Aging and Genes

Yaşlanma ve Genler

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ABSTRACT Aging, like many other biological processes, is subject to regulation by genes that reside in pathways that have been conserved during evolution. The insulin/ IGF-1 pathway, mTOR pathway and p53 pathway are among those conserved pathways that impact upon longevity and aging-related diseases such as cancer. Most cancers arise in the last quarter of life span with the frequency increasing exponentially with time, and mutation accumulation in critical genes (e.g. p53) in individual cells over a lifetime is thought to be the reason. One possible cause of aging is the degradation of the telomeres. As the cell divides over and over, these telomeres become shorter and shorter. In Caenorhabditis elegans, the downregulation of insulin-like signaling induces lifespan extension (Age) and the constitutive formation of dauer larvae (Daf-c). This also causes resistance to oxidative stress (Oxr) and other stress stimuli and enhances the expression of many stress-defense-related enzymes such as Mn superoxide dismutase (SOD) that functions to remove reactive oxygen species in mitochondria. The daf 2 gene mutation in C. elegans, a gene, which is comparable with the human IGF-1 Receptor gene and which is involved in antioxidative action, shows a 30 % increased lifespan in that species. In human we found the following: Sequence analysis of the IGF1 and IGF1 receptor (IGF1R) genes of female centenarians showed overrepresentation of heterozygous mutations in the IGF1R gene among centenarians relative to controls that are associated with high serum IGFI levels and reduced activity of the IGFIR as measured in transformed lymphocytes. Thus, genetic alterations in the human IGF1R that result in altered IGF signalling pathway confer an increase in susceptibility to human longevity, suggesting a role of this pathway in modulation of human lifespan. Aging is controlled by different mechanisms, which influence the speed of the aging process and which is determined by interindividual genetic variants. Disorders in the insulin and lipid metabolism, caused by genetic variants of the genes, involved in this part of the metabolism cause higher glycolisation and tissue damage. Sirtuin genes function as anti-aging genes in yeast, Caenorhabditis elegans, and Drosophila. A number of reports have indicated that SIRT3, upon proteolytic cleavage in the mitochondria, is an active protein deacetylase against a number of mitochondrial targets. In mammals, one of the substrates of the SIR2 ortholog, SIRT1, is a regulator of mitochondrial biogenesis, PGC-1alpha. Indeed, the putative SIRT1 activator resveratrol has been shown to stimulate mitochondrial biogenesis and deliver health benefits in treated mice. Sirtuins are NAD+-dependent enzymes that have been implicated in a wide range of cellular processes, including pathways that affect diabetes, cancer, lifespan and Parkinson's disease.

Key Words: Aging, genes, sirtuin

ÖZET Yaşlanma, diğer biyolojik süreçlerde olduğu gibi, evrim süresince korunmuş yolaklarda yer alan genler tarafından regüle edilen bir durumdur. İnsulin/IGF-1 yolağı, mTOR yolağı ve p53 yolağı, kanser gibi yaşlanma ile ilgili hastalıklar ve ömür uzunluğunu etkileyen korunmuş yolaklar arasında bulunurlar. Kanserlerin çoğunun, hayatın son çeyreğinde ortaya çıkmasının nedenleri zamanla frekansın katlanarak artması, bir ömür boyunca p53 gibi kritik genlerde mutasyonların birikmesi olabilir. Yaşlanmanın olası bir nedeni de telomerlerdeki kayıplardır. Hücreler bölünmelerini sürdürdükçe telomerler kısaldıkça kısalır. Caenorhabditis elegans'da insulin-benzeri sinyalleşmenin baskılanması ömrün uzamasını (Age) ve dayanıklı larva (Daf-c) oluşumu için gereken yapılanmayı indükler. Bu durum oksidatif strese (Oxr) ve diğer stres uyaranlarına direnç oluştururken, mitokondride reaktif oksijen türlerinin yok edilmesini sağlayan Mn superoksit dismutaz (SOD) gibi birçok stres-savunma-ilgili enzimin sentezlenmesini de arttırır. C. elegans'da, insan IGF-1 reseptör geni ile kıyaslanabilen bir gen olan, daf 2 gen mutasyonu, bu gen antioksidatif etkili olduğundan bulunduğu türlerde ömrün %30 uzamasını sağlar. İnsanda tespit edilen şudur: 100 yaşını aşmış kadınların IGF1 ve IGF1 reseptör (IGF1R) genlerinin sekans analizi transforme lenfositlerde ölçüldüğü gibi, yüksek serum IGF1 düzeyleri ve azalmış IGF1R aktivitesi olan kontrollerle akraba asırlık kişiler arasında IGF1R genindeki heterozigos mutasyonların aşırı gösterimini ortaya çıkarmıştır. Böylece, değişmiş IGF sinyalleşme yolağında ortaya çıkan insan IGF1R'deki genetik değişiklikler, insan ömrünün modulasyonunda bu yolağın bir rolü olduğunu düşündürecek şekilde, insan ömrünün uzamasına yatkınlıkta bir artış sağlar. Yaşlanma, yaşlanma sürecinin hızını etkileyen ve bireylerarası genetik varyantlarla (değişikliklerle) belirlenen farklı mekanizmalarla kontrol edilir. Genlerdeki genetik varyantlar nedeniyle oluşan insülin ve lipid metabolizma hastalıkları, metabolizmanın bu kısımlarında yüksek düzeyde görülen glikolizasyon ile doku hasarı nedeniyle ortaya çıkarlar. Mayalar, Caenorhabditis elegans ve Drosophila'daki Sirtuin genleri antiaging (yaşlanma karşıtı) genler olarak işlev yaparlar. Mitokondrideki proteolitik yarığın üzerinde SIRT3'ün bulunduğunu bildiren raporlara göre, SIRT3 mitokondriyal hedeflerin çoğuna karşı aktif bir protein deasetilaz olarak görev yapar. Memelilerde SIR2 ortolog'un substratlarından biri olan SIRT1, PGC-1 alfanın mitokondriyal biogenezinin bir regülatörüdür. Gerçekte, SIRT1 aktivatör temsilcisi resveratrol'ün denek farelerde mitokondriyal biogenezi stimüle edip sağlık açısından yararlı sonuçlar oluşturduğu gösterilmiştir. Sirtuinler NAD+ bağımlı enzimler olup; bunların diyabeti, kanseri, ömür uzunluğunu ve Parkinson hastalığını etkileyen yolaklar dahil, hücresel süreçlerin çoğunda etkili oldukları bilinmektedir.

Anahtar Kelimeler: Yaşlanma, genler, sirtuin

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GENERAL

Genes are located on chromosomes which are condensated structures, belonging to the cell nucleus and carry the genetic information. They consist from a biological acid, called DNA.Genes display the genetical decoding of all our physiological processes and structures in our body. The heritance can be expressed by heterozygoty and homozygoty, which means that you can have heritated your genes either from one parent or from both. This has impact on the phenotypic expression. Homozygotie has two same alleles (one gene includes the two alleles, one from your mother, the other one from your father) and in so far it displays a much stronger expression of the mutation as heterozygoty does.

Poymorphisms are nothing else than small point mutations or deletions, or inversions of DNA parts which are distributed over the whole genome. For example: if you stretch the whole DNA of the smallest chromosome (no 22) of your genetic treasure you will get a distance, which stretches from Barcelona to Valencia. In this case you will find a polymorphic DNA every 1 ½ meter.

Though as humans we display alle the same genes, we are so different one from another .The Polymorphism is responsible for the variety of phentotypic markers of human beeing.

Due to our exams we are searching for those genes, which decode for enzymes of our body. This enzymes controll the converting of substances as hormones and the break down of them. Therefore e.g. slow acting enzymes or fast acting enzymes dominate the metabolism, and, depending of what kind of enzyms are acting, we get different amounts of endproducts. Due to hormone metabolism we can estimate from the results of this research if there is accumulation or fast eliminating of hormones after all. This has importance for risks of breast or-prostate cancer.

Genes determine cardivascular maker genes as well. For example polymorphism in blood pressure genes like AGT or ACE, cholesterol metabolism genes, blood coagulation genes have impact on your health. Nevertheless genes only are basic information of your genetic code and the question if you will get those problemes, which are described as your personal risk depends on how your lifestyle is.

Our antiaging therapeutical strategy, should be 1. individual, 2. custom attracted and 3. secure. The great advances in medicine in the last years can realize this idea for the near future. Women ask more and more after a custom attracted and individualized HRT. We can observe a change of paradigm in nearly all medical subject areas. The standard of an individualized therapy for our patients requires more and more comprehensive epidemiological investigations, meta analyses and genetically analysis, which is especially supported by the detection of the human genome.

So we can estimate the profit of a HRT for women and men in order to reduce their CV- risks and sex specifical cancer risk As we know, weight reduction, exercises and reduced cigarette smoking improve the CV- risk, as well as the breast and prostate cancer risk. We also know, that the possibility of CV- diseases increases dramatically in women after menopause and in men with low testosterone levels, with a 10 years latency of women compared with men, while the fertile phase of women apparently displays a protection against those diseases. We detected some polymorphism in your case, which could have impact on the avbove mentioned diseases.

What causes aging? There isn't a simple answer to that. But one important aspect of aging was discovered in the early 1960s by Leonard Hayflick. Hayflick discovered that aging occurs on the cellular level. Cells divide and replicate themselves over the course of our lives. However, each cell has a built-in clock that says how many times it can divide. Beyond a certain point, the cell ceases to be able to divide or repair itself. This loss of the ability to divide is called cellular senescence.

An interesting aspect of research into cellular senescence has shown that the ticks on a cell's internal clock seem predetermined. This is separate from chronological time. Slowing down or stopping the cell divisions does not change the number of times the cell can divide. Cells frozen in liquid nitrogen pick up where they left off and complete the same number of divisions as unfrozen cells, as if they remember the count.

One possible cause of aging is the degradation of the telomeres, pieces of DNA that cap the ends of each chromosome. As the cell divides over and over, these telomeres become shorter and shorter. Eventually, they become so short that the cell can no longer reproduce. Rather than being a passive, haphazard process of wear and tear, lifespan can be modulated actively by components of the insulin/insulin-like growth factor I (IGFI) pathway in laboratory animals. Complete or partial loss-of-function mutations in genes encoding components of the insulin/IGFI pathway result in extension of life span in yeasts,

worms, flies, and mice. This remarkable conservation throughout evolution suggests that altered signaling in this pathway may also influence human lifespan. On the other hand, evolutionary tradeoffs predict that the laboratory findings may not be relevant to human populations, because of the high fitness cost during early life.

In Caenorhabditis elegans, the downregulation of insulin-like signaling induces lifespan extension (Age) and the constitutive formation of dauer larvae (Daf-c). This also causes resistance to oxidative stress (Oxr) and other stress stimuli and enhances the expression of many stress-defense-related enzymes such as Mn superoxide dismutase (SOD) that functions to remove reactive oxygen species in mitochondria. It has been found,[1] that the MnSOD systems in C. elegans fine-tune the insulin-like-signaling based regulation of both longevity and dauer formation by acting not as antioxidants but as physiological-redox-signaling modulators.The daf 2 gene mutation in C. elegans, a gene, which is comparable with the human IGF-1 Receptor gene and which is involved in antioxidative action, shows a 30 % increased lifespan in that species. In human we found the following: Sequence analysis of the IGF1 and IGF1 receptor (IGF1R) genes of female centenarians showed overrepresentation of heterozygous mutations in the IGF1R gene among centenarians relative to controls that are associated with high serum IGFI levels and reduced activity of the IGFIR as measured in transformed lymphocytes. Thus, genetic alterations in the human IGF1R that result in altered IGF signalling pathway confer an increase in susceptibility to human longevity, suggesting a role of this pathway in modulation of human lifespan.[2] Although everyone agrees that average life expectancy has systematically advanced linearly over the last century, it is not realistic to expect that this pace can continue indefinitely. Our data suggest that, without the invention of some new unknown form of medical breakthrough, the *Guinness Book of World Records* benchmark established by French woman Jeanne Calment of 122 years, set back in 1997, will be exceedingly difficult to break in our lifetime.[2]

Aging is controlled by different mechanisms, which influence the speed of the aging process and which is determined by interindividual genetic variants and can be

1. measured

2. influenced by lifestyle and diet

3. individually controlled by knowledge of the genomic and proteomic basics

THIS DIFFERENT MECHANISMS ARE:

1. GENETIC CONTROLLED APOPTOSIS P53 PATHWAY

The p53 pathway is composed of hundreds of genes and their products that respond to a wide variety of stress signals. These responses to stress include apoptosis, cellular senescence or cell cycle arrest. In addition the p53-regulated genes produce proteins that communicate these stress signals to adjacent cells, prevent and repair damaged DNA and create feedback loops that enhance or attenuate p53 activity and communicate with other signal transduction pathways . Aging, like many other biological processes, is subject to regulation by genes that reside in pathways that have been conserved during evolution. The insulin/ IGF-1 pathway, mTOR pathway and p53 pathway are among those conserved pathways that impact upon longevity and aging-related diseases such as cancer. Most cancers arise in the last quarter of life span with the frequency increasing exponentially with time, and mutation accumulation in critical genes (e.g. p53) in individual cells over a lifetime is thought to be the reason. Given the crucial role of the p53 in tumor prevention, this decline in p53 activity at older ages in animals could contribute to the observed dramatic increases in cancer frequency, and provides a plausible explanation for the correlation between tumorigenesis and aging in addition to the accumulation of DNA mutations over lifetime.[3,4] The macrolide rapamycin is used clinically to treat graft rejection and restenosis. Mammalian target of rapamycin (mTOR) is a central controller of cellular and organism growth that integrates nutrient and hormonal signals, and regulates diverse cellular processes. New studies have linked mTOR to several human diseases including cancer, diabetes, obesity, cardiovascular diseases and neurological disorders. Recent data have also revealed that mTOR is involved in the regulation of lifespan and in age-related diseases. These findings demonstrate the importance of growth control in the pathology of major diseases and overall human health, and underscore the therapeutic potential of the mTOR pathway.[5]

2. OXIDATION-AND INDIVIDUAL ANTIOXIDATIVE CAPACITIES

Energy formation and individual genetic differences regarding the enzymatic and hormonal signal ways, which are involved into the metabolism: The insulin signalling pathway couples feeding and nutritional status in mammals to the tempo and mode of metabolism in most tissues of the animal. The Insulin – like- pathway regulates

longevity and metabolism in different species. This regulation may be mechanistically related to longevity incerase caused by caloric restriction and may be linked to the free radical formation of the Insulin- like pathway.

Cumulutative evidence from studies of gene mutations suggests, that reduced IGF-1 and Insulin signalling leads to delayed aging and increased longevity.

3. CHOLESTEROL METABOLISM

Age is associated with an enhanced low density lipoprotein (LDL) oxidation and atherosclerosis, thus, subjects over 80 years without cardiovascular disease provide a model to investigate the protective factors against atherosclerosis. Serum paraoxonase (PON1), an high density lipoprotein (HDL)-bound enzyme, prevents LDL oxidation. There is a strong evidence that in healthy Sicilians ageing may be characterized by a low frequency of PON1 (-107)T 'risk' allele and by an high frequency of favourable genotypes such as (-107)CC, influencing PON1 activity and HDL-C levels.[6] The following genes are involved in longevity:

Apo E2/3/4 Polymorphism

Apo E4 (Frequency 17%) is correlated with high LDL and low HDL-cholesterol levels (type V) Carrier of this mutation display a pre- dsiposition fpr Alzheimer disease (late onset) .Apolipoprotein E (ApoE) is a protein component of several plasma lipoproteins, including triglyceride-rich chylomicrons and very-low-density lipoprotein (VLDL) particles. As a ligand for the low-density lipoprotein (LDL) receptor, ApoE plays a major role in plasma lipoprotein metabolism. Two SNPs within the human APOE gene form 6 common genotypes, leading to 3 major isoforms of ApoE, ApoE2, -E3, and -E4. The genotypes are as follows (according to their frequency): E3/E3, E3/E4, E3/2, E4/E4, or the very rare E2/E4, and E2/E2 genotypes. Carriers of the ApoE4 allele have higher levels of total and LDL cholesterol and therefore have a higher risk of cardiovascular disease. Further, the ApoE4 allele has been related to dementias, most commonly Alzheimer's disease.

Apolipoprotein A1

Apolipoprotein A1 is one of the central cholesterol carriers, which particpate in the catabolism of HDL-particles. Low HDL concentrations, especially in association with high LDL-Cholesterol levels (ratio LDL/HDL > 3,5) have an incerased risk for MI, stroke and atherosclerosis. This is caused by an decreased efflux from cholesterol out of the vessel walls.(inflammation, oxidationà reaction cell proliferation, wall thickness,, calcification, tightened and hardened vessels à infarction by cutting oxygene supply to the tissue (brain, heart muscle). One mutation is the ApoA1 deficiency, which leads to very low HDl-levels. In our case we search for the good alternative, the pos. –75 G>A polymorphismm.This mutation is important due its correlation to high level of HDL-Cholesterol in the following case: AA (homocygoty) = reaction to unsaturated fatty acids (olive oil) improves HDL-C. levels and in so far it has life extension effects. The GG genotype in opposite reacts with decreased HDL-levels or unchanged levels administering unsaturated FA to the diet and can cut the life expectancy. Centenarians in the island of Sardinia have the constellation very often as well as inhabitants of the village of Limone (lago di garda, Italy) In so far you have a good Apo A1 polymorphism and olive oil would improve your HDl-levels.The human apolipoprotein AI (apoAI) constitutes the major protein component of high-density lipoprotein (HDL, the so-called "good" cholesterol). Because apoAI plays an important role in the reverse transport of cholesterol, low ApoAI/HDL serum levels constitute a well-known risk factor of coronary artery disease (CAD). In the human APOA1 gene, a relatively frequent promoter polymorphism modulates the expression of apoAI. Important interactions, at least for women, between this polymorphism, dietary habits and HDL level are known.

Carriers of the variant allele can increase their serum HDL in response to increased dietary uptake of polyunsaturated fatty acids.

CETP-B1/B2 Polymorphism

The mutation alters the Cholestryl Ester Transfer Protein (CETP), an enzyme involved in regulating lipoproteins and their particle size. Compared with a control group representative of the general population, centenarians were three times as likely to have the mutation (24.8 percent of centenarians had it vs. 8.6 percent of controls) and the centenarians' offspring were twice as likely to have it.

CETP affects the size of "good" HDL and "bad" LDL cholesterol, which are packaged into lipoprotein particles. The researchers found that the centenarians had significantly larger HDL and LDL lipoprotein particles than individuals in the control group. The same finding held true for offspring of the centenarians but not for control-group members of comparable ages.

Evidence increasingly indicates that people with small LDL lipoprotein particles are at increased risk for developing cardiovascular disease, the leading cause of death in the United States and the Western world. Dr. Barzilai and his colleagues believe that large LDL particles may be less apt than small LDL particles to penetrate artery walls and promote the development of atherosclerosis, a major contributor to heart disease and stroke. Their study found that HDL and LDL particles were significantly larger in those offspring and control-group members who were free of heart disease, hypertension and the metabolic syndrome (a pre-diabetic condition that increases risk for cardiovascular disease).The CETP (Cholestery-ester-transfer-protein) is a key enzyme of the HDL-cholesterol metabolism. By transferring cholesterol rom HDL to apolipoprotein B containing LDL and VLDL, CETP can confer higher risk of coronary heart disease by specific reduction of cholesterol content within HDL in favor of arteriogenic LDL und VLDL particles. Men with CHD and heterzogoty in this gene have 30% increased CETP and 13% decreased HDL-C levels.In case of a homozygotie B2 this is not the case. The progredience of CHD correlates with the increase of the numbers of the B1 alleles. A common polymorphism (I405V) in exon 14 of the cholesteryl ester transfer protein (CETP) gene has been recently associated to healthy aging in Ashkenazi Jewish. In order to study this genetic effect in long-lived individuals with a different ethnicity, we analyzed the allele and genotype distributions of the CETP polymorphism a sample of Italian centenarians. Our result does not confirm the association between the I405V CETP variation and the healthy aging phenotype described in the Ashkenazi Jewish population and suggests that other gene environment interactions contribute to longevity.

4. GLYCOLISATION

The non enzymatically formation of sugar bounds leads to increased molecular instability, forming cross- linbking of the DNA and posttranslational transcripts. Disorders in the insulin and lipid metabolism, caused by genetic variants of the genes, involved in this part of the metabolism cause higher glycolisation and tissue damage.

5. METHYLATION, ACETYLATION AND DEACETYLATION

The methylated CpG dinucelotids of DNA regulate the genetic activity, coupled with deacytilation or acetylation of the chromation, suggesting that the switch on and off of a particular gene is determined by this methylated or demethylated dinucleotids, while the NAD dependent acetylation or deacetylation regulates the chromatin packing or depacking (silencing of the genome), including SIR proteins as regulator. Furthermore methylation is involved in cancer formation and neurotransmitter and steroid hormone elimination, as wll homocysteine formation, suggesting a most important role of methylating and de-methylating enzymes in the aging process. Genetic variants of the methylating enzymes lead to individual expression pattern of this enzymes.

Acetylation is only one of a number of types of covalent histone modification that regulate gene transcription. Further investigation of other chromatin-remodelling mechanisms, such as methylation and phosphorylation, will determine whether there is a general histone code for circadian-clock regulation .The involvement of histone modification in the regulation of gene transcription has been widely demonstrated. Histone modification, specifically histone acetylation, is important in the regulation of the mammalian circadian clock. The key proteins that regulate the circadian clock (Clock and Bmal1) drive the transcription of three period genes (Per1, 2 and 3) and two cryptochrome genes (Cry1 and Cry2). The transcript levels of all five genes cycle over a 24-hour period. Paradoxically, the binding of Clock/Bmal1 to the Per promoters remains relatively constant, whereas the strongest binding to the Cry1 promoter corresponds to the lowest levels of Cry1 expression. In this paper, In the search for what might regulate this dynamic H3 acetylation, the authors found that p300 — a protein with histone-acetylation activity — forms a complex with Clock in mouse liver cells. Based on their immunoprecipitation data, the authors propose that, during the day, p300/Bmal1/Clock binds to the promoter, leading to H3 acetylation, polII recruitment and transcription of the Per genes. At night, dissociation of p300 from Clock/Bmal1, together with deacetylase activity associated with the complex, results in promoter deacetylation and inhibition of transcription.

But what brings about the night-time dissociation of p300? Transcription of circadian-clock genes is under the negative control of the Cry proteins. The authors used a luciferase reporter assay to show that Cry1 and Cry2 inhibit p300/Clock/Bmal1-driven transcription from the Per1 promoter. They propose that Cry proteins achieve this inhibition by destabilizing the p300/Clock/Bmal1 complex.

Another regulation mechanism is the methylation of genes, especially the methylated groups transferred to the base cytosine, which is mostly sensitive to methylation groups.

About 70% of all CpG Dinucleotids are methylated

Hypermethylation in CpG islands is a common event in carcinogenesis

The transcriptional silence of tumour suppressor genes by promoter CpG islands Hypermethylation can contribute to oncogenesis

CpG islands are approximately 1-kb-stretches of DNA containing clusters of CpG dinucleotiods that are normally unmethylated in normal cells and are often located near to the 5´ends of genes. Methylation of promoter CpG islands is associated with a closed chromatin structure and transcriptional silencing

Effects of Disturbed Methylation on Cell Function

- Disturbed DNA-REPLICATION: silencing or hyperreplication
- Activation of oncogene genes (RAS; farnelysation of P21 (RAS)
- Hypermethylation of tumor suppressor genes

6. LONGEVITY GENES

Many recently discovered genes, known by such cryptic names as *daf-2, pit-1, amp-1, clk-1* and *p66Shc*, have been found to affect stress resistance and life span in laboratory organisms, suggesting that they could be part of a fundamental mechanism for surviving adversity. Sirtuin genes function as antiaging genes in yeast, Caenorhabditis elegans, and Drosophila. The NAD requirement for sirtuin function indicates a link between aging and metabolism, and a boost in sirtuin activity may in part explain how calorie restriction extends life span. In mammals, one of the substrates of the SIR2 ortholog, SIRT1, is a regulator of mitochondrial biogenesis, PGC-1alpha. Indeed, the putative SIRT1 activator resveratrol has been shown to stimulate mitochondrial biogenesis and deliver health benefits in treated mice. Sirtuins are NAD+-dependent enzymes that have been implicated in a wide range of cellular processes, including pathways that affect diabetes, cancer, lifespan and Parkinson's disease. To understand their cellular function in these age-related diseases, identification of sirtuin targets and their subcellular localization is paramount. SIRT3 (sirtuin 3), a human homologue of Sir2 (silent information regulator 2), has been genetically linked to lifespan in the elderly. However, the function and localization of this enzyme has been keenly debated. A number of reports have indicated that SIRT3, upon proteolytic cleavage in the mitochondria, is an active protein deacetylase against a number of mitochondrial targets. In stark contrast, some reports have suggested that full-length SIRT3 exhibits nuclear localization and histone deacetylase activity. Recently, a report comparing SIRT3-/- and SIRT+/+ mice have provided compelling evidence that endogenous SIRT3 is mitochondrial and appears to be responsible for the majority of protein deacetylation in this organelle. In this issue of the Biochemical Journal, Cooper et al. present additional results that address the mitochondrial and nuclear localization of SIRT3. Utilizing fluorescence microscopy and cellular fractionation studies, Cooper et al. have shown that SIRT3 localizes to the mitochondria and is absent in the nucleus. Thus this study provides additional evidence to establish SIRT3 as a proteolytically modified, mitochondrial deacetylase.[7]

But our own two laboratories have focused on a gene called *SIR2*, variants of which are present in all organisms studied so far, from yeast to humans. Extra copies of the gene increase longevity in creatures as diverse as yeast, roundworms and fruit flies, and we are working to determine whether it does the same for larger animals, such as mice.

As one of the first longevity genes to have been identified, *SIR2* is the best characterized, so we will focus here on its workings. They illustrate how a genetically regulated survival mechanism can extend life and improve health, and growing evidence suggests that *SIR2* may be *the* key regulator of that mechanism

Over the course of a lifetime, cell loss from apoptosis may be an important factor in aging, particularly in nonrenewable tissues such as the heart and brain, and slowing cell death may be one way Sirtuins promote health and longevity. A striking example of Sirt1's ability to foster survival in mammalian cells can be seen in the Wallerian mutant strain of mouse. In these mice, a single gene is duplicated, and the mutation renders their neurons highly resistant to stress, which protects them against stroke, chemotherapy-induced toxicity and neurodegenerative diseases. Wallerian gene mutation in these mice increases the activity of an enzyme that makes NAD, and the additional NAD appears to protect the neurons by activating Sirt1.

7. THE CALORIE CONNECTION

Restricting an animal's calorie intake is the most famous intervention known to extend life span. Discovered more than 70 years ago, it is still the only one absolutely proven to work. The restricted regime typically involves reducing an individual's food consumption by 30 to 40 percent compared with what is considered normal for its species. Animals ranging from rats and mice to dogs and possibly primates that remain on this diet not only live longer but are far healthier during their prolonged lives. Most diseases, including cancer, diabetes and even neurodegenerative illnesses, are forestalled. The organism seems to be supercharged for survival. The only apparent trade-off in some creatures is a loss of fertility.

Understanding the mechanisms by which calorie restriction works and developing medicines that reproduce its health benefits have been tantalizing goals for decades.

Calorie restriction does not slow metabolism in mammals, and in yeast and worms, metabolism is both sped up and altered by the diet. We believe, therefore, that calorie restriction is a biological stressor like natural food scarcity that induces a defensive response to boost the organism's chances of survival. In mammals, its effects include changes in cellular defenses, repair, energy production and activation of programmed cell death known as apoptosis. We were eager to know what part Sir2 might play in such changes, so we looked first at its role during calorie restriction in simple organisms

In yeast restricting food availability affects two pathways that increase Sir2 enzymatic activity in the cells. On one hand, calorie restriction turns on a gene called *PNC1*, which produces an enzyme that rids cells of nicotinamide, a small molecule similar to vitamin B3 that normally represses Sir2. Consistent with the idea that calorie restriction is a stressor that activates a survival response, *PNC1* is also stimulated by other mild stressors known to extend yeast life span, such as increased temperature or excessive amounts of salt. A second pathway induced in yeast by restricted calories is respiration, a mode of energy production that creates NAD as a by-product while lowering levels of its counterpart, NADH. It turns out that not only does NAD activate Sir2, but NADH is an inhibitor of the enzyme, so altering the cell's NAD/NADH ratio profoundly influences Sir2 activity.

In organisms as complex as fruit flies, calorie restriction does require *SIR2* to extend life span. And because the body of an adult fruit fly contains numerous tissues that are analogous to mammalian organs, we suspect that calorie restriction in mammals is also likely to require *SIR2*.

Yet if humans are ever to reap the health benefits of calorie restriction, radical dieting is not a reasonable option. Drugs that can modulate the activity of Sir2 and its siblings (collectively referred to as Sirtuins) in a similar manner will be needed. Just such a Sirtuin-activating compound, or STAC, called resveratrol has proven particularly interesting. Resveratrol is a small molecule present in red wine and manufactured by a variety of plants when they are stressed. At least 18 other compounds produced by plants in response to stress have also been found to modulate Sirtuins, suggest?-ing that the plants may use such mole?-cules to control their own Sir2 enzymes.

Resveratrol-fed flies not only live longer, despite eating as much as they want, but they do not suffer from the reduced fertility often caused by calorie restriction. This is welcome news for those of us hoping to treat human diseases with molecules that target Sir2 enzymes. But first we want a better understanding of the role of Sir2 in mammals.

The mammalian version of the yeast *SIR2* gene is known as *SIRT1* ("*SIR2* homolog 1"). It encodes a protein, Sirt1, that has the same enzymatic activity as Sir2 but that also deacetylates a wider variety of proteins both inside the cell nucleus and out in the cellular cytoplasm. Several of these proteins targeted by Sirt1 have been identified and are known to control critical processes, including apoptosis, cell defenses and metabolism. The potential longevity-enhancing role of the *SIR2* gene family seems, therefore, to be preserved in mammals. But not surprisingly in larger and more complex organisms, the pathways by which Sirtuins achieve their effect have grown considerably more complicated as well.

Increased Sirt1 in mice and rats, for example, allows some of the animals' cells to survive in the face of stress that would normally trigger their programmed suicide. Sirt1 does this by regulating the activity of several other key cellular proteins, such as p53, FoxO and Ku70, that are involved either in setting a threshold for apoptosis or in prompting cell repair. Sirt1 thus enhances cellular repair mechanisms while buying time for them to work resveratrol and another STAC, fisetin, were shown to prevent nerve cells from dying in two different animal models (worm and mouse) of human Huntington's disease. In both cases, the protection by STACs required Sirtuin gene activity.

The protective effect of Sirtuins in individual cells is becoming increasingly clear. NAD levels rise in liver cells under fasting conditions, prompting increased Sirt1 activity. Among the proteins Sirt1 acts on is an important regulator of gene transcription called PGC-1, which then causes changes in the cell's glucose metabolism. Thus, Sirt1 was found to act both as a sensor of nutrient availability and a regulator of the liver's response. Similar data have given rise to the idea that Sirt1 is a central metabolic regulator in liver, muscle and fat cells because it senses dietary variations via changes in the NAD/NADH ratio within cells and then exerts far-reaching effects on the pattern of gene transcription in those tissues. This model would explain how Sirt1 may integrate many of the genes and pathways that affect longevity Sirt1, in turn, also regulates fat storage in response to diet. Indeed, Sirt1 activity is increased in fat cells after food limitation, causing fat stores to move from the cells into the bloodstream for conversion to energy in other tissues. We surmise that Sirt1 senses the diet, then dictates the level of fat storage and thus the pattern of hormones produced by fat cells. This effect on fat and the signals it sends would, in turn, set the pace of aging in the entire organism and make Sirt1 a key regulator of the longevity conferred by calorie restriction in mammals. It would also closely link aging and metabolic diseases, including type 2 diabetes, associated with excess fat. Intervening pharmacologically in the Sirt1 pathway in fat cells might therefore forestall not only aging but also specific ailments.

Another critical process modified by Sirt1 is inflammation, which is involved in a number of disorders, including cancer, arthritis, asthma, heart disease and neurodegeneration. Sirt1 inhibits NF-B. Sirt1 regulates production of insulin and insulinlike growth factor 1 (IGF-1) and that those two powerful signaling molecules, in turn, seem to regulate Sirt1 production as part of a complex feedback loop. The relation between Sirt1, IGF-1 and insulin is intriguing because it explains how Sirt1 activity in one tissue might be communicated to other cells in the body. Moreover, circulating levels of insulin and IGF-1 are known to dictate life span in various organisms—worms, flies, mice, possibly ourselves.

8. DNA REPAIR

The recent hypothesis that common variants (single nucleotide polymorphisms or SNPs) in the population may contribute significantly to genetic risk for common diseases permits a conceptually straightforward approach to identifying age-related disease-causing mutations. Functional variants of DNA replication and repair genes might be expected to be highly significant to cancer and aging since replication must proceed with high fidelity in a cellular environment where an estimated 10000 nucleotides are damaged daily. Single-strand breaks (SSB) are one of the results of DNA damage either by methylation, oxidation, reduction or fragmentation of bases by ionizing radiation, and arise in cells directly by disintegration of damaged sugars or indirectly as intermediates of base excision repair. Studies have demonstrated a role for XRCC1 both in vitro and in vivo during the repair of SSB. A number of SNPs have been identified for the XRCC1 gene, and several have been associated with age-related diseases, especially cancer.[8]

9. INFLAMMATION

Aging is an inflammatory disease**,** causing a link between different age related diseases like bowel disease, cardiovascular disease, Alzheimer and Parkinson disease, osteoarthritis, dental and gum inflammation and cancer. The genetic variants of interleukins or PPAR gamma and alpha receptor cause different expression of pro-inflammatory cytokines or reduced activity of the nuclear receptors, leading to increased pro-inflammation and morbidity or mortality. The total burden of infection at various sites may affect the progression of atherosclerosis and Alzheimer's disease (AD), the risk being modulated by host genotype. The role of lipopolysaccharide (LPS) receptor TLR4 is paradigmatic. It initiates the innate immune response against gram-negative bacteria, and TLR4 single nucleotide polymorphisms (SNPs), such as +896A/G, known to attenuate receptor signaling, have been described. This SNP shows a significantly lower frequency in patients affected by myocardial infarction or AD. Thus, people genetically predisposed to developing lower inflammatory activity seem to have less chance of developing cardiovascular disease (CVD) or AD.[9]

Increased rate of inflammation has been observed to be associated with aging. This is manifested, e.g. as increased blood levels of proinflammatory cytokines, such as interleukin-6 (IL-6). The production of IL-6 is, at least partially, genetically determined the single nucleotide polymorphism (SNP) at the promoter (-174G/C) being decisive. Consequently, some studies have demonstrated that the -174G/C genotype frequencies are different in very old persons as compared to younger ones. The frequency of -174 allele G was clearly higher in the survivors (n = 114) than in the non-survivors (n = 171).

Inflammation

Aging is a chronic inflammatory disease leading to the cardiovascular diseases, Alzheimer D. and Cancer, as well as Parkinson Disease (IPS).The(Interleukin) IL 10 CC 1082 is associated with increased production of IL 16 and highly represented among centenarians. The IL G>A 1082 genotype is correlated to a higher pro inflammatory state and a predictor for disability in aging people. The Interferon gamma snp is a predictor for increased inflammation in women.

Interleukin- 6

Interleukin 6 (IL-6) is a multifunctional cytokine and is involved in both the amplification of and the protection against the inflammation in response to infection and tissue injury. A polymorphism within the promoter region (G>C Pos. –174) has been suggested to modulate IL6-plama levels.

Furthermore, IL-6 gene expression is regulated by other cytokines, transcription factors, and several hormones, e.g. estradiol.

Just like the the G>A Pos. –1082 polymorphism in the interleukin 10 gene (IL-10) described below, for this IL-6 polymorphoism there is also scientific evidence, that certain genotypes can be associated with an increased life expectancy.[10]

Significance of Genetic Variants of IL-6 Gene: from Physiology to Risk Assessment

Basic Informations:

IL-6 is secreted by leukocytes, endothelial cells, muscle cells and adipocytes.

IL-6 secretion is stimulated by TNF-alfa, IL-1, bacterial endotoxins, catecholamines. IL-6 stimulates H-P-Adrenal axis in inflammation; stimultes GH secretion and inhibits TSH. IL-6 is a regulator of Acut Phase responses associated with Insulin resistant conditions.

Ciculating IL-6 is higher in obese, T2DM patients with MS characteristics.

SNPs: -174G>C variant influence transcriptional activity in healthy individuals,

-174GG carriers have higher IL-6 levels in UK, Native Americans and Caucasians and Spanish population.

- 174C was associated with obesity in Swedish and French Canadians.

SNP –572G>C and –597G>A also influences IL-6 secretion, the effect of these 3 SNPs are additive, the GGG-haplotype produces the highest amount in vitro.

Interleukin 10

The cytokine interleukin 10 (IL-10) physiologically limits and down-regulates inflammation. Age related diseases are initiated or worsened by systemic inflammation; conversely, genetic variations determining increased production of anti-inflammatory cytokines have been shown to be associated with successful aging: A polymorphism within the promoter region has been shown to regulate IL-10 levels. An adenine (A) at the site –1082 in the promoter region of the IL-10 gene is associated with low and guanine (G) with high production of IL-10.

In a randomized, double-blind, placebo-controlled, crossover study, 24 postmenopausal women with hypercholesterolemia received 25 g of soy protein or a placebo daily for 6 weeks, with treatment periods separated by 1 month. Markers of vascular inflammation were measured by enzyme-linked immunosorbent assay methods, including: soluble interleukin-2 receptor (sIL-2r), E-selectin, P-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1). There was no effect of soy protein in comparison with placebo on the inflammatory markers: the sIL-2r level was 942.2 +/- 335.3 pg/mL with soy protein and 868.5 +/- 226.9 pg/mL with placebo (P =.311); E-selectin was 39.6 +/- 16.5 ng/mL with soy protein and 42.1 +/- 17.6 ng/mL with placebo (P =.323); P-selectin was 157.9 +/- 67.9 ng/mL with soy protein and 157.5 +/- 47.6 ng/mL with placebo, (P =.977); ICAM-1 was 266.0 +/- 81.3 ng/mL with soy protein and 252.5 +/- 82.7 ng/mL with placebo (P =.435); VCAM-1 was 402.7 +/- 102.1 ng/mL with soy protein and 416.4 +/- 114.8 ng/mL with placebo (P =.53). CONCLUSIONS: Consumption of 25 g of isolated soy protein daily for 6 weeks does not substantially affect markers of vascular inflammation in postmenopausal women with hypercholesterolemia. Interleukin –10 is potent to mediate downregulation of immune response[11] 1082 GàA polymorphism (1082A/A) results in lower IL-10 secretion.IL-10 has protective effects in atherosclerosis.[12] Reduced secretion may contribute to AD.The 1082 G/G genotype is correlated to centenarian men, but not in women.[13] Combination of 1082 IL-10 G/G and TNF alpha –308G/G phenotype is widely distributed< among centenarians.[14]

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