

The use of genetically engineered model systems for research on human aging

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1. ABSTRACT

A major goal in the field of aging research is to identify molecular mechanisms of aging at the cellular level, which are anticipated to form the basis for the development of age-associated dysfunctions and diseases in human beings. Recent progress in research into model organisms of aging has allowed determining precise molecular mechanisms and genetic determinants of the aging process, which appear to be conserved in evolution and some of which apply to human aging as well. The consortium of the authors focuses on aging mechanisms at the cellular level, and exploits the potential of genetic analyses in lower eukaryotic model organisms for a better understanding of regulatory pathways implicated in aging processes. We have established a new database (GiSAO), which provides a unique resource for the analysis of genome-wide expression patterns as being regulated by senescence, apoptosis and oxidative stress in our model systems. This has led to the identification of candidate genes, which are being tested for their impact on lifespan regulation in yeast, the fruit fly *Drosophila melanogaster* and the nematode *C.elegans*.

2. THE USE OF GENETICALLY ALTERED MODEL ORGANISMS FOR AGING RESEARCH

2.1. Rationale

2.2. General approach of the consortium

Maximum life expectancy has been steadily rising for at least 150 years in the developed countries, and is expected to keep on doing so (1). It can be anticipated that this development will cause a major social and economic burden for these societies, unless we will be able to promote healthy aging. There is general consensus that in order to improve the quality of life and health of elderly people, huge efforts in basic research have to be undertaken to understand mechanisms of human aging, and to design new interventions to preserve health into old age. A major problem to reach this ambitious goal is based on the fact that human aging is not easily amenable for modern molecular genetic analyses, and that genetic experiments involving human beings are unethical. In addition, any interventions that would considerably increase human lifespan, even if they were developed now, would take decades to be tested for their efficiency. In view of these problems, research on model organisms and model systems

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has been proposed and carried out on a broad scale in the international scientific community. It is hoped that the use of short-lived organisms will allow discerning the molecular mechanisms underlying the aging process, since some of these mechanisms may be conserved and govern human aging as well. As a backup for this approach, many researchers have used human cellular models for aging, based on cellular senescence, that can be reached by either extended passaging of primary cells, or through the application of a variety of signals, such as oxidative stress, irradiation, the expression of oncogenes, and many other stimuli. As a major drawback for the interpretation of experiments with such model systems, the question remains whether aging at the cellular level, studied *in vitro*, is guided by the same mechanisms as the ones modulating the rate of aging *in vivo*.

To bridge this gap, our consortium not only seeks to identify mechanisms and candidate genes in the context of human cell culture studies but also sets out to test whether these mechanisms i) are conserved in other species and ii) contribute to organismic aging in lower model organisms which are amenable to genetic analysis. This approach bears the promise to identify the relevant human candidate genes and regulatory mechanisms that modulate human aging, with the ultimate goal to design new interventions which eventually retard aging. In addition, a better understanding of mechanisms conserved between model organisms and human cell culture systems will have a positive impact on our understanding of the biology of aging in general.

Genetic experiments in lower eukaryotic model organisms have identified genes that modulate the lifespan of different species, which are referred to as gerontogenes (2). This work has led to the identification of several evolutionarily conserved pathways of aging and current emphasis is on better understanding how the human homologues of these pathways contribute to aging in human beings. However, significant differences in physiology exist between human beings and all short-lived model organisms including the mouse; therefore, the extrapolation of data obtained with model organisms for research on human aging has proven difficult. The current project is based on a genome-wide comprehensive analysis of gene expression differences associated with cellular senescence in a variety of human tissues. The information obtained from these experiments needs to be filtered, in order to identify those changes that are relevant for the aging process. On the one hand, the conservation of gene expression changes between different human tissues will elucidate conserved changes that have a high probability to be relevant for aging. Second, candidate genes that are identified in human cell culture senescence models are subsequently tested for their impact on the lifespan of yeast, flies and worms. The expectation is that genes that i) are co-regulated with aging in different human tissues and ii) modulate lifespan in lower eukaryotes, may be important for human aging. Therefore, a large set of gene expression data from various human and mouse model systems of aging was collected, mostly based on *in vitro* senescence models using cells from various tissues. This major effort

laid the basis for the creation of a unique gene expression data bank referred to as the GiSAO database (“Genes involved in Senescence, Apoptosis and Oxidative stress”). In this data repository, genes that are differentially regulated in response to natural aging or in stress-induced senescence, as well as genes implicated in signaling and execution of apoptotic cell death, are organized as a searchable set of data, which allows the identification of candidate genes that are relevant for the aging of more than one human cell type. This data bank allows us to link the identity of human candidate genes to the relevant orthologues in lower eukaryotic model organisms, such as yeast, worms and flies. Genes that are identified as candidates by RNA profiling in mammalian cell culture models and for which a homologous yeast gene exists, are functionally analyzed in yeast lifespan experiments. In a pilot study, we have already tested a selected number of human candidate genes and as a first proof of concept for our working hypothesis, we have identified yeast orthologues for two specific human candidate genes. It could be shown that disruption of these genes extends yeast lifespan (Jansen-Dürr, Madeo & Fröhlich, unpublished). Similarly, a pilot study with gene knockdown in *Drosophila*, has identified a conserved gene, which restricts lifespan, since siRNA-mediated knockdown of its orthologue in *Drosophila* extends lifespan (Minois & Jansen-Dürr, unpublished). In the future, raw data sets within the GiSAO database will be aggregated together with additional information gained by functional analyses and clustered in order to identify additional candidate genes that may play a role in human cellular senescence and possibly in aging.

The work described above illustrates the usefulness of the concept, i.e. to do comparative analyses in human cells and model organisms. However, the original concept was centered on yeast and mammalian systems without taking into account model organisms that are located in between these two extremes in the evolutionary scale. The upcoming years will also include work on flies and worms, as being described in the following section. To tackle this question, a library of transgenic RNAi *D. melanogaster* lines targeting almost the entire fly genome (3) will be used to test the effect of down-regulation of candidate genes at the organismal level in a multicellular model species. This will be most informative for those human and mouse genes, for which yeast homologues do not exist. Selected candidate genes, for which orthologues in *C.elegans* have been identified, will also be knocked down by RNA interference in this organism. Genes, which upon knockdown in *C.elegans* or *Drosophila* lead to lifespan extension, will be further characterized in human cellular models. Primary cells from human tissue (e.g. skin fibroblasts) will be used as a model system to evaluate the functional significance of human candidate genes. The rationale of our approach for the identification of candidate genes and their validation is shown in Figure 1. The proposed screening procedure combines expression profiling in human aging models with genetic analyses in model systems. Other groups have carried out genome-wide gene knockdown and gene knockout screens with the goal to establish a comprehensive set of molecular

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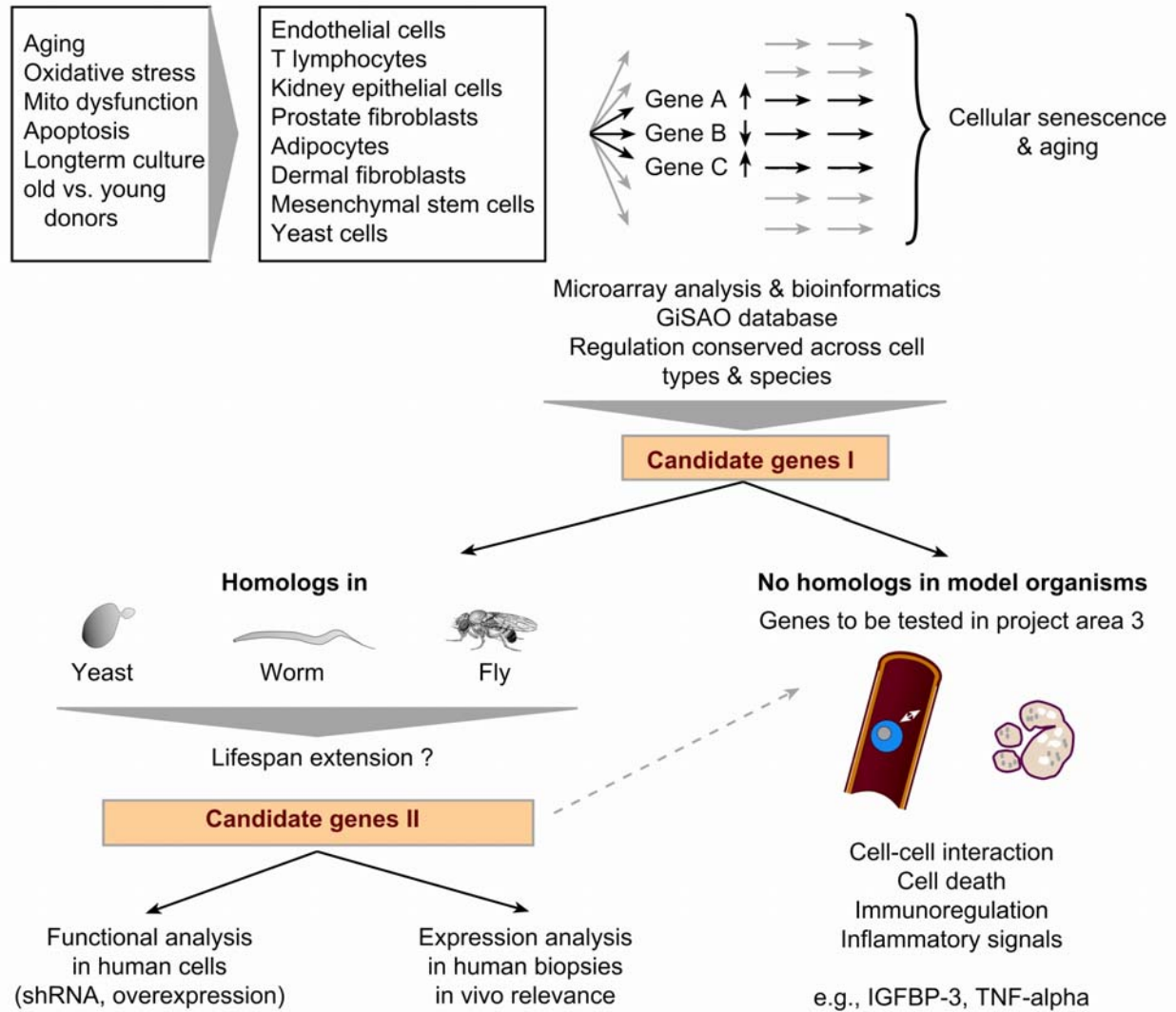


Figure 1. Identification and validation of candidate genes that play a role in senescence, apoptosis and oxidative stress. Cells from different human tissues were grown until they reached the senescent phenotype. In addition, cells were treated with oxidative stress or mitochondrial inhibitors to induce premature senescence. Differential gene expression analysis was also carried out for young and senescent yeast cells and for yeast strains, where apoptotic regulators were eliminated by gene disruption. RNA samples were prepared at the beginning and at the end of each treatment and genome-wide changes in gene expression were analyzed by Affymetrix technology. Gene expression data were organized as datasets of the GiSAO database and analyzed by bioinformatic tools. This yielded a short list of about two hundred genes that are commonly regulated in most, if not all, of the experimental systems; particular emphasis was put on genes that are co-regulated between yeast and human cell types. For mammalian candidate genes orthologous genes in the yeast, worm and fly were determined. For genes, where homologs in yeast or *Drosophila* could be identified, a pilot study of gene disruption has been performed. In this experiment, genes were knocked out (yeast) or knocked down (*Drosophila*) and the influence on the lifespan of the corresponding species was analyzed. This revealed already two candidate genes, which upon disruption extend the lifespan and therefore can be expected to play a role in regulating the rate of aging. A pilot study will be carried out with RNAi of selected candidate genes in *C. elegans*. Genes that are identified by these functional screens (referred to as candidate genes II) will then be analyzed for their function in human aging, applying cell culture studies as well as expression analysis in human biopsies. Candidate genes for which no orthologs in the lower eukaryotic model organisms can be identified, will be functionally tested in human cell culture systems, applying cell interaction and co-cultivation experiments.

pathways controlling lifespan in both yeast (4) and *C.elegans* (5). Since during evolution new regulatory pathways emerged that do not exist in lower eukaryotic organisms, as best exemplified by the adaptive immune

system, the analysis based on lower eukaryotic model systems is at risk to miss out regulatory pathways that control human longevity. We therefore addressed the question in a slightly different way. Based on data derived

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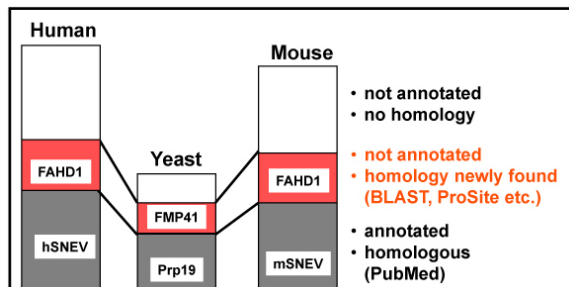


Figure 2. GiSAO database. Gene expression data derived from Affymetrix-based RNA profiling in various models of cellular senescence are collected in the GiSAO (Genes involved in Senescence, Apoptosis and Oxidative stress) database. In the current version, orthologues found in the genomes of *Homo sapiens*, *Mus musculus* and *Saccharomyces cerevisiae*, are assembled in a way to allow cross-species comparisons. The inclusion of information related to orthologous genes in *C. elegans* and *D. melanogaster* is in preparation. Genes fall in one of the three categories “annotated & homologous”, “not annotated, homology newly found” and “not annotated, no homology”, corresponding to their present status. It can be expected that new evolutionary conserved pathways underlying cellular ageing will be discovered through this analysis.

from a genome-wide comprehensive analysis of gene expression differences associated with cellular senescence in a variety of human tissues, emerging evidence of novel pathways will then be validated by genetics in model organisms. To our knowledge, such approach is not followed up by any other group.

2.3. The GiSAO database

This database represents an ensemble of genes, which were found differentially regulated in response to natural aging or through stress-induced senescence, as well as genes implicated in apoptotic signaling. In essence, GiSAO.db is a centralized, web-based database for the storage and retrieval of genuine information regarding samples, methods, experimental details concerning expression of aging-related genes, corresponding annotation and evolutionary conserved genetic orthology. Currently more than 100 whole genome array data sets, candidate gene lists, gene orthology cross references with respect to *S. cerevisiae*, *C. elegans*, *D. melanogaster*, *M. musculus* and *H. sapiens*, and results of experimental analyses are stored in GiSAO.db, together with linkage to gene annotation and the direct access to KEGG pathways. This design allowed identifying candidate genes that appear to be relevant during aging with respect to more than one human cell type. A critical feature of this data bank was to link the identity of human candidate genes to their true orthologues in yeast, worm, fly and mouse. This feature greatly supports mutual comparison of biological properties of evolutionarily conserved genes in the context of senescence, apoptosis and oxidative stress (Figure 2). Genes that have been identified as candidates by RNA profiling and for which yeast and/or fly homologues exist, can now be functionally analyzed in yeast

and fly lifespan experiments, which in due course will provide provisional evidence for novel molecular mechanisms that control distinct aging processes in eukaryotes.

Data obtained from mRNA expression profiling, proteome analysis, functional analyses, as well as data from publicly available resources provided a starting point for our research in order to define conserved pathways involved in the interplay of aging, senescence, oxidative stress and cell death. In a systematic approach, data from a variety of human cellular aging models, which had either been subject to *in vitro* or *in vivo* aging and/or oxidative stress, were analyzed applying the following criteria: i) differential expression in a maximum number of model systems with respect to aging or stress; ii) elucidation of those genes that are highly conserved in evolution and therefore have true orthologous counterparts in yeast, fly, mouse and human.

Lifespan and stress resistance of yeast strains deficient in those orthologous genes, which are coding for non-essential genes, can be readily determined. The mutants are being tested for resistance or hypersensitivity to five different oxidants and heat shock as well as for their sensitivity to inducers of cell death (H_2O_2 , acetate, amidodarone) and for properties related to chronological aging such as apoptotic markers, necrosis, and the induced production of reactive oxygen species (ROS). Since mother cell-specific lifespan can only be determined by separation of individual daughter cells performing micromanipulation these tests are cumbersome and time consuming and only a few candidates can thus be examined as a first screen. Then, selected gene deletion strains are taken to the more laborious determination of mother cell-specific lifespans. Further in depth characterizations include heterologous expression of the mammalian genes in yeast, GFP-tagged variants for intracellular localization of the proteins, and the dependence of the corresponding gene function regarding known aging pathways such as TOR or major death pathways.

In *Drosophila melanogaster*, which is phylogenetically closer to mammals than is yeast, specific down-regulation of gene transcription by means of stable RNAi expression will be validated with respect to life and health span extension. Those genes that show an increased life and/or health span will be further studied in order to determine the dependence of molecular pathways they are involved in as well as by performing complementation with the human orthologue. The latter will be carried out in knock-out or mutant yeast strains in order to test whether the phenotype can be rescued.

Exploiting the knowledge of GiSAO, candidate molecules that appear to be involved in the cross-talk between different human cell types were revealed by their complementary altered expression pattern during cellular aging. This observation strongly suggests that interaction and / or communication may no longer occur in an appropriate manner, thus leading to decreased signaling and/or cell death. Moreover, proneness to necrotic cell death rather than apoptosis may ultimately provide additional pro-inflammatory stimuli. The goal of the

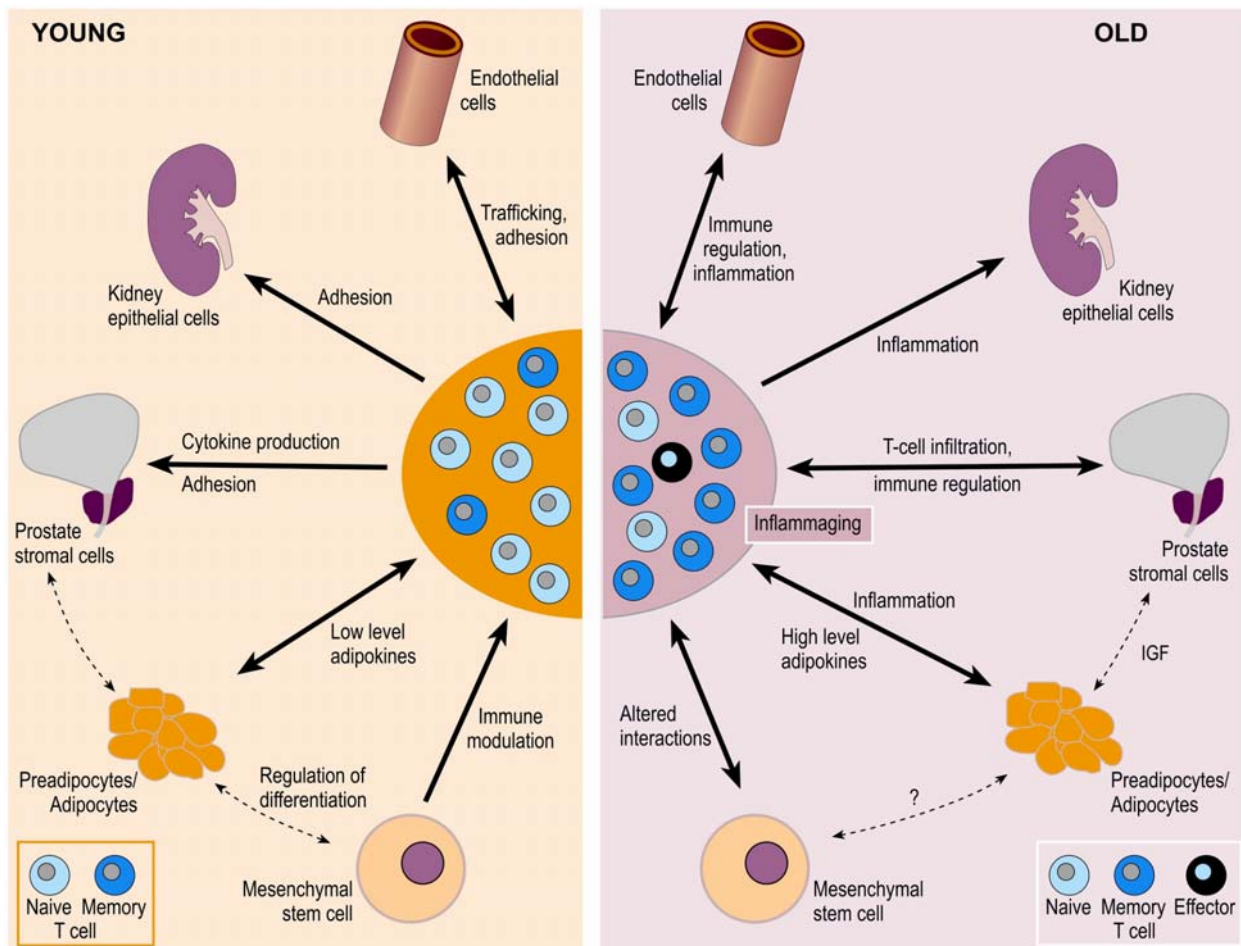


Figure 3. Age-associated changes in cell-cell interaction. At young age, the T-cell compartment is primarily filled with naïve cells and a few memory cells, whereas at old age the frequency of memory cells is largely increased and effector cells also represent a large part of the T-cell compartment. In response to this change in the T-cell repertoire, changes in cytokine production and the expression of T-cell markers on the surface undergoes dramatic changes, which are believed to influence the phenotype of cells in the periphery. Reciprocally, senescence-associated changes in the phenotype and gene expression in adipocytes, endothelial cells, prostate stroma cells, kidney cells and haematopoietic stem cells are believed to alter the interaction of these cells with T-cells, but also certain regulatory interactions between peripheral cells of different tissues. Age-associated changes in these interactions will be studied.

intended integrated data analysis is to predict the outcome of senescence-associated alterations in the profile of pro-inflammatory mediators in our cellular model systems. Chronic inflammation supports the development and progression of age-related diseases. However, the underlying molecular mechanisms are still poorly understood. Various human cell types communicate with leukocytes in the young organism to maintain tissue homeostasis. Hence, we are putting the hypothesis forward that age-associated gene expression changes in various interacting cell types drive age-associated degeneration of immune responses as well as the promotion of inflammatory processes. We therefore investigate interactions of T cells with other cell types in more detail and thus the role of molecules are currently being investigated with special respect given to altered interactions between T cells and other cell types during cellular aging and senescence. In this context, cellular

interactions between T cells and: i) endothelial cells (cellular adhesion and immune regulation), ii) prostate stroma cells (trans-differentiation and proliferation), iii) kidney epithelial cells (pro-inflammatory phenotype), iv) pre-adipocytes / adipocytes (increased TNF-alpha, lack of IGF-I), or v) mesenchymal stem cells (immune modulation / suppression, T cell differentiation) are being studied in order to reveal whether necrosis contributes to increased inflammatory responses in the elderly and/or whether inflammatory molecules may induce premature senescence in these systems. We assume that the cellular secretome either leads to or mediates pro-inflammatory signals (Figure 3).

To support the experimental work, we first applied integrated data analysis to predict the outcome of senescence-associated alterations in the production profile of secretory factors in general, in particular pro-

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inflammatory mediator molecules in T cells, and their reciprocal counter-parts in interacting cell types. Next this assumption is being experimentally studied using established *in vitro* model systems in a fashion by which cellular adhesion and distinct interactive processes will be characterized by applying co-cultivation techniques.

3. EXAMPLES FROM THE NFN CONSORTIUM

The authors of the current communication have formed a national research network (referred to as NFN) for aging research in Austria, which is supported by the Austrian science foundation (FWF). The approach described above has been successfully used in the current research network and this is illustrated in two examples in the following section.

3.1. Example 1: Apoptosis and other forms of programmed cell death in aging yeast and human cells

3.1.1. Senescence phenotypes in yeast and human cells

Mother cell-specific aging in yeast gained broad interest in the 1990s, when the group of Jazwinski (6) showed that this model system of aging depends on mitochondrially generated ROS. At the same time the group of Guarente (7, 8) showed that mother cell-specific aging can be triggered by ERCs (extrachromosomal ribosomal circles) and longevity depends on the SIR2 gene, which was shown later to be the link of this aging model to caloric restriction (9). While ERCs are specific to yeast and do not exist in human cells, the sirtuin gene family is highly conserved and its link to aging and to caloric restriction has been shown for higher cells (9). Besides mother cell specific aging (replicative aging), chronological aging can also be studied in yeast. Whereas results of RNA profiling studies suggest that both processes are guided by different molecular mechanisms, cells undergoing replicative aging and chronological aging share certain mechanistic features, such as the increase in both ROS production and apoptotic cell death.

Hence, we have compared the phenotypes of senescent terminal yeast mother cells with senescent human umbilical vein endothelial cells (HUVEC) (10-12). We found a surprising degree of phenotypic similarity, including an increase in cell size, irregular nuclear morphology, rearrangements of the actin cytoskeleton, and increased ROS production, as shown by electron spin resonance (ESR) measurements and several redox-sensitive dyes. Strikingly, we found an increased propensity to undergo apoptotic cell death in both senescent yeast cultures and senescent HUVECs. This is in contrast with senescent human fibroblasts, which develop apoptosis resistance, suggesting that the precise process by which senescent cells reach the end of their lifespan depends on the cell type (13).

3.1.2. Cell death and aging/senescence

Whether apoptosis is an aging or an anti-aging mechanism has been subject of considerable debate in the field. Knockout of the apoptosis regulator p66Shc in the mouse was shown early on to extend lifespan, suggesting that apoptosis may restrict organismic lifespan under some

conditions (14). More recently, BAX, an important player in apoptosis, has been linked to age-related changes in apoptosis. Changes in apoptotic proteins like BAX could contribute to aging-associated atrophy in both the skeletal muscle (15) and in the aging heart (16) of rats. It is not known how these processes are mechanistically related to human aging, but a role of BAX or other mitochondrially localized pro-apoptotic proteins in human aging is conceivable. For example, Endonuclease G (EndoG), when released from mitochondria acts as an apoptotic nuclease, has been discussed to play a crucial role in a plethora of human diseases. Of note –and similarly to Bax attributed changes in aging- EndoG translocation has been associated with age-induced muscle degeneration during sarcopenia (17), and overexpression of EndoG results in fly muscle degeneration (Madeo, unpublished results). However, more work will be required to clarify the role of mitochondrial apoptosis regulators in the aging process in yeast, and to investigate whether similar mechanisms are involved in aging of higher organisms. The pathway for EndoG-mediated cell death was unknown until recently. Combining yeast chronological aging, genetics and biochemical protein interaction assays, a pathway of EndoG interaction partners required for cell death induction during the aging process was established (18). Doing so, we determined components of the permeability transition pore, as well as histone H2B to interact with EndoG and to be necessary for cell death upon EndoG overexpression. Thus, a pathway in which mitochondrial pore opening, and chromatin association are successively involved in EndoG mediated death has been pictured. We also found that deletion of the yeast homologue of the translationally controlled tumor protein (TCTP) prolongs replicative lifespan. The wild type protein has anti-apoptotic functions when translocated to mitochondria.

A further aim of these studies is to explore whether necrosis, known to occur in various aged human tissues, is regulated by similar mechanisms in mammalian cells compared with yeast. Yeast will be used to analyse the causative molecules and mechanisms leading to necrotic cell death in detail. The most promising candidates will then be investigated in higher systems. We will analyze the molecular mechanisms and genetic determinants of senescence-associated necrosis and compare the data obtained in human cells with yeast necrosis studies. The role of cell death in aging, as it will be addressed, is shown in Figure 4.

3.1.3. Identification of evolutionarily conserved gerontogenes

We have also approached the question of the biochemical and genetic mechanism (s) of aging by identifying new genes which when mutated can lengthen the mother cell-specific lifespan of yeast.

Our approach was to test all the yeast deletion mutants created in genes that were shown by microarray experiments to be differentially expressed in old vs. young cells. All these mutants were screened for their resistance or sensitivity to five different chemicals that create oxidative stress when applied externally. The rationale is

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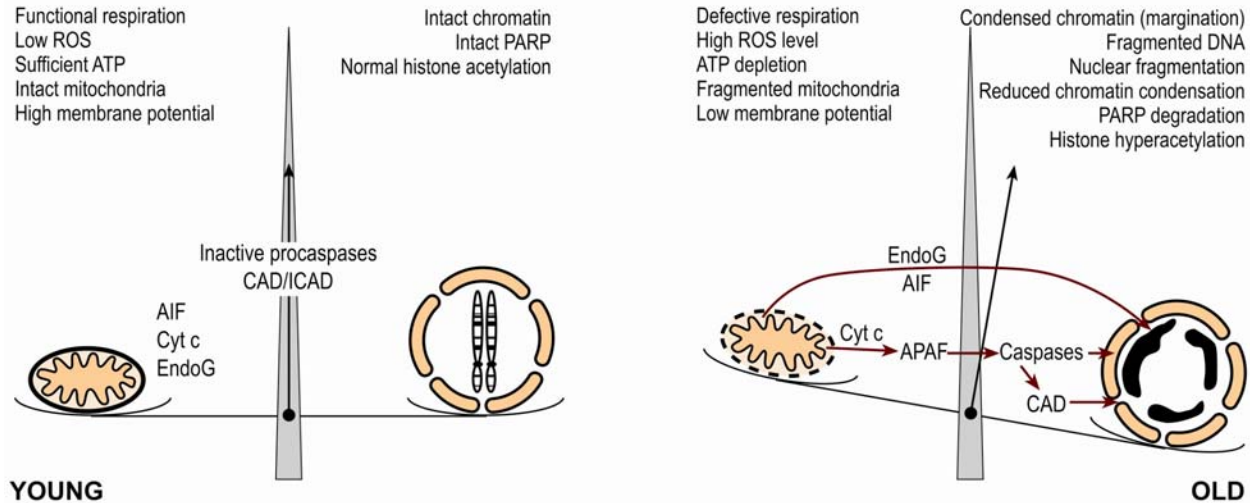


Figure 4. The highway to death. Release of mitochondrial factors results in destruction of the nucleus via several pathways – in yeast and in mammals cytochrome C, AIF and Endonuclease G perform pro-life vital functions in the intact mitochondria of young healthy cells. During aging or under stress-induced premature aging, these proteins leak from damaged mitochondria or are actively released via specific pores. Subsequently, EndoG and AIF migrate into the nucleus. In vertebrates, cytochrome C recruits APAF1 to form the apoptosome, resulting in formation of active caspases and as a result, activation of nuclease CAD. These processes lead to DNA fragmentation, chromatin condensation and nuclear disintegration. Histone hyperacetylation is associated with necrotic cell death.

that long-lived mutants are often oxidative stress resistant, not only in the yeast system but also in metazoan model systems. In a second approach, the most highly conserved genes identified in more than two model systems in the GiSAO database were all tested for their mother cell-specific lifespan (ongoing unpublished work). In a third approach we used studies of the apoptosis specific mitochondrial proteome of yeast and identified a highly conserved eukaryotic gene, TCTP (19). In the meantime this protein was also found in the aging mitochondrial proteome of *Drosophila* and *C. elegans*. It was found that the yeast homolog of this protein plays a highly interesting role in aging as well as in apoptosis. The protein is located in the cytoplasm in growing cells, but after applying oxidative or other stresses, concomitantly with the transformation of the mitochondrial network of the cells to many small roundish mitochondria, this protein is translocated to the mitochondria, thus confirming the proteomic results. The protein sits at the outer surface of the mitochondria and co-localizes with the so called „stress granules“ of the yeast cell (unpublished). The protein interacts with microtubules, as shown by a systematic investigation of the deletion mutant's sensitivity for benomyl. The deletion mutant is long-lived. We called this yeast TCTP homolog Mmi1 (19). Overexpressing Mmi1p in yeast has an anti-apoptotic effect (unpublished). A comparison of the properties of yeast, *Podospira*, and human TCTP (MMI1) is currently under way. The human TCTP protein exerts its anti-apoptotic effect after transfer to the nucleus (20).

3.2. Example 2: Modulation of aging by SNEV/Prp19 in yeast, human cell cultures and a mouse model

SNEV (Senescence evasion factor, Acc. Nr. NP_055317), also known as PRP19 (pre-mRNA processing

19), hPso4 (human psoralen sensitivity 4) and hNMP200 (human nuclear matrix protein 200), has originally been identified as mRNA that is downregulated in HUVECs after entry into replicative senescence (21), and as a novel nuclear matrix protein (22). SNEV is downregulated in senescent human endothelial cells, skin fibroblasts and renal proximal tubular epithelial cells (RPTECs), and upregulated in several tumor cell lines, suggesting that it correlates with proliferative capacity. Indeed, stable overexpression of SNEV, resulting in 2-3 fold increase of SNEV protein level, triggered a significant delay of replicative senescence, which did not alter endothelial characteristics (23). The detailed characterization of SNEV that followed this initial finding led to the discovery of multiple properties of SNEV, as outlined below. Most of its functions seem to be highly conserved from yeast to man, since its orthologue in *S. cerevisiae*, called Prp19p or Pso4p, also works in pre-mRNA splicing (24, 25), the ubiquitin-proteasome system (26), and DNA repair (27).

In proteomic approaches aimed at identifying the proteins that compose the human spliceosome, SNEV was identified as one of around 300 splicing factors. Immunoprecipitation of CDC5L subsequently identified SNEV as a member of the CDC5L complex, which associates with the spliceosome in an ATP-dependent manner at the second step transesterification reaction (28). SNEV forms homo-oligomers which can be inhibited by peptides consisting of the SNEV self-interaction domain. Addition of these peptides inhibits spliceosome formation before its catalytic activation (29). This is consistent with SNEV's presence in three pre-catalytic complexes, the B* complex (30), the BAU1 complex (31), and the B complex (32). Therefore, a pre-catalytic role for SNEV might include formation of a scaffold that allows assembly of

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other splicing factors, or ubiquitination of other proteins necessary for spliceosome assembly, as SNEV shows ubiquitin ligase activity.

SNEV acts as an E3 ubiquitin ligase *in vitro*, in reactions containing UbcH3 as E2 ligase (33). The catalytic activity thereby depends on the presence of the U-box that constitutes the N-terminus of SNEV and leads to polyubiquitination by K48, the classical proteasome degradation signal (33). In addition, the U-box domain of SNEV physically and directly interacts with the $\beta 7$ subunit of the proteasome (26), and SNEV's mouse homologue interacts with mSUG1, a regulatory subunit of the proteasome (34). However, SNEV appears not to be a prime substrate for the proteasome, since it is not degraded *in vitro* by purified proteasomes at least under the standard conditions used (26, 34). Since inhibition of proteasome activity increases co-localization of the proteasome with SNEV, as well as that of ubiquitin with SNEV in HeLa cells, SNEV possibly "escorts" its substrate (s) to the proteasome for destruction (26). However, we cannot exclude that post-translational modifications (e.g. phosphorylation), or binding to so far unknown factors, would not result in proteasomal degradation of SNEV. In this regard it is of note, that SNEV is ubiquitinated upon DNA damage and that SNEV oligomerization is changed by its ubiquitination (35).

While it is commonly agreed upon the importance of proteasome activity (36, 37) and ubiquitination (38) during aging, it is not clear if SNEV influences cellular life span by affecting the function of the ubiquitin/proteasome system. We expect that the identification of the still unknown substrate (s) of SNEV's E3 activity, will help to dissect the role of SNEV during cellular aging.

SNEV is not only ubiquitinated upon DNA damage but also plays a direct role in DNA damage repair. It interacts with terminal deoxynucleotide transferase (TdT) in human lymphoid cells and binds sequence independently to double- but not to single-stranded DNA (39). In keeping with this DNA binding capacity, SNEV was found in a protein complex binding to the fetal hemoglobin promoter in murine mouse erythroleukemia cells (40). Furthermore, SNEV and three other proteins of the CDC5L-associated complex (CDC5L, PLRG1 and SPF27) (28), together with Werner protein (WRN), are necessary for early steps of DNA interstrand cross-link repair (41).

WRN (Werner protein) encodes a DNA helicase of the RecQ family with exonuclease activity that is mutated in patients suffering from Werner's syndrome, a premature aging syndrome (42-44). *In vitro* cultivated cells derived from Werner patients also show a decreased replicative life span (45), while – as mentioned above – stable overexpression of SNEV has the opposite effect. Since SNEV overexpression also correlates with increased resistance to the chemotherapeutic drug bleomycin and the glutathione-depleting reagent BSO (23), it is intriguing to think that the DNA interstrand cross link repair function

might be responsible for conferring longer life to cells (reviewed in (46)).

3.2.1. SNEV knock-out is early embryonic lethal

Considering all of the essential functions mentioned above, it is not too surprising that disruption of the SNEV gene in the mouse is lethal already at day 3.5. of gestation. The inner cell mass of isolated blastocysts does not proliferate anymore and largely increased apoptosis is observed (47). However, while no overt phenotype has been observed so far, SNEV^{+/-} mice derived fibroblasts that contain only 50% of SNEV mRNA enter replicative senescence significantly earlier than those from wild type litter mate controls, again pointing to the crucial role of SNEV in influencing replicative life span (47). Surprisingly, the heterozygous SNEV^{+/-} mice show also a decrease in frequency and self renewal capacity of hematopoietic stem cells (48). A similarly low level of SNEV mRNA was observed in hematopoietic stem cells of the short lived senescence accelerated mouse strain SAMP8 compared with the long lived control SAMR1 mice, which again is accompanied by a decrease in their frequency and self renewal (48), findings that would suggest immune defects in the SNEV^{+/-} mice similar to that observed in the SAMP8 mice (49). However, further investigations in this regard are necessary.

4. CONCLUSION

The work carried out in the consortium so far has shed new light on the regulation of programmed cell death and its role for aging processes in both yeast and human cells. We have also identified genes that are implicated in longevity assurance processes in an evolutionarily conserved manner; for example SNEV and its yeast homolog were shown to contribute to aging in yeast, mouse and human systems. While the comparative analyses in the model systems mentioned above have identified additional candidate genes, whose molecular and functional characterization is currently underway, in future work we will also carry out genetic analyses in worms (*C.elegans*) and flies (*D.melanogaster*), which will allow to address longevity effects of additional human genes, for which homologues were not found in yeast. Information contained in the GiSAO database will provide a unique basis for a set of experiments where the influence of aging and senescence on the interaction between immune cells and other cell types will be studied, with the ultimate goal to provide new insight into mechanisms of age-associated functional impairment of the immune system.

5. ACKNOWLEDGEMENTS

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Abbreviations: GiSAO: Genes involved in Senescence, Apoptosis and Oxidative stress; SNEV: senescence evasion factor; EndoG: endonuclease G

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