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SCIENTIFIC IDEAS OF LIFE EXTENSION



The review is prepared by the «Science for Life Extension» Foundation
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The purpose of this review is to advocate different research directions in one way or another associated with studying the fundamental mechanisms of aging and searching for the ways to extend human lifespan and healthspan.

The initiative to promote the research is proposed solely by the «Science for Life Extension» Foundation, but not by the investigators. The Foundation is in no way representing the scientists, but considers the listed research areas essential and extremely valuable. We would like to inform the society about the need to increase the amount of scientific investigations in these fields.

Chairman of the Board of Trustees
of the «Science for Life Extension» Foundation

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01

The possibility to reprogram human somatic cells into induced pluripotent stem cells opens a broad perspective for regenerative medicine. Stem cells obtained with the use of this technology are able to differentiate into almost any type of cells.

IMPROVEMENT OF INDUCED PLURIPOTENT STEM CELLS PRODUCTION AND APPLICATION METHODS

Production of induced pluripotent stem cells

Until recently the reprogramming method was based on the use of viral vectors that modify the host cell genome by incorporating the necessary genes. But this method turned out to be risky due to induction of tumorigenesis.

However, in May 2009 the group of **Sheng Ding** from the Scripps Institute (California), showed that it was possible to obtain the generation of iPS cells using another method (1). They totally avoided genetic interference – instead of genes they incorporated proteins capable of reprogramming the cell. As a result, murine cells became stem cells and were not transformed into cancer cells. Now the researchers are planning to carry out experiments on human tissues.

Modeling pathogenesis

The application of human induced pluripotent stem cells offers a new strategy for modeling human diseases and gaining new insights into human pathogenesis and treatment. In the **Lorenz Studer** Laboratory (Developmental Biology Center, Memorial Sloan-Kettering Cancer

Center) a model was created of a rare but fatal peripheral neuropathy - familial dysautonomia using the patient's cells obtained via reprogramming (2). This model allowed the investigators to make a hypothesis regarding the mechanism of this neuropathology. **In the future it will be possible to select a drug for the treatment of this disease.**

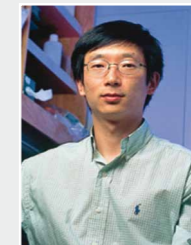
Ways to solve the problem of high cancer incidence

Paul Knoepfler from UC Davis School of Medicine offers possible ways of solving this serious problem (3):

1. The idea is to use the stem cells to produce progenitor or precursor cells of the desired lineage and then **transplant progenitors separated from potentially oncogenic cells.**
2. The stable genetic introduction into stem cells of a gene such as thymidine kinase, capable of **selectively destroying undifferentiated cells, which tend to form tumors.**
3. **Using killer antibodies directed against antigens** present on the cell membrane of human stem cells.
4. **Genetic modification of induced pluripotent stem cells in order to reduce their oncogenic potential.**

Publications:

- 1 Hongyan Zhou *et al.* «Generation of Induced Pluripotent Stem Cells Using Recombinant Proteins», *Cell Stem Cell*, 2009
- 2 Studer *et al.* «Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs», *Nature*, 2009
- 3 Knoepfler P., «Deconstructing Stem Cell Tumorigenicity: A Roadmap to Safe Regenerative Medicine», *Stem cells*, 2009



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02

Adult stem cells provide the basis for regeneration of aging tissue. Their dual ability for self-renewal and multilineage differentiation is controlled by direct interaction with a specific microenvironment – the so called “stem cell niche”, which consists of “regular” somatic cells subjected to aging (1). It is essential to understand how to “rejuvenate” the stem cell niche in order to maintain the proliferative activity of the stem cells.

PREVENTION OF STEM CELL NICHE AGING

The niche undergoes aging, damage and errors accumulate with time. This leads to the loss of functionality, in particular – to stem cell proliferative potential reduction.

As a result, the number of stem cells decreases and so does the production of stimuli that regulate the stem cells differentiation pathways.

Development of the artificial niche

Erin Lavik’s lab, Yale University, is studying neural stem cell niche with the goal to develop therapies to treat spinal cord injuries, glaucoma and retina degeneration.

In particular, the researchers carried out an interesting work on **engineering an artificial neural stem cell niche using synthetic polymers**.

The project turned out to be quite successful, but unfortunately, too expensive to use it for the lab purposes.

Nevertheless, the point was proven that this approach – engineering an artificial stem cell niche – is possible in principle.

A model to study stem cell niche

The *Drosophila* sperm are generated from germline stem cells that cluster around a well-defined ‘hub’ of non-dividing, non-germline cells that maintain germline stem cells and coordinate sperm development. This all makes them the stem cell niche cells.

A team led by **Leanne Jones** at the Salk Institute in La Jolla, California, was studying the *drosophila* germline cells, from which the sperm is formed later on. The investigators made a surprising observation that this niche is being actively replenished by a population of non-germline stem cells. It is essential to find the answer to the pressing question for regenerative medicine: How stem cells create and maintain the niches that in turn sustain other stem cells. It seems Professor Jones found a suitable model to investigate this issue. The experiments of the group revealed that the somatic stem cells give rise to two lineages: sperm-encapsulating cyst cells and hub cells that form the niche (2).

This work offers an accessible system for studying both the mechanisms that guide cell transition between fates and the way these mechanisms change with age.

Publications:

- 1 Wagner et al. «Aging of hematopoietic stem cells is regulated by the stem cell niche», *Experimental gerontology*, 2008.
- 2 Voog et al., «Multipotent somatic stem cells contribute to the stem cell niche in the *Drosophila testis*», *Nature*, 2008.



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03

The telomerase is an enzyme that elongates and maintains telomeres (chromosome end regions) allowing the cell to proliferate unrestrictedly. Telomere elongation could help solving the problem of aging if it would be possible to eliminate the risk of cancerous transformation of such cells. The combination of genetic methods and cell therapy provides an opportunity to achieve this goal.

LIFESPAN EXTENSION AND ANTI-CANCER THERAPY WITH THE HELP OF TELOMERASE REACTIVATION AND STEM CELLS TRANSPLANTS

Telomerase becomes inactive in differentiated somatic adult human cells and it keeps its activity in germline and stem cells and also in the majority of cancer cells.

Telomerase reactivation prolongs lifespan in cancer resistant mice

In 2008 **Maria Blasco** from the Spanish National Cancer Institute showed that Telomerase Reverse Transcriptase overexpression in cancer resistant mice slows down aging and extends median lifespan (1). Therefore, **in the case of proper oncogenesis control it might be possible to decelerate aging via slowing down the replicative aging of the cells in an organism.**

Possible cancer therapy

The introduction of telomerase is proposed as a method to combat aging via cell therapy and a possible method to regenerate tissue,

while telomerase inhibition and telomere shortening is suggested as a possible therapy to defeat cancer with intact p53. Researchers thus face the challenge of understanding the complex processes, which regulate the potential benefits of both telomerase inhibition and activation (2).

Prevention of cancer and aging

To test the hypothesis **that transplanting hematopoietic stem cells with long telomeres can improve survival and organ homeostasis**, the international research team led by **Zhenyu Ju** (Sino-German Laboratory for Aging and Regenerative Medicine, Beijing, China) together with **Leonard Rudolph** (Max-Planck-Research Group on Stem Cell Aging, Ulm, Germany) will conduct series of experiments transplanting the hematopoietic stem cells from the wild-type mice into telomere dysfunctional mice.

The main purpose of the project is to find out whether intrinsically mortal stem cells can maintain a proliferating tissue indefinitely if periodically replenished.

If the answer is yes, this will motivate exploring such treatments as part of an exceptionally robust cancer-prevention therapy.

Publications:

- 1 Blasco M. et al. «Telomerase Reverse Transcriptase Delays Aging in Cancer-Resistant Mice», *Cell*, 2008
- 2 Shawi M «Telomerase, senescence and ageing», *Mech Ageing Dev.*, 2008



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04

“Arrested” cells are the cells that stopped in different phases of their cell cycle. Accumulation of such cells in the organism leads to promotion of the aging processes and to tumorogenesis. This is the reason why it is extremely important to find the way to eliminate arrested cells.

ELIMINATION OF SENESCENT («ARRESTED») CELLS

All cells in the organism produce specific molecules – cytokines, which carry signals, activate the processes of proliferation (dividing), differentiation, apoptosis etc. Through the secretion of various regulatory molecules senescent cells can influence other cells triggering their aging processes.

Secreting harmful molecules by senescent cells promotes aging

Human cells senescing because of DNA damage secrete a myriad of factors associated with inflammation and malignancy. This state of a cell – senescence-associated secretory phenotype – develops slowly over several days and only after DNA damage.

The group of **Judith Campisi** from Buck Institute showed that the particular state when the cell secretes harmful molecules occurs only after establishment of persistent DNA damage signaling, but not after transient DNA damage responses, which, nevertheless, initiate cytokine expression via specific proteins. Dr. Campisi highlights **the necessity of finding the way to effectively identify and eliminate such harmful arrested cells.**

Application of viral vectors to develop the senescence-associated secretory phenotype

The scientists from the University of Massachusetts (**Paul Kaufman**) in collaboration with Buck Institute (**Judith Campisi**) and Lawrence Berkley Laboratory (**Priscilla Cooper**) **worked out a model to create senescent cells lineages.**

This model can simplify investigation of the secretory phenotype and possible ways of eliminating senescent cells.

The researchers describe a collection of 59 viral vectors that comprise an integrated system for expression of complementary DNAs, small hairpin RNAs or microRNAs, required for turning on or off the genes responsible for production of particular proteins of interest **(2).**

In other words, **this model enables turning the non-proliferative cells into senescent cells with the secretory phenotype.** It is essential for further search for the methods to eliminate arrested cells, which contribute to tumor formation and aging.

Publications:

- 1 *Campisi et al., «Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion», Nature Cell biology, 2009*
- 2 *Campeau et al., «A Versatile Viral System for Expression and Depletion of Proteins in Mammalian Cells», Plos One, 2009*



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05

The frequency of infectious and autoimmune diseases as well as tumors tends to grow with age.

This is partially caused by the age-related defects of the immune system.

To strengthen the body defense it is possible to use engineering methods.

ENGINEERING IMMUNE CELLS

The correlation of a wide range of age-related diseases and deterioration of immune system functioning let scientists suggest that the aging of immune system limits lifespan. Present-day technologies open up opportunities for strengthening the immune system using engineering methods.

Engineering anticancer T cells

Under conditions of chronic antigenic stress (chronic infection, cancer, autoimmunity) accumulation of dysfunctional T cells may be detrimental. **Engineering T cells can contribute to fighting such chronic diseases.**

To provide functional longevity for human clones of T cells it is necessary to work out strategies other than telomere maintenance, such as strengthening the defense against free radicals and improvement of lymphocyte DNA repair.

To obtain a sufficient number of cells for adaptive immunotherapy of cancer, it is necessary to explore such ways as enforced expression of certain heat shock proteins and proteasome components, and interference with the expression of negative regulatory receptors expressed by T cells (1).

Engineering B cells

The technology of reprogramming human somatic cells into induced pluripotent stem cells opens up new vistas for vaccination. **Somatic cells can be induced into iPSC and transformed into immune system cells**, which are then reprogrammed into memory B cells by way of antigen introduction into the culture medium. These B cells are able to release antibodies to various pathogens (2). The obtained memory cells are transferred back to the patient thus strengthening his defense against various infections.

Engineering dendritic cells

Dendritic cells are a complex of antigen-presenting cells which initiate and regulate immune response. Doctor **Angus Thomson** from the University of Pittsburg is studying a new method which may be used for therapy of tissue rejection and autoimmune diseases, as well as for maintenance of immune functions in an aging organism. He suggests **ways to control maturation and differentiation of dendritic cells and to engineer new cells** (3).

Publications:

- 1 Pawelec et al., "Engineering anticancer T cells for extended functional longevity", *Annals of the New York Academy of Science*, 2004.
- 2 Li et al., "Cell based vaccination using transplantation of iPSC-derived memory B cells", *Vaccine*, 2009
- 3 Lu L, Thomson AW., "Manipulation of dendritic cells for tolerance induction in transplantation and autoimmune disease", *Transplantation*, 2002



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06

Female reproduction period is limited by the number of female germ cells – oocytes. The number of these cells decreases with age and the quality declines as well. A part of all the oocytes follows the apoptosis path (self-destruction) under the influence of alfa-kinases. Experiments on how the kinases of this class impact oocyte numbers and quality are needed. It is also essential to study their influence on the organism as a whole. Finding the way to extend female fertility period may lead to life extension.

FEMALE GERM CELL APOPTOSIS AND ITS INFLUENCE ON AGING OF THE ORGANISM

The age-related decrease in the number of ovarian follicles critically impairs the female reproductive capability. During the fetal period, the number of ovarian follicles in an ovary reaches approximately seven million at 20 gestational weeks.

This number begins to decrease at 24 gestational weeks, and declines to about forty thousand at birth. The number continues to decline after birth and disappears almost completely before menopause.

Pentosidine accumulation plays a role in age-related oocytes apoptosis

The loss of ovarian follicles is termed follicular atresia, which is mediated by apoptosis of the constituent cells. Scientists in **Hiroaki Ohta's** group from the Tokyo Women's Medical University believe **there is a sex hormone-independent apoptotic mechanism involved in aging.**

The researchers hypothesized that the age-related increased oxidative stress amplifies carbonyl stress, alters cell signaling, and deprives oocytes of normal functions of the endoplasmatic reticulum and proteasome, leading to apoptosis induction of the cells. The investigators also associate the apoptosis with pentosidine accumulation (the end glycation product – a compound forming due to arginine, lysine and pentose cross-linking).

Determining the cellular and molecular mechanisms of toxic cell signaling in age-related oocyte apoptosis should improve understanding of the aging and infertility processes and to develop appropriate therapeutic strategies **(1).**

Apoptosis is crucial for oocyte quality maintenance

Female germ cells – oocytes arrest development at the end of prophase of meiosis I and remain quiescent for years.

Over time, the quality and quantity of these oocytes decreases, resulting in fewer pregnancies and an increased occurrence of birth defects. Researchers **Sara Andux** and **Ronald Ellis** from UMDNJ School of Osteopathic Medicine demonstrated **mutations that block all cell deaths result in a severe, early decline in oocyte quality, and this effect increases with age (2).**

Researchers conclude that competition for resources is a serious problem in aging germ lines, and that apoptosis helps alleviate this problem.



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Publications:

- 1 Matsumine M. et al., "Pentosidine Accumulation in Human Oocytes and Their Correlation to Age-Related Apoptosis", *Acta Histochem. Cytochem*, 2008
- 2 Andux, Ellis, «Apoptosis maintains oocyte quality in aging *Caenorhabditis elegans* females», *PLoS Genetics*, 2008

07

Functions lost during the aging process can be recovered. In order to do that it is necessary to increase responsiveness of the senescent cells toward a variety of agonists, including growth factors. The aging phenotypes of hyporesponsiveness and morphological alteration are shown to be adjusted by modulation of the several membrane-associated molecules.

LOWERING CAVEOLIN EXPRESSION PROMOTES FUNCTIONAL RECOVERY OF THE CELL AGING PHENOTYPE

The major goals of aging research are focused on the development of a replacement strategy of the aged organs or cells, based on immortalizing tools, stem cells, or artificial substitutes.

Reduction of caveolin status in senescent cells leads to restoration of the responsiveness and shape

Professor **Ryu** from the Seoul National University College of Medicine proposed a new concept of functional recovery on the basis of the functional restoration of the responsiveness of the senescent cells toward a variety of agonists, including growth factors (1). The aging phenotypes of hyporesponsiveness and morphological alteration are shown to be readily adjusted by modulation of the several membrane-associated molecules, named gatekeeper molecules, among which caveolin is one of the major determinants.

Caveolin is the essential component of the caveolae, responsible for regulation of signal transduction, endocytosis and transcytosis, and

cytoskeletal arrangement. The caveolin status is associated strictly with cellular transformation, if depleted, and with senescent phenotype, if overexpressed.

Therefore, simple reduction of caveolin status in senescent cells leads to restoration of the functional responsiveness to mitogenic stimuli and even of the cellular shape. So, **the gatekeeper molecules, represented by caveolin, may play the prime role in determination of the senescent phenotypes.**

These results show that the replace principle would not necessarily be the essential one, but the restore principle can be somehow substituted for the betterment of the aged cells and organisms.

Caveolin as a target for preventing age-related diseases

Free radicals play a role in aging and age-related human diseases, including pulmonary emphysema. Cigarette smoke represents a source of oxidants. Scientists from the group of Dr. **Ferruccio Galbiati**, Pittsburg University, demonstrated that cigarette smoke extracts promote stress-induced premature senescence in wild type but not caveolin-1 null lung murine fibroblasts.

In vivo studies show that caveolin-1 expression is necessary for cigarette smoking-induced senescence of lung fibroblasts and pulmonary emphysema.

Therefore, **caveolin-1 is a novel therapeutic target for the treatment and/or prevention of pulmonary emphysema (2).**



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Publications:

- 1 Ryu et al., "Functional efficiency of the senescent cells: replace or restore?", *Ann N Y Acad Sci.*, 2004
- 2 Bartholomew et al., «Caveolin-1 regulates the antagonistic pleiotropic properties of cellular senescence through a novel Mdm2/p53-mediated pathway», *Cancer Research*, 2009

08

Autophagy is the process of clearing the «cellular junk» – damaged organelles and defective proteins. Autophagy effectiveness is impaired in aging cells. One of the reasons is reduction of chaperone proteins availability that play an important role in the autophagy processes. That's why it is crucial to find the ways of maintaining chaperone availability in the cells.

CHAPERONE-MEDIATED AUTOPHAGY ACTIVATION

Chaperone-mediated autophagy is a type of autophagy contributing to the removal of altered proteins by the lysosomes as part of the cellular quality-control systems.

Chaperones facilitate the membrane transportation of substrate proteins into lysosomes, where they are degraded afterwards.

Maintaining the number of chaperone proteins

The group of **Ana Maria Cuervo** (Albert Einstein College of Medicine, Bronx, New York) demonstrated that **chaperone-mediated autophagy activity declines in aged organisms**.

The scientists have proposed that this failure in cellular clearance could contribute to the accumulation of altered proteins, the abnormal cellular homeostasis and, eventually, the functional loss characteristic of aged organisms.

If the chaperone availability necessary for this type of autophagy is increased the intracellular accumulation of damaged proteins is lowered **(1)**.

Maintaining the number of lysosomal receptors

Also, **maintaining the lysosomal receptor abundance for chaperone-mediated autophagy can contribute to efficient autophagic activity until late in life**.

Ana Maria Cuervo showed that preservation of autophagic activity is associated with lower intracellular accumulation of damaged proteins, better ability to handle protein damage and improved organ function **(2)**.

Pharmacological activation of heat shock protein expression

The scientists in the **Alice Liu** laboratory in Rutgers University (New Jersey) discovered that clinically relevant concentrations of the FDA-approved drug riluzole significantly increased the heat shock protein hsp70 synthesis by inducing the expression of the corresponding gene.

The scientists **demonstrated the effect of the cellular protection systems enhancement as a response to heat shock**. It is now not known if riluzole targets a step in the chaperone-mediated autophagy pathway or that it targets the heat shock protein-1 as a substrate for this pathway.

Analysis of the effects of riluzole in an *in vitro* chaperone-mediated autophagy-mediated protein degradation system should help to provide some answers to these questions **(3)**.



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Publications:

- 1 Morimoto, Cuervo, «Protein homeostasis and aging: taking care of proteins from the cradle to the grave», *The Journals of Gerontology*, 2009
- 2 Zhang, Cuervo, «Restoration of chaperone-mediated autophagy in aging liver improves cellular maintenance and hepatic function», *Nature Medicine*, 2008
- 3 Yang J et.al., «Riluzole Increases the Amount of Latent HSF1 for an Amplified Heat Shock Response and Cytoprotection», *Plos One*, 2008

09

The processes of aging and autophagy (degradation of a cell's own components) are closely connected. They are regulated by the same signaling factors. Thus, to maintain autophagy activity at a young level it is necessary to define possible ways of controlling signaling mechanism interaction using SIRT1, mTOR, FoxO3, NF-kappaB and p53. It is these factors that determine the lifespan.

STIMULATION OF AUTOPHAGY HAS A POSITIVE EFFECT ON LONGEVITY

Autophagy is involved in cellular protein and organelle degradation. During aging, the efficiency of autophagic degradation declines and intracellular waste products accumulate. In the nematode *Caenorhabditis elegans*, there is clear evidence that lifespan is linked to the capacity to regulate autophagy.

Recent studies have revealed that the same signaling factors regulate both aging and autophagocytosis, thus highlighting the role of autophagy in the regulation of aging and age-related degenerative diseases (1).

Stimulation of autophagocytosis by rapamycin

The group of Professor **Aris** from the University of Florida showed that autophagy regulates amino acid homeostasis which is necessary for maintenance of yeast chronological life span (2).

The researchers also made a conclusion that **it is possible to use rapamycin for pharmacological stimulation of autophagy** (3).

Stimulation of autophagy by antilipolytic drugs

Speaking about studies involving rodents, the results of Bergamini's research group (University of Pisa) showed that the stimulation of

macroautophagy reduces the total level of low-density lipoproteins and high-density lipoproteins to young values, and triglycerides level becomes even lower. In this case the researchers used 3,5-dimethylpyrazole as an antilipolytic drug which activated macroautophagy (4).

Induction of autophagy by spermidine

Doctor **Francesco Madeo** and his colleagues from the University of Graz extended the lifespan of yeast, flies, worms and human immune cells by induction of spermidine, a natural polyamine whose intracellular concentration declines during human aging (5).

In addition, **spermidine administration potently inhibited oxidative stress in aging mice**. Induction of this substance alters the acetylation status of the chromatin, which leads to significant upregulation of various autophagy-related transcripts, triggering autophagy.

The authors conclude that enhanced autophagy is crucial for polyamine-induced suppression of necrosis and enhanced longevity.

Publications:

- 1 Salminen, Kaamiranta, "Regulation of the aging process by autophagy", *TMM*, 2009
- 2 Alvers et al., "Autophagy and amino acid homeostasis are required for chronological longevity in *Saccharomyces cerevisiae*". *Aging Cell*, 2009
- 3 Alvers et al., "Autophagy is required for extension of yeast chronological life span by rapamycin", *Autophagy*, 2009



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- 4 Bergamini et al., "Stimulation of autophagy by antilipolytic drugs may rescue rodents from age-associated hypercholesterolemia", *Rejuvenation Research*, 2009
- 5 Eisenberg et al. "Induction of autophagy by spermidine promotes longevity", *NCBiology*, 2009

10

Damaged, non-functional and aggregated proteins are accumulating with age in a cell. It is happening to a large extent, because one of the main organelles responsible for protein waste degradation – proteasome – starts to function worse and worse. Proteasome activity enhancement methods development might allow to slow down aging processes and prolong lifespan of the organism.

REDUCTION OF PROTEASOME INHIBITION

The proteasome is responsible for «cutting» damaged proteins into amino acids. The age-related inhibition of the proteasome occurs, which is the reason of «protein junk» accumulation.

Heat shock proteins prevent the proteasome inhibition

Qunxing Ding and **Jeffrey Keller** from the University of Kentucky revealed that the proteasome plays a key role in the aging processes and that the **reduction of proteasomal protein degradation leads to lifespan decrease**.

The scientists obtained data proving that heat shock proteins overexpression leads to preventing oxidative stress-mediated proteasome inhibition. Investigators from the same group confirmed that impairments in the function of purified proteasomes occurs in the earliest stages of Alzheimer's disease, and directly support a role for oxidative inactivation contributing to these declines **(1)**.

Thus, **reduction of proteasomal oxidative status, for example, via heat shock proteins might be beneficial**.

Caloric restriction favours proteasome activity

In the works by **Bertrand Friguet** from the University of Paris the **beneficial effect of caloric restriction was shown on age-related proteasome alterations**.

The peptidylglutamyl-peptide hydrolase activity, which is decreased with age in rats fed the standard diet, was restored in the rats that had fewer proteins in their diet **(2)**.

Blocking proteasome inhibition may lower the levels of proinflammatory molecules

Oxidative stress and inflammation are implicated in the pathogenesis of many age-related diseases. The group of **Fu Shang** from Tufts University (Boston) demonstrated that **oxidative inactivation of the proteasome is a molecular link between oxidative stress and overexpression of interleukin (IL)-8** (a cytokine that causes inflammation).

A novel signaling cascade was elucidated that leads to up-regulation of IL-8 in response to proteasome inactivation.

Blocking any of the stages of this signaling pathway will lead to preventing the proteasome inactivation that may lower the production of proinflammatory molecules in the cell **(3)**.



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Publications:

- 1 *Cecarini et al.*, «Oxidative inactivation of the proteasome in Alzheimer's disease», *Free Radical Research*, 2007
- 2 *Friguet et al.*, «Dietary self-selection can compensate an age-related decrease of rat liver 20 S proteasome activity observed with standard diet», *The Journals of Gerontology*, 1998
- 3 *Fernandes et al.* «Proteasome inactivation promotes p38 mitogen-activated protein kinase-dependent phosphatidylinositol 3-kinase activation and increases interleukin-8 production in retinal pigment epithelial cells», *Molecular Biology of the Cell*, 2009

11

Lipofuscin is the end product of intracellular lipid and protein oxidation. It is also known as the “age pigment”, as during aging it accumulates in nonproliferating cells, especially in large neurons in brain regions responsible for motor functions. To slow down age-related diseases it is necessary to work out methods of lipofuscin cleavage and study the ways to reduce its accumulation in cells.

REDUCTION OF LIPOFUSCIN ACCUMULATION

Lipofuscin is insoluble in water and not degradable by lysosomal enzymes or the proteasomal system. It accumulates in various cell types, including heart, liver, kidney, and neuronal tissue.

Lipofuscin accumulation depends on the functionality of mitochondrial repair systems, the proteasomal system, and the functionality and effectiveness of the lysosomes (1).

The increasing amount of Lipofuscin in a single postmitotic cell is associated with its life span and, consequently, with the whole organism aging.

That is why working out of methods for lipofuscin cleavage and prevention of its accumulation in cells will help to slow down aging and progression of age-associated diseases.

Using recombinant enzymes for replacement therapy of lipofuscinosis

To work out methods for prevention of lipofuscin accumulation, such a neurodegenerative disease as infantile neuronal ceroid lipofuscinosis is used as a model. It is caused by the gene mutation

which encodes the lysosomal enzyme, and, as a result, lipofuscin accumulates in neurons. A possible way of treatment is **enzyme-replacement therapy where missing enzymes are delivered through the cerebrospinal fluid.**

Application of this method by Doctor **Beverly Davidson** from the University of Iowa resulted in the attenuated neuropathology in mice (2). The results of this study demonstrate that intraventricular enzyme delivery to the CNS is feasible and may be of therapeutic value.

Synthesis of lysosomal enzyme low-molecular activators for lipofuscin cleavage

Doctor **Sandra Lee Hofmann** from the Southwestern Medical Center, University of Texas, showed in her research that the drug cysteamine (a simple aminothiol used in the treatment of cystinosis) may be effective in treatment of infantile neuronal ceroid lipofuscinosis.

Thiolate ion is reactive in the age pigment cleavage reaction.

Besides, the amino group probably facilitates the penetration of its molecule into lysosomes. In the course of research it was discovered that application of cysteamine not only cleaves lipofuscin in cells, but also prevents its accumulation to a certain degree (3).

Thus, improvements are needed in the design of small molecules able to reduce lipofuscin content in cells.



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SANDRA LEE HOFMANN

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Publications:

- 1 Jung et.al., “Lipofuscin: formation, distribution, and metabolic consequences”, *Annals of New York Academy of Science*, 2007
- 2 Chang et.al., “Intraventricular enzyme replacement improves disease phenotypes in a mouse model of late infantile neuronal ceroid lipofuscinosis”, *Molecular Therapy*, 2008
- 3 Lu JY, Hofmann SL., “Inefficient cleavage of palmitoyl-protein thioesterase (PPT) substrates by aminothiols: implications for treatment of infantile neuronal ceroid lipofuscinosis”, *J Inherit Metab Dis.*, 2006

12

Mobile dispersed genetic elements are DNA fragments that are able to move in a genome within a chromosome or between chromosomes. This results in deterioration of gene functions. Genetic instability, in its turn, is a factor of cell aging. To slow this process down, it is necessary to work out methods preventing the transposition of mobile dispersed genetic elements.

SUPPRESSION OF TRANSPOSITION OF MOBILE DISPERSED GENETIC ELEMENTS

Genetic instability being the consequence of a mobile element penetration leads to deregulation of gene expression and, as a result, to **age-related deterioration of cell physiology, cessation of cell growth and, eventually, to cell death or blast-transformation.**

Studying of the mechanisms regulating transposition of mobile dispersed genetic elements in a cell will contribute to determination of therapeutic targets for fighting premature aging and many pathological genome instability-related processes in an organism.

Inactivation of mobile dispersed genetic elements transposition using RNA silencing

Cells can regulate the activity of their mobile genetic elements using RNA silencing (suppression of gene expression with the help of short RNAs). A new class of such RNAs – piRNAs connected with the Piwi protein family – **is involved in suppression of mobile elements activity.** It was shown that Piwi proteins reduce the activity of different mobile elements in the germ cells of not only fruit flies, but also in mammals, fish and other organisms.

Alexei Aravin identified the first natural small RNA silencing mechanism in the germ lines of fruit flies (1) and found a new class of small RNAs called piRNAs, which opened up new opportunities for studying epigenetic mechanisms of transposon inactivation in mammals (2). Further studies led to discovery that piRNA pathway provides cells with a programmable immune system against mobile genetic elements capable of both long-term memory of invasions and acute response to active mobile elements (3).



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Introduction of biotin into diet positively affects genome stability and slows down aging process

Biotin is a water-soluble vitamin of group B, also known as vitamin H, vitamin B7 and coenzyme R. Covalent binding of biotin with histones is mediated through holocarboxylase synthetase. The group of Professor **Zempleni** from University of Nebraska showed that introduction of high doses of biotin into a diet of adult people results in an increased level of biotinylated histones in lymphocytes and a reduced number of retroelement transpositions (4).

Thus, **histone biotinylation is a new diet-dependent way of epigenetic regulation of genome stability and the related aging process.**



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USA

Publications:

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There is a number of genes, inhibition of which leads to prolonged lifespan. Such genes are called longevity genes.

It is necessary to identify all the genes of this type and find out how to regulate them correctly. In this case it will be possible to significantly increase lifespan and healthspan.

EPIGENETIC AND PHARMACOLOGIC REGULATION OF GENES AFFECTING LONGEVITY

The most important of the longevity genes are – *TOR* (target of rapamycin) and *PI3K* (phosphoinositide 3-kinase). There is also a number of genes overexpression of which leads to prolonged lifespan. These are the stress resistance genes: heat shock proteins, antioxidant enzymes, DNA and proteins repair, proteasome components, calpain genes, autophagy proteins, innate immunity proteins and xenobiotic detoxication enzymes genes. Proper control of these genes may lead to increased lifespan and improvement of its quality.

Ribosomal Protein S6 Kinase 1 Signaling Regulates Mice Life Span

Dominic Withers from the London University College in collaboration with such scientists, as **Linda Partridge** and **David Gems** discovered that deletion of ribosomal S6 protein kinase 1 (S6K1), a component of the nutrient-responsive mTOR (mammalian target of rapamycin) signaling pathway, led to increased life span and

resistance to age-related pathologies, such as bone, immune, and motor dysfunction and loss of insulin sensitivity (1). The experiments show that **this kinase gene regulates mammalian healthspan**. It is possible that therapeutic manipulations of this gene and also adenosine monophosphate-activated protein kinase might mimic caloric restriction and could provide protection against a wide range of age-related diseases.

Genistein, a soy isoflavone, activates expression of antioxidant genes

Consuelo Borrás from the University of Valencia reported that estrogens up-regulate longevity-associated genes. Her research team studied the effects of genistein, a soy isoflavone (this substance has a similar structure to the female hormone estradiol), on the expression of antioxidant longevity-related genes (2). **Genistein increased production of the antioxidant enzyme manganese-superoxide dismutase.**

These molecular studies may provide a basis for determining the effects of genistein and other soy protein-derived products on longevity in both animals and humans.

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The thymus is a gland located in the upper portion of the chest cavity just behind the sternum. The thymus is responsible for T-lymphocytes formation. A significant loss of thymic functionality with aging – involution – results in less efficient immune response. Development of age-related thymic involution retarding methods will slow the aging processes accompanied by reduction in T cell output, occurrence of lipid insertions in the connective tissue and adipose tissue development.

DEVELOPMENT OF METHODS RETARDING AGE-RELATED INVOLUTION OF THYMUS

Possible interventions to slow down thymic involution: application of *IL-7* (interleukin-7), *KGF* (keratocyte growth factor), steroid blockade, stem cells and transplants, inflammation inhibition, pituitary hormones (1).

Ghrelin, a so called hunger hormone, can be used as a possible factor causing growth hormone synthesis.

Ghrelin administration causes increase in thymocyte number and size.

Control of the supply of Insulin-like growth factor-1 maintains immune homeostasis

Professor **de Vallejo** from the University of Pittsburgh showed that controlling the availability of insulin-like growth factor-1 (IGF-1) in the thymus could be one of the ways to maintain immune homeostasis during postnatal development and aging (2). The tissue availability of IGF-1 is controlled by pregnancy-associated plasma protein A (PAPPA). Homozygous deletion of this protein in mice leads to their lifespan extension. More significantly, **PAPPA deletion results in the resistance to age-dependent atrophy of the thymus, and to the maintenance of diverse, highly functional T cells in extended old age mice.**

Caloric restriction increases the thymocyte density in the core and medulla

Professor **Dixit's** research group from Louisiana State University compared T-cells from the 26-months-old caloric restricted mice and the control group. Researchers found out that the former proliferate better and express more interleukin-2. They came to a conclusion that **reducing proadipogenic signaling in thymus via CR may promote thymopoiesis during aging** (3).

Zinc diet supplementation can promote thymopoiesis

Compromised zinc status associated with aging may be an important contributing factor in reduced thymopoiesis and impaired immune functions. **Zinc supplementation improves thymopoiesis, which is proved by increased total thymocyte numbers** (4). In the study of Professor **Ho** from the University of Oregon improved thymic output was mediated in part by reducing the age-related accumulation of immature CD4(-)CD8(-)CD44(+)/CD25(-) thymocytes, as well as by decreasing the expression of stem cell factor, a thymosuppressive cytokine.

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Hormones involved in some manifestations of aging are growth hormone, insulin-like growth factor-1 (IGF-1), melatonin, dehydroepiandrosterone (DHEA), sex hormones and thyroid hormones.

Hormone replacement therapy may prevent or delay some aspects of aging.

HORMONAL REGULATION AND HORMONE REPLACEMENT THERAPY (ESTROGEN, GROWTH HORMONE, IGF-1)

Changes in the levels of growth hormone, insulin-like growth factor-1 (IGF-1), melatonin, dehydroepiandrosterone (DHEA), sex hormones and thyroid hormones are associated with changes in body composition, visceral obesity, muscle weakness, osteoporosis, urinary incontinence, loss of cognitive functioning, reduction in well being, depression, and sexual dysfunction. Hormone replacement therapy may alleviate the debilitating conditions of secondary partial endocrine deficiencies by preventing or delaying some aspects of aging **(1)**.

Estrogen therapy lowers mortality in older women

Hormonal deficiency during menopause may alter immune response, but estrogen therapy can alleviate this decline, because estrogens are likely to play an important role in the immune function. As shows the 22-year longitudinal study named the Leisure World Cohort Study long-term **estrogen therapy is associated with lower all-cause mortality in older women (2)**.

Growth hormone replacement therapy improves immunity and slows down aging

Growth hormone is not only responsible for growth in the childhood, but also remains important for the adult metabolism. Growth hormone secretion decreases with age, which is a marker of aging, therefore many age-related changes are the features of AGHD (Adult Growth Hormone Deficiency).

Age-related changes in the communication between the neuroendocrine and the immune system have been scarcely studied.

Aging in mammals is associated with an impairment of the immune response, especially regarding lymphocyte functions.

Furthermore, the endocrine system is also affected by aging, one of the most significant changes being the decrease in the secretion of several hormones such as growth hormone.

Scientists from **Monica de la Fuente's** group from the Complutense University of Madrid **(3)** revealed that the **administration of growth hormone can reduce or even reverse the age-related changes** observed in such key immune function parameters, as chemotaxis, lymphoproliferative response to the mitogen concanavalin A, interleukin-2 release and natural killer cell activity.

Therefore, growth hormone replacement therapy can delay or slow down some aspects of the aging process by increasing the immune function.



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Publications:

- 1** Fukai et al., "Hormone replacement therapy-growth hormone, melatonin, DHEA and sex hormones", *Nippon rinsho*, 2009.
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Aging is part of a developmental genetic program that begins at birth and ends at death **(1)**. The aging process not only involves pathways of deterioration, but also mechanisms of repair and remodeling including the induction of novel physiologic pathways **(2)**. Unraveling mechanisms of tissue remodeling with advancing age, and the identification of genetic factors linked to longevity are critical to the development of alternative strategies to help maintain physiological homeostasis and promote healthy aging.

TARGETING BIOCHEMICAL AND GENETIC PATHWAYS OF EXCEPTIONAL AGING

T cell repertoire remodeling

Older adults aged 65 years and up exhibit wide heterogeneity of health phenotypes, ranging from the very frail with poor vaccine responses, to those who are functionally unimpaired with fairly good vaccine responses. Many older adults are exceptionally aging well beyond the median life spans of many human populations.

Since protective immunity is a determinant of fitness, exceptional aging is likely to be due to mechanism(s) of immunological homeostasis that is distinct to old age. Indeed, whereas T cell-mediated immunity is classically elicited only when the clonotypic T cell receptor (TCR) recognizes specific antigen; clonal lineages of T cells in old age acquire a variety of receptors normally expressed on natural killer (NK) cells **(3)**.

Expression of NK-related receptors on T cells of older adults suggest that T cells of the aged are functionally distinct from the clonotypic T cells of younger people. Recent studies by Professor **de Vallejo** show that these unusual NK-like T cells are functionally versatile that can be activated in either TCR-independent or TCR-dependent manner **(4)**.

Longevity Genes

Longevity and exceptional aging tend to be familial. In recent years, studies in both humans and animals, have shown particular genes are linked to longevity.

Work by Dr. **Willcox** show that a polymorphism of FOXO 3A gene is strongly associated with human longevity **(5)**, a finding that has now been recapitulated in at least three populations.

FOXO 3A is a genetic component of the insulin and insulin-like growth factor signaling system (IIS), deletion of various IIS molecules in invertebrates have been shown to increase lifespan.

Identification of genes linked to longevity is vital to proper design of clinical interventions to improve health care of the oldest old **(6)**.

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Development and perfection of organ growing methods can fix the problem of lacking donor organs for transplantation.

Today, the best known methods are growing of organs on a foreign “skeleton” using patients’ own cells and bioprinting.

REGENERATIVE MEDICINE: GROWING ORGANS

Transplantation of trachea grown on patient’s stem cells

In Spain **the first operation was performed where a trachea cleared from donor cell antigens was transplanted to a human (1).**

At the first stage the scientists from the University of Padua (Italy) used a 7 centimeter piece of trachea to produce a tissue skeleton consisting of collagen. At the same time the cell culture was prepared that was grown on the patient’s bronchial epithelium.

To obtain chondrocytes – cartilaginous cells – the specialists from the University of Bristol used stem cells from the patient’s bone marrow.

At the next stage the prepared connective-tissue skeleton was lined with bronchial epithelium cells and covered with cartilaginous cells, which was followed by placing of the organ to the bioreactor in the University of Milan. 96 hours later the epithelial cells and chondrocytes became “fused” with the skeleton, and in the course of the operation led by **Paolo Macchiarini** from the Hospital Clinic of Barcelona the patient’s left primary bronchus was removed and replaced with the transplant.

The left lung began functioning at once and continues to do so without using immunosuppressants.

Growing urinary bladder

Doctor **Anthony Atala** and his colleagues from the Wake Forest Institute for Regenerative Medicine **were the first to grow an organ in the laboratory, an engineered urinary bladder (2).** The scientists worked out a method of progenitor cell isolation and subsequent growing of separate layers where either muscle or epithelial cells prevailed. Then separate layers were combined. The artificially grown implant was attached to patients’ urinary bladders starting in 1998. During several years following operation the patients were observed to have gradual improvement in health.

At present the scientists are working on the method that would enable growing of 22 tissue types, including blood vessels, kidney and trachea cells.

Bioprinting of blood vessels

Professor **Gabor Forgacs** and his laboratory in the University of Missouri (Organ Printing project) **produced blood vessels and nerve grafts as well as pieces of heart tissue using a 3D bioprinter** filled with “ink” saturated with cells (3).

This printer builds the desired “construction” layer by layer according to the instructions of a computer. In all other experiments aimed at growing even a simple heart patch it is necessary to make an “assembly skeleton” which would define the form of the future organ or transplant. The advantage of the new method is that such a skeleton is not needed at all – the form of a vessel, piece of liver or heart muscle is defined by the computer program controlling the printer.



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Publications:

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The current understanding of the mechanisms of aging allows us to start its pharmacologic suppression in humans, using drugs clinically approved for other diseases.

It is also essential to determine minimal effective concentrations and rational combinations of age-suppressive drugs.

Rapamycin, metformine, MEK inhibitors, resveratrol and some other agents can be referred to as such. Simultaneously, it is necessary to search for new aging suppressant drugs.

PHARMACOLOGIC SUPPRESSION OF AGING AND AGE-RELATED DISEASES

Inhibition of mTOR pathway, as well as activation of sirtuins, FOXO, PTEN and AMPK extends life span in diverse species from yeast to mammals. And the aging-suppressive effect of caloric restriction can in part be explained by inhibiting the same TOR signaling pathway. We have shown that rapamycin and U0126, inhibitors of mTOR and MEK, also suppress cellular senescence.

Fight against age-related diseases

As shown in a number of pre-clinical trials rapamycin (its target is TOR) is recommended for the therapy of almost all age-related diseases. That proves this drug has a geroprotective effect. Also metformin, an anti-diabetic drug, which acts in the TOR pathway, retard aging and extend lifespan in mice. Modulators of sirtuins and TOR have been discovered that mimic calorie restriction and mitigate certain age-related diseases.

Clinical trials of anti-aging drugs

Clinical trials of rapamycin are planned at Roswell Park, USA, as a drug for preventing breast cancer, colon polyps and also planned

is prophylactic prevention of cancer by low doses of rapamycin.

Rapamycin has preclinical indication for most age-related diseases (such as atherosclerosis, age-related macular degeneration, neurodegeneration). Phase II clinical trials could be started immediately on humans.

Search for rational and minimal effective concentrations of geroprotectors and their combinations

It is crucial to carry out investigation of effects of rapamycin and other known anti-aging drugs and also anti-cancer drugs influencing the cancer and aging signaling pathways on normal cells of different tissues (fibroblasts, smooth-muscle cells, endothelial cells, adipocytes, neurocytes, glia, beta-cells, retinal cell line), as well as in fast-aging mice and cancer-prone mice.

Screen for new age-suppressive drugs

In order to identify new age-suppressant drugs it is necessary to carry out a **screening among natural products and among existing drugs (short term clinical implications) and also chemical libraries (medium term clinical implications)**. A separate task – development of drugs that will selectively eliminate senescent fibroblasts, fat cells and glial cells.

Therefore, a screen of libraries of chemical compounds in arrested non-senescent fibroblasts versus terminally senescent fibroblasts is needed. After sorting the agents that selectively kill terminally-senescent fibroblasts their testing is needed.



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Publications:

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Deletions or mutations of various genes in different species – from worms to mice – may slow down aging and extend lifespan. Some of these genes can be put together in a unified TOR (target of rapamycin) pathway. Inhibition of this pathway might become an effective way to retard aging.

TOR METABOLIC PATHWAY INACTIVATION VIA RAPAMYCIN

TOR metabolic pathway is activated by nutrients, growth factors and insulin and it promotes protein synthesis and cell growth. Rapamycin can be a good inhibitor of TOR and slow down aging.

Rapamycin slows down cellular aging

Rapamycin is shown to decelerate the loss of proliferative potential (and thus, aging) of the cell in culture (1). It is important that rapamycin does not force the cell into proliferation when its cell cycle is arrested by p21 and rapamycin also does not remove the cycle arrest, but only maintains the proliferative capacity.

The mechanism of cellular senescence inhibition by rapamycin is in principle different from the already known mechanisms of “reversing aging”. Until now reversing was based upon genetic manipulation letting the cell “avoid”, escape the cell cycle arrest.

But cell cycle activation is known to cause cancer without suppressing aging.

On the contrary, **cell growth inhibitors like rapamycin suppress aging processes without making the cell proliferate.**

Rapamycin extends lifespan in mice

Inhibition of the TOR signalling pathway by genetic or pharmacological intervention extends lifespan in invertebrates, including yeast, nematodes and fruitflies.

However, whether inhibition of mTOR signalling can extend lifespan in a mammalian species was unknown.

The authors of the article in Nature showed that rapamycin, an inhibitor of the mTOR pathway, extends median and maximal lifespan of both male and female mice when fed beginning at 600 days of age (2).

Rapamycin led to lifespan increase of 14% for females and 9% for males. The effect was observed in three independent experiments in different laboratories (**David Harisson**, Jackson Lab, **Randy Strong**, University of Texas, **Richard Miller**, University of Michigan). In a separate study, rapamycin fed to mice beginning at 270 days of age also increased survival in both males and females.

These results were based on an interim analysis conducted near the median survival point. **Rapamycin may extend lifespan by postponing death from cancer, by retarding mechanisms of aging, or both.**

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Investigations of existing compounds and drugs intended for treatment of various diseases sometimes reveal their geroprotective properties. We already know that a number of nootropics, anti-diabetics and hormones have aging-suppressant properties. It is therefore important to conduct large-scale screening of a sufficiently large range of potential geroprotectors – both individually and in various combinations and concentrations.

STUDYING GEROPROTECTIVE PROPERTIES OF MELATONINE, METFORMIN AND PEPTIDES

Melatonin: regulation of biorhythms

It is shown that in laboratory rodents while keeping in conditions of constant light the pineal gland function is suppressed, resulting in reduction of melatonin secretion, development of anovulation and increase in tumor frequency.

Women living in high latitudes with usually broken light regime (“white nights”, “polar night”), also frequently demonstrate development of anovulatory syndrome, mastopathy and breast cancer. **The role of circadian production of pineal hormone melatonin in these processes is poorly studied.**

The potential connection between light treatment and melatonin with oncogenes expression responsible for the development of breast cancer and clock genes expression controlling circadian rhythms is also not sufficiently studied. Currently some clinical observations suggest that the light regime can affect the development of tumors in humans. The ideas about the important role of free radicals in the molecular mechanisms of aging and carcinogenesis have been widely developing in the last decades. Therefore **it seems to be very interesting to study the levels of free radical processes and antioxidant system state under different light regimes.**

Metformin: reducing the risk of cancer

In clinical observations it was found that the **use of metformin and other biguanides reduces by more than a third total mortality, mortality from heart attacks and complications from diabetes, improves cancer patients’ survival and reduces the risk of breast cancer in patients with type 2 diabetes.**

In experiments on laboratory rodents the geroprotective effect of antidiabetic drugs was revealed. This effect was associated with lower incidence of spontaneous tumors. In various models of chemical and radiation carcinogenesis it was found that biguanides retard the development of induced tumors and inhibit the growth of a number of transplanted tumors **(3).**

Peptides: induction of protein synthesis

Peptides isolated from organs of young animals when injected into the organism are able to induce protein synthesis, contributing to the restoration of basic functions that have been violated in the process of aging.

It was shown that prolonged use of peptides in animals **leads to a significant increase of life expectancy by 20–40% and to the attainment of specific limit of life expectancy.** It was also found that short peptides (di-, tri- and tetrapeptide) can complementarily interact with specific DNA binding sites in the promoter area of genes, leading to the separation of the chains of the double helix and to the activation of RNA polymerase **(5).**



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Publications:

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In young organisms the rate of glycation (reaction between proteins and sugar molecules) is so low that its products are removed from cells. However, modified proteins tend to accumulate with age, which leads to age-related changes in cells and tissues – changed structure, deteriorated vital functions. That is why it is so important to develop drugs for slowing down the process of protein glycation.

DEVELOPMENT OF DRUGS ABLE TO SLOW DOWN PROTEIN GLYCATION

Negative factors like free radicals, nonenzymatic glycosylation, chemical and physical environmental factors cause specific changes in the structure of macromolecules. Sugar molecules binding to such macromolecules results in formation of massive cross-coupling bonds – glycosylation products. Their amount in an organism increases with age.

Possible ways of intervention into this process:

- 1) prevention of glycation end products formation, chemical bonding of glucose and its neutralization;
- 2) prevention of cross-linkage formation between glycated proteins and adjacent protein molecules;
- 3) facilitation of proteolysis stimulating glycated protein breakdown.

Pharmacological breakdown of protein cross-linkage

A group of scientists headed by Doctor **Josephine Forbes** is studying complications of glycation and diabetes in Baker Medical Research Institute in Melbourne, Australia. They are searching for ways to create pharmacological drugs that will enable separation of protein and sugar molecules for breaking down cross-links.

Creation of such drugs will reduce the amount of glycation end products in tissues (1). It is possible that, **ultimately, proteins will be able to restore and return to their normal state**, which will revoke the negative effects of glycation.

Genetic regulation of glycation end product receptor prevents formation of harmful protein complexes

High levels of oxidative stress and inflammation are associated with cardio-vascular diseases and are connected with glycation end products. They interact with various receptors including AGER1.

Helen Vlassara from Mount Sinai School of Medicine (New York) showed in her research that overexpression of AGER1 receptor stimulates removal of glycation products and blocks oxidative stress and inflammation.

This results in **increased body defense against arterial hyperplasia and inflammation caused by injury (2)**. This is what makes it so important to find ways to control the expression of this receptor.

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Studies of geroprotective properties of carnosine will clarify the effects of free radicals on the regulation of gene expression involved in longevity control, and establish the fundamental genome patterns implemented in the processes of aging and death. Application of SkQ is the best mechanism of preventing the generation of mitochondrial ROS (reactive oxygen species).

PREVENTING THE EFFECTS OF OXIDATIVE STRESS

Studying the changes in stress resistance genes expression under the influence of carnosine

It is shown that carnosine can repair (rejuvenate) cells, contributing to certain genes activity in fibroblast culture (1). However, such studies have never been conducted on the organism level.

Recently, it was shown that carnosine is able to increase longevity and the quality of life of senescence-accelerated mice, and also to hinder the development of senile traits in OXIS rats, in which the processes of aging are determined by the activation of oxidative stress.

Carnosine was also shown to be an effective factor in lifespan increasing of the fruit fly *D. melanogaster* (2). Now scientists have to determine which genes' activation (or suppression) contribute to this effect. In addition, it is necessary **to compare the geroprotective effectiveness of carnosine and its derivatives and to establish their primary gene targets.**

The obtained data will clarify the effects of free radicals on gene expression regulation involved in longevity control, and to establish the fundamental genome patterns implemented in the processes of aging and death.

Studying the effects of SkQ1 – antioxidant-dissociator found in mitochondria

SkQ1 is an effective antioxidant. Its goal It removes the **excess of mitochondrial reactive oxygen species appearing in the process of aging.** In the heart mitochondria *in vitro* it was shown that SkQ1 can recover the respiratory chain, i.e. to serve as a renewable antioxidant of repeated action. Its properties are studied under the guidance of Academician RAS **Vladimir Skulachev** involving members of the Institute of Physical and Chemical Biology, and a number of Moscow State University faculties, as well as more than 30 research institutes in Russia, Sweden, USA and Ukraine.

The results showed that SkQ1 is able to inhibit apoptosis in HeLa cells and human fibroblasts induced by H₂O₂, to increase the median life expectancy in fungi *Podospora anserina*, crustaceans *Ceriodaphnia affinis*, *Drosophila* and mice – up to two times.

In mice and rats it was demonstrated that SkQ1 decelerates development of such features of aging as the involution of the thymus and spleen follicular cells, reduction the lymphocytes-neutrophils ratio in the blood, as well as osteoporosis, cataracts, retinopathy, glaucoma, baldness, graying, loss of estral cycles in females and sex attraction in males, hypothermia etc.

The researchers concluded that **this antioxidant reduces mortality in the early and middle stages of aging and prevents the emergence of a large group of senile defects (3).**



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Publications:

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Caloric restriction (reducing the food intake by 30-40% without malnutrition) is proven to delay aging and extend lifespan in yeast, worms, mice, rats and primates.

Can caloric restriction extend human lifespan? Long-term research is needed to answer this question.

CALORIC RESTRICTION PROLONGS LIFE

Caloric restricted and methionine restricted diets

Lifespan can be extended in rodents by restricting calories or by providing food low in methionine.

It is shown by the group of **James Harper** that a period of food restriction limited to the first 20 days of life, via a 50% enlargement of litter size, shows extended median and maximal life span relative to mice from normal sized litters.

Moreover, methionine restricted diet initiated at 12 months of age also significantly increases longevity **(1)**.

Furthermore, mice exposed to a caloric restricted diet show changes in liver messenger *RNA* patterns, in phosphorylation of *Erk*, *Jnk2*, and *p38 kinases*, and in phosphorylation of mammalian target of rapamycin and its substrate *4EBP1*. Such changes were not observed in liver from age-matched methionine restricted mice. These results introduce new protocols that can increase maximal life span.

Possibly, **the spectrum of metabolic changes induced by low-calorie and low-methionine diets may become a further research and application agenda.**

Caloric restriction extends lifespan in primates

The findings of a 20-year longitudinal adult-onset caloric restriction study in rhesus monkeys show that **low-calorie diet prolongs life in primates (2)**.

In a population of rhesus macaques maintained at the Wisconsin National Primate Research Center, moderate caloric restriction lowered the incidence of aging-related deaths. At the time point reported, 50% of control fed animals survived as compared with 80% of the caloric restricted animals. Furthermore, caloric restriction delayed the onset of age-associated pathologies. Specifically, caloric restriction reduced the incidence of diabetes, cancer, cardiovascular disease, and brain atrophy.

Such interventions, as caloric restriction need long-term studies in humans. Moreover, in order to achieve some results caloric restriction will be needed in younger adults those who do not have the symptoms of aging.

But such investigations are likely to have some short-term beneficial effects if people with age-related diseases would be involved in these experiments.

Publications:

- 1 Sun et al., «Life-span extension in mice by preweaning food restriction and by methionine restriction in middle age», *the Journals of Gerontology*, 2009
- 2 Colman et.al., «Caloric restriction delays disease onset and mortality in rhesus monkeys», *Science*, 2009



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Mathematical modeling is used for working out methods to increase longevity based on system analysis of aging mechanisms and ways of their regulation (1). One of the insufficiently studied methods is the determination of optimal synergies – results of organism exposure to fixed-dose combinations of various factors, both negative and positive.

MATHEMATICAL MODELING OF OPTIMAL SYNERGIES FOR LONGEVITY

Today, mathematical modeling is very promising for searching new approaches to increase longevity.

Modeling oxidative stress

The oxidative stress theory connects the rate of organism aging with the rate of oxygen consumption and production of ROS (reactive oxygen species damaging cells). The accumulating oxidative damage impairs the homeostatic ability of the organism. The oxygen effective level in mitochondria drops. Based on the oxidative stress theory **it is possible to develop a general scheme of organism aging and lifespan.**

Working out of human organism nominal model

It is necessary to give special attention to the poorly studied synergy effect in the aging processes of certain organism systems. Aging of each physiological system results in reduction of a certain resource in the model. The combined aging of systems leads to the synergetic effect, and the organism dies considerably earlier. **To increase longevity it is necessary to work out methods for neutralization of negative synergies effect.**

Modeling external factors

The task is to model all known factors affecting the process of normal aging (genetic factors, environmental factors and lifestyle) and study the influence of each of them and their combinations on an organism. A promising trend in studying of synergies effect on lifespan **is studying mortality in organisms with several diseases (combined morbidity) (2).** Sometimes one disease weakens the negative effect of the other. For example, some autoimmune diseases (such as asthma) reduce the probability of death from cancer in aged organisms.

Optimal choice of environment and lifestyle

Application of present-day approaches to modeling of aging and lifespan will enable **quantitative estimation of synergetic effects** and, in particular, the role of such factors as age-associated exacerbation of injuries received earlier, aggravations of various diseases or manifestation of autoimmune diseases.

Publications:

- 1 Novoselstev V., Michalski A. «Mathematical modeling and aging: research program». *Advances of Gerontology*, 2009.
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- 4 Novoselstev V. «Mathematical modeling in Biology: systems capable of living and dying». «Automation and Telemechanics», 2006.



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The naked mole rat (*Heterocephalus glaber*) is a small rodent living in dry savannas and semideserts of Kenya, Ethiopia and Somalia. These animals are insensitive to pain and acids, resistant to increased CO₂ concentrations, and never have cancer or atherosclerosis. What is the most important is that naked mole rats live almost 10 times longer than similar species and don't display age-associated changes for a long period of time. The task facing scientists is to understand the reasons for these unique qualities.

STUDYING NAKED MOLE RAT – ANIMAL WITH NEGLIGIBLE SENESENCE

The body composition of naked mole rats remains practically unchanged from 2 to 24 years; females show no decline in fertility even when well into their third decade of life. The mortality rate in these animals doesn't increase with age. Obviously, physiological and biochemical processes in naked mole-rats evolved to dramatically extend their healthy lifespan (1).

Studying cell resistance

Roshelle Buffenstein from the University of Texas found that **fibroblasts (connective tissue cells) of naked mole rats are resistant to cadmium, methyl-methane sulphonate, paraquat, heat and low-glucose medium**. However, the sensitivity of cells to endoplasmic reticulum stress indicates that the changes resulting from denaturation can affect cell survival and aging rate (2).

Thomas Parc from the University of Illinois, Chicago, and several colleagues from the USA and Germany published a work in the peer-reviewed open-access journal PLoS Biology demonstrating that **the skin of naked mole rats is resistant to acid and capsaicin** (a component of chili peppers), as well as to high temperatures (3).

Naked mole rats lost reactions to these irritants during evolution. At the same time they perceive environment temperature, have tactile sensitivity and feel pain when pricked or pinched. The resistance of cells to stress might be one of the key reasons for disease resistance and long lifespan of these animals.

Studying cell hypersensitivity

Vera Gorbunova and **Andrey Seluanov** from the University of Rochester found the fibroblasts of naked mole rats to be hypersensitive to contact inhibition. Contact inhibition is a process of arresting cell growth when two or more cells come into contact with each other.

Contact inhibition is also an important anti-cancer mechanism.

Scientists suggest that the hypersensitivity of naked mole rat cells is what makes them resistant to tumors. It is planned to study hypersensitivity to contact inhibition and understand the ways to control it.

Publications:

- 1 Buffenstein R, «Negligible senescence in the longest living rodent, the naked mole-rat: insights from a successfully aging species», *the Journal of Comparative Physiology*, 2008
- 2 Salmon et.al. "Fibroblasts from naked mole-rats are resistant to multiple forms of cell injury, but sensitive to peroxide, ultraviolet light, and endoplasmic reticulum stress», *The Journals of Gerontology*, 2008
- 3 Park et.al., «Selective inflammatory pain insensitivity in the African naked mole-rat (*Heterocephalus glaber*)», *Plos Biology*, 2008



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SCIENTIFIC IDEAS OF LIFE EXTENSION

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