. *Biochem Biophys Res Commun*, 273, 793-8. (2000)
147. Haigis, M. C., R. Mostoslavsky, K. M. Haigis, K. Fahie, D. C. Christodoulou, A. J. Murphy, D. M. Valenzuela, G. D. Yancopoulos, M. Karow, G. Blander, C. Wolberger, T. A. Prolla, R. Weindruch, F. W. Alt & L. Guarente: SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. *Cell*, 126, 941-54 (2006)
148. Mostoslavsky, R., K. F. Chua, D. B. Lombard, W. W. Pang, M. R. Fischer, L. Gellon, P. Liu, G. Mostoslavsky, S. Franco, M. M. Murphy, K. D. Mills, P. Patel, J. T. Hsu, A. L. Hong, E. Ford, H. L. Cheng, C. Kennedy, N. Nunez, R. Bronson, D. Frendewey, W. Auerbach, D. Valenzuela, M. Karow, M. O. Hottiger, S. Hursting, J. C. Barrett, L. Guarente, R. Mulligan, B. Demple, G. D. Yancopoulos & F. W. Alt: Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell*, 124, 315-29 (2006)
149. Haigis, M. C. & L. P. Guarente: Mammalian sirtuins--emerging roles in physiology, aging, and calorie restriction. *Genes Dev*, 20, 2913-21 (2006)
150. Wang, C., L. Chen, X. Hou, Z. Li, N. Kabra, Y. Ma, S. Nemoto, T. Finkel, W. Gu, W. D. Cress & J. Chen: Interactions between E2F1 and SirT1 regulate apoptotic response to DNA damage. *Nat Cell Biol*, 8, 1025-31 (2006)
151. Sinclair, D. A. & L. Guarente: Extrachromosomal rDNA circles--a cause of aging in yeast. *Cell*, 91, 1033-42. (1997)
152. Kaeberlein, M., M. McVey & L. Guarente: The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. *Genes Dev*, 13, 2570-80. (1999)
153. Weitao, T., M. Budd & J. L. Campbell: Evidence that yeast SGS1, DNA2, SRS2, and FOB1 interact to maintain rDNA stability. *Mutat Res*, 532, 157-72 (2003)
154. Tsukamoto, Y., J. Kato & H. Ikeda: Silencing factors participate in DNA repair and recombination in Saccharomyces cerevisiae. *Nature*, 388, 900-3. (1997)
155. Martin, S. G., T. Laroche, N. Suka, M. Grunstein & S. M. Gasser: Relocalization of telomeric Ku and SIR proteins in response to DNA strand breaks in yeast. *Cell*, 97, 621-33 (1999)
156. Mills, K. D., D. A. Sinclair & L. Guarente: MEC1-dependent redistribution of the Sir3 silencing protein from telomeres to DNA double-strand breaks. *Cell*, 97, 609-20. (1999)
157. McAinsh, A. D., S. Scott-Drew, J. A. Murray & S. P. Jackson: DNA damage triggers disruption of telomeric silencing and Mec1p- dependent relocation of Sir3p. *Curr Biol*, 9, 963-6. (1999)
158. Astrom, S. U., S. M. Okamura & J. Rine: Yeast cell-type regulation of DNA repair. *Nature*, 397, 310- (1999)
159. Lee, S. E., F. Paques, J. Sylvan & J. E. Haber: Role of yeast SIR genes and mating type in directing DNA double-strand breaks to homologous and non-homologous repair paths. *Curr Biol*, 9, 767-70. (1999)
160. Maas, N. L., K. M. Miller, L. G. DeFazio & D. P. Toczyski: Cell cycle and checkpoint regulation of histone H3 K56 acetylation by Hst3 and Hst4. *Mol Cell*, 23, 109-19 (2006)
161. Celic, I., H. Masumoto, W. P. Griffith, P. Meluh, R. J. Cotter, J. D. Boeke & A. Verreault: The sirtuins hst3 and Hst4p preserve genome integrity by controlling histone h3 lysine 56 deacetylation. *Curr Biol*, 16, 1280-9 (2006)
162. Brachmann, C. B., J. M. Sherman, S. E. Devine, E. E. Cameron, L. Pillus & J. D. Boeke: The SIR2 gene family, conserved from bacteria to humans, functions in silencing, cell cycle progression, and chromosome stability. *Genes Dev*, 9, 2888-902. (1995)
163. Garcia-Salcedo, J. A., P. Gijon, D. P. Nolan, P. Tebabi & E. Pays: A chromosomal SIR2 homologue with both histone NAD-dependent ADP-ribosyltransferase and deacetylase activities is involved in DNA repair in Trypanosoma brucei. *Embo J*, 22, 5851-62 (2003)
164. Yuan, Z., X. Zhang, N. Sengupta, W. S. Lane & E. Seto: SIRT1 regulates the function of the Nijmegen breakage syndrome protein. *Mol Cell*, 27, 149-62 (2007)
165. Tissenbaum, H. A. & L. Guarente: Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. *Nature*, 410, 227-30. (2001)
166. Berdichevsky, A., M. Viswanathan, H. R. Horvitz & L. Guarente: C. elegans SIR-2.1 interacts with 14-3-3 proteins to activate DAF-16 and extend life span. *Cell*, 125, 1165-77 (2006)
167. Wang, Y., S. W. Oh, B. Deplancke, J. Luo, A. J. Walhout & H. A. Tissenbaum: C. elegans 14-3-3 proteins regulate life span and interact with SIR-2.1 and DAF-16/FOXO. *Mech Ageing Dev*, 127, 741-7 (2006)
168. Wang, Y. & H. A. Tissenbaum: Overlapping and distinct functions for a Caenorhabditis elegans SIR2 and DAF-16/FOXO. *Mech Ageing Dev*, 127, 48-56 (2006)
169. Wang, F., M. Nguyen, F. X. Qin & Q. Tong: SIRT2 deacetylates FOXO3a in response to oxidative stress and caloric restriction. *Aging Cell*, 6, 505-514 (2007)
170. Moynihan, K. A., A. A. Grimm, M. M. Plueger, E. Bernal-Mizrachi, E. Ford, C. Cras-Meneur, M. A. Permutt & S. Imai: Increased dosage of mammalian Sir2 in pancreatic beta cells enhances glucose-stimulated insulin secretion in mice. *Cell Metab*, 2, 105-17 (2005)
171. Bordone, L., M. C. Motta, F. Picard, A. Robinson, U. S. Jhala, J. Apfeld, T. McDonagh, M. Lemieux, M. McBurney, A. Szilvasi, E. J. Easlon, S. J. Lin & L. Guarente: Sirt1 regulates insulin secretion by repressing UCP2 in pancreatic beta cells. *PLoS Biol*, 4, e31 (2006)
172. Lemieux, M. E., X. Yang, K. Jardine, X. He, K. X. Jacobsen, W. A. Staines, M. E. Harper & M. W. McBurney: The Sirt1 deacetylase modulates the insulin-

like growth factor signaling pathway in mammals. *Mech Ageing Dev*, 126, 1097-105 (2005)
173. McBurney, M. W., X. Yang, K. Jardine, M. Hixon, K. Boekelheide, J. R. Webb, P. M. Lansdorp & M. Lemieux: The mammalian SIR2alpha protein has a role in embryogenesis and gametogenesis. *Mol Cell Biol*, 23, 38-54 (2003)
174. Cheng, H., R. Mostoslavsky, S. i. Saito, J. P. Manis, Y. Gu, P. Patel, R. Bronson, E. Appella, F. W. Alt & a. K. F. Chua: Developmental defects and p53 hyperacetylation in Sir2 homolog (SIRT1)-deficient mice. *Proceedings of the National Academy of Sciences*, 100, 10794-10799 (2003)
175. Sohal, R. S. & R. Weindruch: Oxidative stress, caloric restriction, and aging. *Science*, 273, 59-63 (1996)
176. Panowski, S. H., S. Wolff, H. Aguilaniu, J. Durieux & A. Dillin: PHA-4/Foxa mediates diet-restriction-induced longevity of C. elegans. *Nature*, 447, 550-5 (2007)
177. Clancy, D. J., D. Gems, E. Hafen, S. J. Leevers & L. Partridge: Dietary restriction in long-lived dwarf flies. *Science*, 296, 319 (2002)
178. Lakowski, B. & S. Hekimi: The genetics of caloric restriction in Caenorhabditis elegans. *Proc Natl Acad Sci U S A*, 95, 13091-6 (1998)
179. Dempsey, J. L., M. Pfeiffer & A. A. Morley: Effect of dietary restriction on *in vivo* somatic mutation in mice. *Mutat Res*, 291, 141-5 (1993)
180. Aidoo, A., R. A. Mittelstaedt, M. E. Bishop, L. E. Lyn-Cook, Y. J. Chen, P. Duffy & R. H. Heflich: Effect of caloric restriction on Hprt lymphocyte mutation in aging rats. *Mutat Res*, 527, 57-66 (2003)
181. Barja, G.: Free radicals and aging. *Trends Neurosci*, 27, 595-600 (2004)
182. Bartke, A., J. C. Wright, J. A. Mattison, D. K. Ingram, R. A. Miller & G. S. Roth: Extending the lifespan of long-lived mice. *Nature*, 414, 412 (2001)
183. Bonkowski, M. S., J. S. Rocha, M. M. Masternak, K. A. Al Regaiey & A. Bartke: Targeted disruption of growth hormone receptor interferes with the beneficial actions of calorie restriction. *Proc Natl Acad Sci U S A*, 103, 7901-5 (2006)
184. Wood, J. G., B. Rogina, S. Lavu, K. Howitz, S. L. Helfand, M. Tatar & D. Sinclair: Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature*, 430, 686-689 (2004)
185. Chen, D., A. D. Steele, S. Lindquist & L. Guarente: Increase in activity during calorie restriction requires Sirt1. *Science*, 310, 1641 (2005)
186. Lamming, D. W., M. Latorre-Esteves, O. Medvedik, S. N. Wong, F. A. Tsang, C. Wang, S. J. Lin & D. A. Sinclair: HST2 mediates SIR2-independent life-span extension by calorie restriction. *Science*, 309, 1861-4 (2005)
187. Longo, V. D. & B. K. Kennedy: Sirtuins in aging and age-related disease. *Cell*, 126, 257-68 (2006)
188. Rogina, B. & S. L. Helfand: Sir2 mediates longevity in the fly through a pathway related to calorie restriction. *Proc Natl Acad Sci U S A*, 101, 15998-6003 (2004)
189. Kaeberlein, T. L., E. D. Smith, M. Tsuchiya, K. L. Welton, J. H. Thomas, S. Fields, B. K. Kennedy & M. Kaeberlein: Lifespan extension in Caenorhabditis elegans by complete removal of food. *Aging Cell*, 5, 487-94 (2006)
190. Bass, T. M., D. Weinkove, K. Houthoofd, D. Gems & L. Partridge: Effects of resveratrol on lifespan in Drosophila melanogaster and Caenorhabditis elegans. *Mech Ageing Dev*, 128, 546-52 (2007)
191. Shi, T., F. Wang, E. Stieren & Q. Tong: SIRT3, a mitochondrial sirtuin deacetylase, regulates mitochondrial function and thermogenesis in brown adipocytes. *J Biol Chem*, 280, 13560-7 (2005)
192. Cohen, H. Y., C. Miller, K. J. Bitterman, N. R. Wall, B. Hekking, B. Kessler, K. T. Howitz, M. Gorospe, R. de Cabo & D. A. Sinclair: Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science*, 305, 390-2 (2004)
193. Al-Regaiey, K. A., M. M. Masternak, M. Bonkowski, L. Sun & A. Bartke: Long-lived growth hormone receptor knockout mice: interaction of reduced insulin-like growth factor i/insulin signaling and caloric restriction. *Endocrinology*, 146, 851-60 (2005)
194. Rodgers, J. T., C. Lerin, W. Haas, S. P. Gygi, B. M. Spiegelman & P. Puigserver: Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature*, 434, 113-8 (2005)
195. Gerhart-Hines, Z., J. T. Rodgers, O. Bare, C. Lerin, S. H. Kim, R. Mostoslavsky, F. W. Alt, Z. Wu & P. Puigserver: Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha. *Embo J*, 26, 1913-23 (2007)
196. Kim, D., M. D. Nguyen, M. M. Dobbin, A. Fischer, F. Sananbenesi, J. T. Rodgers, I. Delalle, J. A. Baur, G. Sui, S. M. Armour, P. Puigserver, D. A. Sinclair & L. H. Tsai: SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer's disease and amyotrophic lateral sclerosis. *Embo J* (2007)
197. Outeiro, T. F., E. Kontopoulos, S. M. Altmann, I. Kufareva, K. E. Strathearn, A. M. Amore, C. B. Volk, M. M. Maxwell, J. C. Rochet, P. J. McLean, A. B. Young, R. Abagyan, M. B. Feany, B. T. Hyman & A. G. Kazantsev: Sirtuin 2 inhibitors rescue alpha-synuclein-mediated toxicity in models of Parkinson's disease. *Science*, 317, 516-9 (2007)
198. Suzuki, K. & T. Koike: Mammalian Sir2-related protein (SIRT) 2-mediated modulation of resistance to axonal degeneration in slow Wallerian degeneration mice: a crucial role of tubulin deacetylation. *Neuroscience*, 147, 599-612 (2007)
199. Baur, J. A., K. J. Pearson, N. L. Price, H. A. Jamieson, C. Lerin, A. Kalra, V. V. Prabhu, J. S. Allard, G. Lopez-Lluch, K. Lewis, P. J. Pistell, S. Poosala, K. G. Becker, O. Boss, D. Gwinn, M. Wang, S. Ramaswamy, K. W. Fishbein, R. G. Spencer, E. G. Lakatta, D. Le Couteur, R. J. Shaw, P. Navas, P. Puigserver, D. K. Ingram, R. de Cabo & D. A. Sinclair: Resveratrol improves health and survival of mice on a high-calorie diet. *Nature*, 444, 337-42 (2006)
200. Lagouge, M., C. Argmann, Z. Gerhart-Hines, H. Meziane, C. Lerin, F. Daussin, N. Messadeq, J. Milne, P. Lambert, P. Elliott, B. Geny, M. Laakso, P. Puigserver & J. Auwerx: Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell*, 127, 1109-22 (2006)
201. Bellizzi, D., G. Rose, P. Cavalcante, G. Covello, S. Dato, F. De Rango, V. Greco, M. Maggiolini, E. Feraco, V. Mari, C. Franceschi, G. Passarino & G. De Benedictis: A

novel VNTR enhancer within the SIRT3 gene, a human homologue of SIR2, is associated with survival at oldest ages. *Genomics*, 85, 258-63 (2005)
202. Rose, G., S. Dato, K. Altomare, D. Bellizzi, S. Garasto, V. Greco, G. Passarino, E. Feraco, V. Mari, C. Barbi, M. BonaFe, C. Franceschi, Q. Tan, S. Boiko, A. I. Yashin & G. De Benedictis: Variability of the SIRT3 gene, human silent information regulator Sir2 homologue, and survivorship in the elderly. *Exp Gerontol*, 38, 1065-70 (2003)
203. Vaquero, A., M. Scher, D. Lee, H. Erdjument-Bromage, P. Tempst & D. Reinberg: Human SirT1 Interacts with Histone H1 and Promotes Formation of Facultative Heterochromatin. *Mol Cell*, 16, 93-105 (2004)
204. Narita, M., S. Nunez, E. Heard, A. W. Lin, S. A. Hearn, D. L. Spector, G. J. Hannon & S. W. Lowe: Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. *Cell*, 113, 703-16 (2003)
205. Alcendor, R. R., S. Gao, P. Zhai, D. Zablocki, E. Holle, X. Yu, B. Tian, T. Wagner, S. F. Vatner & J. Sadoshima: Sirt1 regulates aging and resistance to oxidative stress in the heart. *Circ Res*, 100, 1512-21 (2007)
206. Chua, K. F., R. Mostoslavsky, D. B. Lombard, W. W. Pang, S. Saito, S. Franco, D. Kaushal, H. L. Cheng, M. R. Fischer, N. Stokes, M. M. Murphy, E. Appella & F. W. Alt: Mammalian SIRT1 limits replicative life span in response to chronic genotoxic stress. *Cell Metab*, 2, 67-76 (2005)
207. Chen, W. Y., D. H. Wang, R. C. Yen, J. Luo, W. Gu & S. B. Baylin: Tumor suppressor HIC1 directly regulates SIRT1 to modulate p53-dependent DNA-damage responses. *Cell*, 123, 437-48 (2005)
208. Pruitt, K., R. L. Zinn, J. E. Ohm, K. M. McGarvey, S. H. Kang, D. N. Watkins, J. G. Herman & S. B. Baylin: Inhibition of SIRT1 reactivates silenced cancer genes without loss of promoter DNA hypermethylation. *PLoS Genet*, 2, e40 (2006)
209. Wales, M. M., M. A. Biel, W. el Deiry, B. D. Nelkin, J. P. Issa, W. K. Cavenee, S. J. Kuerbitz & S. B. Baylin: p53 activates expression of HIC-1, a new candidate tumour suppressor gene on 17p13.3. *Nat Med*, 1, 570-7 (1995)
210. Brunet, A., L. B. Sweeney, J. F. Sturgill, K. F. Chua, P. L. Greer, Y. Lin, H. Tran, S. E. Ross, R. Mostoslavsky, H. Y. Cohen, L. S. Hu, H. L. Cheng, M. P. Jedrychowski, S. P. Gygi, D. A. Sinclair, F. W. Alt & M. E. Greenberg: Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science*, 303, 2011-5 (2004)
211. Bradbury, C. A., F. L. Khanim, R. Hayden, C. M. Bunce, D. A. White, M. T. Drayson, C. Craddock & B. M. Turner: Histone deacetylases in acute myeloid leukaemia show a distinctive pattern of expression that changes selectively in response to deacetylase inhibitors. *Leukemia*, 19, 1751-9 (2005)
212. Hida, Y., Y. Kubo, K. Murao & S. Arase: Strong expression of a longevity-related protein, SIRT1, in Bowen's disease. *Arch Dermatol Res*, 299, 103-6 (2007)
213. Kuzmichev, A., R. Margueron, A. Vaquero, T. S. Preissner, M. Scher, A. Kirmizis, X. Ouyang, N. Brockdorff, C. Abate-Shen, P. Farnham & D. Reinberg: Composition and histone substrates of polycomb repressive group complexes change during cellular differentiation. *Proc Natl Acad Sci U S A*, 102, 1859-64 (2005)
214. Fu, M., M. Liu, A. A. Sauve, X. Jiao, X. Zhang, X. Wu, M. J. Powell, T. Yang, W. Gu, M. L. Avantaggiati, N. Pattabiraman, T. G. Pestell, F. Wang, A. A. Quong, C. Wang & R. G. Pestell: Hormonal control of androgen receptor function through SIRT1. *Mol Cell Biol*, 26, 8122-35 (2006)
215. Dryden, S. C., F. A. Nahhas, J. E. Nowak, A. S. Goustin & M. A. Tainsky: Role for Human SIRT2 NAD-Dependent Deacetylase Activity in Control of Mitotic Exit in the Cell Cycle. *Mol Cell Biol*, 23, 3173-85 (2003)
216. Hiratsuka, M., T. Inoue, T. Toda, N. Kimura, Y. Shirayoshi, H. Kamitani, T. Watanabe, E. Ohama, C. G. Tahimic, A. Kurimasa & M. Oshimura: Proteomics-based identification of differentially expressed genes in human gliomas: down-regulation of SIRT2 gene. *Biochem Biophys Res Commun*, 309, 558-66 (2003)
217. Inoue, T., M. Hiratsuka, M. Osaki, H. Yamada, I. Kishimoto, S. Yamaguchi, S. Nakano, M. Katoh, H. Ito & M. Oshimura: SIRT2, a tubulin deacetylase, acts to block the entry to chromosome condensation in response to mitotic stress. *Oncogene*, 26, 945-57 (2007)
218. Ashraf, N., Zino, S., Macintyre, A., Kingsmore, D., Payne, A.P., George, W.D. & P.G. Shiels: Altered sirtuin expression is associated with node-positive breast cancer. *Br J Cancer*, 95, 1056-61 (2006)
219. De Nigris, F., Cerutti, J., Morelli, C., Califano, D., Chiariotti, L., Viglietto, G. & A. Fusco: Isolation of SIR-like gene, SIR-T8, that is overexpressed in thyroid carcinoma cell lines and tissues. *Br J Cancer*, 86, 917-23 (2002)
220. Beard, B. C., S. H. Wilson & M. J. Smerdon: Suppressed catalytic activity of base excision repair enzymes on rotationally positioned uracil in nucleosomes. *Proc Natl Acad Sci U S A*, 100, 7465-70 (2003)
221. Nilsen, H., T. Lindahl & A. Verreault: DNA base excision repair of uracil residues in reconstituted nucleosome core particles. *Embo J*, 21, 5943-52 (2002)
222. Levine, A. J., Z. Feng, T. W. Mak, H. You & S. Jin: Coordination and communication between the p53 and IGF-1-AKT-TOR signal transduction pathways. *Genes Dev*, 20, 267-75 (2006)
223. Peretz, S., R. Jensen, R. Baserga & P. M. Glazer: ATM-dependent expression of the insulin-like growth factor-I receptor in a pathway regulating radiation response. *Proc Natl Acad Sci U S A*, 98, 1676-81 (2001)
224. Schneider, J. G., B. N. Finck, J. Ren, K. N. Standley, M. Takagi, K. H. Maclean, C. Bernal-Mizrachi, A. J. Muslin, M. B. Kastan & C. F. Semenkovich: ATM-dependent suppression of stress signaling reduces vascular disease in metabolic syndrome. *Cell Metab*, 4, 377-89 (2006)
225. Trojanek, J., T. Ho, L. Del Valle, M. Nowicki, J. Y. Wang, A. Lassak, F. Peruzzi, K. Khalili, T. Skorski & K. Reiss: Role of the insulin-like growth factor I/insulin receptor substrate 1 axis in Rad51 trafficking and DNA repair by homologous recombination. *Mol Cell Biol*, 23, 7510-24 (2003)
226. Yang, S., J. Chintapalli, L. Sodagum, S. Baskin, A. Malhotra, K. Reiss & L. G. Meggs: Activated IGF-1R inhibits hyperglycemia-induced DNA damage and

promotes DNA repair by homologous recombination. *Am J Physiol Renal Physiol*, 289, F1144-52 (2005)
227. Calogero, S., F. Grassi, A. Aguzzi, T. Voigtlander, P. Ferrier, S. Ferrari & M. E. Bianchi: The lack of chromosomal protein Hmg1 does not disrupt cell growth but causes lethal hypoglycaemia in newborn mice. *Nat Genet*, 22, 276-80 (1999)
228. Giavara, S., E. Kosmidou, M. P. Hande, M. E. Bianchi, A. Morgan, F. d'Adda di Fagagna & S. P. Jackson: Yeast Nhp6A/B and mammalian Hmgb1 facilitate the maintenance of genome stability. *Curr Biol*, 15, 68-72 (2005)
229. Stambolic, V., D. MacPherson, D. Sas, Y. Lin, B. Snow, Y. Jang, S. Benchimol & T. W. Mak: Regulation of PTEN transcription by p53. *Mol Cell*, 8, 317-25 (2001)
230. Feng, Z., H. Zhang, A. J. Levine & S. Jin: The coordinate regulation of the p53 and mTOR pathways in cells. *Proc Natl Acad Sci U S A*, 102, 8204-9 (2005)
231. Shahrabani-Gargir, L., Pandita, T.K. & H. Werner: Ataxia-telangiectasia mutated gene controls insulin-like growth factor I receptor gene exprssion in a deoxyribonucleic acid damage response pathway via mechanisms involving zinc-finger transcription factors SP1 and WT1. *Endocrinology* 145, 5679-87 (2004)

**Key Words:** Lifespan, Cancer, Sirtuins, SIRT6, Genomic Instability, DNA repair, DNA Damage, Insulin Signaling, Metabolic Homeostasis, Review

**Send correspondence to:** Raul Mostoslavsky, Department of Medicine, The Massachusetts General Hospital Cancer Center, Harvard Medical School, 185 Cambridge St., Boston MA, 02114, Tel: 617-6433146, Fax: 617-6433170, E-mail: rmostoslavsky@mgh.harvard.edu