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Molecular Signal Integration of Aging and Diabetes Mellitus

Irina Sarvilina

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Abstract

DM is considered as the cause of accelerated aging. Numerous biomedical studies have proved the key role of neuroimmune-endocrine interactions in the human body, which trigger the universal molecular pathways in the development of aging and DM (GH/IGF-1, Ras-MAPK, FOXO3A, sirtuin, mTOR, CETP, Timeless gene, TZAP pathways). Modern methods of proteomic and bioinformatic analysis allow us to investigate key genomic-proteomic interactions that underlie diabetic nephropathy (DN) in patients with type 2 DM. The study of the formation and development of DN can become the model for studying molecular pathways of aging of kidney tissue. Future biomedical research based on methods of high-throughput screening (HTS) of a pool of target molecules will lead to great advances in the diagnosis of aging stages and DM, as well as the development of methods for the prevention and therapy of accelerated aging of the human body and various violations of carbohydrate metabolism (1D-2D/MALDI-TOF-MS, HTS, biochips, biosensors).

Keywords: aging, diabetes mellitus, pathway, gene expression, proteomics

1. Introduction

The latest data show that the prevalence of diabetes mellitus (DM) in the world has increased more than in two times, peaking at 415 million by the end of 2015 [1]. In accordance with the current evaluation of the International Diabetes Federation, 642 million patients will be with DM by 2040 [2]. Increased incidence of DM caused the adoption of the United Nations (UN) resolution 61/225 dated 20 December 2006 about DM. In the 2011, political declaration was adopted by the UN to the national healthcare systems to create a multidisciplinary strategy in the area of prevention and control of noninfectious diseases, where particular attention is drawn to the problem of DM as one of the leading causes of disability and mortality [3].

There is significant increase of the prevalence of DM in the Russian Federation. According to the Federal Register of Diabetes Mellitus, at the end of 2016, 4.35 million outpatients with DM (3% of the total population) were registered in Russia, of whom 92% (4 million people) had type 2 DM, 6% (255,000 people) - type 1 DM and 2% (75,000 people) -other types of DM. Actual number of patients with DM remains underestimated, since only identified and reported cases are considered. The results of the large-scale Russian epidemiological study NATION confirmed that only 50% of type 2 DM cases are diagnosed. Actual number of patients with DM can be at least 8-9 million people (about 6% of the population) in Russia [4]. Because of the lack of timely diagnostics of DM, some patients do not receive necessary therapy and have higher risk of the developing of such complications of DM as retinopathy, nephropathy, ischemic heart disease, cerebral ischemia, peripheral angiopathy. These complications are responsible for most cases of disability and mortality of DM.

Today, DM is considered as the cause of accelerated aging [5]. Twenty percent of middle-aged people and 35% of the population of older persons are characterized by varying degrees of impaired glucose tolerance (IGT) and symptoms of insulin resistance. An increase in the frequency of obesity and sedentary lifestyle and the major risk factors for type 2 DM suggests that the prevalence of DM in the world will increase. The management of this disease becomes difficult for persons aged 60, 70 and 80 years. The risk of complications, such as ischemic heart disease, increases with age, as well as damage with age of organs of vision, hearing and physical activity, can amplify in the presence of DM.

The modern stage of the development of researches in the field of DM and aging is interrelated and involves the use of unified technological platforms for molecular diagnostics and pharmacology of stages of aging and DM. Unified technological platforms presuppose the performing of comparative genomic and proteomic studies, the results of which allow to study interrelated pathogenesis of aging and DM. Also new technological platforms are necessary for the development of new prophylaxis and treatment of these interconnected pathological states. The analysis of data from comparative genomic and proteomic studies allows the formation of unified molecular pathological pathways of DM and aging. The chapter presents new technological platforms for the early identification and the development of anti-aging and anti-diabetic agents.

2. Neuroimmune and neuroendocrine communications: aging, metabolic syndrome and diabetes mellitus

Aging is a universal factor for metabolic and immune disorders in humans and related diseases, including DM [6]. So, analysis of the reciprocal effect of neuroendocrine factors and immunological processes at the system level, human organs and tissues is very important. It is the basis for the development of processes of aging and the emergence of DM formation of mechanisms at cellular and molecular levels.

The maintenance of the homeostasis of nervous and immune systems is carried out by comparable number of cellular elements. The integration of nervous and immune systems is due

to the presence of neuronal processes, receptors and neurotransmitters in the nervous system, as well as the presence of highly mobile cell elements and cytokines in the immune system [7]. The search of opportunities of the influence to immune processes through the central nervous system in the order to prevent of aging and metabolic disorders is based on fundamental laws of hierarchical organization of regulatory system, the presence of humoral signals in cell populations, the points of application of the effect in tissues and organs. The information in the nervous system is encoded in the sequence of electrical impulses and in the architecture of neuronal interactions, in the immune system information is stored in stereochemical configuration of molecules and receptors involved in lymphocyte interactions. There was evidence of a common receptor apparatus in the immune system to neuromediators and nervous system to endogenous immunomodulators. Immunological active neuroendocrine substances - thymosin, triiodothyronine (T3) and thyroxine (T4), protimosin, endogenous regulator of protimosin, parathymosin, oxytocin, Th-I antigen and vasoactive intestinal peptide have been found both in the brain and in the thymus, they play significant role in the aging of human immune system [8]. The greatest number of studies are devoted to the participation of interleukin 1 (IL-1), in immunoregulation at the level of immunocompetent cells and in regulation of functions. Interleukin 2 (IL-2) also exerts various effects on the immune and nervous systems mediated by affinity binding to the corresponding cell surface receptors. The activating effect of IL-2 on lymphocytes and macrophages is manifested in the enhancement of the antibody-dependent cytotoxicity of these cells with parallel stimulation of the secretion of tumor necrosis factor α (TNF-α). IL-2 induces proliferation and differentiation of oligodendrocytes, affects the reactivity of the hypothalamus neurons and increases the level of adrenocorticotropic hormone (ACTH) and cortisol in the blood, which together form a stable mechanism of neuroimmune and neuroendocrine network interactions. Cells that are targets for the action of IL-2 are T-lymphocytes, natural killers (NK), and macrophages. IL-2 causes the functional activation of these cell types and the secretion of other cytokines, for example, increases the production of NK cells by interferon γ (IFN-γ) [9]. There are data about the production of nervous cells of IL-1, IL-6 and TNF-α, which are critical components in the development of chronic inflammation with destruction of β-cells of the pancreas in DM type 2 [10]. It is known that glucocorticoids (GCs), androgens, estrogens and progesterone suppress immune responses, and growth hormone (GH), T4 and insulin have a stimulating effect [11, 12]. Cells of the immune system transmit transmembrane signal to receptors for GCs, insulin, GH, estradiol, testosterone, beta-adrenergic agents, acetylcholine, endorphins and enkephalins [13]. All of above-mentioned hormonal factors are involved in the formation of metabolic and immunological changes in conditions of aging and DM. For example, the exogenous administration of contra-insular hormones T3 and T4 alters functional activity of the immune system. This action is realized through cytoplasmic and nuclear receptors in immune cells [14, 15]. The theory of aging suggests that life expectancy has negative relationship with metabolic rate, which is regulated by hormones of energy metabolism. Experimental hypothyroidism increased life expectancy in young rats, whereas hyperthyroidism shortened life expectancy. Several mutant mice in long life experiment had reduced or almost absent thyroid function [16–18]. Hypothyroidism can affect life expectancy by reducing the intensity of metabolism, body temperature and oxygen consumption, resulting in a decrease in the generation of free oxygen radicals and associated oxidative damage in cells. Subclinical hypothyroidism is associated with a reduction

in mortality in women, which was found in families with long life expectancy and is due to polymorphism of the receptor to thyroid stimulating hormone (TSH) [19]. An important fact is that subclinical hypothyroidism is often recorded with type 2 DM [20].

Most of the data indicate the role of insulin as one of the growth factors that support the readiness of lymphoid cells to realize the response to an antigen. The stimulating effect of this hormone is manifested mainly in conditions of the pathology of the immune system with DM. Proliferative activity of lymphoid cells is reduced in patients with insufficient insulin production; first of all, functions of T cells suffer [21]. Antagonistic pleiotropic hypothesis of aging suggests that some pathological pathways that are evolutionarily necessary for the development of the human body and reproductive function become unfavorable with aging of the human body. For example, the increase of the ratio of GH / insulin-like growth factor-1 (IGF-1) is necessary for the growth and maturation during puberty. GH secretion decreases with age, resulting in a corresponding decrease in IGF-1 concentration. Low levels of IGF-1 in the human body are associated with an increased risk of developing type 2 DM [22].

GCs are the most studied and effective participants in pathological changes in neuroimmunoneuroendocrine network interactions that occur in patients with DM. Genes that are targets of GCs are responsible for the synthesis of protein molecules involved in virtually all parts of immunological process in DM [23]. GCs inhibit the synthesis of IL-1, TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-3, 4, 5, 6, 8 and reduce the induction of NO synthase, which leads to the decrease of NO synthesis and pro-inflammatory effect through cyclooxygenase, phospholipase A2, endothelin-1, involved in cardiovascular remodeling in aging and DM [24, 25]. On the other hand, GCs enhance the synthesis of proteins that have an anti-inflammatory effect, including the synthesis of lipocortin-1, which inhibits the activity of phospholipase A2 and the production of leukotrienes (C4, D4, E4), as well as prostaglandin E2 and leukotriene B4 [26]. GCs are inducer of type II receptors for IL-1, which has an anti-inflammatory effect. GCs inhibit the enhanced TNF-α transcription of the IL-8 gene [27]. Feedback in neuroendocrine interaction is carried out by cells that originate from lymphocytes through the hypothalamic-pituitary-adrenal system.

So, the nervous, immune and endocrine systems fulfill their specific functions with the help of identical mechanisms and are interrelated.

Insulin resistance is the main component of the metabolic syndrome and is very often found in elderly patients. Abdominal obesity, which is often found with human aging, is the main cause of insulin resistance and metabolic syndrome [28]. Aging is also associated with an increase in the level of proinflammatory cytokines that interact with insulin. Cytokines are isolated from adipose tissue, and cytokine synthesis increases with age, it is associated with aging. It has been shown that the synthesis of cytokines increases by aging cells [29]. Glucostatic theory was formulated by J. Mayer, who described a feedback system that maintains the level of glycemia. In accordance with this theory, the hypothalamus controls the absorption of nutrients through receptors that respond to changes in glycemia [30].

The interaction of metabolic disorders and the distribution of adipose tissue in the human body constitute links in the vicious circle that can accelerate the aging process and the onset

of DM development. Lipostatic theory postulates the existence of a feedback mechanism between the amount of fat stored by the body, nutritional behavior and fat burning.

The theory predicts the presence of chemical signal produced in adipose tissue, which controls food behavior, physical and metabolic activity [31]. In 1994, leptin was discovered, that is, produced by adipocytes, moves with blood to the brain and acts on the hypothalamic receptors, suppressing the appetite [32]. Decreased leptin concentration leads to the development of obesity and is considered as one of the factors of the pathogenesis of type 2 DM. There is an increased level of cortisol, heat release, the restriction of growth, the lack of reproductive function, unlimited appetite and insulin resistance in mice with the ob/ob genotype. Leptin receptors belong to the family of cytokine receptors of class 1 and are present in the hypothalamus, fatty tissue, liver, skeletal muscles, pancreas, ovaries, prostate, placenta, kidneys and lungs. Leptin reaches the arcuate nuclei of the hypothalamus, interacts with its receptors in centers of hunger and satiety and reduces appetite. The binding of leptin activates the release of adrenaline, which increases the level of cAMP and the activity of protein kinase A through the adrenergic α-3 receptors, and triggers the synthesis of thermogenin, which converts mitochondria of adipocytes into unconjugated state [33].

In the arcuate nuclei of the hypothalamus, energy consumption is controlled by two types of neurons: orexigenic neurons stimulate the appetite by producing and releasing neuropeptide Y (NPY), which acts on the next neuron sending the brain a signal to eat. The concentration of NPY in the blood rises during fasting. It is the high level of peptide NPY that causes obesity in mice od/od and db/db [34]. Anorexigenic neurons of arcuate nuclei of the hypothalamus produce α-melanocyte-stimulating hormone (α-MSH). The release of α-MSH results in the next neuron sending a signal to the brain to stop eating. Mutations in the melanocortin receptor, which is expressed in the brain cells and plays a role in the regulation of appetite, lead to the appearance of obesity and type 2 DM [35]. Insulin acts on the hypothalamic receptors, suppressing appetite by inhibiting the release of NPY by the orexigenic neurons and also stimulating the production of MCH by anorexigenic receptors, reducing food intake and increasing thermogenesis. Leptin makes the liver and muscle cells more sensitive to insulin.

Adiponectin—a protein, consisting of 224 amino acids, is encoded by the ADIPOQ gene and secreted by adipocytes under the action of insulin. Adiponectin regulates energy homeostasis, has anti-inflammatory and anti-atherogenic effects. Its level decreases with obesity and is associated with glucose metabolism. Adiponectin increases the absorption of fatty acids by myocytes and the rate of β-oxidation of fatty acids in muscles, blocks the synthesis of fatty acids and gluconeogenesis in hepatocytes and stimulates the absorption and metabolism of glucose in muscles and the liver. These effects of adiponectin are provided by increasing the level of cAMP and activating cAMP-dependent protein kinase. A low level of adiponectin is characteristic for obesity, DM and cardiovascular diseases [36]. The similarity of adiponectin to TNF-α was found.

TNF-α is one of the key pro-inflammatory cytokines, secreted by macrophages and released by adipose tissue cells. One of the main targets of TNF-α is the adipocytes themselves, where it blocks the transcription of several genes and activates the expression of others. These effects

can lead to insulin resistance, chronic inflammation with systemic consequences for the body. Many genes transcribed by TNF-α are activated by the transcription factor in NF-κB adipose tissue cells.

The peroxisome proliferator-activated receptors (PPARs) alter gene expression, affecting the metabolism of fats and carbohydrates in response to changes in lipid levels in food. Ligands of these transcription factors are fatty acids and their derivatives. PRAPs act on the nucleus of the cell by forming heterodimers with another nuclear receptor—retinoid X receptor (RXR) that binds to regulatory regions of DNA. PPARs include genes necessary for β-oxidation of fatty acids and the formation of ketone bodies during fasting and stimulate the expression of genes encoding proteins that provide β-oxidation and dissipation of energy due to the formation of mismatched mitochondria. In mice with non-functioning receptor, leptin-activated PPAR-γ prevents the development of obesity by stimulating the synthesis of proteins involved in the cleavage of fatty acids and thermogenesis [37].

Ghrelin is a peptide hormone consisting of 28 amino acids produced by P/D1 cells of mucous membrane of the fundus of the stomach. Ghrelin receptors are expressed by neurons in the arcuate nucleus and ventromedial hypothalamus, here the processes associated with the action of ghrelin are mediated: stimulation of the production of releasing hormones, increased appetite, changes in the level of glucose and lipid metabolism, regulation of secretion and contractions of walls of the gastrointestinal tract [38]. It stimulates the release of GH. The concentration of ghrelin in the blood increases before eating and falls immediately after its intake. The concentration of ghrelin in the blood plasma increases with age, which contributes to weight gain in people as they age [39].

Consequently, numerous biomedical studies have proved the key role of neuroimmune-endocrine interactions in the human body, which trigger the universal molecular pathological pathways in the development of aging and DM.

3. The complex role of molecular pathways in the aging process and DM

Human body cells constantly receive signals from the body and the environment, causing processes such as damage, infection and stress. The modern field of research, called "epigenetics," explores how the environment and time affect the functioning of genes and the development, health and aging of humans. Some epigenetic changes are serious triggers for the development of DM or conditions that increase the risk of developing related DM to age. The response to internal and external signals from cells and the production of these signals occurs through biological pathways that are important for the development of aging and DM, including oxidative stress and/or cellular metabolism. Everyday millions of destructive events occur in the DNA structure, but in cells there are powerful mechanisms that protect DNA from damage, and these mechanisms remain active in old age.

Today, the goal is to sequester 100 genes in 1000 healthy elderly people, which can shed light on the inherited variability that underlies the protection of some people from aging diseases, including DM, enabling them to live a healthy life at their age. Sequencing allows

researchers to determine whether the presence of mutation carrier makes the elderly more secure or more vulnerable to the effects of damaging factors. Topol et al. perform study compared genetic sequencing in healthy volunteers, aged 80 years, and persons whose death has been linked to diseases associated with aging, including type 2 DM [40]. Scientists are finding that healthy people have an extremely low probability of genetic variations associated with the development of the disease. This fact proves the idea that protective genes play major role in the successful aging people. Therefore, the identification of molecular bases of protective effect would develop similar medicines, including effective means of preventing type 2 DM. Barzilai et al. sequenced several candidate genes of centenarians, including a variant gene that modifies the mechanism of cholesterol metabolism [41]. Scientists have sequenced genes of IGF1, its receptor and have identified mutations that are unique to women aged 100 years [42]. Calorie restriction increases life span and reduces age-related deterioration of work systems and physiological responses of age-related diseases, including with the development of DM. Restriction of caloric intake in animals in the experiment leads to a decrease in the level of glucose and insulin in the blood plasma and reduces inflammatory responses and the intensity of oxidative stress.

Genetic analysis identified several genes that affect life span and associated with damage to the pituitary development, the decrease of the secretion of GH, food intake and apoptosis. The work of these genes converges in the region of the IGF-1 receptor pathway and reproduces many effects of limiting calorie intake. Although *dwarf* mice having the defect in the synthesis of GH or the IGF-1 signaling pathway are also characterized by an increase of life expectancy, people with signaling defects associated with GH are prone to the development of diseases associated with aging. One of the targets of IGF-1, the signal pathway within the cell, is the repression of proteins responsible for stress resistance, including SOD and heat shock proteins, as well as a decrease in IGF signaling can increase life expectancy by increasing the expression of genes responsible for stress resistance. The mutation of the receptor to IGF-1, a phosphorylation target (p66 Shc), also increases the life span without affecting other organs and systems. When Shc is activated, the levels of intracellular oxygen radicals increase, suppressing the factor FKHRL1, which is involved in apoptosis (**Figure 1**).

Let us consider the scheme of molecular pathological pathway insulin/IGF-1 in humans, where mechanisms of aging and the appearance of type 2 DM are converging.

IGF-1 and IGF-1R provide the activity of proliferative signaling system that stimulates growth in many cell types and blocks apoptosis. In vivo, IGF-1 acts as an immediate response to effects of many growth factors and GH. One of the components of IGF-1, mitogenic signaling, is associated with the tyrosine kinase receptor via Shc, Grb2 and Sos-1, activating the RAS and MAP kinase cascade (raf, Mek, Erk). The end point of the MAP kinase pathway is the modification of the activity of transcription factors, such as the activation of ELK transcription factors. The serum response factor (SRF) and AP-1 provide mitogenic activity of many growth factors. IGF-1R signals for cellular survival and growth in response to IGF-1 and IGF-2. IGF-1R activates three signaling pathways that converge on the phosphorylation process of the BAD protein and block apoptosis. The first pathological pathway is activated by the
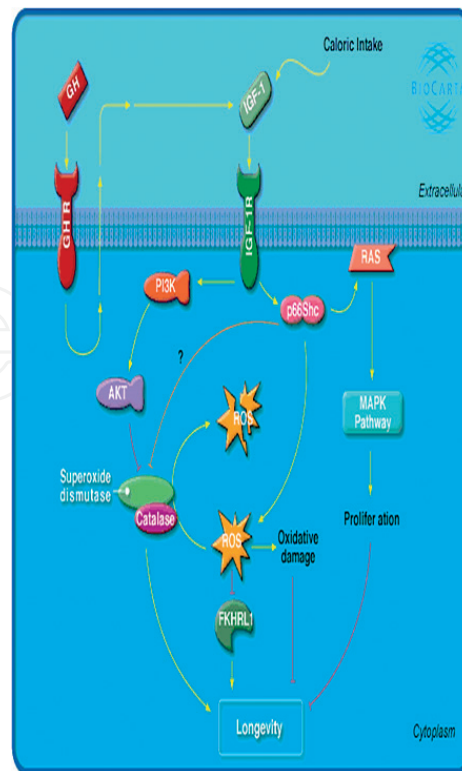
Figure 1. Scheme of participation of IGF-1-signaling in the regulation of life expectancy (database of pathways of BioCarta).

IGF-1R PI3 kinase, and the AKT signaling pathway phosphorylates BAD and blocks apoptosis. The second pathological pathway is activated by IGF-1R involving the Ras-Map-kinase pathway with the blockade of apoptosis. The third pathological pathway involves the interaction of Raf with mitochondria in the response to the activation of IGF-1R. The similarity of these pathological ways blocks apoptosis and increases the response to IGF-1R stimulation. The function of proapoptotic BAD molecule is regulated by the phosphorylation of three sites (ser 112, 136 and 155), which reduces the possibility of BAD heterodimerization by survival proteins of BCL-XL or BCL-2 cells. Phosphorylated BAA binds to 14-3-3 and is sequestered in the cytoplasm. Phosphorylation of ser-136 is associated with activation of Akt and phosphorylation of Ser-112 is due to the activation of the Ras-MAPK pathway. BAD Ser 155 is a large phosphorylation site that induces the formation of growth factors and is protected by inhibitors of protein kinase A.

It is known that FOXO3A gene prevails among long livers and probably determines longer life span, being one of the members of the family of transcription factors that mediate insulin action and resistance to stress. The relationship between polymorphisms of the FOXO3A gene and human life expectancy is presented in eight independent cohorts of centenarians [43]. Cell resistance to stress and cell survival in aging and DM may increase with high expression of the protein of the FOXO3A gene due to its effect on the activation of several members of the family of serum glucocorticoid-regulated kinases.

Genome-wide association studies (GWAS) have identified genes associated with DM and aging [44]. Most of the detected longevity genes have distant effect on one of the three

molecular pathways in the cell: insulin/IGF-1, sirtuins and mTOR. In the 1980s, scientists discovered the first gene that limited the lifespan of *Caenorhabditis elegans* and called it *Age-1*. The effects of the gene *Age-1* are realized through the molecular pathway of insulin/IGF-1: when the activity of the gene *Age-1* decreases, the molecular pathway of insulin/IGF-1 decreases and the life of *C. elegans* lengthens. Recent studies have shown that in people with mutations in the pathway of insulin/IGF-1, the risk of developing DM may be reduced.

The sirtuin pathological pathway in the cell regulates the metabolism of the cell. In the 1990s, scientists from the Massachusetts Institute of Technology discovered an extrocopy of the equivalent of sirtuin, sirtuin 2, which increases the life span of yeast. mTOR pathway (mammalian target of rapamycin) plays a role in the aging processes of various organisms, controlling the rate of protein synthesis, which is important for the functioning of the cell. The inhibition of this pathway in rapamycin mice leads to an increase in life expectancy [45].

The development of DM is associated with hyperlipidemia III and IV types. An example of a gene associated with healthy aging and long life expectancy is the cholesteryl ester transfer protein gene. The homozygous variant 405VV of the *CETP* gene is associated with low concentrations of the CETP protein in the blood, high concentrations of HDL cholesterol and large HDL particles, which determines the protection of the human body from the development of DM, cardiovascular diseases and Alzheimer's disease [46].

The work of biological clock is determined by oscillatory genes and genes responsible for unidirectional movement of time (telomere activity). Oscillatory genes synchronize behavioral and biochemical processes with a day/night cycle. Telomeres, which are repetitive series of DNA sequences that form terminal regions of chromosomes, are shortened during each subsequent division of the cell. The shortened telomeres are registered in various pathological conditions associated with aging. The activity of all processes in human cells is supported by NADH and ATP, synthesized from nutrients. Limiting the intake of calories increases the level of AMP and NAD and healthy life span of animals. Silent Information Controller T1 (SIRT1), NAD-dependent deacetylase, reduces the telomere reduction process, whereas the 1α receptor activator γ, activated by the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), is phosphorylated by kinase AMP and deacetylated SIRT1. Thus, PGC-1α is a key component of circadian oscillator that combines human biological clock and energy metabolism. Reactive forms of oxygen, formed in conditions of genetic mutation of biological clocks, lead to an accelerated reduction of telomeres. The above-mentioned processes are described in patients with DM.

The *Timeless* gene (Tim) was found in Drosophila and encodes a protein that regulates the circadian rhythm [47]. Oscillations of mRNA and protein rhythmically occur in time as part of the work of negative coupling in the transcription-translation system involved in the work of the period (per) gene of the periodic oscillatory protein [48–50].

The timeless protein connects the cell cycle with the circadian rhythm in humans. In the model of "direct coupling," two cycles are separated by key protein, the expression of which is determined by the circadian pattern [51]. The special role of the Tim gene in creating circadian rhythm is realized with the help of the *Cry* gene in humans. The transcription of the *Cry* and *Per* genes is activated by the *CLOCK*/*BMAL1* complex and suppressed by the PER/CRY complex.

Timeless protein in humans (hTIM) is responsible for the production of electrical oscillations emanating from the suprachiasmatic nucleus of the hypothalamus (SCN) and determining for all circadian rhythms in the human body. This protein interacts with the key products of the activity of the oscillation genes *CLOCK, BMAL, PER1, PER2* and *PER3.* Sancar et al. investigated the role of hTIM in the work of cell cycle in humans [52]. It plays integral role in phases of G2/M and the intra-S cell cycle. In the G2/M phase of the cell cycle, hTIM binds the ATRIP subunit to the ATR protein kinase responsible for DNA damage. Binding of hTIM and ATR subsequently leads to phosphorylation of Chk1, resulting in cell cycle arrest or apoptosis. The *Timeless* gene influences to the development of human diseases. DNA damage associated with telomeres is increased in cells with reduced replication of the *Timeless* gene, along with disruption of telomere replication. Swi1 is a protein associated with the Timeless protein, which is responsible for DNA replication in the telomere region [53]. Single nucleotide polymorphism in the *Timeless* gene, which leads to the replacement of glutamine by arginine in the amino acid sequence of the protein, has not demonstrated an association with changes in morning or evening diurnal rhythms in humans [54]. The Timeless protein can be responsible for circadian rhythms in pancreatic β-cells [55]. It is believed that the Timeless protein can be identified as a kinase suppressor with Ras-1-like activity [56].

The telomeric zinc finger-associated protein (TZAP) associated with long telomeres that have low concentration of protective complex competing with TRF1 and TRF2 factors linking telomeric repeats. In telomeres, TZAP causes a purification process that leads to rapid removal of telomere repeats. The regulation of the length of telomeres in human cells has been proposed: reduced concentration of protective complex in long telomeres leads to binding of TZAP protein and initiation of telomeres purification and sets an upper limit of telomere length [57]. Telomere shortening was previously associated with the development of DM in several pilot studies and in two large studies. Zee et al. showed that telomere length was less in the study group of patients with type 2 DM than in the control group (adjusted odds ratio = 1.748) [58].

Salpea et al. performed a study in which it was found that telomere length was less in type 2 DM and this fact corresponded to a high level of oxidative stress in these patients. Short telomeres are an independent predictor of the progression of diabetic nephropathy (DN) in patients with type 1 DM in the early onset of the disease [59, 60]. Astrup et al. showed that short telomere length is the predictor of all causes of death in type 1 DM [61]. Short telomeres were detected in arterial wall cells in patients with type 1 and type 2 DM [62]. Patients with IGT demonstrated shorter telomere length compared to healthy controls, and patients with DM and severe atherosclerosis showed the presence of shorter telomeres compared with patients with DM without atherosclerosis. The presence of obesity and insulin resistance was associated with the length of telomere leukocytes in the adult population [63]. The study found direct causal relationship between telomerase activity and insulin secretion, as well as glucose tolerance: the TERC-/- mutation showed ITG, which was caused by impaired glucose-stimulated insulin secretion from pancreatic islet cells due to a decrease in pancreatic cell size and replication damage of producing insulin β-cells [64].

Klass showed that the life expectancy of *C. elegans* could vary with the presence of the mutation of the *Age-1* gene, and this effect is associated with caloric restriction [65]. Later,

Johnson showed that life expectancy increased to 65% due to the mutation of the *Age-1* gene to greater extent than caloric restriction [66]. The *Age-1* gene encodes catalytic subunit of class I phosphatidylinositol 3-kinase (PI3K). Ten years after Johnson's research, the analysis of the *daf-2* gene was performed, and Cynthia Kenyon demonstrated an increase in the half-life of *C. elegans* [67].

Despite the fact that long livers can be characterized by unique set of genes, future biomedical research based on methods of high-performance screening of a pool of target molecules will lead to great advances in the diagnosis of aging stages and DM, as well as the development of methods for the prevention and therapy of accelerated aging of the human body and various violations of carbohydrate metabolism.

4. New technological platform for diagnostic and predictive pharmacology of aging stages and diabetes mellitus

Research in the field of aging and diabetology is related to recent discoveries in genomics and proteomics, new analytical equipment allows identifying biomarkers of aging and DM, the development of new drugs occurs through high-throughput screening of target molecules in human body [68].

At present, we have created unified technology platform for diagnostics and predictive pharmacology of aging and DM, taking into account interdisciplinary approach, including complex solution of problems of genomics, proteomics and metabolomics in the range of universal molecular pathways [69].

The technological platform for diagnostics and predictive pharmacology of DM includes three components taking into account a unique instrument base:

- *Scientific component*: Search for target biomarkers (α-β-subunit of insulin receptor, tyrosine kinase, MEK1/2-MAPK-cascade, Shc-Grb2-SOS-Ras–Raf mitogen, atypical isoforms of protein kinases, molecular cascade of GH/IGF-1, etc.) with aging and DM as the basis for a new level of diagnosis; the development of cellular technologies in the treatment of DM in combination with the development of new drugs based on targeted biomarkers; *in vitro* and *in vivo* studies of individual PK processes of drugs for the prevention of aging and treatment of DM; combining information on phenotypic manifestations of drug effects on the basis of pharmacoproteomic profile with the results of PK studies; PK/PD modeling; personalization of therapy for stages of aging and each type of DM.

- *Technological component*: New methods of genomic, proteomic, pharmacokinetic, pharmacoprotein, pharmacogenomic studies; biomodeling; software on bioinformatics and for the registration of ADR; methods of visualization of molecular PD effects of drugs in biological fluids and body tissues; creation of bank of biosamples.

- *Medical component*: The introduction of technological platform for molecular diagnostics and drug monitoring in aging and DM, diagnostic of ADR, new biomolecular methods

of control and prevention of aging and complications of DM in the clinic; clinical trials of drugs; economic evaluation of the platform.

Methods of genomic and proteomic analysis of human biosamples at various stages of aging and aging-related DM are used most widely in biomedical research (**Figure 2**).

Modern systems for high-throughput screening (HTS) of molecules in cellular structures (for Image-Based High Content Screening, High Content Analysis and High Content Imaging) allow to investigate the proliferation and cytotoxicity, cell viability, cell cycles, the expression of nuclear, cytoplasmic proteins and plasma membrane proteins, mitochondrial mass, phospholipidosis, signaling pathways, the increase and the decrease of nuclear sizes, apoptosis and fragmentation of nuclei. These systems allow performing complex analysis of cellular structures in real time, obtaining universal biological information about the development of aging processes and related diseases at the molecular level in the cell. Model of diabetic cardiomyopathy has been developed with the help of Operetta High Content Screening (Perkin Elmer, UK)—the system of high-performance screening of cell structures. The ways of pharmacological influence on the key targets of the development of this complication of DM have been developed. The model of this state in vitro was developed taking into account reproduced environmental conditions and genetic factors from human pluripotent stem cells of cardiomyocytes.
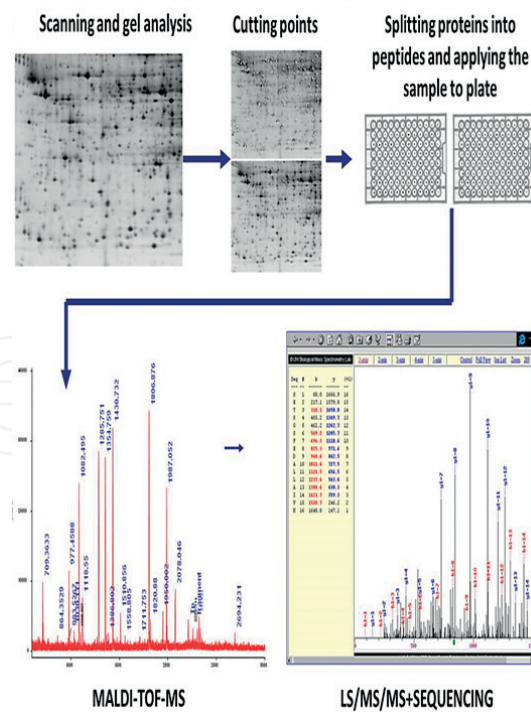

Figure 2. Molecular pattern of patient's biosample, obtained by methods of proteomic analysis (two-dimensional polyacrylamide gel electrophoresis, MALDI-TOF-MS, HPLC / MS / MS).

Biochemistry of DM was obtained, which stimulates the development of the phenotype of cardiomyopathy and which allowed us to analyze structural and functional changes in cardiomyocytes. The cardiomyopathic phenotype was reproduced definitively in specific cells of patients and determined by the initial clinical status. *In vitro* model was included in the stages of screening platform that identifies drugs that prevent the development of the phenotype of cardiomyopathy [70].

Thus, the current stage in the development of biomedical research in the field of aging and DM is associated with the introduction of new technology platform for HTS of molecules in human cells and the pharmacology of the aging stages and all types of DM.

The newest biomedical tools of the twenty-first century are biological microchips (biochips, DNA microarrays). Developed biological microchips make it possible to realize in an accessible form very complex integrative approaches of genomics, proteomics and selomics. An important medical application of biochips is the early diagnosis of DM and the development of new therapy, as well as the correlation of diagnostic markers of DM with diagnostic markers of stages of aging of the human body. Researchers conduct on-chip simultaneous analysis of tens of thousands of genes and compare the expression of these genes in healthy and diseased cells. Biochips are also an indispensable tool for biomedical research, which can in one experiment recognize the influence of various factors (drugs, proteins, nutrition) on the work of tens of thousands of genes. The effectiveness of biochips is due to the possibility of parallel carrying out a huge number of specific reactions and interactions of molecules of biopolymers, such as DNA, proteins and polysaccharides, with each other and low molecular ligands. The task is to quickly and effectively determine the concentration of the desired compound, for example, glucose for people with DM.

The use of protein chips for search of markers of aging and DM is promising direction. The following two tasks are of particular interest: simultaneous qualitative and quantitative determination of large number of proteins in cells of various tissues or in various functional states; study of interactions of cellular proteins with each other and other cellular ligands (DNA, low molecular compounds).

Bioelectronic devices are able to raise the quality of medical analyzes to new level, they will contribute to a one-stage definition of stages of aging and early diagnosis of DM.

Highly sensitive oligonucleotide microarrays were used to evaluate mRNA levels and identify transcriptional profiles of fibroblast cultures obtained from donors of different ages. The mRNA levels were measured in actively dividing fibroblasts isolated from young, middle-aged and elderly patients, as well as patients with progeria. The study identified genes, the expression of which is associated with phenotypes of different age groups and diseases. The aging process is based on a mechanism that includes an increasing number of errors in the mitosis of dividing cells at the post-productive stage of human life [71].

The progress and development of biosensors used in the clinic was related to the development of glucose biosensor in the 1960s and obtained on the basis of early integration of the redox enzyme glucose oxidase with an oxygen electrode [72]. New materials, sensory

configurations and technical innovations have been proposed for the determination of glucose [73–75]. Currently, glycemic control is based on the study of blood glucose, which still requires frequent blood sampling with a certain degree of inconvenience. Despite the intensive application of the existing method of blood glucose testing, precise monitoring of glucose fluctuations during the day cannot yet be performed. Therefore, today, subcutaneous biosensors are used, which measure glucose periodically during the day [76, 77]. However, the creation of an accurate implantable biosensor for glucose, acceptable for patients with DM, is an open question. Today, the most promising results were obtained on biosensors, which are based on amperometric detection of hydrogen peroxide formed by enzymes immobilized on electrodes. Updike et al. created a biosensor for glucose, implanted subcutaneously, with maximum duration of up to 5 months [78].

Interstitial levels of lactate should reflect its systemic level when hypoxia appears in the tissues. There is an opportunity to control lactate *in vivo* in the interstitium; however, it is difficult to introduce monitoring methods into the clinic because of their unreliability due to the influence on the level of lactate of a lot of endogenous factors. Several types of biosensors for the determination of lactate are presented in the literature. A microfluidic biochip was developed, which is integrated with a highly sensitive fiber-optic biosensor of glucose. Experimental results showed that the biochip determines an ultra-low glucose concentration (1 nM) [79]. Due to the fact that DM as an aging-related disease progresses and due to complex and stepwise processes of malfunctioning of the pancreatic β-cells at the molecular level that can be registered in the blood, early detection of DM requires the use of supersensitive systems for detecting molecular changes. To this end, a protein microchip was developed, including the use of polyfluorophor technology. The innovative system is characterized by high sensitivity: the possibility of the determining of biomarkers at the level of femtograms in 10 µl of the biosample is 92% within 10 minutes [80].

It is believed that the stages of aging and related diseases, including DM, are characterized by their bar code—a change in the level of transcription of a set of genes specific for this disease. It is assumed that in the future, according to the barcode, changes in the expression in particular set of genes can be diagnosed with specific diseases and the stages of their development and, consequently, develop targeted treatment regimen.

At present, bioinformatics is ready to provide data about tens of thousands of new drug targets, predicting the function of genes and deciphering the sequence of proteins. Promising bioinformatic developments are presented in such sections of medicine as gerontology and diabetology. The main directions of bioinformatics are distinguished, depending on the objects of study: sequence bioinformatics, structural bioinformatics and computer genomics. The main task of bioinformatics in the development of new drugs is to provide technologies that allow the formation of target targets for directed action of drug having specific structure for this target.

Today, an extended analysis is available, including the molecular pathways presented in most databases; so, large array of information about altered proteins can be obtained, including their expression and/or post-translational modifications in molecular pathways. Comparative analysis of two web products for the analysis of pathways and intermolecular

interactions—Ingenuity Pathway Analysis (IPA) and STRING—is published. Data about key proteins, participating in molecular pathological pathways (Wnt, APP, insulin signaling, mitochondrial apoptosis, tau-phosphorylation), were obtained on the basis of medical literature and data of proteomic analysis of the HEK293 cell line.

Information about protein interactions in complexes is found in databases such as MINT, BioGRID, IntAct or HRPD. It is possible to provide high percentage of predicted protein-protein interactions and interactions based on literature data (PubMed database). Widely used web resource for the analysis of inter-protein interactions STRING is not only a database but also linked to several other resources with large volume of literature sources [81]. The Cytoscape graphical tool allows us to create network interactions of high degree of complexity. Recently, web platform has been launched that integrates data on molecular pathways for the development of pathological processes and the analysis of intermolecular interactions, including six different databases (KeGG, Bio-Carta, Gene Ontology, Reactome, Wiki, NCI pathways) and interacts functionally with database on molecular activity of proteins (Interpro) and database of complex information about proteins (Corum).

Identification of the protein in the study of the biosample should be accompanied by a detailed analysis of its primary, secondary and tertiary structure, as well as its post-translational modifications and intermolecular interactions (BLAST search engine). The amino acid sequence of the protein can be analyzed in software products such as Pfam, Interpro, SMART or DAVID [82], whereas sequence analysis of post-translational protein modifications can be performed using algorithms such as MotifX or PhosphoMotif [83].

Modern methods of proteomic and bioinformatic analysis allow us to investigate key genomic-proteomic interactions that underlie DN in patients with type 2 DM. The study of the formation and development of DN can become the model for studying molecular pathways of aging of kidney tissue [84].

We carried out prospective comparative cohort study with parallel design for the search of molecular prognostic markers of DN of different stages using methods of proteomics and bioinformatic analysis on the basis of the Department of Nephrology of the Dagestan State Medical University (Makhachkala, Dagestan, Russia) and the Department of Nephrology of the Rostov State Medical University (Rostov-on-Don, Russia), MC "Novomeditsina" (Rostov-on-Don, Russia) [85]. It included 205 patients with T2DM and DN (stages 1–4). Patients corresponded to the criteria for the DN classification proposed by the Committee on Diabetic Nephropathy [86]. The duration of DN was 10.5 years. Molecular phenotyping of biosamples (urine) was processed with methods of proteomics: the prefractionation, the separation of proteins with standard sets (MB-HIC C8 Kit, MB-IMAC Cu, MB-Wax Kit, «Bruker», USA) and matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS/MS, Ultraflex II, «Bruker», USA). The partially identified sequences were then submitted to "BLAST protein-protein" and screened against the *Homo sapiens* Swissprot database to check if this identification matched the MASCOT-identification (Matrix Science). The data of the molecular interactions and functional features of proteins were received with STRING 10.0 database.

Protein name	Group 1 (n = 42)	Group 2 (n = 48)	Group 3 (n = 65)	Group 4 (n = 50)	Control group (n = 30)	MW (Da)	Functional process (sources: InterPro, Entrez, SWISS-PROT, NRDB, PDB, KEGG
TGF-β1	18 PCG-1 = 0.001	27 PCG-2 = 0.000	52 PCG-3 = 0.000	48 PCG-4 = 0.000	2	44,341	Pro-fibrotic and anti-inflammatory activities, the regulation of tubular EMT
E-cadherin	22 PCG-1 = 0.000	38 PCG-2 = 0.000	62 PCG-3 = 0.000	49 PCG-4 = 0.000	1	97,456	The regulation of tubular EMT; the maintenance of epithelial integrity, cell phenotype; the progression of renal fibrosis
Cystatin C	15 PCG-1 = 0.001	40 PCG-2 = 0.000	60 PCG-3 = 0.000	45 PCG-4 = 0.000	1	15,799	Cysteine proteinase inhibitor, tubular damage marker
Collagen IV	4 PCG-1 > 0.05	32 PCG-2 = 0.000	48 PCG-3 = 0.000	42 PCG-4 = 0.000	1	164,038	Constituent of mesangial matrix, marker of the phase of compromised renal filtration function
MMP 9	8 PCG-1 > 0.05	32 PCG-2 = 0.000	60 PCG-3 = 0.000	49 PCG-4 = 0.000	1	78,458	Potent modulator of ECM turnover and also of shedding of syndecans
Fibronectin	4 PCG-1 > 0.05	35 PCG-2 = 0.000	52 PCG-3 = 0.000	43 PCG-4 = 0.000	1	262,625	Adhesive glycoprotein, locally stimulated mesangial and epithelial cell production
NGAL	10 PCG-1 = 0.043	42 PCG-2 = 0.000	62 PCG-3 = 0.000	48 PCG-4 = 0.000	1	22,588	Kidney development; it loses through the damaged glomerulus, injured tubular cells produce NGAL as a compensatory mechanism against intracellular oxidative stress and complement- induced apoptosis.
Ceruloplasmin	12 PCG-1 = 0.006	37 PCG-2 = 0.000	42 PCG-3 = 0.000	35 PCG-4 = 0.000	1	122,205	Marker of damaged glomerulus

Protein name	Group 1 (n = 42)	Group 2 (n = 48)	Group 3 (n = 65)	Group 4 (n = 50)	Control group (n = 30)	MW (Da)	Functional process (sources: InterPro, Entrez, SWISS-PROT, NRDB, PDB, KEGG
β2-microglobulin	6 PCG-1 > 0.05	37 PCG-2 = 0.000	62 PCG-3 = 0.000	49 PCG-4 = 0.000	1	11,774	The indicator of incipient DN; detecting injured epithelial cells in the proximal tubules
Podocin	23 PCG-1 = 0.000	45 PCG-2 = 0.000	63 PCG-3 = 0.000	49 PCG-4 = 0.000	1	42,201	Podocyte-specific protein, interact with the PI3K/AKT-signaling pathway for maintenance of functional integrity
MCP-1	11 PCG-1 = 0.011	39 PCG-2 = 0.000	48 PCG-3 = 0.000	46 PCG-4 = 0.000	1	2583	Chemotactic factor for monocytes; regulates the memory T lymphocytes, NK cells; increases with TNFα and IL-6 in damaged kidneys

Table 1. Qualitative profile of urine proteins in T2DM patients with DN.

All changes in patients with DN are associated with a higher expression of urine proteins in the progression of epithelial-to-mesenchymal transition (EMT) and changes in the extracellular matrix (ECM) in kidneys in T2DM patients with DN (**Table 1**). Proteomic analysis helps in the detection of differences in the component composition of the urine proteins in patients with DN of varying stages compared with the control group. Molecules interact among themselves and with other molecules as participants in universal pathways in T2DM patients with DN, which are the key elements for EMT formation and changes in ECM: Smad, p38 MAPK, TLRs, Wnt, mTOR, Notch, small GTPase and Hedgehog and PI3K/AKT-signaling pathways.

Each protein molecule in the functional group interacts with other protein molecules. For example, the molecular interactions of NGAL are presented in **Figure 3**. The concentration of NGAL increases in the urine of T2DM patients with DN. The study identified the biomarkers of tubular damage that have a key role in the development and progression of DN. The research into signaling pathways and molecules that are involved in ECM formation may help in developing strategies to prevent DN. Molecular pathways for the development of DN constitute a model for the study of molecular pathways in the development of aging of kidney tissue.
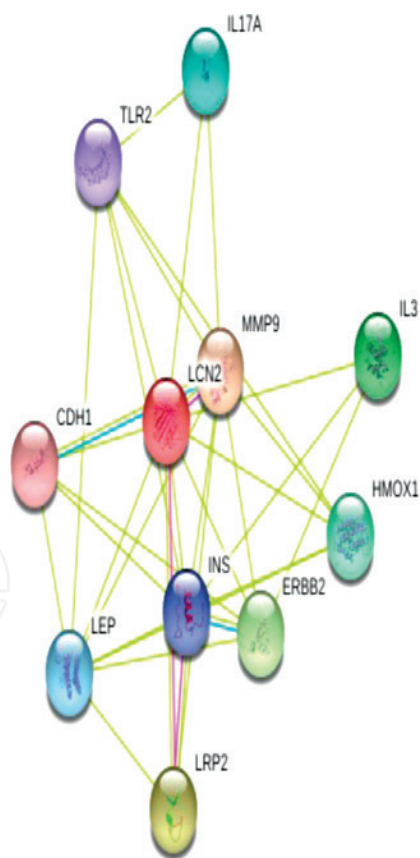

Figure 3. Molecular interactions of NGAL (STRING 10.0 database). LCN2, lipocalin-2; MMP-9, matrix metallopeptidase 9; LRP2, low density lipoprotein-related protein 2; ERBB2, erythroblastic leukemia viral oncogene homolog 2 (neuro/glioblastoma derived oncogene homolog); IL3, interleukin 3 (colony-stimulating factor, multiple); HMOX1, heme oxygenase (decycling) 1; IL-17A, interleukin 17A; LEP, leptin; INS, insulin; TLR2, toll-like receptor 2 and CDH1, cadherin 1, type 1, E-cadherin (epithelial cadherin).

Thus, the future progress in biomedical research of the aging stages of the human body and DM is associated with the development of experimental genomics, transcriptomics, proteomics and selomics and with the development of typical human development scenario, starting from the postnatal period on the basis of modern technological platforms. The development of methods for the systematic analysis of molecular interactions in cell and the subsequent study of their functional activity makes it possible to discover new pathways for the development of human pathology associated with aging.

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