CASPASE 3 AND BCL-2 EXPRESSION ALONG AGING IN ADRENAL ZONA RETICULARIS AFTER DEXAMETHASONE ADMINISTRATION

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The administration of dexamethasone enhances cell death rate in deeper layers of the adrenal cortex (zona reticularis, ZR). In previous structural studies, it was verified that the apoptotic index, estimated by the percentage of Tunel positive nuclei, was more pronounced in younger ages when compared to older ages. However, beyond the final, structural, events of apoptosis, it is important to verify the expression of other factors involved in apoptosis activation.

Wistar male rats aged 2, 6, 12, 18 and 24 months (n=2-3), were divided into 2 groups. One group was injected with dexamethasone phosphate (DEX), 4 mg/Kg i.m. for 3 consecutive days; the controls received a similar volume of saline; at day 4, the animals were sacrificed, the adrenals processed for caspase 3 (C3) and bcl-2 immunocytochemistry, using fluorochromes for detection.

Dexamethasone injection did not result in ZR structural changes at any age, when compared to controls. In both groups, C3 and bcl-2 localized to the cytoplasm of ZR parenchyma cells and its transition to zona fasciculata, but was absent from other layers of the cortex as well as from the medulla.

The adrenals of DEX injected rats, at all ages, exhibited more C3 and bcl-2 labelled cells than controls. Apparently, higher labelling occurred at 6 and 12 months and there was a decrease thereafter. At all ages, bcl-2 cells appeared to outnumber C3 labelled cells. Co-localization of bcl-2 and C3 labelling was observed throughout the ZR in a substantial number of cells.

These findings indicate that bcl-2 and caspase 3 are activated in adrenal cortex during cell death consequent to dexamethasone administration, in an age-related pattern similar to the final apoptotic events.

CHARACTERIZING THE STRESS RESPONSE TO DIFFERENT MODELS OF PROTEIN MISFOLDING

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Aging is characterized by a progressive accumulation of cytotoxic and proteotoxic damage caused by environmental stress. Molecular chaperones are ubiquitous, highly conserved proteins responsible for the maintenance of protein folding homeostasis in cells. Damaged, misfolding proteins are readily recognized by the complex chaperone system in cells and are either refolded/repaired at the level of conformation or targeted predominantly for the proteasomal degradation if the damage is irreparable. Damaged proteins accumulate in postmitotic cells, and in cells harboring of severe inheritable mutations (such as polyQ proteins) and may have profound cytotoxic effects, including transcription factor deactivation and cytoskeletal derangements, apoptosis induction. These pathologies have wide clinical implications from neurodegeneration to aging.

To better understand the role of protein structure and the role of molecular chaperones in these processes, we have overexpressed several protein-misfolding models in Cos-7 cells and currently study there aggregation properties and the induction of the stress response by sedimentation of detergent insoluble fractions, western blotting and immunofluorescence analysis. Of these, GFP::degron, a C-terminal fusion of a 16-residue “degron” peptide to GFP, was found to form iuxtanuclear aggregates resembling to aggresomes and markedly induced the major stress protein Hsp70. Results of our further ongoing studies will be presented.

PROTEOTOXIC STRESS INDUCES CLUSTERIN OVER-EXPRESSION THROUGH HEAT-SHOCK FACTOR 1 AND PROTEIN STABILIZATION

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Clusterin/Apolipoprotein J (CLU) is a heterodimeric secreted glycoprotein expressed in a wide variety of tissues and found in all human fluids. CLU induction has been reported under various stress conditions such as heat shock, oxidative stress, ultraviolet or ionizing radiation and treatment with chemotherapeutic drugs. The aim of our work was to examine the effect of proteotoxic stress on CLU expression and to determine whether CLU constitutes a proteolytic substrate of the proteasome. We used the U-2 OS osteosarcoma cell line and the WI38 human embryonic lung fibroblast primary cell line. Total, as well as partial inhibition of the proteasome increased both CLU mRNA and protein levels in both cell lines. Since transcriptional elements of heat-shock factor 1 (HSF-1) and Activator Protein-1 (AP-1) are found in CLU promoter, we were then interested in identifying whether one of these two transcription factors mediates this CLU mRNA up-regulation. Pre-treatment of the cells with KNK437, a HSF-1 inhibitor, abolished CLU mRNA or protein induction in U-2 OS cells, whereas it prevented CLU mRNA up-regulation but further increased CLU protein levels in WI38 cells. Furthermore, pre-treatment of the cells with 6-gingerol, an AP-1 inhibitor, failed to abolish the increase observed after proteasome inhibition on CLU protein levels in both cell lines. Therefore, HSF-1, and not AP-1, may be responsible for the mRNA CLU up-regulation in both cell lines. The observed CLU protein stabilization after proteasome inhibition is currently under investigation. Regarding the possible proteasome-mediated degradation of CLU, pulse-chase experiments revealed that the intracellular form of CLU accumulated upon proteasome inhibition, whereas the secretion of CLU was not affected. Also we are investigating whether CLU is ubiquitinated and subsequently degraded by the proteasome. The results so far indicate that CLU up-regulation under proteotoxic stress is probably not only transcriptionally mediated, but also post-translationally, possibly due to reduced degradation of CLU by the proteasome.

EVIDENCE OF MITOCHONDRIAL LON PROTEASE SPECIFIC SUBSTRATES BASED ON A PROTEOMIC APPROACH

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Mitochondrial dysfunction has been implicated in the aging process as well as a number of age-related diseases associated with increased levels of mitochondrial derived reactive oxygen species and oxidative damage. Protein degradation represents the final step by which oxidatively modified proteins can be eliminated. In the cytosol, the proteasome constitutes the main proteolytic machinery involved in the elimination of oxidized protein. Previous studies have shown that mitochondrial matrix contains ATP-stimulated proteolytic activity mostly carried by the Lon protease that has been implicated in the degradation of oxidized, dysfunctional, and misfolded proteins. We have also reported an age-related impairment of Lon protease function in aged rat organs such as liver and heart. In order to establish the specific role of the Lon protease and to find physiological substrates, we have used proteomic based approaches to identify proteins that are specifically degraded by the Lon protease. We hypothesized that Lon specific degradation oxidized protein substrates should accumulate in mitochondria deleted for Lon when challenged with oxidative stress. Therefore, mitochondria isolated from yeast (ΔLon) and exposed to various concentrations of H_2O_2 were used in this study. Specific mitochondrial matrix proteins that are potential endogenous substrates of Lon protease were obtained and characterized. Our results also suggest that protein carbonylation is a signal for recognition and proteolysis by the Lon protease.

PARP-1 REGULATES TELOMERE-LENGTH IN A REVERSIBLE MANNER

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Telomere dysfunction is an important tumour suppressor mechanism and mediator of cellular senescence in human cells. Many tumours activate telomerase to counteract somatic telomere shortening, and telomere maintenance is a prerequisite for tumorigenesis. Telomeres are specific DNA-protein complexes at chromosomal ends protect from irregular repair activities, achieved in part by formation of an unusual structure, the t-loop. T-loop formation is facilitated by the interaction of the telomeric DNA-sequence with TRF-1 and TRF-2, whose functions are bending and bridging of the DNA and stabilising the t-loop, respectively. In order to maintain telomeres by telomerase, t-loops need to be unfolded, probably by dislodging TRFs from DNA. This is achieved by poly(ADP-ribose)polymerases, with tankyrases modifying TRF-1 and PARP-1/-2 interacting with TRF-2. Although telomere length-regulation has mainly been assigned to tankyrases, we wanted to shed more light on the impact of PARP-1 on telomere maintenance, as there have been conflicting results in the literature. Therefore, we treated hamster and human cells with the pan-PARP inhibitor 3-aminobenzamide and measured telomere length by quantitative-fluorescence-in-situ-hybridisation and southern blotting. Telomeres shortened dose-dependently within one week. Time course experiment revealed fast initial reduction (450bp/population doubling) to a new level at roughly 75% of controls after 48 hrs, stable for at least four weeks. After release from 3AB, telomeres were elongated back to control length. Re-elongation was telomerase-dependent, as fibroblasts showed no re-gain of telomeric tracts. In order to address which PARP is responsible for this effect, we transfected HeLaS3 cells with specific siRNAs against PARP-1 and PARP-2 and monitored telomere development. PARP-1 silencing led to the same effect as 3AB; PARP-2 siRNA induced a minor shortening if at all. We conclude that PARP-1 is the major effector of 3AB-mediated telomere regulation and that other PARPs – although in vitro inhibited by 3AB – are of minor or no importance *in vivo*.

EFFECTS OF CHONDROITIN SULFATE ON H_2O_2-INDUCED SENESCENT HUMAN SKIN FIBROBLASTS AND COLLAGEN SYNTHESIS IN HaCaT KERATINOCYTES

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The ability of human skin to rejuvenate itself diminishes with the passage of time, resulting in increased fragility. This increased fragility reflects both reduced growth of skin cells and loss of connective tissue, such as collagens, proteoglycans or elastin.

Chondroitin sulfate (ACS) is a glycosaminoglycan, which exerts anti-inflammatory and anabolic effects on chondrocyte matrix molecules, promotes cell-cell and cell-matrix interaction, and facilitates growth factor response. Surprisingly, much less is known about its biological properties in other tissues such as dermis and epidermis.

In order to evaluate ACS on skin aging, we have developed an *in vitro* model of stress-induced premature senescence (SIPS), which is closely related to chronological-aging. We have evaluated the effects of ACS on matrix metalloproteinases (MMP) and ECM components, which are dysregulated during skin aging. We have also studied the effect of ACS on collagen synthesis in HaCaT keratinocytes.

By using quantitative RT-PCR, we clearly showed that MMP-1 and -3 are up regulated, and type I collagen and elastin slightly decreased in SIPS fibroblasts. We have also showed that ACS strongly inhibited MMP over-expression and slightly induced type I collagen and elastin mRNA level. On the other hand, we have also demonstrated that, in HaCaT keratinocytes, ACS stimulated collagen synthesis, a component of basal membrane, which is quantitatively reduced during aging. This result suggests that ACS could counteract the structural changes observed in basal membrane.

Alterations of skin observed during skin aging are both due to modification of dermal matrix and structural changes in the basal membrane, probably giving a less effective tissue regeneration. Our findings illustrated the anti-aging properties of ACS on two different targets. ACS is able to reduce the exacerbated catabolic activity in dermis, by reducing MMP expression, and to stimulate the anabolic activity in epidermis, by stimulating collagen synthesis.

THE DYNAMICS OF HUMAN AUTOSOMAL TELOMERES

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Human telomeres are composed of the DNA sequence TTAGGG reiterated into arrays of upto 20kb, which together with associated proteins cap the ends of human chromosomes. The loss of telomeric sequences with ongoing cell division ultimately results in the loss of telomeric function and the triggering of replicative senescence in many human cell types. The accumulation of senescent cells as a function of age may contribute to age-related tissue deterioration and disease.

The analysis of the human 2p, 11q, 12q, 17p and XpYp telomeres using Single Telomere Length Analysis (STELA), a technology which determines telomere length from single DNA molecules, revealed two key mechanisms that result in telomere loss; gradual erosion as a consequence of end-replication losses, and large-scale length changes termed human Telomere Rapid Deletion (hTRD). With the exception of 17p, these dynamics were conserved amongst the telomeres analysed; the telomere of 17p, however, was more stable with a striking paucity of hTRD, and displayed a trend towards being the shortest telomere. Telomerase-expressing cancer cells and germ line cells displayed both allelic variation and chromosome specific telomere length maintenance and hTRD events, indicating the presence of *cis*-acting factors that govern both telomeric stability and chromosome-specific telomere length in the presence of telomerase.

Erosion rates as a function of PD were conserved at the different ends within the same cell strain, but increased as the cell populations approached replicative senescence. Furthermore the erosion rates differed between different cell strains. For example the IMR90 fibroblasts telomeres eroded at a significantly higher rate than any of the other cell strains. We have been investigating the nature of the strain specific differences in rates of telomere erosion rates and hTRD, recent data concerning these aspects of our work will be presented.

HUMAN LONGEVITY NETWORK: CONNECTION TO AGE-RELATED DISEASES

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Analysis of established longevity-associated proteins and their partners in humans revealed that they form a continuous protein-protein interaction (PPI) network (Budovsky et al. Longevity network: Construction and implications. Mech Aging Dev, 2006). This network (further denoted as HLN, Human Longevity Network) is characterized by the presence of highly connected nodes (hubs). We hypothesized that the involvement of hubs in age-related diseases (ARDs) may be one of the ways by which the HLV proteins with multiple interactions may affect the longevity. We found that the hubs of the HLN are involved in at least one ARD, with many being involved in several ARDs including cancer, atherosclerosis, type II diabetes, neurodegenerative diseases, osteoporosis, arthrosis and sarcopenia. Of note, many proteins which expression is changed in ARDs were found to be nodes and hubs of HLN. Similarly, analysis of DNA microarray data reported for humans of different age, revealed that considerable portion of the HLN genes undergo age-related changes in their transcriptional activity. These observations support our hypothesis and suggest that common mechanisms may stand behind both aging and ARDs. Thus, the constructed HLN provides a useful framework for analysis of the possible links between the ARDs, aging and longevity. The results suggest that the HLN nodes and especially hubs exhibiting age-related changes in their expression, may be primary targets for longevity-promoting interventions.

IDENTIFICATION OF BIOMARKERS OF MUSCLE AGING AND SENESCENCE

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Muscle loss is the most common phenomenon of normal healthy aging and frequently leads to frailty and loss of independance in the elderly . It is important to understand the basic cellular mechanisms underlying this impairment. Decrease in muscle strength is associated with a decrease in cross sectional area of the muscle fibres, a decrease in capillary bed density as well as an increase in fibrotic tissue. A proteomic analysis is currently being carrried on biopsies of human skeletal muscle from young and old individuals in order to identify biomarkers of normal aging. Satellite cells are adult muscle stem cells which are responsible for muscle growth and muscle repair. These cells are closely associated with the muscle fibre located outside the muscle fibre sarcolemma but beneath the basement lamina. We have shown that the number and quality of these satellite cells available for both repairing and maintaining muscle mass decreases during aging suggesting that regeneration may be compromised in older individuals. We have also shown that these cells can make only a limited number of divisions and that this number decreases as a function of age. and following transplantation into immunodeficient mice cells from older individuals make less fibres than cells from young individuals. We are currently using a proteomic approach to characterise the secretome and the proteome of young and senescent muscle stem cells. In addition we have shown that the myogenic program is modified as cells reach senescence.

N-GLYCOMIC CHANGES IN BLOOD PROTEINS DURING HUMAN AGING: A NEW FUNCTIONAL AGING BIOMARKER?

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Due to the rapidly increasing number of elderly people in many countries, there is a need for innovative treatments for age-related diseases. Therefore, in addition to studying aging mechanisms, the identification of candidate aging biomarkers to measure age-related changes may be of great value not only to gerontologists, but also to people in general, by preventing aging-related diseases through development of anti-aging medicines.

Glycosylation is not a random phenomenon but is highly reproducible in a given physiological state. Important changes in cellular processes, such as aging and age-related diseases, may be expected to result in alterations in the glycan profiles of secreted glycoproteins. Our recently developed DSA-FACE based N-glycan analysis system is a high throughput technology platform designed to determine the N-glycan profiles of proteins in serum and other body fluids. By using this method, we were able to show that three N-glycan sugar structures (NG0A2F, NG0A2FB and NA2F) are altered with aging, not only in total serum but also in IgG, demonstrating the involvement of the glycosylation machineries in liver and B-cells in aging. This observation led us to hypothesize that physiological age could be monitored by an N-glycan fingerprint. Three glycan markers in a patient with premature aging disease (Werner syndrome) were comparable to those of centenarians, reinforcing their use as a functional biomarker. Our data indicate that measurement of the N-glycan level could provide a noninvasive surrogate marker for general health or for age-related disease progression, and for monitoring the improvement of health after therapy.

PROTEASOME MANIPULATION AND ITS FUNCTIONAL EFFECTS ON CELLULAR SENESCENCE, STRESS RESPONSE AND LONGEVITY

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The proteasome is the major cellular proteolytic machinery responsible for the maintenance of cellular homeostasis. Alterations of proteasome function have been recorded in various biological phenomena including cellular response to oxidative stress, aging and replicative senescence, while recently it has been implicated to longevity.

Here we report that proteasome can be manipulated, either up-regulated or down-regulated, resulting in different functional effects on aging, survival and longevity of the cells. Once proteasome is activated through stable overexpression of beta 5 catalytic subunit or hUMP1/POMP accessory protein, proteasome activities are stimulated, due to elevated amount of assembled proteasome. These increased levels of assembled proteasome result to enhanced cellular capacity to cope better with oxidative stress, mainly through an up-regulated rate of proteolysis. Furthermore, stable transfectants exhibit an extended lifespan and a delay of senescence. In contrast, upon replicative senescence of normal primary fibroblasts, the proteasome is down-regulated resulting to lower amount of assembled and thus, functional proteasome. Additionally, when proteasome is partially inhibited in young primary human fibroblasts (remaining activity in levels similar to the ones found in replicative senescent fibroblasts), the cells exhibit an irreversible senescence-like phenotype within 2 weeks following the treatment. By taking advantage of cell lines with absent p53 and/or Rb pathways, we have identified which of these pathways is mainly responsible for this accelerated appearance of senescence, rendering cells capable of escaping from this phenotype. In conclusion, these data demonstrate the central role of the proteasome during cellular senescence, response to stress and longevity.

CHASING THE SUBSTRATE(S) OF THE E3 UBIQUITIN LIGASE SNEV$^{PRP19/PSO4}$

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The ubiquitin proteasome system is responsible for one of the cell's most important cyclical processes, regulated protein degradation. This process regulates as diverse functions as cell division, DNA repair and the immune defence. Ubiquitination is catalyzed by three enzymes termed E1, E2 and E3, where E3 regulates the specificity of the reaction by binding directly to substrates. E3-substrate interactions are implicated in an increasing number of diseases, but despite their biomedical importance, a very small fraction of E3 enzymes have been linked to specific substrates.

The human E3 ligase SNEV$^{Prp19/Pso4}$ has been identified in a screening for genes downregulated in replicatively senescent human endothelial cells. So far, SNEV$^{Prp19/Pso4}$ has been proven to be involved in a variety of essential cellular pathways like pre-mRNA splicing, DNA repair, ubiquitin/proteasome associated protein degradation and lifespan regulation. However, knowing no ubiquitination substrate, an essential prerequisite for understanding its molecular functions is still lacking.

A high-throughput luminescence assay based on the AlphaScreen™ technology provides a quantitative, effective and sensitive method to detect ubiquitination substrates of yeast E3 ligases in vitro. Therefore, the assay is conducted with Prp19, the yeast homologue of SNEV$^{Prp19/Pso4}$. Since SNEV$^{Prp19/Pso4}$ and Prp19, as well as the complexes they are contained in, are highly conserved, also their substrates are assumed to be conserved. The assay has proven to be capable of discovering biologically relevant substrates of E3 enzymes and allows for a direct comparison of the relative level of ubiquitination between different proteins.

Currently, a number of purified yeast proteins are being screened for ubiquitination by Prp19. Complementing this approach, we have established a stable His_6-ubiquitin cell line for purification of differentially His_6-ubiquitin tagged proteins after overexpression or knock-down of SNEV$^{Prp19/Pso4}$. The identification of a target would shed light on the interrelation of the different cellular processes SNEV$^{Prp19/Pso4}$ is involved in.

CHARACTERISATION OF PROTEASOME GLYCATION DURING AGING USING PHAGE DISPLAYED ANTIBODIES

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The proteasome complex is the major cellular component that degrades damaged and misfolded proteins outside lysosomes. During aging the enzymatic activity of the proteasome decreases although the amount of proteasome subunits seemingly remains constant, suggesting that the reduced activity is caused by changes to the proteasome or its regulators. Non-enzymatic post-translational protein modifications, e.g. glycations, giving rise to advanced glycosylated endproducts (AGEs) increases during aging and these modified proteins are usually targeted for degradation by the proteasome. However, proteasome subunits as well as their regulatory subunits also undergo glycation, which might cause the observed reduction in enzymatic activity. Although antibodies recognizing the glycation on proteins are available as well as proteasome subunit specific antibodies, the possibility of identifying glycated proteasome subunits with a single antibody would be beneficial and in some cases essential for the quantification and localization of specific glycated subunits.

Utilizing the powerful phage display technique, we have selected antibodies specific for glycated proteasome subunits. Purified recombinant proteasome subunits were glycated *in vitro*, immobilized on an immunotube, and used for a single round of selection with a phage displayed antibody library containing $1.38 \cdot 10^8$ clones. The selected antibodies were screened for specificity to individual glycated proteasome subunits without showing cross-reactivity to non-glycated subunits or other glycated proteins. The antibodies showed an increase in glycated proteasome subunits during aging of cells grown in cultures as determined by ELISA. In Addition, stainings of permeabilized paraformaldehyde fixed cells showed a clear increase in glycated proteasome subunit expression during aging, while antibodies against non-glycated subunits remained stable. The glycated proteasome subunits were most abundant in the cytoplasm where damaged and misfolded proteins are known to accumulate. This study clearly demonstrates that proteasome subunits are glycated in vivo. However, the possible functional changes of glycations remains to be elucidated.

ANALYSIS OF POLY(ADP-RIBOSE)-PROTEIN INTERACTIONS USING A NOVEL CHIP APPROACH

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Poly(ADP-ribosyl)ation is an immediate cellular response to genotoxic insults catalyzed by the family of poly(ADP-ribose) polymerases (PARPs). PARP-1 is the founding member and accounts for 90% of poly(ADP-ribose) (PAR) formation. PARP-1 activity is triggered by DNA strand breaks leading to the synthesis of long and branched chains of PAR under consumption of NAD^+. Several years ago our lab established a positive correlation between cellular PAR formation capacity and mammalian lifespan. Moreover, it has been recently shown that PARP1 interacts physically and functionally with Werner Syndrome protein.

Aim of our project is to establish a poly(ADP-ribose) microarray to study the interaction of PAR and specific binding partners like histone H1, p53 and XPA with regard to chain length and branching.

PARP-1, XPA and p53 were overexpressed in Sf9 cells and purified. *In vitro* synthesized PAR was end-labeled using the carbonyl-reactive linker biocytin hydrazide. ELISA experiments using neutravidin coated plates confirmed the successful covalent modification with biotin. Following separation of polymer by high resolution HPLC the collected fractions were characterized on modified sequencing gels revealing discrete ADP-ribose chains from 6-60 ADP-ribose units. Subsequently, selected fractions were purified by avidin affinity chromatography and used for PAR-protein studies in solution. Both XPA and p53 promote complex formation with long ADP-ribose chains (55mer; $K_{D\text{-}XPA}=3.21x10^{-7}M$ and $K_{D\text{-}p53}=1.31x10^{-7}M$), whereas only p53 was able to bind short polymer (15mer; $K_{D\text{-}p53}=2.46x10^{-7}M$) as monitored by EMSA. Additionally, we observed that p53 induces the formation of at least three specific complexes with long PAR chains. Furthermore, fractionated PAR was successfully immobilized on a C3amino-slide. Bound polymer was detectable down to 4 fmol in a chain-length dependent manner using commercial antibodies, underlining the sensitivity and functionality of the produced chips. This novel microarray platform will permit the characterization of PAR-protein interactions in a high-throughput manner.

MITOCHONDRIAL DNA BASE COMPOSITION AND LONGEVITY IN DIFFERENT TAXA OF VERTEBRATES

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Maximum lifespan (MLS) has long been used in search of evolutionary determinants of longevity. Due to a key position in energy metabolism, generation of reactive oxygen species and regulation of cell death, mitochondria may contribute to MLS determination. With this in mind, we examined possible links between mtDNA base composition and MLS in 275 species from different taxa of vertebrates including 128 mammals, 27 birds, 26 reptiles, 22 amphibians, and 72 fish. We found that increased guanine and decreased adenine or thymine contents on mtDNA H-strand were associated with elevated MLS in most orders of mammals analysed. Partial correlation analysis revealed a dominant role of guanine in determination of correlative links between MLS and mtDNA base composition in mammals. Accumulation of thermodynamically more stable G-C pairs would result in an increased resistance of mtDNA to denaturating factors. This could be particularly relevant for endotermic organisms. Indeed, a clear trend in adenine-to-guanine substitution on H-strand and thymine-to-cytosine substitution on L-strand is observed from short-lived to long-lived mammals. These processes could reach 'saturation' in birds, with their higher body temperature and metabolic rate, presumably explaining lack of correlation between MLS and mtDNA base composition in birds. No significant correlations were also observed for two classes of terrestrial exothermic vertebrates, amphibians and reptiles. Fish exhibited another pattern of mtDNA-MLS relationships including a positive correlation of MLS with thymine, a negative one with cytosine or adenine, and a lack of correlation with guanine. In fish, guanine may play a dual role which is masked by reciprocal effects of thymine and cytosine on MLS. Further research is necessary for clarifying the evolutionary pattern and "cause-and-effect" relations between MLS and mtDNA.

EXPRESSION AND ACTIVITY OF SOD, CATALASE AND GLUTATHIONE PEROXIDASE AND OXYGEN CONSUMPTION IN AGING MICE

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There is a growing consensus on a dual role of reactive oxygen species (ROS). Acting as either regulatory or damaging factors, ROS could significantly modify aging pattern. According to existing estimates, 1-10% of consumed oxygen is converted into superoxide. It means that a chain $O_2 \rightarrow O_2^{\cdot-} \rightarrow H_2O_2 \rightarrow H_2O$, regulated by SOD, catalase and glutathione peroxidase (GPx), could be the main pathway of ROS metabolism. Due to certain proportionality, oxygen consumption (Vo_2) could be used as an index of superoxide generation.

Objective**.** The present study was undertaken in order to evaluate the age-related patterns of correlative and cluster relationships between a battery of prooxidant and antioxidant variables.

Material and Methods**.** Vo_2, the levels of mRNA (RT-PCR) and activities of SOD, catalase, GPx and glutathione reductase were determined in the liver of 62 young adult (3-5 months) and 58 old (23-26 months) male C57Bl/6 mice.

Results and Conclusions. The mean values of most studied variables, except Vo_2 and catalase mRNA, did not significantly change in aging. However, distribution analysis revealed declined proportion of individuals with a higher expression of SOD and catalase but not GPx in old mice compared with young animals. Positive correlations were found between SOD, catalase, and GPx mRNA levels in both age groups, assuming that coordinated expression of antioxidant genes could be essential for successful aging. Surprisingly, there were practically "zero" correlation between mRNA level and activity of SOD or catalase. In contrast, the expression and activity of GPx correlated negatively in young mice, whereas positively in old animals. Tree-clustering indicated the enzymatic pair catalase-GPx which is primarily responsible for H_2O_2 metabolism, as a key "target" of antioxidant defense modification in aging.

GENOME-WIDE PARALLELS BETWEEN DNA REPAIR-DEFICIENT, LONG LIVED AND NATURALLY AGED MICE

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To unravel the role of genome surveillance pathways in longevity-associated processes, we compared genome-scaled, liver expression profiles across a series of DNA repair-deficient mouse models (i.e. $Csb^{m/m}$-$Xpa^{-/-}$, $Ercc1^{-/-}$ and $Ercc1^{-/D}$) and mice that live substantially long either due to genetic manipulation (i.e. Ames, Snell and Ghr-/- dwarf mice), treatment (i.e. caloric restriction) or both (calorically restricted Ames dwarfs). We find the majority of genes associated with endocrine, metabolic and dietary regulation to demonstrate shared expression patterns across these mouse models. Subsequent analysis revealed these processes to be also significantly regulated in naturally aging mice. We propose that unrepaired cytotoxic DNA damage in DNA repair-deficient progeroid mutants induces a highly conserved metabolic response that reallocates resources from growth to somatic preservation aimed at extending lifespan by limiting the deleterious effects of arrested transcription, cellular senescence and death that likely underlie accelerated and natural aging.

EFFECTS OF BLUE ALGAE PIGMENTARY EXTRACT ON H_2O_2-INDUCED SENESCENT HUMAN SKIN FIBROBLASTS

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The mechanical properties of the dermis are determined, primarily, by the extracellular matrix (ECM). These mechanical properties change dramatically as a function of age, as a direct result of the known age-related changes in the molecules of the dermal ECM: collagens, elastin or matrix metalloproteinases (MMP).

In order to evaluate blue algae pigments extracts (from *Phormidium uncinatum*) on skin aging, we have developed an *in vitro* model of stress-induced premature senescence (SIPS).
The antioxydant capacity of the pigmentary extract was evaluated by chemiluminescence: a potent scavenging superoxide anions activity was shown. By using quantitative RT-PCR, we clearly showed that catabolic enzymes are up regulated in SIPS human fibroblasts, since we measured an increase of MMP-1 and MMP-3 expression. Moreover, we observed a decrease of mRNA level of two ECM components: type I collagen and elastin. We have also showed that pre-incubation of SIPS human fibroblasts with pigmentary extract strongly reduced senescence effects.

On the other hand, we demonstrated that Fibroblast Growth Factor-2 (FGF2) concentration is reduced in SIPS fibroblasts and stimulated by pigmentary extract preincubation. We have also studied the effect of conditioned medium (CM) from SIPS fibroblasts, preincubated or not with pigmentary extract, on HaCaT keratinocytes proliferation. Our results showed that CM issued from SIPS fibroblasts inhibited HaCaT keratinocytes proliferation. Interestingly, CM issued from SIPS fibroblasts preincubated with pigmentary extract restored HaCaT keratinocytes proliferation. One of the mechanisms involved in this effect could be a stimulation of FGF2 synthesis by pigmentary extract.

In conclusion, our results demonstrated the anti-aging properties of blue algae pigmentary extract. Aging affect, both, the morphology and function of skin. Remarkably, our finding indicate that blue algae pigmentary extract is able to counteract the age dependent decline or surexpression of molecular signals regulating an active exchange between epithelial and mesenchymal cells.

REVITALIZATION AND LIFESPAN EXTENSION BY XENOGENIC FETAL MATERIALS: LABORATORY RODENT AND CELL CULTURE STUDIES

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Parenteral administration of xenogenic fetal materials to old rats had been shown by Kment and coworkers in the 1960ies and 1970ies to compensate for at least some age-related losses of physiological capacity in aged rats. In a number of studies in the 1980ies, then commercially available lyophilized homogenates of fetal sheep testis given subcutaneously (s.c.) to aged rats produced statistically significant long-acting revitalizing effects on a number of age parameters of connective tissue, skin, aorta, liver, kidney, heart, spontaneous and reactive motor activity, running capacity, learning and memory, plasma testosterone as well as on cohort survival after the age of 24 months. A fetal sheep mesenchymal preparation (Resistocell®) had even more pronounced revitalizing effects on old male rats including significantly elevated testosterone levels. In another survival study, female OF-1 mice showing age-related expression of lymphatic leucosis were given a single block of 5 s.c. injections of Resistocell® or Ringer's solution, respectively, at the age of 50%-survival. Survival curves separated within weeks: the last controls died at the age of 700 days whereas the last animals of the treated group attained an age of 1100 days. In order to elucidate the mechanism of this effect, we are using the YAC-1 mouse lymphoma cell line. A series of cell culture proliferation studies showed two opposite effects of the complex material: a clearly dose-related stimulatory effect at low concentrations, and a proliferation-blocking effect at higher concentrations which seems to arrest the cell cycle of the lymphoma cells (probably in G_1 or G_0) or sends them into apoptosis. These effects are abolished by heat denaturation and were not obtained by addition of xenogenic albumin. First studies with a cell death detection test point to high apoptotic activity induced by the fetal material. Hypothetical signal transduction mechanisms and testing strategies are discussed.

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MANIPULATING MITOCHONDRIAL LON PROTEASE IN THE AGING MODEL PODOSPORA ANSERINA: EFFECTS ON LIFESPAN, MTDNA STABILITY AND OXIDATIVE DAMAGE

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The "free radical theory" predicts that the age-related accumulation of ROS-generated damage of biomolecules including nucleic acids, lipids and proteins is causatively involved in organismic aging [1]. Mitochondria are the main source of ROS generation and consequently are highly prone to damage. As part of a defence system, these organelles contain proteases which recognize damaged proteins and degrade them helping to keep a population of functional mitochondria until a certain critical threshold of damage is reached. LON protease is one of a few mitochondrial proteases involved in this kind of a mitochondrial 'protein quality control' system. This highly evolutionary conserved serine protease, located in the mitochondrial matrix, acts as an ATP-dependent hetero-oligomeric complex and removes preferentially oxidized proteins to prevent the accumulation of aggregates [2]. For the mammalian LON protease another function has been described: the binding to specific mtDNA regions, RNA and to DNA polymerase gamma, suggesting that LON is directly involved in mtDNA metabolism [3]. In order to investigate the role of LON in *Podospora anserina*, a filamentous fungus extensively used as an experimental aging model [4], the open reading frame coding for a protein with high homology to known LON proteases, termed *PaLon*, was subsequently cloned and initially characterized. Quantitative Real-Time PCR analysis revealed increased levels of *PaLon* in senescent cultures of the wild-type compared to those in juvenile cultures. However, LON protease activity measurements with matrix proteins resulted in the demonstration of no increased protease activities in senescent cultures. These contradictionary results may be the result of age-related damage of PaLON and subsequent degradation of the protein. In order to increase PaLON activity in older cultures, *P. anserina* mutants overexpressing *PaLon* were generated and characterized according to lifespan, oxidative damage and different molecular pathways (e.g., mtDNA stability).

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MINOR CHANGES IN A STRESS CHAPERONE-MORTALIN HAVE MAJOR IMPACT ON AGING AND CANCER BIOLOGY

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Mortalin (mthsp70/Grp75) is a heat uninducible stress chaperone. Some of the established features of mortalin include its various subcellular sites, multiple binding partners and differential subcellular distribution in normal and immortal cells. It was shown to retard nuclear translocation, transcriptional activation and control of centrosome-duplication functions of p53, and contribute to human carcinogenesis. It functions as an adaptive protein in a variety of stress response mechanisms. Interestingly, minor structural changes can abolish its chaperone activity and have drastic biological consequences as seen in zebrafish model of Myelodysplastic syndrome and in old age pathologies, such as Alzheimer and Parkinson disease. Functional role of mortalin in cell proliferation control mechanisms will be addressed.

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QUANTUM DOTS FOR SENESCENCE INDUCING-siRNA SCREENING AND *IN VIVO* IMAGING

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Quantum dots (QD) are fluorescent semiconductor nanocrystals that are emerging as superior alternatives to the conventional organic dyes for biological applications. We found that they are more stable and provide better resolution than the conventional fluorescent dyes in protein imaging. Based on this, we employed a visual assay to screen for siRNAs that induce senescence in cancer cells and have identified ten candidates. We also prepared a conjugate of QD with an internalizing antibody and demonstrate that the QD-antibody conjugate efficiently gets internalized into the cells and was visible even after multiple cell divisions. We demonstrate that the internalized QD (iQD) is nontoxic to cells and provide a sensitive tool for long-term molecular imaging.

NONPATHOLOGICAL SENESCENCE ARISES FROM UNSUITABLE EXTERNAL INFLUENCES

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Recent findings prove extrinsic origin of cellular aging (Conboy et al., 2005). We extend this principle to the whole organism. External factors induce organisms' vital activities with different effectiveness to self-maintenance**.** Let us consider the two-parameter Gompertz's mortality rate and assume that these parameters depend on the third parameter *P* characterizing environmental pressure (total external influences). Biological consideration allows for the conclusion that there is a range of environmental conditions (parameter *P*), which corresponds to adequate vital activity, i.e. reasonable amount of environmental pressure plays a stimulating role for organism's functioning and at this range of optimal functioning an organism can completely renew itself, i.e. it is ageless. However, this range is not optimal for survival since environment induces heavy death toll in population of such organisms. In other words, mortality rate of such organisms is high because of environmental reasons (extrinsic mortality). To reduce this mortality the organism chooses a less aggressive environment. It makes compromise sacrificing optimal functioning (complete renewal) for the benefits of less aggressive environment. As a result of such strategy the organism's renewal becomes incomplete and the senescence generates an age-related increase of the mortality rate. However, this increase because of senescence is compensated by more significant mortality decline due to external (environmental) causes. The optimal balance is kept by evolutionary forces, which optimize average fitness of the population of organisms. Taking this consideration into account one can assume that in current (compromised) situation the Gompertzian exponential parameter (which reflects contribution of the rate of individual aging) must decline when environmental pressure increases. At the same time age-independent part of Gompertz's mortality rate (another Gompertzian parameter) must increase when the environmental pressure increases. Thus there is distinct reciprocal relationship between these two parameters, which was repeatedly observed since B.Strehler and A.Mildvan (Science 1960, **132**, 14).

IDENTIFICATION OF ZEBRAFISH MUTANTS WITH SENESCENCE-ASSOCIATED BIOMARKERS

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Many genetic or environmental manipulations that alter lifespan in model organisms also alter survival following acute stresses such as oxidative damage, genotoxic stress, and thermal stress. Thus in flies and worms mutations which enhance lifespan also increase resistance to oxidative stress. This is also true for most of the small number of mutations which increase lifespan in mice. In lower organisms this coupling of stress responses and aging mechanisms has proved a useful tool in identifying new genes that affect the aging process without the need for performing lengthy lifespan analyses. Therefore, it is quite possible that this approach may also be applied to the identification of zebrafish aging mutants and pharmacological agents that slow the aging process or even extend lifespan through enhanced resistance to oxygen radicals or other stresses. To facilitate high-throughput mutant and drug screens in zebrafish aging, we have developed a senescence-associated β-galactosidase (SA-β-gal)-based colormetric and fluormetric assays that use uptake of either X-gal or a fluorescent dye FDG, as a marker of premature senescence in zebrafish embryos. We have first verified that the signal intensity of SA-β-gal is dramatically increased both in aging fish and in embryos exposed to oxidative stress. We have next validated the assay by demonstrating that known signaling molecules or genetic mutations, which would be expected to modulate oxidative stress response, are linked to SA-β-gal induction in stressed embryos. We are further performing a screen using chemical oxidants to search for potential aging mutants in zebrafish. In this screen, chemical-induced oxidative stress is adopted to identify mutant fish, which either enhance or suppress activity of the SA-β-gal elicited by chemical oxidants as sensitizer. We have already isolated several candidate mutants that show enhanced response to oxidative stress. We propose that this novel method of mutant analysis will accelerate the discovery of new types of zebrafish mutants and genes associated with senescence as well as stress response and will also contribute to pharmacological interventions in aging as well as oxidative stress.

EXPOSURE TO γ-IRRADIATION PROVOKES ACCELERATED SENESCENCE IN HUMAN LUNG FIBROBLASTS THAT ENHANCE THE GROWTH OF MALIGNANT LUNG EPITHELIAL CELLS IN VITRO AND IN VIVO

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Cellular senescence is considered to be a potent anticancer mechanism. However, it has been proposed that senescent stroma cells may enhance the growth of adjacent malignant epithelial cells. Exposure of tumours to repeated low doses of γ-irradiation is a common treatment regime in several tissues. However, the effect of this stress to the neighboring stromal cells and the interaction of the latter with cancer cells has not been adequately investigated. In this study, we have exposed confluent cultures of human lung fibroblasts, derived from normal or cancer-associated regions, to repeated subcytotoxic doses of 4 Gy of γ-irradiation. We have found that a single dose immediately activates a DNA damage response, as shown by the activation of the ATM/Chk2/p53/$p21^{WAF1}$ axis, leading to an intense cell cycle arrest. After a series of doses (total dose approx. 50 Gy), followed by cell subculturing, cellular senescence was accelerated, as shown by morphological alterations, growth arrest, $p21^{WAF1}$ upregulation and senescence-associated β galactosidase staining. Next, we studied the effect of these prematurely senescent cells on the growth of human malignant lung cell lines (A549, HT-1080 and H1299). Medium conditioned by young and prematurely senescent cells has no major effect on the proliferation of all three cell lines. However, in co-culture studies we have found that the growth of cancer cells was strongly enhanced when cultured on senescent cells. In addition, in immunocompromised mice γ-irradiation-induced senescent cells, similarly to replicative senescent fibroblasts, intensely promoted A549 cells to form tumours. These findings support the concept that replicative- or stress-induced- senescence may play a role in tumourigenesis late in life.

THE ROLE OF NF-κB IN CELLULAR SENESCENCE

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Normal cells divide for a limited number of generations, after which they enter a state of irreversible growth arrest termed cellular senescence. In addition to telomere length, the pRb and p53 pathways are also involved in regulating cellular senescence. Induction of senescence due to telomere erosion initiates a DNA double strand break (DSB) checkpoint response, which involves activation of the kinases ATM and Chk2 and their downstream effector p53. Further, ARF which is induced during senescence leads to the induction of p53 phosphorylation at Ser15, a target site of ATM and also to the modulation of NF-κB function by repressing the anti-apoptotic function of Rel(p65). Induction of ATM is required for IKK activation in response to DSBs, through its interaction with NEMO. Conceivably, the DNA damage-induced NF-κB response is essential for cell survival, pointing towards a role of NF-κB in cellular senescence.

In addition to telomere shortening, excess mitogenic signaling or oncogenic stress are also inducers of senescence. Oncogenic Ras promotes uncontrolled mitogenesis but when expressed in primary cells including normal human diploid fibroblasts (HDFs) provokes a permanent cell cycle arrest with features of senescence, through the induction of DNA damage checkpoint response.

Some reports showed that senescent HDFs exhibited reduced NF-κB binding activity and protein levels, others observed no changes. Here, we showed that the in vitro senescence of IMR-90 fibroblasts resulted in a reduction in the levels of functional nuclear NF-κB. To investigate whether NF-κB was involved in cellular senescence, a super repressor (IκBαSR) and RNA interference was used. Suppression of the NF-κB signaling pathway or knocking-down IKKβ but not IKKα provoked premature senescence of IMR-90. Further activation of NF-κB, through constitutive expression of a mutant IKKβ (IKKβT) rescued IMR-90 from *Ha-RasV12*-induced premature senescence, suggesting that NF-κB plays a critical role in cellular senescence of HDFs.

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INDIVIDUAL AGING AS A CONCEPTUAL AND PRACTICAL CHALLENGE

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The statement that it is not possible to modify aging processes in humans is common in scientific symposiums and conferences. Factually, the situation is much more optimistic.

We define that aging is the gradual and random accumulation of damage in the organism that increase the probability of death (see Kirkwood, 1999). It is an operational definition of the aging process as probability can be measured. According this concept there is no sharp dividing line between age-related changes and age-associated pathologies in humans and aging processes can be controlled.

Aging processes can be modified from individual and demographic (average of the population) points of view. More effective can be individual measures as they take into account the individuality of aging: the genetic background of the person and his lifestyle factor. The lifestyle factor depend on environment and the individual development of the organism during his life (different quality of life, living and working conditions, professional and occupational activity, family and sexual life, friends, food, income, etc.).

There are several ways to take into account human individuality. The subject should take into account unpleasant sensations and feelings, as they are special defense signals that have developed in organisms for informing about needs of changing the environment and activity of these organisms. Another prospective prevention strategy is using chemical substances in case of risk factors to health.

Our studies have shown that postponing aging changes by 20 years is possible at present if to take into account human individuality.

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CHAPERONES AND THE EPIGENETICS OF LONGEVITY
HORMONAL REVERSAL OF CHAPERONE GENE SILENCING

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That aging and longevity are epigenetic phenomena can be supposed from the inherent immortality of embryonic stem cells.

That the molecular chaperones are of crucial importance for the definition of longevity appears from the involvement of chaperones as common denominators in the mechanisms of cellular immortalization in vitro, as well as in syndromes of premature aging (1-2).

Evidence have been presented to suggest that the phenotype of aging, as well as the associated decrease in cancer resistance, results from an increase in the methylation of gene promoters and deacetylation of histones, leading to a progressive silencing of housekeeping genes including the molecular chaperones(3).

The great hormonally determined differences in longevity of genetically identical social insects or nematodes, demonstrates the presence of hormonal mechanisms, with influence on chaperone expression, of possible significance for the assurance of homeostasis and longevity also in mammals.

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POLYMORPHISM OF MITOCHONDRIAL DNA CONTROL REGION AND ITS ASSOCIATION WITH AGING IN THE LATVIAN POPULATION

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Recent observations in different human populations suggest that the accumulation of point mutations in the coding and control (non-coding) regions of mitochondrial DNA (mtDNA) may be important in human aging. In particular, 150T polymorphism in the mtDNA non-coding region has been associated with longevity in some European and Asian populations, e.g. Finns, Italians, and Japanese. However, this polymorphism was not universally observed; among Polish centenarians was found correlation between aging and polymorphisms at sites 73 and 152 in the control region.

Material of the research was DNA isolated from the blood cells (leukocytes). Objects for the polymorphism of mtDNA control region and aging association study were 299 healthy unrelated Latvians aged from 18-40 years, 49 elderly individuals aged from 74-89 years, and 16 centenarians. Both hypervariable segments (HVS-I and HVS-II) of mtDNA control region were analyzed in three mentioned above aged groups.

Large-scale screening of the mtDNA control region from subjects of an Latvian population revealed a homoplasmic C150T transition near an origin of heavy mtDNA-strand synthesis in approximately 14.3% of 49 subjects 74-89 years old, but, in contrast, in only 5.7% of 299 younger individuals ($P = 0.05$). However, among centenarians this certain polymorphism was not found. The possible explanation, on one hand, is a small sample number of studied centenarians, and on another hand, the genetic peculiarities of long-lived individuals.

Our findings suggest that 150T polymorphism in the mtDNA control region is associated with aging in the Latvian population.

TRANSLOCATION OF A SAM-DEPENDENT O-METHYLTRANSFERASE TO MITOCHONDRIA DURING AGING OF *PODOSPORA ANSERINA*

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In order to elucidate age-dependent changes in the composition of the mitochondrial subproteome in the filamentous fungus *P. anserina*, a differential proteome analysis was performed. Mitochondrial protein samples of juvenile and senescent strains were analysed by isoelectric focusing combined with subsequent 2D-SDS-PAGE and MALDI mass spectrometry. The analyses included radioactive labeling, separation by 2D-gel electrophoresis and mass spectrometry-based protein identification. One protein previously demonstrated to increase in abundance in total protein samples of senescent *P.anserina* strains represents a putative SAM-dependent O-methyltransferase (Averbeck et al, 2000). The newly identified localisation in mitochondrial preparations was verified by biochemical and cell biological analyses. A gfp-fusion of the O-methyltransferase was found to translocate to mitochondria during aging. Current experiments focus on the function of this protein. Overexpression strains were constructed and we are in the process of generating 'knock-out' strains. Lifespan, O-methyltransferase-activity and mtDNA stability will be investigated in these strains. In parallel, a tagged form of the protein will be isolated after cloning the cDNA into a vector for heterologous expression in *E.coli*. The isolated enzyme will be used for the production of antibodies for further investigations.

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OXIDATIVE PHOSPYLATION PROVIDES A VALUABLE TOOL FOR IDENTIFYING THE AGING MITOCHONDRIAL PHENOTYPE

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Damage of mitochondria might be the cause and the target of the aging process. In the heart the oxidation of substrates and the synthesis of ATP are shared between two types of mitochondria: subsarcolemmal (SSM) and interfibrillar (IFM). Previously, we reported that oxidative capacity decreases with aging in IFM whereas SSM remain unaffected. Further investigation localized the aging defects in complexes III and IV of the mitochondrial electron transport chain (ETC). During our 6 year study we have performed oxidative phosphorylation assays on SSM and IFM from control groups of 6 month (N=97) vs. 24 month (N=97) Fisher 344 rats. To determine whether there are differences between the two age groups statistical analysis of the oxidative phosphorylation data was performed. We carried out t-test comparisons of means for independent samples. All tests were specified as two-tailed with significance level of 0.05. Our results show that aging significantly decreased by 17.5% state 3 (ADP stimulated) respiration in SSM with glutamate (complex I substrate), whereas respiration with durohydroquinone (complex III substrate) and TMPD-ascorbate (complex IV substrate) remain unaltered. In contrast, in IFM aging significantly decreased by 30% state 3 with glutamate, by 12% with durohydroquinone and by 19% TMPD-ascorbate. Uncoupled respiration with glutamate was decreased by 20% in SSM and by 35% in IFM, suggesting that the defects are localized in the oxidation of substrates rather than phosphorylation of ADP. Our results confirm the defect in IFM in the aged heart. We conclude that the two populations of heart mitochondria develop specific reactions to the aging process. Oxidative phosphorylation analysis is a valuable tool for identifying the aging phenotype in isolated mitochondria.

DISTRIBUTION CHANGE OF WD40 REPEAT PROTEIN 1 IN ARTIFICIALLY INDUCED SENESCENT PC12 CELLS

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Background: WDR1 is thought be correlating with polymerization and depolymerisation of actin protein. Though WDR1 protein under various circumstances was not elucidated up to the present time. In this regard, we tried to see a change in the distribution of WDR1 protein within artificially induced senescent PC 12 pheochomocytoma cells for the first time.

Methods: PC12 pheochromocytoma cells (ATCC CRL-1721) were grown in the culture media including 1 uM 3' –Azido-3' –deoxythymidine (AZT, Sigma-Aldrich, USA). The senescence of the cells was confirmed by senescence detection kit (Calbiochem, San Diego, CA) immunocyochemical study by using WDR1 antibody was also performed in the cells treated with AZT during 0, 75, 153 days.

Results: WDR1 protein was mainly observed within the cytoplasm of the cells not treated with AZT. However the distribution of the same protein was change into the nucleus after 153 day-AZT treatment.

Conclusion: The distribution of WDR1 protein was changed into nucleus in the artificially senescent PC12 cells.

MAINTENANCE OF SELF-RENEWAL CAPACITY AND STEMNESS OF MESENCHYMAL STEM CELLS

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It is generally accepted that in higher organisms' stem cells exhibit a longer lifespan than specialized somatic cells do. It is however still unclear of whether the potential proliferative capacity of tissue stem cells correlates with longevity of the individual donor. We study adult mesenchymal stem cells (MSC) and particularly address the following question:
« Do the stem cells fade with age or is it the niche that grows old? »

However, despite the actual spatial MSC abundance within the organism, little is known about their corresponding *in vivo* niches.It is of general interest in the aging field, how the altered extracellular milieu affects cell and tissue function.

O_2 is fundamental for life However, physiological O_2 levels are rarely taken into account when explanting and culturing primary cells and normal atmospheric O_2 tension will already put them under high oxidative stress (~20% O_2). *In vivo*, mesenchymal progenitors were found enriched in the vicinity of trabecular bone, a region of low O_2 tension. We asked the question of whether a significantly reduced oxygen tension may have a profound impact on cellular processes, in particular regarding lifespan and stemness of MSC.

Experimental results indicate that MSC age in a cell autonomous manner. Freshly explanted MSC reflect the chronological age of their donor. When adjusting O_2 to physiological levels during *ex vivo* cultures the explanted mesenchymal progenitors displayed characteristic variations. Lowering O_2 content in cultures does not inhibit the proliferation capacity of MSC but prolongs lifespan. More over, it is a simple protective measure to block MSC differentiation. Whether O_2 is a decisive signal to propagate stem cell fate or whether it is necessary for the differentiation of mesenchymal progenitor cells *in vivo* and moreover whether MSC derived from elderly donors are less stress resistant is currently being carefully studied.

SERUM PROTEIN LINKED N-GLYCOMIC CHANGES DURING HUMAN AGING: A NEW FUNCTIONAL AGING BIOMARKER?

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Background: Glycosylation is not random but is highly reproducible in a given physiolocal state. It is well accepted that the N-linked oligosaccharides of glycoproteins play important biological roles by influencing the functions of glycoproteins. Significant changes in cellular processes, such as aging and aging-related diseases, may be expected to result in alterations to the glycan profiles of secreted glycoproteins. N-glycan profiling is expected to be used as an aging biomarker predicting the condition of human health.

Methods: DSA-FACE based N-glycan analysis system is a high throughput technology platform and is designed to detect N-glycan profiles from glycoproteins in serum or other body fluids. 219 serum or plasma of the healthy people in different age groups and 129 plasma of the centenarian were used by N-glycan analysis.

Results: The levels of three N-glycan sugar structures (NG0A2F, NG0A2FB and NA2F) in blood are altered with aging. There is an increasing abundance of agalactosylation (NG0A2F, NG0A2FB) and a decreasing abundance of core fucose digalatosylated biantennary (NA2F). This demonstrates that the alteration of N-glycosylation is associated with aging processes.

Conclusion: The measurement of N-glycan level could provide a noninvasive marker for man's health condition or the forecasting of disease progression upon aging, and for efficacy of anti-aging treatment or following up the patient´s condition after therapy.

SCREENING FOR LIFESPAN EXTENSION GENES IN HUMAN DIPLOID FIBROBLASTS

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Onset of cellular senescence in human primary fibroblasts is regulated by intrinsic and extrinsic factors. Intrinsic senescence is the result of telomere shortening, while extrinsic senescence in human cells is mainly regulated through the p16/pRb pathway. In screens using mouse embryonic fibroblasts, several genes of the polycomb family, such as Bmi1 and Ezh2, have been identified which extend the lifespan of these cells through changes in the regulation of the extrinsic pathway. One of these genes, Bmi1, also extends the lifespan of human lung fibroblasts (WI38, which senesce partly through extrinsic factors), but has no effect in human foreskin fibroblasts (BJ, which senesce through the intrinsic pathway). We recently found that Ezh2, like Bmi1, is not capable of extending lifespan in BJ cells.

Considering the differences in regulation of senescence between human and mouse fibroblasts, we developed a screen to identify lifespan-extending genes directly in human fibroblasts. In this screen we used IDH4 cells, primary lung fibroblasts in which a dexamethasone inducible T-ag reversibly regulates senescence. We analyzed the effect of Bmi1 or Ezh2 over-expression in these cells but neither gene was capable of overriding the induced senescent state, thus emphasizing the difference in the regulation of senescence between human and mouse cells. We introduced a cDNA library through retroviral transfection into IDH4 cells, and selected over 100 clones that escape the induced senescent state. Although most of the clones re-expressed T-ag at high levels, we are currently pursuing several clones with T-ag expression levels similar to senescent IDH4 cells. The results of the analysis of these clones, which should contain cDNA's of genes that are involved in the regulation of human senescence, will be presented.

UP-REGULATION OF THE SENESCENCE BIOMARKER CLUSTERIN/APOLIPOPROTEIN J CONTRIBUTES TO HUMAN CANCER CELLS RESISTANCE ACQUISITION TO CHEMOTHERAPEUTIC DRUGS

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Clusterin/Apolipoprotein J (CLU) is a heterodimeric glycoprotein that has been implicated in aging and in numerous physiological disturbance states including *in vivo* cancer progression. To investigate the involvement of CLU in human cancer cells response to chemotherapy, a variety of experimental models has been applied. Several reports demonstrated CLU gene induction in cancer cells after exposure to chemotherapeutic drugs, including doxorubicin (DXR). The aim of our work was to address the role of CLU during osteosarcoma (OS) cells adaptation and eventual resistance to chemotherapeutic drugs. To this end, we developed multi-drug resistant OS cell lines by continuous exposure to gradually increasing, clinically relevant, concentrations of DXR. The created DXR-resistant (DXR-R) OS cells expressed increased protein levels of the marker multi-drug resistant 1 (MDR1) and appeared to be cross-resistant to various unrelated cytotoxic agents. However, pharmacological inhibition of MDR1 sensitized only partially the multi-drug resistant OS cells to DXR. Molecular analysis showed that the multi-drug resistant OS cells had elevated CLU mRNA and protein amounts. Subsequent functional analysis revealed that the increased CLU levels were related to the development and maintenance of OS cells multi-drug resistance, since either knock-down of the CLU mRNA via small interfering RNA or neutralization of the CLU protein by the use of a specific antibody resulted in sensitization of the multi-drug resistant OS cells to chemotherapeutic agents. Therefore, CLU may represent a predictive marker, which correlates to response of cancer cells to chemotherapy.

MODULATION OF ANGIOGENESIS BY HEAT SHOCK IN MORTAL AND IMMORTAL HUMAN ENDOTHELIAL CELLS

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Angiogenesis is a physiological process involving the growth of new blood vessels from pre-existing vessels, by the active participation of vascular and microvascular endothelial cells. Since there is an age-related decrease in angiogenesis, our aim is to study the effects of various stimulatory and inhibitory factors on blood vessel formation. Our labs have previously shown a variety of anti-aging and beneficial hormetic effects of repeated mild heat shock on human fibroblasts and keratinocytes. This approach is now extended to human vascular and microvascular endothelial cells, using normal human vascular endothelial cells (HUVEC) undergoing aging *in vitro,* and an immortal human microvascular cell line, HMEC-1. A standardized tube-formation assay by growing cells on commercially available basement membrane extract (Matrigel matrix), is employed to analyse the kinetics and extent of new blood vessel formation in response to 1 hr heat shock at various temperatures (39°C to 43°C). Formation of vascular tubes is monitored by light microscopy and quantitative comparisons are made by using reliable imaging programs. Results from our ongoing studies comparing age-related changes in tube formation in serially passaged HUVEC and immortal HMEC-1 in response to single or multiple exposures to mild and severe heat shock will be presented. Additionally, some data on the effects of other potential modulators of angiogenesis, such as sugars, cytokinins and stress hormones will be presented.

HORMONES AND AGING RESULTS OF THE EXPLORATIVE PROJECT IN THE GERMAN NATIONAL GENOME RESEARCH NETWORK II (NGFN-2), GENETIC AETIOLOGY OF LONGEVITY

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Recent data have illustrated that hormones at age- and sex-specific levels can alter the development of cells by regulating their transcriptome. An *in vitro* model on endogenous skin aging has been developed, which can be applied to all skin cell types and may serve to enhance our understanding on skin aging. Human SZ95 sebocytes were incubated under a hormone-substituted environment consisting of growth hormone, insulin-like growth factor-I, 17β-estradiol, progesterone, testosterone and dehydroepiandrosterone in concentrations corresponding to 20- (f20) and 60- (f60) year-old women. SZ95 sebocytes at f60 showed a significantly lower content of sebaceous lipids in contrast to the cells receiving the f20 treatment. This is the first *in vitro* evidence that skin xerosis occurring with age may be attributed to lack of circulating hormones. Furthermore, expression profiling employing a cDNA microarray composed of 15,529 cDNA transcripts identified 899 genes with altered expression levels at f20 versus f60. Among them, genes were identified which are involved in several biological processes such as DNA repair and stability, mitochondrial function, oxidative stress, cell cycle and apoptosis, ubiquitin-induced proteolysis and transcriptional regulation processes that play a substantial role in endogenous aging. Interestingly, genes at key positions in the pathomechanism of neurodegenerative diseases also showed differential expression between f20 versus f60 treated SZ95 sebocytes. Subsequently, aging-associated genes from the 899 ones, which showed an upregulated expression at f60 treated cells have been selected and their inhibition has been performed by means of small interfering RNA (siRNA). Verification of the inhibition was performed at RNA and protein level via TaqMan PCR and Western blotting, accordingly. The goal of our further work is to examine the alterations of the biologic activity of the siRNA-transfected cells in order to gain greater insights into the role of the respective genes in the aging process.

NUTRACEUTICAL STRATEGY IN AGING: TARGETING HEAT SHOCK PROTEIN AND INFLAMMATORY PROFILE VIA IL-6 POLYMORPHISM UNDERSTANDING

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Antioxidant nutrients have been implicated in processes associated with aging and inflammatory damage. Moreover, aging is associated with a decreased expression of HSP at the cellular level. The aim of this study was to assess the inflammatory profile and polymorphism of healthy elderly subjects and the possible influence of a nutraceutical supplementation. Forty sedentary, normofolemic, normolypemic subjects aged 66-74 y old (69 ± 3) were recruited. Subjects were randomly divided in two groups matched as for life-style, alcohol/tobacco use and none was taking any vitamin/mineral supplements or medications.. One group was given an ISO9001-certified fermented papaya preparation (FPP, Osato Research Institute, Gifu, Japan) 9g/day by mouth in the morning while the control group shall receive same amount of placebo (flavoured powdered sugar). Treatment were carried out in a cross-over manner with a 3 months supplementation period followed by a 6-week washout period between treatments. A group of 10 healthy young subject (24-33 y/old) was also considered. IL-6 promoter-174 G/C polymorphism genotype was determined by an allele specific PCR Blood samples was withdrawn at entry and on a monthly basis to test: Redox Status **(**SOD**,** GSH, GSH-Px and GSSG), IL-6, ultrasensitive (hs-) CRP and serum Hsp70 (inducible form) concentration. Unlike redox balance, hs-CRP and IL-6 were higher in elderly subjects ($p<0.05$ vs young). The serum concentration of HSP70 showed a trend inverse correlation with markers of inflammation in the whole elderly population but significantly in -174 G/C-negative subjects (r: 0.62, $p<0.05$). Nutraceutical intervention brought about a normalization of inflammatory parameters ($p<0.05$) with a parallel rise of HSP70 ($p<0.05$). Our finding suggest that even in apparently healthy elderly, a pro-inflammatory profile plays as a down-regulating factor for inducible HSP70, expecially in -174 G/C-negative genoptype. A nutraceutical intervention seems to beneficially modulate such phenomenon and has to be taken into account for longer term observation.

OXIDATIVE STRESS-INDUCED RPE CELL SENESCENCE: A MODEL FOR AGE-RELATED MACULAR DEGENERATION (AMD)

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It has been hypothesized that cumulative oxidative stress plays a role in retinal aging and in the pathogenesis of age-related macular degeneration (AMD). The major risk factor for AMD is age and the retinal pigmented epithelium (RPE) is the prime target for the early development of the disease, due to the presence of extracellular material deposits, called drusen, beneath RPE. The mechanisms of RPE cell senescence and degeneration in AMD remain unknown. Therefore, we investigated the role of oxidative stress in RPE cell senescence and characterize the molecular changes underlying this process.

Human RPE cells were submitted to chronic exposure of the chemical oxidant t-BHP. Gene expression was analyzed by a cDNA microarray and by qPCR. Induction of stress, senescence and signal transduction associated proteins were analyzed by western blotting and ELISA tests. Functional tests were realized to assess the effect of senescence on RPE intrinsic properties: transepithelial permeability, angiogenic factor secretion and adhesion.

High, transient and cumulative t-BHP-mediated oxidative stress induced RPE cell senescence, characterized by 1- cell hypertrophy, 2- incapacity to proliferate after serum stimulation, 3- Senescence-Associated β Galactosidase activity, and 4- cell cycle arrest in G2. Thirty-seven genes associated with stress and senescence were found differentially expressed. Stress-induced premature senescence (SIPS) affected RPE cell permeability, phototransduction enzyme expression, adhesion and angiogenic factor secretion, all these process being altered in the retina of AMD patients. Furthermore, proteins accumulating in drusen of AMD patients were found overexpressed in SIPS-treated RPE.

In conclusion, our study shows that chronic oxidative stress induces RPE cell premature senescence characterized by physiological, structural and biochemical changes, and provides a useful model to study the mechanisms and markers of senescence during RPE aging. Therefore, this model could be of interest in screening therapeutic strategies against age-related RPE degeneration, such as AMD.

EFFECTS OF INTER-INDIVIDUAL VARIATION ON ZINC-REGULATED GENE EXPRESSION IN AGING

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Nutrition plays a critical role in health and disease in vulnerable populations. Understanding the complex nature of the interaction between dietary nutrients and individual variation in gene expression, whilst a relatively unexplored area of nutritional science, is important in identifying individuals or groups of individuals that would benefit from a nutritional intervention. Although mild zinc deficiency, which is prevalent in vegetarians, diseased individuals, and the general aging population, depresses immunity and increases risk of disease in later life, zinc intervention trials in the general population have produced conflicting results. Since heterogeneity of the elderly population may impact on response to dietary zinc, nutrigenomic approaches may aid in identifying subsets of the elderly population with increased risk of zinc deficiency who might receive benefit from a dietary zinc intervention. In the current study, we used gene array technology to investigate the effects of *in vitro* zinc supplementation on gene expression of peripheral blood mononuclear cells isolated from elderly donors. Ingenuity™ pathway analysis identified several genetic networks altered during human aging which appeared responsive to zinc, including inflammatory, stress, and metabolic signalling pathways. Many of these zinc-regulated genes, including IL-6, are important in human health and disease. Elderly individuals with IL-6 polymorphisms resulting in enhanced IL-6 production displayed a differential response to dietary zinc, including decreased transcription of pro-inflammatory cytokines and alterations in metabolic regulatory pathways. These data indicate that elderly individuals with a pro-inflammatory phenotype might have increased risk of zinc deficiency and may benefit from zinc supplementation.

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COMBINED LIPID GENE POLYMORPHISMS ARE ASSOCIATED WITH SUBSTANTIAL TRIGLYCERIDE AND HDL-CHOLESTEROL ALTERATION AND POOR PHYSICAL FUNCTIONING IN OLDER PEOPLE

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Circulating cholesterol fractions and triglycerides are well established as independent risk factors for vascular disease and exceptional survival. There are polymorphisms in human Apolipoprotein E (ApoE), apolipoprotein A-V (ApoA5), cholesteryl ester transfer protein (CEPT), lipoprotein lipase (LPL) and hepatic lipase (LIPC) that are each well validated as associated with small changes in specific lipid levels. An analysis of combined effects of these polymorphisms on lipid levels, disease markers and functioning in older people is needed.

We studied 977 subjects from the InChianti population based cohort aged 65 years and over. Associations between each established polymorphism and serum concentrations of total cholesterol, HDL-C, LDL-C and triglycerides were studied, and risk allele counts were constructed for each measure. Associations with disease and physical performance (using the Short Physical Performance Battery score – SPPB - based on walk speed, chair stand and balance tests) were examined.

Polymorphisms in two genes altered total and LDL cholesterol, three altered HDLs and four altered triglycerides: the latter two gene sets overlapped.

We found a strong association between the risk allele count for triglycerides with serum triglyceride: 16.2% with 0-2 risk alleles, 25.6% with 3 alleles and 34.6% with 4-6 alleles were either borderline high or above recommended triglyceride levels (>150 mg/dL). Those with 4-6 alleles therefore had an odds ratio = 2.69 (95% CI: 1.71 – 4.22, $p = 1.7 \times 10^{-5}$) for having triglyceride levels above 150 mg/dL, compared to those with 0-2 alleles. The risk allele score was also associated with having diagnosed peripheral arterial disease (p= 0.017), and poorer performance on the tested SPPB score OR=1.21(95% CI: 1.04 – 1.41, p=0.016).

The accumulated effect of lipid gene variability is associated with substantial changes in risks of elevated triglyceride levels. These effects are associated with poor age related physical functioning, confirming observational studies of the effect of triglycerides on aging outcomes.

CYTOKINES AND MMPS/TIMPS PRODUCTION BY CULTURED DERMAL PAPILLARY AND RETICULAR FIBROBLASTS FROM DONORS OF DIFFERENT AGE SUGGEST PREFERENTIAL PAPILLARY DERMIS MODIFICATIONS DURING SKIN AGING

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Intrinsic skin aging is characterized by a marked atrophy of the dermal connective tissue with a progressive loss of elasticity and dermal papillae that are associated with reduction and disorganization of the components in the extracellular matrix.

The adult human dermis is divided into the superficial papillary dermis and the deep reticular dermis respectively populated by papillary and reticular fibroblasts embedded in extracellular matrix. Because the production of extracellular matrix by fibroblasts is controlled by growth factors and cytokines, and because MMPs are known to play an important role in matrix remodeling, we hypothesized that a variation of cytokines and MMPs/TIMPs profiles, secreted by papillary and reticular dermal fibroblasts, could play an important role in dermal changes occurring during normal skin aging *in vivo*.

Matched pairs of papillary and reticular human fibroblasts, obtained from breast skin of women of different ages were isolated and cultured on plastic. The amounts of interstitial cytokines, MMPs and TIMPs secretion were assessed by ELISA and compared as a function of fibroblast population and donor's age.

Our results showed that IL-6, VEGF, KGF, MMP-1, MMP-2, MMP-3, TIMP-1 and TIMP-2 released by papillary fibroblasts seemed to increase with age whereas their release by reticular fibroblasts seemed to be constant. In contrast with these, MCP-1 release by reticular fibroblasts seemed to decrease with age although the release by papillary fibroblasts seemed to be unchanged. These results can be discussed both in terms of changes in the properties of the cells or changes in cell populations as a function of age. This study indicates that aging could preferentially affect fibroblast function in the superficial dermis.

A COMMON GENETIC VARIANT IN CANCER GENE p16^{INK4A} IS STRONGLY ASSOCIATED WITH PHYSICAL FUNCTION IN OLDER PEOPLE

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P16^{ink4a} and ARF play critical roles in regulating the Rb and p53 tumour suppressor pathways, are expressed from the same genetic locus and are frequently deleted in a variety of human cancers. Mutations in p16 account for many familial cases of melanoma. Expression of p16 is strongly age related.

Initially 938 women aged 65 to 80 years from the EPIC study (Norfolk UK) were genotyped for common polymorphisms in p16 and related genes. One SNP was significantly associated with physical functioning and was then genotyped in 1916 additional EPIC participants, 765 InChianti study and 419 Iowa-EPESE participants. Associations were tested with the SF36 physical performance subscale plus the Short Physical Performance test Score in 'white Caucasian' origin subjects aged 65 to 80yrs.

Of 25 SNPs in 5 related genes initially tested, the rs2811712 SNP at the p16/ARF locus was associated with reduced impairment (p= 0.018). This association was replicated in the additional EPIC samples (p-value=0.017) and the InChianti Study (p=0.038), and on one sided significance in the Iowa-EPESE; for the overall association p=7.4x10^{-5}. The prevalence of severely limited function declined from 12.7% in the p16 common homozygotes through 8.8% (heterozygotes) to 5.3% (rare homozygous).

The p16 / ARF locus and cancer control / cell senescence pathways play an important role in physical aging. There are substantial effects of a common variant in this cancer linked gene on physical functioning and disability in older people.

REGULATION OF T-CELL RESPONSES TO ACUTE AND CHRONIC VIRUSES IN OLD RODENTS

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T-cell immunity undergoes profound changes in senescence, including, but not limited to, decline in naive T-cell production, increased compensatory proliferation of the remaining naive T-cells, consumption of naive T-cells by repeated and/or persistent pathogenic assaults, and decreased efficacy of T-cell signaling. We investigated the interplay of acute or chronic viral infection and regulation of homeostasis and immunological memory in old mice. We provide evidence for antigen-independent disturbances in T-cell homeostasis and repertoire that can lead to increased vulnerability to pathogens. Moreover, we show that chronic, but not acute, viral infections directly contribute to additional disturbances in T-cell homeostasis and memory maintenance. Relative importance of antivirals and of restorative T-cell intervention is discussed in light of these results.

ABNORMAL MITOSIS AND ABERRANT NUCLEAR MORPHOLOGY IN H_2O_2-INDUCED PREMATURE SENESCENT HUMAN FIBROBLASTS

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Aberrant nuclear morphology such as irregular shapes of nuclear outline and micronuclei formation or nuclear fragmentation is often observed in replicatively or prematualy senescent cells. The mechanisms leading to the production of such aberrant nuclear morphology accompanying cellular senescence are not well understood. Because recent research suggests abnormal regulation of mitosis is closely related with cellular senescence, mitosis failure in senescent cells might be responsible for the production of such abnormal nuclei.

In this study, premature senescence was induced in normal human fibroblasts by brief exposure to hydrogen peroxide (H_2O_2) and mitoses were recorded with a time-lapse fluorescent microscopy up to 7 days after the exposure. As a result, abnormal mitoses leading to the production of polyploid cells with aberrant nuclear morphology were often observed several days after exposure to H_2O_2. The abnormal mitoses observed include an exit from mitosis without chromosome disjunction and cytokinesis, which produced polyploid interphase cells. This process is known as mitotic slippage. In other cases, cytokinesis failure after chromosome disjunction produced binucleate cells. In some cases, chromosomes segregated abnormally without chromosome disjunction and resulted in multiple fragmented nuclei. Cell cycle analysis with laser scanning cytometer showed accumulation of a subpopulation with polyploid DNA content after exposure to H_2O_2. Cells with aberrant nuclear morphology were mainly recognized in such a subpopulation with polyploid DNA content. Immunofluorescent staining of α- and γ-tubulin showed the increased frequency of mitotic cells with malformed spindles or abnormal centrosome number after exposure to H_2O_2. Ill-aligned chromosomes in prophase or metaphase cells were also often observed.

The observations in this study suggest that the mitotic failure suchlike mitotic slippage result in polyploid cells with aberrant nuclear morphology often observed in senescent cells. Implication of defective mitotic spindles in abnormal mitosis was also suggested.

THE POPULATION DYNAMICS AND ALTERED PHENOTYPE OF VASCULAR SMOOTH MUSCLE CELLS

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The accumulation of senescent cells with an altered phenotype is a plausible primary cause of the aging of mitotic tissues. Senescent cells are produced as a result of tissue turnover by the operation of stochastic processes which trigger permanent exit from the cell cycle. Thus the rate at which such cells are produced and their phenotype once senescent are crucial in determining whether replicative senescence can play a role in the aging of any given tissue.

We have undertaken an analysis of the population dynamics of human vascular smooth muscle cells in culture. The growth fraction, senescent fraction and apoptotic fraction were measured across the lifespan of the culture. Over 40 population doublings, we observed a gradual loss of the growth fraction, a non-linear increase in the senescent associated beta-galactosidase positive fraction of cells and minimal apoptosis. These population dynamics differ markedly from those observed by us some years ago in human vascular endothelium.

In parallel with this, we sought to characterise the phenotype of senescent (42 population doubling) human vascular smooth muscle cells relative their growing (14 population doubling) counterparts using Affymetrix microarrays. Three different analytical methods were used to identify genes that were differentially regulated between the young and senescent populations. In each case a *p*-value threshold was selected at which < 0.5% of the resultant probeset list would be expected to be false positives. In addition, a minimum effect size of at least 2-fold differential regulation was required.

A total of 333 probesets were called as differentially expressed by these methods. These were also strikingly overrepresented in a list of known genes involved in atherosclerotic or vascular calcification processes manually created by review of the relevant literature prior to our data collection. Thus senescent vascular smooth muscle cells are plausible causal agents for cardiovascular aging.

THE CORRELATION BETWEEN THYROID FUNCTION AND SERUM LEVELS OF LIPID PROFILES, HOMOCYSTEINE, LEPTIN, FIBRINOGEN, AND CRP AMONG HYPERTHYROID PATIENTS.

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The present study was undertaken to determine whether thyroid hormones affect lipid profiles, erithrocyte sedimentation rate (ESR), serum total homocysteine (t-hcy), leptin, fibrinogen, C-reactive-protein (CRP) levels in patients with hyperthyroidism.

Twenty three hyperthyroid patients (mean age 41.782 ± 2.363) were included in the study. Serum levels of homocysteine, leptin, fibrinogen, CRP, total-cholesterol (T-Ch), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholestreol (LDL-C) and ESR were measured and body mass indices (BMI) were calculated before and after treatment.

Pretreatment t-hcy (p=0.001), T-Ch (p=0.001), LDL-C (p=0.001), HDL-C (p=0.001) levels and BMI of the hyperthyroid patients were significantly lower than the post-treatment levels. However, fibrinogen and ESR decreased after the treatment. Serum leptin and CRP did not change significantly during the treatment. Pre and post treatment T-Ch and LDL-C were negatively correlated with fT_3 levels (r = -0.588, p=0.01, r = -0.543 p= 0.01, r = - 0.543, p= 0.01, r = -0. 653, p= 0.01 respectively). Pre-treatment HDL-C was inversely correlated with TSH (r = - 0. 423, p= 0.05). Pre-post- treatment LDL-C were negatively correlated with fT_4 levels (r= - 0. 536, p= 0.001, r = - 0. 422, p= 0.05 respectively). Pre-treatment T.Ch was inversely correlated with fT_4 (r = -0. 590, p=0.01). t-hyc, leptin , sedimentation and BMI did not correlate with TSH, fT_3 and fT_4.

Hyperthyroid state is associated with high plasma fibrinogen and ESR levels. Elevated plasma fibrinogen and ESR levels may be a possible expalnation fort he high cardiovascular morbidity among effected subjects. These changes may reflect low-grade inflammation or disturbances in coagulation in hyperthyroidism

ROS-DEPENDENT 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE DEREGULATION DURING AGING

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Aim: It has been observed that the age-related total activation of the hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoAR) is due to a lack of regulation by phosphorylation/dephosphorylation process. Such fully activation has been correlated with ROS increase but the responsible mechanism is still unknown. So, aim of this work was to study the mechanism underlying ROS induced impaired regulation of HMG-CoAR verifying the involvement of the main modulators of reductase short term regulationIn particular in a well characterised cellular model, the HepG2 cell line after high ROS content induction and in aged rat liver, the role of AMP dependent kinase (AMPK) and protein phosphatase 2A (PP2A) has been studied.
Methods: To induce ROS content as high as in aged rat liver, HepG2 cell line were treated with 200μM H_2O_2. ROS content was measured by fluorescence analysis. HMG-CoAR activation state was evaluated by a radioisotopic method. The activation state of the AMPK and the level of PP2A was measured by western blotting. H_2O_2-induced signal transduction pathway was evaluated by western blotting and the downstream effects by using specific inhibitors. Protein association was revealed by co-immunoprecipitation.
Results: In HepG2 cell line the HMG-CoAR results completely activated by ROS. AMPK activation state is high even if the enzyme isn't able to phosphorylate HMG-CoAR; PP2A level doesn't change. The p38/MAPK phosphorylation rises and the use of a p38 inhibitor or of some phosphatase inhibitors prevents ROS-induced HMG-CoAR full activation. Both in H_2O_2 treated cell and aged liver the association of PP2A with HMG-CoAR is well detectable.
Conclusion: The presented data show that ROS increase is able to induce HMG-CoAR full activation enhancing the reductase-PP2A association through p38 activation and subsequent dephosphorylation of the enzyme. On the contrary the modified activity of the AMPK seems to be not involved in this deregulative process.

CHRONIC AGGREGATION OF AN ER PROTEIN: EFFECTS ON PROTEIN HOMEOSTASIS AND THE CYTOSOLIC STRESS RESPONSE

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Protein conformational diseases are important models to understand the role of protein homeostasis in the aging of postmitotic tissues. One such model is alpha1-antitrypsin (AAT) deficiency, characterized by the accumulation of a misfolding mutant (PiZ) inside the lumen of the hepatic endoplasmic reticulum (ER). The mutant protein harbors a Glu-Lys change, which induces a disruption of a salt bridge, accompanied by an inability to pass the ER quality control. Both human patients and PiZ transgenic mice have similar symptoms of hepatic failure, culminating in cirrhosis and hepatocellular carcinoma.

The accumulation of misfolded proteins along the secretory pathway induces the unfolded protein response (UPR). However, work from our and from other laboratories using either PiZ transgenic mice or overexpressing cell lines could neither detect induction of ER chaperones, nor the activation of UPR mediators. Transgenic mice showed an interaction of PiZ with protein disulfide isomerase (PDI), and a decreased protein disulfide reductase activity. Moreover, showing a crosstalk between the ER and the cytosolic compartment, both antioxidant and protein repair enzymes (thioredoxin and methionine sulfoxide reductase A) and the heat shock proteins (Hsp70 and Hsp90) were upregulated.

To further elucidate the role of an ER protein in the general protein homeostasis at the cellular level, we engineered a transgenic liver cell line with inducible expression of the wild type alpha-1-antitripsyn and the PiZ mutant. Results of our ongoing studies will be presented.

OXYGEN FREE RADICALS IN SENESCENCE: ARE THEY SIGNAL TRANSDUCERS?

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Oxidative stress, which can be generated by dysfunctional mitochondria, results in DNA damage and accelerated telomere shortening. Thus, mitochondrial superoxide generation, which increases as human fibroblasts approach senescence, contributes to telomere-dependent senescence. However, to a large extent mitochondrial dysfunction and cellular ROS production is a consequence of cell senescence. We show that induction of growth arrest by either telomere uncapping or by telomere-independent, genome-wide DNA damage results in increased mitochondrial biogenesis and dysfunction including mitochondrial superoxide production, cellular ROS increase and compromised calcium dynamics.

We also show that ROS induction is dependent on mitogen signalling in particular p38 MAPK and TGFβ/NADPH oxidase pathways. In addition, frequencies of nuclear DNA damage foci are significantly reduced by inhibition of the same signalling pathways, suggesting that secondary ROS generation contributes to long-term DNA damage signalling and, thus, stability of the senescent phenotype.

MEFs from late generation Terc-/- mice senesce even under low ambient oxygen in a telomere-dependent manner and show high mitochondrial superoxide production. At the same time, MEFS from late generation Terc-/- p21-/- double-knockout mice do not senesce and retain low mitochondrial and cellular ROS levels, indicating that the signalling towards mitochondrial dysfunction and ROS induction emerges downstream from p21. Interestingly, both tissues and MEFs of Terc-/- p21-/- mice show lower frequencies of non-telomeric γ-H2A.X foci than those from Terc-/-, confirming the idea of a positive feed-back loop between DNA damage signalling and mitochondrial ROS production. Moreover, inhibition of p53, a transcriptional activator of p21, by siRNA leads to less ROS generation and less DNA damage foci formation.

In conclusion, we propose that mitochondrial dysfunction and ROS production are part of the senescent phenotype, are triggered via mitogen signalling pathways that emanate downstream from p21 and contribute to the long-term maintenance of senescence via a stable activation of DNA damage response.

ANALYSIS OF SITE SPECIFIC OXIDATION IN MITOCHONDRIAL ACONITASE

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Mitochondrial matrix proteins are sensitive to oxidative inactivation and oxidized proteins are known to accumulate during aging. Mitochondrial aconitase plays a key function in cellular energy production and loss of its activity has a major impact on the organism. Reactive oxygen species can lead to the inactivation of enzymes by the irreversible conversion of side chain residues of arginine, lysine, proline and threonine into carbonylated (ketone and aldehyde) counterpart products. Thus these carbonylated residues can be used as biomarkers of oxidative damage. We have investigated the oxidation sites in porcine aconitase in its apoenzyme and holoenzyme forms in order to identify the specific sites of oxidation and whether there are differences regarding the presence or absence of the iron sulfur cluster (prosthetic group). Oxidized aconitase was derivatized using biotin hydrazide for site specific detection by mass spectrometry approaches (MALDI-TOF/TOF). The protein carbonyl content was measured using the 2,4-dinitrophenylhydrazine derivatization method, followed by quantification by HPLC. Oxidized BSA was used as model protein to study derivatization efficiency and to establish the method. The results have been compared to verify differences between the oxidation sites depending on the presence or absence of the prosthetic group. The divergence regarding these sites highlights the role of the metal group during the oxidation process and the implications of the mechanisms of the metal catalyzed oxidation *in vivo*.

GENETIC BACKGROUND DETERMINES THE TYPE OF IMMUNE RESPONSE AND ITS MODULATION BY DHEA, BUT NOT THE TRENDS IN AGE DEPENDENT CHANGES IN MICE

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In most cases the attempts to correct the age-dependent changes in immune system has different results in different experimental conditions which may be caused by genetic background.

Objective. This study examines the influence of genetic background and aging on immune cell reactivity in vivo and in vitro by assessing the plaque forming cells (PFC), proliferative response (PHA and Con A), lymphoid cell subpopulation and cytokine production by splenocytes in intact and DHEA treated C57BL/6 and BALB/c female mice of different ages.

Results. The young C57BL/6 mice had greater proliferative response to Con A, and smaller number of PFC in the spleen in comparison with BALB/c. These differences was accompanied by greater Con A induced IL-2 production by splenocytes in vitro, and smaller CD4+/CD8+ ratio in the spleen of C57BL/6 mice, that supposed the mainly (Th1)-type response in this line. Single DHEA injection 1 day before experiment led to the inversion changes in splenocyte subpopulation: the CD4+/CD8+ ratio and IL-2 production was decreased in the spleen of C57BL/6 mice and increased in BALB/c, the number of Con A activated CD25+ splenocytes was increased in C57BL/6 and decreased in BALB/c mice. These changes were not accompanied by any changes in functional tests which may suggest different regulatory mechanisms of DHEA action in different lines. The old C57BL/6 mice had similar differences in splenocyte subpopulations in comparison with BALB/C as in young mice. But had not any differences in functional tests, and in DHEA action.

Conclusion. Immune cells from young mice both strains responded to antigen in vivo, and to mitogens in vitro in different manner. But old mice had similar age dependent decrease of lymphocyte functions irrespective of genetic background. This fact may testify that aging process is regulated by very conservative genes, which is possessed in most inbred lines.

INFLUENCE OF ORCHECTOMY ON BONE MINERAL DENSITY IN MALE RATS OF REPRODUCTIVE AGE

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The aim of the present study is to evaluate the influence of orchectomy on bone mineral density and bone mineral content in male rats of reproductive age.

There were inspected 16 male rats of reproductive age, "Vistar" line. 10 rats (mass-0,18±0,005 kg) made up a control group (CG); 8 animals of experimental group (mass-0,20±0,006 kg) have undergone orchectomy (ORC).

Bone mineral density (BMD) and bone mineral content (BMC) were measured using dual energy X-ray densitometry (DEXA) and «Experimental animals» software. Examination was made before orchectomy and over 30 days after operation (Tab. 1). The index was calculated according to the formula: Δ BMD(%) = (Δ BMD/BMD ref.) x 100

Table 1. Dynamics of bone mineral density and bone mineral content in male rats of reproductive age after orchectomy.

Group	BMD ref.	Δ BMD	Δ BMD (%)	BMD ref.	Δ BMC	Δ BMC (%)
CG	0,105 ± 0,002	0,019 ± 0,009	19,33 ± 9,82	9,65 ± 0,26	2,44 ± 0,28	25,88 ± 3,48
ORC	0,113 ± 0,002	-0,003 ± 0,003	-2,87 ± 2,43	11,62± 0,31	-0,30± 0,31	-2,41 ± 2,65
F	5,84	3,89	4,01	7,12	32,52	26.7
p	0,015	0,047	0,041	0,009	< 0,00001	< 0,00001

Annotation**:** M±m; CG - animals of control group; ORC – animals with orchectomy; BMD ref. - initial indexes of bone mineral density of the entire body; BMC - initial indexes of bone mineral content of the entire body; F - Fisher index.

The orchectomy leads to a substantial decrease of bone mineral density and bone mineral content in male rats of reproductive age, allowing this method to be used for creation of experimental model of osteoporosis.

COMMON GENETIC VARIATION IN INTERLEUKINS: ASSOCIATIONS WITH SERUM CONCENTRATIONS AND PHYSICAL FUNCTIONING IN OLDER PEOPLE

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Interleukins IL-1RN, IL-6r and IL-18 are important cytokines, implicated in aging processes. Some of these interleukins or their receptors or antagonists already provide targets for treatments. We hypothesized that interleukin related genotypes alter serum concentrations and thereby alter inflammatory profiles, age related physical functioning and the development of frailty.

We studied 1273 subjects (730 aged 65-80 years) from the InChianti population based cohort and 999 subjects from wave 6 of the Iowa-EPESE cohort, aged 65 to 80 years. In the InChianti study, serum concentrations of the investigated cytokine markers were measured, plus other inflammatory markers. We selected polymorphisms covering more than 80% of normal variation in each target gene using data from the hapmap project. Associations with physical performance (slower 7 meter walk time, SPS score physical performance tests), other inflammatory markers and metabolic traits were examined.

For each cytokine investigated we observed strong statistical evidence of functional effects of polymorphisms within these genes on respective protein levels. A single polymorphism in *IL-6r* explained 21% of variation in IL-6r levels ($p = 5.1x10^{-62}$) and was also associated with circulating IL-6 levels ($p = 1.9x10^{-4}$). Similar observations were made for *IL-1RN* ($p=4.9x10^{-6}$) and *IL-18* ($p=1\times10^{-5}$). These polymorphisms had a range of effects on other inflammatory and metabolic markers. An *IL-18* polymorphism was associated with physical functioning tested using walk time test (combined p=0.001) and SPS scores (combined p=0.019) in both the InCHIANTI and Iowa studies, and was found to reside on the same haplotype, previously implicated in cardiovascular mortality in the Atherogene study. This result requires further replications.

Common genetic variation in selected interleukin related genes may play an important role in explaining differences between older people in the development of inflammatory processes in aging.

CD4/CD8 RATIO, NUTRITION AND LONGEVITY IN BELFAST ELDERLY LONGITUDINAL FREE-LIVING AGING STUDY (BELFAST)

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A competent immune system is important in good quality aging and longevity. A CD4/CD8 ratio < 1 has been associated with reduced survival in some human studies, and may describe an 'immunophenotype' associated with poor outcome. In a previous study we showed CD4 lymphopenia in nonagenarians to be associated with poor nutrition. In this study we asked if CD4/CD8 ratio in **B**elfast **E**lderly **L**ongitudinal **F**ree-Living **A**ging **ST**udy (**BELFAST**) subjects determined survival and whether it related to nutrition.

CD4/CD8 ratio was measured in 223 community living, apparently well subjects, 92, >90 years, 25, 80-90 years and 27, 60-80 years with other younger healthy blood donors. CD4 and CD8 lymphocytes were defined by flow cytometry, and nutrition measured by anthropometry or biochemical methods. Mean CD4/CD8 ration was 2.1 (SD1.2), median 1.9, with a non significant decreasing trend with age (p=0.08). Females had non significantly higher CD4/CD8 ratios compared to males including for nonagenarians -CD4/CD8 ratio in males>90 years was 1.8 compared to 2.1 for female nonagenarians. CD4/CD8 ratio was weakly associated with nutritional factors including skin fold thickness (p=0.04), vitamin A categories (p=0.05) and selenium (p=0.01) and subject dependency index (p=0.06). With data split into 2 groups (CD4/CD8 count <1.3 and >1.3), Kaplan Meier test showed no differences between survival (p=0.82).

In apparently well **BELFAST** subjects the CD4/CD8 ratio is maintained across all the age groups including nonagenarians and was weakly related to some nutritional measures. Octo/Nonagenarian males have non-significantly lower CD4/CD8 ratio compared to females. Kaplan Meier survival analysis showed no survival advantage for nonagenarians showing higher CD4/CD8 ratio.

SIGNIFICANCE OF DISTRESS MANAGEMENT IN EUPHENICS. DIRECT CONNECTIONS WITH AGING DECELERATION

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In the actual and future civilizations, the human aging will have a decisive influence in re-configuration of concepts about life in general and especially about human being. The new outlooks must be convergent and harmonious with phenomena ecology of globalization and must furnish new solutions and strategies in science, biology, medical field and society. Euphenics modulation by distress control has immediate and long-term implications in geronto-geriatrics. They can be explain and put into practice by two new concepts, which were introduced by the authors. There are the negative life tetrad as entropic cascade (distress → impairment → aging → disease) and the reverse, the positive life tetrad as anti-entropic cascade (eustress associated with good life styles → diminuation of wear and tear, as well as of functional and structural damages → aging deceleration and normal senescence → decrease, as frequency and intensity, of polypathologies of old people). In these prospects, distress control becomes a multilevel and complex phenomenon. It should unify the rational interventions on surroundings and decrease of external stressors (physical, chemical, biological etc.) with strong, pertinent and continuous changes in human mentality and behaviour harmonization, associated to diminution of psychic distress (cognitive, affective etc.) and elimination of wrong life styles. In addition, nutrigenetics and genetic nutritioneering have strong benefic, anti-entropic and reconstructive effects. They reduce negative impact of distress on human being and can modify inherited traits (by improving gene expression and even reprograming the genes), and like this contribute to a healthier, longer and more time active life. Synergistic new connections, conceptions and strategies at bio-medical, health care, anthropological, social and political levels can solve these urgent problems with final desideratum: improvement of environment, health and life quality.

FROM EUPHENICS TO EUGENICS. FROM ENVIRONMENTAL CONTROL TO EXTENSION OF HUMAN LIFE AND PERFORMANCE

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Environment is in continuous changing (bad or good). Also, the humans are still in evolution (bad or good). But, in our days, the surroundings configuration and human evolution depend in the greatest measure of human behaviours and actions. So, the human interventions on the outside (environment) and on the inside (ourselves) are and will be decisive in the near future. These can determine the normality of surroundings and humans with harmonious inter-connections, or abnormality and destruction (aggressive environment and fragile human beings) with total disaccord and antagonism between them. Therefore, to exemplify these complex inter-relations, we introduced a new concept: the negative life tetrad (distress → impairment → aging → disease). During ontogenesis, this outlook has a negative impact on humans and turns into a strong entropic cascade. Moreover, environment devastation with its stupid and harmful use, all kind of stressors, stressful and risky life with irrational life styles and stress-related disorders increase human impairment and damages (functional and structural), accelerate senescence and pathological aging, and finally bring about age-associated diseases with polypathologies of elderly people. But, the knowledge and intelligent actions on the negative life tetrad can create global strategies to improve, to unify and to better control these three fields: environment, euphenics and eugenics. In this way, rational use of euphenics, associated with elimination of bad life styles and assuming of reasonable and good life styles, can contribute to impairment decrease, aging deceleration and diseases diminution (as frequency and intensity) in old persons. The ideas from Joshua Lederberg's paper "Molecular biology, eugenics and euphenics", published 44 years ago in Nature, vol. 198, no. 4897, pp. 428-429, May 4, 1963 can be now and in the near future successfully apply for euphenics and eugenics control, also for their positive interrelations, with final aim: health improvement and aging deceleration.

ANALYSIS OF SITE SPECIFIC OXIDATION IN MITOCHONDRIAL ACONITASE

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Mitochondrial matrix proteins are sensitive to oxidative inactivation and oxidized proteins are known to accumulate during aging. Mitochondrial aconitase plays a key function in cellular energy production and loss of its activity has a major impact on the organism. Reactive oxygen species can lead to the inactivation of enzymes by the irreversible conversion of side chain residues of arginine, lysine, proline and threonine into carbonylated (ketone and aldehyde) counterpart products. Thus these carbonylated residues can be used as biomarkers of oxidative damage. We have investigated the oxidation sites in porcine aconitase in its apoenzyme and holoenzyme forms in order to identify the specific sites of oxidation and whether there are differences regarding the presence or absence of the iron sulfur cluster (prosthetic group). Oxidized aconitase was derivatized using biotin hydrazide for site specific detection by mass spectrometry approaches (MALDI-TOF/TOF). The protein carbonyl content was measured using the 2,4-dinitrophenylhydrazine derivatization method, followed by quantification by HPLC. Oxidized BSA was used as model protein to study derivatization efficiency and to establish the method. The results have been compared to verify differences between the oxidation sites depending on the presence or absence of the prosthetic group. The divergence regarding these sites highlights the role of the metal group during the oxidation process and the implications of the mechanisms of the metal catalyzed oxidation *in vivo*.

mtDNA INHERITED VARIABILITY IN FRONTOTEMPRAL DEMENTIA

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Frontotemporal dementia (FTD) is a complex neurodegenerative syndrome, whose clinical hallmarks are behaviour and/or language dysfunction caused by a focal degeneration mainly affecting frontal and temporal brain regions. FTD is characterized by a high level of heterogeneity which causes difficulties in disentangling the genetic factors affecting susceptibility to FTD.

In the last years, an increasing number of studies investigated the role of mitochondrial DNA (mtDNA) variability as a contributing factor to the pathogenesis of neurodegenerative diseases (Howell et al., 2005 *Trends Genet* 21:583–586). However, if mtDNA variability contributes to FTD has not been explored yet. Aim of our study was to fill this gap.

We collected in Calabria (southern Italy) a sample of 114 unrelated FTD patients (68 sporadic and 46 familial cases) and 180 ethnically, age and sex matched healthy controls. By sequencing the entire mtDNA HVS-I region of the D-loop, we identified 174 haplotypes: 24 and 27 were exclusive of familial and sporadic FTD, respectively; 99 were exclusive of the controls; the remaining 8 haplotypes were shared between patients and controls. However, significant statistical differences were not found between sporadic or familial FTD patients and controls.

Then, based on RFLP analysis of the coding region and sequencing of the HVS-I and HVS-II regions, the patterns of mtDNA haplogroups and sub-haplogroups were investigated. Again, no statistically significant differences were found between patients and controls.

On the whole, the data show that mtDNA inherited variability is not associated with FTD, at least in the Calabrian population. At present, studies are in progress which explore the role of mtDNA epigenetic variability on such a disease.

SPATIAL ANALYSIS OF THE DISTRIBUTION OF MALE LONGEVITY IN CALABRIA (ITALY)

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Female/Male (F/M) ratio among ultra-nonagenarians is significantly lower in Calabria (southern Italy) than in other Italian and European areas, due to a reduced male mortality after the age of 60. In order to better understand the role played by biological and environmental factors on this phenomenon, we investigated whether the geographic distribution of long-lived subjects is uniform throughout Calabria or it is concentrated in specific areas. We carried out a Spatial Analysis (by Geographic Information System software) of the distribution across Calabria of the Male Nonagenarian-Rate (mNR), a modified version of the Centenarian-Rate index (Robine et al. 2006, Popul. Stud 60: 99-113).

We identified two areas of exceptional male longevity: Zone1 (on the Aspromonte mountains) and Zone2 (near the town of Cosenza). Zone1 is characterized by a very low F/M ratio among nonagenarians (1:1), while in the Zone2, despite the high mNR, this ratio (1:2) is not different from the regional value due to the female NR which is the highest in the region.

It is important to notice that Zone1 is located in a mountainous rural area with difficult communications. By contrast, Zone2 is included in an economically and socially developed area. The recent observation that Sardinian centenarians are clustered in restricted areas characterized by geographic isolation and endogamy, suggested us to explore the effects of population inbreeding on male longevity in Calabria by surname abundance analysis (Cavalli Sforza et al., 2004 Princeton University Press, Princeton). This analysis revealed a low surname abundance, and hence a high inbreeding level, corresponding to Zone1 but not to Zone2.

In conclusion, male (and female) longevity in Zone2 may be related to improved economic and social conditions. As to Zone1, male longevity may be related to specific biological features of the area. Population inbreeding is likely to be one these features.

ROLE OF TP53 CODON 72 POLYMORPHISM IN CARDIOVASCULAR DISEASES: A STUDY ON ITALIAN POPULATION

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Cardiovascular Diseases (CVD), such as acute coronary syndrome (ACS) and stroke represent one of the main causes of morbidity and mortality in the elderly. Apoptosis of cardiomyocytes is an important mechanism in the pathogenesis of the ischaemic damage, and a key role in such mechanism is played by p53, the product of TP53 gene. A common polymorphism at codon 72 of TP53 gene determines an Arginine (R) to Proline (P) aminoacidic substitution in p53. Our previous data indicated that such polymorphism has an *in vivo* relevance in patients with ACS by modulating serum levels of Troponin I and CK-MB, two markers related to the extension of the ischaemic damage. In this study we evaluated the frequency of CVD and the genotypes of TP53 (codon 72) IL-6 (-174 G/C) and APOE (□2/□3/□4) genes in an Italian population recruited in the framework of the InCHIANTI epidemiological study. We studied a total of 792 subjects (350 men and 442 women) aged more than 60 years. Multivariate analysis indicates that TP53 RR genotype is associated with CVD, especially in males. A similar trend was observed for APOE □4+ subjects, but not for IL-6 (-174 GG). An additive effect on CVD risk of the three considered genotypes was also evident. This study confirmed the importance of APOE for CVD and revealed a new association between TP53 codon 72 polymorphism and CVD. In order to confirm this new association, a case-control study was then performed on another population of the same geographic area. The study was performed on 460 patients with ACS and 477 age- and sex-matched controls, all genotyped for TP53 codon 72 polymorphism. The analysis confirmed an increased risk for CVD in RR subjects and a protective role for PP genotype. TP53 codon 72 RR genotype can thus be considered a risk factor for CVD in the Italian population.

CMV INFECTION DEEPLY SHAPES THE EFFECTOR CD4+ T CELL COMPARTMENT DURING AGING

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In recent years it has become apparent that CMV chronic infection contributes to a number of modifications that characterize immunosenescence. Most of the work on CMV and immunesenescence has been carried out mainly on CD8+ T cells while CD4+ T cells has been neglected.

The present study focused on phenotypical and functional evaluation of effector CD4+ T cells against CMV in a cohort of subjects of different ages (25-100 yrs). The immunophenotype of CMV seropositive compared to CMV seronegative subjects, revealed that effector CD28-CD4+ T cells were detectable only within CMV positive subjects and rose significantly with age. To evaluate functional CMV-specific CD4+ responses, in terms of both intracellular IFN-γ production and exhibition of degranulation marker (CD107a), we stimulated freshly drawn PBMC with mixtures of peptides spanning two immunogenic CMV proteins (pp65 and IE-1). IFN-γ producing CD4+ T cells in response to anti-pp65 protein were significantly increased, both as percentages and absolute numbers, in the old subjects when compared to young subjects, whereas anti-IE-1 CD4+ T-cell responses were uniformly low, at all ages. This supremacy of pp65 proved to be functionally relevant not only in terms of intracellular cytokine production but also in terms of potential cytotoxic activity as the group of oldest subjects had about 1/3 of CD4+ T-cells responding to pp65 that coexpressed CD107a.

Our data indicate that during aging CMV infection, similar to that observed in CD8+ T cell subset, shapes the effector CD4+ T cell compartment by a progressive expansion of effector CD28-CD4+ T cells and a large accumulation of pp65-specific CD4+ T-cells exhibiting markers of cytotoxic activity.

TELOMERASE OVER-EXPRESSION PROTECTS MITOCHONDRIA

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Telomerase is a unique reverse transcriptase with its main function to maintain telomeres. Over-expression of hTERT, the catalytic subunit of human telomerase into normal somatic cells counteracts telomere shortening and replicative senescence. Recently additional functions for hTERT have been described such as conferring increased resistance to DNA damaging and apoptosis-inducing agents, supporting activation of stem cells, etc.

We measured the production of free radical species (ROS) , mitochondrial membrane potential, mitochondrial mass, copy number and mt DNA damage under normal conditions and increased oxidative stress (hyperoxia, hydrogen peroxide treatment). Microarray analysis has been performed in order to characterise changes in gene expression which might contribute to the decreased oxidative stress levels in hTERT-immortalised fibroblasts

When challenged under chronic mild hyperoxia, hTERT overexpressing cells displayed a higher proliferation and delayed gamma-H2A.X damage foci formation than parental fibroblasts. This was related to decreased levels of oxidative stress, especially mitochondrial superoxide, and a higher mitochondrial membrane potential (MMP). Additionally, we found that hTERT overexpressing cells display a lower mitochondrial mass, mtDNA copy number and mt DNA damage, and changes in the expression of a multitude of genes involved in mitochondrial biogenesis, Ca^{2+}-related signalling, energy metabolism and apoptosis regulation were found in cells overexpressing hTERT. Many changes in gene expression where opposite to those found during senescence.of normal fibroblasts. This suggests that hTERT expression improves mitochondrial function and diminishes retrograde response.

Fractionation experiments and immunocytochemistry showed that telomerase is translocated from the nucleus to the cytoplasm and especially to mitochondria under acute and chronic mild oxidative stressing agreement with published data (Haendeler 2003, 2004; Santos et al., 2004, 2006). However, in contrast to some published data (Santos et al., 2004, 2006) we found that hTERT overexpression protected mitochondrial DNA from damage by acute (H_2O_2) or chronic (mild hyperoxia) oxidative stress.

Our data suggest that telomerase protects mitochondria from oxidative stress-induced dysfunction by lowering mtDNA damage, decreasing mitochondrial ROS production and increasing MMP.

DOUBLE FACED EXPRESSION OF PD-1 ON CD8+ T CELLS.

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Advanced age is characterized by a decline of immune competence, thought to be related to an exhaustion of T-cell capacities. Recent work suggests that the expression of PD-1 on virus specific CD8+ T-cells reflects a state of functional exhaustion, resulting in a suboptimal control of viral replication. To gain further insights into the significance of PD-1 expression on CD8+ T-cells, we have performed a detailed analysis of its expression on CD8+ T-cell subsets in humans.

We show that PD-1 expression has two facets *in vivo*. On the one hand, it is linked to T-cell differentiation: PD-1 is up-regulated on the majority of early/intermediate differentiated subsets, which include HIV- and EBV-specific CD8+ T-cell populations and is down-regulated as CD8+ T-cells reach late stages of differentiation, exemplified by CMV-specific CD8+ T-cells. On the other hand, it is also linked to T-cell activation: within the PD-1 positive cell population, PD-1 over-expression occurs along with the up-regulation of activation markers like CD38 or HLA-DR. Although PD-1 was recently defined as a biological marker of exhaustion, our data show a tight correlation with differentiation and activation. It is important to consider these findings when assessing the expression of PD-1 on T-cells, in particular in the context of aging, where higher frequency of activated and highly differentiated subsets has been described. High levels of PD-1 on CD8+ T cells may not reflect their inability to be functional, but their state of differentiation and activation. This is particularly important to keep in mind in the context of aging, since immunosenescence and exhaustion are often associated.

CORRECTION OF PROLIFERATION AND DRUG SENSITIVITY DEFECTS IN THE PROGEROID WERNER'S SYNDROME BY HOLLIDAY JUNCTION RESOLUTION

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The progeroid Werner's syndrome (WS) represents the best current model of human aging. It is caused by loss of the WRN helicase/exonuclease, resulting in high levels of replication fork stalling and genomic instability. Current models suggest that characteristic WS phenotypes of poor S phase progression, low proliferative capacity, and drug hypersensitivity are due to accumulation of alternative DNA structures at stalled or collapsed forks during DNA replication, and Holliday junction resolution has been shown to enhance survival of cis-platin treated WS cells. Here, we present a direct test of the hypothesis that the replication/repair defect in unstressed WS cells is due to an inability to resolve recombination intermediates. We have created isogenic WS cell lines expressing a nuclear-targeted bacterial Holliday junction endonuclease, RusA, and show that Holliday junction resolution by RusA restores DNA replication capacity in primary WS fibroblasts and enhances their proliferation. Furthermore, RusA expression rescues WS fibroblast hypersensitivity to replication fork-blocking agents camptothecin and 4NQO, suggesting that the hypersensitivity is due to inappropriate recombination at DNA structures formed when the replication fork arrests or collapses at 4NQO- or camptothecin-induced lesions. This work is the first to demonstrate that Holliday junction accumulation in primary Werner syndrome fibroblasts results in their poor proliferative capacity, and to rescue WS hypersensitivity to camptothecin and 4NQO by Holliday junction resolution

BLOM7 ALPHA IS A NOVEL PROTEIN INTERACTING WITH RNA AND THE REPLICATIVE SENESCENCE-RELATED PROTEINS SNEV AND HIC5

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Blom7 alpha is a novel protein and was discovered as binding partner of $SNEV^{Prp19/Pso4}$, an essential splicing factor and U-box E3 ligase. Furthermore $SNEV^{Prp19/Pso4}$ increases the replicative lifespan of human endothelial cells. Interestingly different independent biochemical methods including GST-pulldown assays, Co-Immunoprecipitations and Colocalisation in HeLa cells suggest also an interaction of Blom7 alpha with Hic5, a protein which is able to induce senescence in immortalised cell lines on its own. Here were present a basic biochemical characterisation of this highly interesting novel protein.

Blom7 alpha contains two KH-modules, which are known as putative RNA binding domains. In order to identify endogenous RNA interacting with Blom7 alpha SELEX (systematic evolution of ligands by exponential enrichment) against a human placenta DNA library was performed. The pool after eight rounds was sequenced. The results suggest an interaction with RNA coding for the hypervariable region of the Immunoglobuline kappa light chain. The fact that Blom7 alpha binds this RNA which undergoes a highly sophisticated splicing process and interacts with other splicing factors further supports our hypothesis of Blom7 alpha being a splicing factor itself.

Most interestingly Blom7 alpha seems also to interact with ribosomal RNA as we could show in various Co-Immunoprecipitation experiments, followed by RNA-isolation and RT-PCR (reverse transcription polymerase chain reaction) with specific primers for 28S and 18S rRNA. In order to elucidate if this interaction is direct, EMSAs (electrophoretic mobility shift assays) are currently carried out.

Taken together these findings give us a first glimpse at the various functions of a novel protein, possibly linking transcription, pre-mRNA splicing, translation and regulatory mechanisms for the induction and repression of replicative senescence.

DIRECT AND CELL AUTONOMOUS SUPPRESSION OF THE IGF-1/GH GROWTH AXIS BY DNA DAMAGE

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DNA damage has been suggested as a prime cause of aging. The IGF-1/GH (insulin-like growth factor I/growth hormone) somatotroph axis has been shown to regulate aging from worms to mammals. Recently, it has been shown that mouse models for premature aging syndromes caused by defects in nucleotide excision repair show an attenuation of the IGF-I/GH growth axis. Here we report that the suppression of the IGF-I/GH growth axis is a direct response to DNA damage and that this response is cell autonomous. Furthermore, cells derived from prematurely aging animals show an exacerbated and prolonged somatotroph repression upon DNA damage. We, therefore, suggest that any cell that accumulates DNA damage during its lifespan can induce an aging response, which in turn would contribute to the aging of the organism. Furthermore, we have developed a cellular *in vitro* assay for studying an aging response to environmental factors, which will open novel perspectives for intervention and modulation of aging.

CYTOMEGALOVIRUS-SPECIFIC $CD8^+$ T CELLS DECREASE THE CELLULAR RESPONSE TO INFLUENZA VIRUS IN ELDERLY PERSONS

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The immune response to Influenza is typically decreased in elderly persons which leads to a severe course of disease and death. Not only aging but also latent cytomegalovirus (CMV) infection may contribute to their unresponsiveness to Influenza infection by causing premature immune senescence. We now show that elderly persons who are chronically infected with CMV have a higher frequency of CMV-specific $CD8^+$ T cells whereas their fraction of Influenza (FLU)-specific $CD8^+$ T cells is significantly smaller compared to young persons ($p<0,01$). During in vitro co-stimulation of purified $CD8^+$ T cells from 8 elderly (>60 years) and 6 young (<35 years) persons with the Influenza $M1_{58-66}$ peptide and the CMV_{pp65} peptide, the propagation of FLU-specific $CD8^+$ T cells is inhibited by the expansion of CMV-specific $CD8^+$ T cell clones. This dominance of CMV-specific $CD8^+$ T cells is not due to higher precursor numbers, but due to a higher affinity of their T cell receptors (TCR) to the antigenic peptide/MHC complex. CMV-specific $CD8^+$ T cells respond to lower peptide concentrations and have an increased capacity to bind peptide/MHC multimers than corresponding FLU-specific $CD8^+$ T cells. They also show a limited clonal diversity and use a very restricted set of TCR V-beta chains, indicating a preferential growth capacity of a few selected clones. A depletion of these certain T cell populations before specific stimulation in turn leads to an improvement of the Influenza-specific $CD8^+$ T cell response. From these data we conclude that latent CMV infection represents a risk factor for elderly persons to have a diminished immune response to Influenza.

AGING AND REJUVENATION IN A COLONIAL ASCIDIAN

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Many invertebrates show an extreme capacity of adult tissue renewal. This capacity is reflected in asexual reproduction during colonial life-cycles and during regeneration of lost body parts. Although telomeres are the key to immortality in cells, this is still not clear in whole animals where many factors contribute to determine life-span. The question of aging has been raised for sexually reproducing animals, cloned animals like the sheep Dolly, for plants and the plankton *Volvox*, but there is basically no information about this in natural clones.

This project aims to understand development, the role of stem cells and aging in colonial marine invertebrates. The model for the study is the colonial ascidian *Diplosoma listerianum*. This ascidian reproduces both sexually by standard larva–to-adult development and asexually in the adult phase by forming two buds that grow out from the intestinal area. These buds grow by massive cell proliferation to form an anterior branchial basket containing a new brain and a posterior gut region. Although wild *Diplosoma* mature sexually in a few weeks and typically survive less than a year in nature, individual cultured clones of *Diplosoma* have been kept in reproductive isolation for over 15 years. In this project, we have investigated the possibility of senility in these clones. We will compare unmated (virgin) representatives of old lab clones, with their recently mated clonemates and their young offspring.

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OXIDATIVE STRESS FAILS TO INDUCE PROTEIN MISFOLDING IN REPLICATING CELLS

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A hallmark of aging and a number of degenerative diseases is the increase in oxidized proteins. In these states misfolded and aggregated protein species also accumulate, especially in postmitotic cells. Hydrophobic surface exposure of misfolded proteins is a potent inducer of the stress response, signalling the conformational state of the proteome towards the maintenance mechanisms. However, the causal relationship between oxidative stress and protein misfolding is an open question.

In the present study we investigated whether a localized protein damage induced by oxidative agents (H_2O_2 and iron-ascorbate) would lead to protein unfolding/aggregation and stress response in replicative lymphocytic and epithelial cell cultures. We found that in contrast to heat shock, oxidative stress was unable to induce an increase in hydrophobic surface exposure in live cells, cell extracts and purified proteins. Similarly, we could not observe an increase in protein aggregation, a direct consequence of protein unfolding. Moreover, oxidative stress did not lead to HSF-1-dependent transcription of the major heat shock genes, another sensitive marker of protein unfolding. These results suggest that short term oxidative stress does not lead to bulk protein misfolding in replicating cells and raise important questions about the role of free radicals in protein homeostasis in aging postmitotic cells.

TRICHOSTATIN A ALTERS A-TUBULIN AND HISTONE ACETYLATION LEVELS AND DIFFERENTIALLY INDUCES APOPTOSIS IN FOUR LEUKEMIC CELL LINES.

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It has been widely recognized that histone deacetylases (HDACs) are promising targets for therapeutic interventions intended to reverse aberrant epigenetic states associated with cancers. Consequently there has been considerable effort to investigate the effects of histone deacetylase inhibitors (HDACIs) in numerous types of cancers. One of the most effective and well-studied histone deacetylase inhibitors is trichostatin A. This agent has been used in anticancer regimens. Trichostatin A inhibits class I and II histone deacetylases which results in the accumulation of acetylated histones and chromatin remodeling events. Changes in chromatin structure affects gene expression and in this respect, histone deacetylase inhibitors can alter programmed biological processes. Thus, depending on the cell type, treatment with these inhibitors may induce apoptosis, cell cycle arrest, differentiation and even the senescent phenotype. However, it has also been shown that aside from histones, other nonhistone proteins, such as transcription factors, as well as the microtubule protein, α-tubulin, are also acetylated. In light of the fact that microtubules are involved in various morphological changes as well as in apoptosis, the study of the effect of trichostatin A on α-tubulin acetylation in relation to histone acetylation may be of unique interest. In this respect, the present study has focused on these three parameters, α-tubulin and histone acetylation and induction of apoptosis after treatment with trichostatin A in four leukemic cell lines as well as in physiological human peripheral blood lymphocytes. To this end, with respect to the above, the effect of different incubation times with this agent, were analyzed. The results showed that trichostatin A has a differential effect on the levels of histone H4 and tubulin acetylation in the different cellular systems analyzed and more importantly, that these levels are related to the levels of induced apoptosis.

EFFECT OF THE HISTONE DEACETYLASE INHIBITOR, TRICHOSTATIN A, IN PERIPHERAL BLOOD LYMPHOCYTES AS A FUNCTION OF DONOR AGE

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The histone deacetylase inhibitor, trichostatin A, is a promising agent for the treatment of certain types of cancers, alone, or in synergistic combination with other anticancer agents. One of the advantages of the use of histone deacetylase inhibitors, such as TSA, is that its effects have been found to be more potent towards cancer cells as compared to normal cells. The effect of anticancer agents on the immune system and on lymphocytes in particular, is of major importance to the success of anticancer regimens. In this respect information regarding the effect of such agents on normal lymphocytes as compared to malignant cells may be of significant value for the successful designing of clinical protocols. Moreover, the parameter of age may also be a factor in the differential effects of such protocols. Histone deacetylase inhibitors lead to the accumulation of acetylated histones and depending on the cell type, these inhibitors may induce either apoptosis, or cell cycle arrest or differentiation. In light of the above, in the present study, we have analyzed the effect of trichostatin A in human peripheral blood lymphocytes as a function of donor age. Previous work from our lab has shown that TSA induces the accumulation of core histone H4 acetylation and apoptosis in human peripheral blood lymphocytes. We have also observed an increase in histone H4 acetylation as a function of donor age. However the influence of age on the degree of apoptosis in lymphocytes has not been investigated. Toward this end in the present work we have investigated whether there is a differential age-related effect of TSA in the induction of apoptosis in correlation with the accumulation of acetylated histone H4.

CELL SENSITIZATION AFTER siRNA-MEDIATED APOLIPOPROTEIN J/CLUSTERIN KNOCK DOWN AND POTENTIAL BIOMEDICAL APPLICATIONS

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Clusterin/Apolipoprotein J (CLU) is an enigmatic glycoprotein that is differentially expressed in many severe diseases including aging and cancer progression. We have shown that CLU is a senescence biomarker that exerts a pro-survival function. siRNA-mediated CLU knock down in human osteosarcoma (OS) cells induces growth retardation, higher rates of endogenous cellular death and sensitizes OS cells to stress. Nevertheless, the molecular basis of these effects remained elusive. Here we demonstrate that effective and sustained CLU depletion from OS cells by siRNA induces late morphological alterations, growth arrest at the G_1/S checkpoint and activation of the mitochondrial axis of apoptosis that engages caspase-9. Also, CLU knock-down resulted in down regulation of the BH pro-survival (bcl-2 and bcl-X_L) proteins and activation of p53 and its downstream targets, namely $p21^{WAF1/CIP1}$ and bax. Stable over-expression of either bcl-2 or the V143A p53 dominant negative mutant in U-2 OS cells attenuated CLU knock down mediated apoptosis. In addition the V143A p53 mutant abolished the growth retardation effect. Parallel investigation of the CLU knock down effects in the Sa OS p53-null cellular context revealed the induction of mild apoptosis, via bcl-2 and bcl-X_L down-regulation, but no effect on cellular growth, indicating that p53 is essential for the growth inhibitory effect but it acts as an enhancer of the apoptotic outcome. Supportively, reinforced expression of either p53 or p21 in tetracycline-inducible $p53^{WT}$ or $p21^{WT}$ Sa OS cell lines restored the growth retardation effect in both cell lines and significantly enhanced apoptosis in the Sa OS Tet-53 cells. We suggest that the CLU-specific siRNA oligonucleotides used, may prove valuable agents during anti-tumor therapy or at other pathological conditions where CLU has been implicated.

MODELLING TELOMERE-DEPENDENT SENESCENCE WITH SSDNA OLIGONUCLEOTIDES

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Telomere uncapping is known to induce senescence by activating a DNA damage response. However, it is still unclear what structural features of uncapped telomeres activate signalling kinases like ATM. One hypothesis is that the exposure of the telomeric single-stranded G-rich 3' overhang triggers a DNA damage response and is, thus, equivalent to telomere uncapping.

We compared the effects of two short single-stranded oligonucleotides, $(TTAGGG)_2$ and $(CCCTAA)_2$. 48h after treatment of human cells with G-rich oligonucleotides, mimicking the exposure of the telomeric 3' overhang, resulted in growth arrest and induction of DNA damage foci containing γ-H2AX with a frequency similar to senescent cells, whilst C-rich oligonucleotides at the same concentration had no effect. G-oligonucleotide treatment was also accompanied by an increased production of reactive oxygen species, which has been associated with the senescent phenotype. Oligonucleotides did not co-localize with γ-H2AX foci, instead the induced DNA damage foci were preferentially localized at telomeres.

BrdU incorporation assays showed that the effect of G-oligonucleotides on γ-H2AX foci formation was cell-cycle dependent; entry of cells into S phase was necessary for subsequent DNA damage foci formation. We are now testing the possibility that G-oligonucleotides compete with the telomeric 3'overhang for Pot1 binding, resulting in its titration from telomeres. Together, our results suggest that short G-rich single-stranded oligonucleotides are partial models for uncapped telomeres: They are probably sufficient to titrate essential factors like Pot1 away from telomeres and so trigger telomere uncapping, but are not recognized as substrate for DNA damage signaling kinases themselves.

REACTIVE OXYGEN SPECIES CAN BE CONTROLLED BY THE SECRETORY GLYCOPROTEIN, CLUSTERIN, FROM SIDE POPULATION CELLS IN THE LACRIMAL GLAND: A NEW INTERVENTION FOR AGE-RELATED DRY EYE DISORDERS

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This study was performed to determine the function of the secretory glycoprotein, clusterin, a side population (SP) cell-specific gene and its role in restoring secretory function of the lacrimal gland (LG). SP cells were purified from the LG of heterozygous enhanced green fluorescent protein (EGFP)-transgenic (GFP-Tg) mice of a C57BL/6 background. Two weeks after irradiation, the SP cells were injected with a microinjector directly into the LG of each experimental mouse. Eight weeks after the injection the mice were sacrificed for analysis and the engrafted cells were identified by the presence of GFP. The engrafted cells were then transplanted into mice with irradiation-induced hypofunction in the LG. Using the fluorescent indicator dichlorofluorescein diacetate, intracellular levels of ROS were assayed. Two months after the transplantation, secretions from the LG in the recipient mice were restored; however, since no outgrowths were produced and the transplanted cells were only sparsely distributed, it is likely that soluble factors secreted by the SP cells, and not reconstituted cells, restored the functions of the residual glands. We found that clusterin is the SP cell-specific secretory glycoprotein, and it can rescue cell death through the suppression of ROS accumulation induced by irradiation or oxidative stress. Our results indicate that the advantages of SP cell transplantation are attributable to the functions of the SP cell-derived soluble factor, clusterin, but not to the cell transplantation-mediated reconstitution of the glands. We propose that SP cells in each organ might also have specific factors that prevent cell stress, including oxidative stress. Here we provide initial evidence suggesting the possibility of clinical application of the SP cell-related factor, clusterin, to treat oxidative-stress related aging diseases, including age-related dry eye disorders.

COMPUTER AIDED DESIGN OF INHIBITORS FOR SFRP PROTEINS AS A WAY TO OBTAIN STEM CELLS SELF RENEWAL AGENTS

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Adult stem cells persist in dormant state in microenvironment called a stem cell niche. The activity of stem cells and switch from dormant state to differentiation or self renewal is regulated by signaling pathways in the stem niche. One of these signaling pathways is Wnt-Frizzled-β-catenin signaling pathway. It was shown that activation of Wnt proteins leads to activation of stem cells proliferation. This signaling pathway is also active in different types of cancer. The activity of this pathway is regulated in the intercellular space by several groups of protein inhibitors. The main family of such inhibitors is an SFRP family. Activation of the dormant stem cells self renewal and further differentiation can be used for the renewal of tissues. In present work we have constructed molecular models of SFRP protein oligomers and performed computer aided drug design of SFRP inhibitors, which can potentially activate Wnt signaling pathway and the stem cells proliferation.

LATENT INFECTION WITH CMV AND AGING PER SE LEAD TO DISTINCT CHANGES IN $CD8^+$ AND $CD4^+$ T CELL SUBSETS IN THE ELDERLY

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One of the most profound age-related immunological changes is a decrease in the number of antigen-inexperienced naïve T lymphocytes combined with an increase of antigen-experienced memory and effector cells. It has been shown that latent infection with Cytomegalovirus (CMV) strongly increases those age-related changes in the composition of T cell subsets. However, no clear-cut distinction has so far been made between strictly age-related and CMV-induced changes.

We therefore analyzed $CD4^+$ and $CD8^+$ naïve ($CD45RA^+CD28^+$), memory ($CD45RA^-CD28^+$) and effector ($CD28^-$) T cells in a large cohort of CMV-positive (n = 164) and CMV-negative (n = 87) elderly persons and showed that percentages of $CD8^+$ as well as $CD4^+$ effector T cells were higher, but percentages of naïve and memory cells lower in CMV-positive compared to CMV-negative elderly donors. Additionally $CD8^+$ and $CD4^+$ effector T cells were correlated with other T cell subpopulations. We found that negative correlations within $CD8^+$ T cell subsets are present in both CMV-positive and CMV-negative elderly. In contrast correlations within $CD4^+$ T cell subpopulations and a positive correlation between $CD8^+$ and $CD4^+$ effector T cells were found in CMV-positive individuals only.

Our results demonstrate that different T cell subsets compete for space within the $CD8^+$, but not the $CD4^+$ T cell population and that CMV induces changes in the $CD4^+$ compartment that differ from the solely age-related changes seen in CMV-negative elderly. We conclude that aging per se and latent infection with CMV lead to distinct changes in $CD8^+$ and $CD4^+$ T cell subsets and that the CMV-status of a population has to be taken into account when the effect of aging on the composition of the T cell pool is analyzed.

A GENOME APPROACH TO INTER-INDIVIDUAL VARIATIONS IN CANCER SUSCEPTIBILITY AMONG ATOMIC-BOMB SURVIVORS

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Immunological capacity attenuates along the human aging process, resulting in an increased risk of various diseases including cancer. We have observed that the aging-associated attenuation of immunological capacity was further accelerated among Atomic-bomb (A-bomb) survivors in Hiroshima and Nagasaki in a radiation dose-dependent manner. We have also observed that an increased mutability found in A-bomb survivors exposed to high dose was associated with increased cancer incidence in a follow-up study. Noteworthy are great inter-individual variations in selected immunological phenotypes and mutability in A-bomb survivors, indicating that a wide variation among individual's aging process, a prominent aspect of human aging, may be in part explained by individually-differing immunological and DNA-repair capacities. Aiming to elucidate an involvement of genetic factors in individually-differing responses to radiation exposure, we intensively investigated the effects of aging in the radiation-associated development of cancer in terms of various genetic polymorphisms examined in case-control studies within an RERF cohort of A-bomb survivors. Single nucleotide polymorphism (SNP) and variable number of tandem repeat (VNTR) analyses of immune-related and DNA-repair genes are underway with 3,667 subjects including 1,444 cancer cases. The preliminary results included significant associations between IL-10 or TP53 haplotyping and cancer risks in A-bomb survivors. Our findings proposed a possibility that the aging effects on relationship between radiation and cancer risks were modulated by genetic factors of individuals.

DEVELOPING PRODUCTS FOR AGE RELATED DISEASES

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DNage is a biotechnology company that focuses on the development of products for medical and health problems associated with aging. The company originated from the Erasmus Medical Center in Rotterdam that has performed groundbreaking research in the field of DNA repair/damage and its connection to the process of aging. DNage has exclusive access to this technology including a range of unique, rapidly aging mouse models for the development of therapies to reduce the effects of aging. Recently, DNage was acquired by Pharming Group NV and is based in Leiden, the Netherlands.

A link between accumulation of DNA damage and the rate of aging is now well established. Instead of focussing on DNA damage per se, we are following a unique approach by inhibiting specific DNA repair pathways in vitro and in vivo. This allows us to investigate the effects of DNA damage by both endogenous and exogenous factors and to identify compounds that prevent DNA damage and abate aging pathology. The accelerated aging phenotype of the mouse models further facilitates rapid identification of therapeutic targets and biomarkers that mark the onset and progression of aging diseases. Moreover, by using conditional knockout mouse models, we are able to study the aging of specific organs and tissues without the confounding effects of general aging pathology on the body.

DNage has filed several patent applications and has active programs in the fields of Osteoporosis, Neurodegeneration, Metabolic diseases and Premature Aging Syndromes. In addition, the company is currently establishing corporate partnerships on other aging diseases and in the field of nutritional and clinical food applications. Its first product, Prodarsan®, a mixture of small molecules with a strong safety record, is currently in preclinical testing for use in premature aging diseases such as Cockayne Syndrome. For additional information, please visit our website www.dnage.nl