NEW GENERATION TRANSGENIC MODELS IN MODERN PERSONALIZED MEDICINE

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ABSTRACT

The review considers the main trends of investigation in model organisms (Danio Rerio, Mus musculus) in modern personalized medicine, presents the main approaches to transgenesis, allowing more accurate modeling of specific human pathologies. Existing model systems for studying atherosclerosis, dyslipidemic disorders, neurodegenerative diseases are described. Three-dimensional cellular technologies applicable within a personalized approach to a patient are mentioned.

Key words: animal models, Danio rerio, dyslipidemia, neurodegeneration, three-dimensional cell culture, transgenesis.

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INTRODUCTION

Transgenic animals are used in laboratories as biomodels in medical research. More than 90% of them are genetically modified rodents, mainly mice (Mus musculus). The use of genetically modified animals is an important tool for the study of human diseases; they are used to understand the function of individual genes and genomes in the context of susceptibility to various diseases, their causes and progression, as well as to create alternative approaches for the treatment of pathologies. In this review, we will discuss both the methods of transgenesis that have already become classical, and new approaches to obtaining transgenic animals that are most often used in fundamental and applied medicine, as well as consider in detail some pathologies where modified animals are the main tool and key to understanding molecular and cellular anomalies that lead to diseases of vertebrates, including humans.

REVIEW OF CLASSICAL AND NEW APPROACHES TO TRANSGENESIS

The use of transgenic models has become widespread in biology and medicine. One of the earliest tools of genetic engineering was the method of conventional gene knockout, which allows obtaining a line of animals that completely lack the functional product of the gene at all stages of ontogenesis. Thanks to the activities of several consortia, the goal of obtaining knockouts of all known mouse genes has been practically achieved; the production of transgenic organisms of this type for experimental animals of other species is intensively developing [1]. However, the traditional conventional gene knockout technique has a number of limitations. In the course of large-scale phenotyping programs of transgenic mice, it was found that knocking out about 30% of genes in the homozygous state leads to a lethal phenotype and another 7% to reproductive defects [2]. Also, such animal lines do not allow studying the role of the gene during a single period of ontogenesis or within a certain morphological structure.

Methods of creating transgenic stable animal lines are numerous and diverse, depending on the goals and objectives of researchers. These include gene knockout to determine, for example, the signaling pathway, the induction of protein overexpression and expression that allow to alter cellular processes, visualize cells or destroy them by the formation of toxic products carried out with the help of directed mutagenesis, transgenes or viral transduction [3—5].

Most approaches to transgene expression in a particular cell type require a promoter that controls the transcription of the gene in the cell. There are several ways to activate the promoter in a specific cell type. If promoter sequences specific to the cell type of interest are known, they can be used to create a construction and then randomly insert it into the mouse genome [6]. However, this approach often requires the creation and screening of many transgenic lines, as the level of transgene expression and transmission efficiency may vary depending on the number of copies of the transgene in the genome and their integration sites [7].

Gene expression in the cell of interest can also be achieved by a “knockout” when a transgene is inserted into a genomic coding sequence of a cell-specific gene with using a specific mutagenesis. This approach provides the most accurate expression of the transgene, since all the internal regulatory elements of the promoter are preserved, but it leads to an undesirable homo- or heterozygous knockout of a cell-specific gene. This can be avoided if the transgene is inserted together with the IRES element (internal ribosome entry site), which provides internal translation initiation, and both genes are expressed in parallel [8].

Another method for direct expression of a transgene is transgenic constructions based on bacterial, yeast or other artificial chromosomes (BAC, YAC or PAC). Artificial chromosomes contain large fragments of genomic DNA, and a particular clone will include a coding sequence of a specific gene and promoter elements [9, 10]. This approach has two advantages: firstly, large genomic fragments can contain most or all of the promoter regions that control the expression of transgene in the cell of interest; and secondly, the endogenous coding sequence of a specific gene does not change unless the transgene is accidentally integrated into the coding sequence of the gene.

Direct control of transgene expression requires activating the promoter in the cell of interest at a certain time. With the help of induced transgene expression, such strict spatial and temporal control can be carried out. The widely used Tet-On and Tet-Off approaches allow activating transgene expression in the cell of interest at a certain time [11, 12]. The Tet-Off and Tet-On systems use tetracycline transactivator protein (TTA) capable of binding to DNA in certain sequences of the TetO operator. tTA activation can be suppressed (Tet-On) or activated (Tet-Off) by tetracycline and its derivatives and thereby prevent activation of specific genes [13].

As a fundamentally different approach to editing, site-specific recombinases are used, allowing manipulations directly at the genome level in the experimental organism. The most common system for generating non-conventional knockout animals is the Cre-Lox system [14]. Cre recombinase, an enzyme found in bacteriophage P1, specifically recognizes sequences called loxP sites. This enzyme cuts out the sequence located

between two LoxP sites and stitches the ends of the original DNA. Thus, in a model organism, a gene or its fragment is enclosed between LoxP sites, and the process of its restriction is controlled by Cre expression.

The use of site-specific recombinases also opens up new opportunities for creating models for genetic tracing, a technique that allows identifying descendants of cells expressing the gene of interest. A typical model system consists of two genetic structures. The first one is represented by the coding sequence of Cre-recombinase located under the promoter of the gene of interest. The second structure consists of a gene encoding a reporter and a floxed (located between two loxP sites) stop codon located in front of it. Thus, in cells in which the promoter of the gene of interest is active, recombination and co-expression of the reporter gene occurs. A further way to modify the system was to create a fusion protein — CreERT and its modifications — recombinases, activated by human estrogen receptor agonists [15]. Such a system allows you to start recombination in a certain period of time by introducing the corresponding ligand without affecting the cells expressing the gene of interest in other periods of ontogenesis.

A further way to modify this method is to create new chimeric recombination inducer proteins based on Cre. One of the promising options is a fusion protein from Cre and dihydrofolatereductase E. Coli [16]. In this case, low-toxic drug trimethoprim can be used as an inducer, for which there is no endogenous receptor in the mouse body. Along with the development of genetic constructs of recombination inducers, reporter ones are also developing. The existence of many variants of LoXP sites and fluorescent proteins with different spectral characteristics and intracellular localization made it possible to create a number of complex animal reporter lines, such as Brainbow, Zebrabow, Confetti and others, in which recombination leads to a random combination of coexpression of several reporters, which allows experiments such as clonal analysis [17], connectoma studies and neural network development processes [18].

Not only fluorescent proteins can be placed in the coding sequence of the recombinase target structure. One of the effective methods of studying the role of individual cell types in the development of the organism — genetic ablation — is based on the induction by recombinase of the expression of diphtheria toxin that causes cell death [19].

A number of classical methods of neurobiology have received additional opportunities thanks to the constructed sensor proteins. For example, there are mice lines where calcium-sensitive fluorescent proteins that are sensitive to transmembrane potential and peroxides are expressed instead of reporter proteins [20]. Thus, it is possible to apply classical methods of recording neuronal activity in application to a specific type or population of cells identified by Cre expression.

A well-characterized marker of neuronal activity is the expression of IEG (immediate early genes) genes, such as c-foc, c-jun and Arc. The recombinases expressed under the promoters of these genes make it possible to identify neuronal ensembles involved in the implementation of behavioral acts in mice [20].

The Cre-Lox system is also the basis for many model constructions in optogenetic studies in neurobiology. In this case, recombinase induces the expression of photoreactive proteins. Initially, the most common were photoactivated ion channels found in a number of bacteria. Fusion proteins from photoreactive and enzymative-active parts are becoming increasingly widespread, which allows inducing intracellular biochemical reactions by the directed action of light. In combination with the above-mentioned constructions, including Cre under the IEG promoter, it becomes possible to reactivate neural networks that previously participated in the course of the behavioral act of interest [21] .

An alternative method is the use of the Flp-FRT system, which, however, has received less development. Among the problems, the thermolability of the Flp enzyme and the relatively low efficiency of recombination are highlighted, which makes it impossible to use it in homoiothermic organisms [22]. In the course of further modifications of the recombinase, these problems were partially resolved, but its effectiveness is still inferior to Cre-Lox.

A similar principle to Cre-Lox is used in tracing with the application of Dre-rox, which has not yet become widespread. The combination of several recombination systems in one model organism makes it possible to create complex controlled models, visualize several types or populations of cells simultaneously, provided that several genes are co-expressed simultaneously [23].

DANIO — UNIQUE BIOMODELS FOR STUDYING PATHOLOGIES

Brachydanio rerio models are more cost-effective than rodent models due to the lower cost of maintaining the model organism. The advantages include simplicity of maintenance and the possibility of obtaining a large number of embryos in a short period of time. Housing and selecting systems are much simpler. Theoretically, one pair of danio fish can produce thousands of genetically identical embryos. The specificity of reproduction makes it possible to investigate rare genetic events and conduct parallel testing on a large homogeneous sample [24].

External fertilization makes embryo manipulation much more accessible than in mammals. In addition, the

transparency of Danio Rerio embryos makes it possible to study organogenesis in vivo, in particular when using specific lines and vital dyes, including fluorescent marking.

In addition, the embryos of danio fish develop extremely quickly: by 24 hours after fertilization, most stages of organogenesis are completed, which makes it possible to visualize in real time all stages of the development of the organism [25, 26].

It is important to note that in addition to this rapid anatomical development, neural, hormonal and paracrine connections are also established and provide homeostasis already at the early stages of development [27-29].

The small size of the embryos of danio fish and adult individuals can also be an advantage in laboratory conditions by reducing the consumption of valuable reagents during screening studies. The small size also facilitates whole-tissue [30], whole-organ and whole-organism transcriptomic [31, 32], proteomic [33] and other "omix" analyses [34], as well as whole-organ clonal analysis [35] and cell-cell mapping [36].

Despite the fact that the common ancestors of fish and humans diverged about 450 million years ago, it is known that about 82% of the genes responsible for genetic diseases are represented by orthologous genes in danio fish [37]. The ease of editing the genome and the abundance of orthological genes in these animals made it possible to create accurate biomodels on danio.

Editing the fish genome using programmable nucleases lets make double-stranded breaks in the DNA region of interest, which leads to a target mutation resulting in the inactivation of the gene of interest or changing its operation.

Fish studies play a central role in the development and application of genome editing technologies: initially, the editing of the fish genome was carried out using ZFN (zink finger nucleases) technology — specially designed specific proteases that can be targeted at the desired DNA region and make changes to the genome with high accuracy.

The next step in the development of genome editing and obtaining model fish is the emergence of TALEN (Transcription Activator-Like Effector Nucleases) technology: the advantage of this type of endonucleases is that they can be designed to bind to any DNA sequence of interest and that they can cause a wide range of mutations in fish with an efficiency of over 98.5% [38]. CRISPR (Clustered Regulatory Interspaced Short Palindromic Repeats) / Cas9 technology is the most modern approach in genome editing, it allows quick and accurate genome editing and is the most common technique for danio [39]. These technologies are aimed at inactivating genes by inserting or making small inserts, which makes it possible to develop lines of fish with homozygous mutations of model pathologies in just 2 generations [40]. Thus, blood diseases were modeled on fish for the first time [41], biomodels of cardiovascular [42] and neurodegenerative diseases were obtained [43].

A distinctive feature of using fish in personalized medicine is obtaining humanized models of diseases. As a rule, the humanized model is based on increasing the functionality of the target genes. The simplest method is a temporary increase in gene expression, which allows us to trace the relationship between the overexpression effect in a biomodel and the physiological effect of a human mutation [40].

For example, overexpression of NRAS mRNA in danio embryos simulates the process in which overexpression of an orthologous gene in humans leads to the development of Noonan syndrome [44]. Similar studies show that the overexpression of the PTDSS1 gene associated with the development of Mayevsky syndrome is associated with skeletal abnormalities in danio hatchlings [45].

Humanized fish are actively used in studies of neurodegeneration processes, for example, as a simulation of the toxic effects of synucleins, in particular human α-synuclein involved in the development of Parkinson's disease [46]. The accumulation of α-synucleins in neurons was associated with neurodegenerative processes, the mechanisms of which were unclear. With the help of the GAL4/UAS system, which allows activating the genes of interest artificially, it has been shown that overexpression of human α-synuclein in neurons leads to a toxic effect and degeneration of nerve cells. A similar approach, which consisted in artificial creation of sequences in the C9orf72 gene responsible for the synthesis of glycine-alanine repeats, also made it possible to create a model of amyotrophic lateral sclerosis in danio fish [47]. Dominant hereditary diseases and the effect of various mutations on the penetrance of the pathological sign can be studied on fish. For example, spinal cerebellar ataxia type 3 is caused by the death of neurons in the cerebellum and is caused by a mutation of the potential-dependent potassium channel Kv3.3 gene. The childhood and adult forms of this disease are associated with mutations and hyperexpression of various alleles of the gene, which leads to the formation of different phenotypes in model fish. The infantile, severe form of the disease leads to migration disorder of motor neurons, while the adult form leads to axonal branching disorder of motor neurons [48].

TRANSGENIC ANIMALS FOR THE STUDY OF ATHEROSCLEROSIS AND DYSLIPIDEMIC DISORDERS

An ideal model of dyslipidemic disorders and atherosclerosis in animals for biomedical and pharmaceu-

tical research should have the appropriate potential for extrapolating data to humans. Basically, such models are based on the induction and acceleration of the formation of an atherosclerotic plaque through the use of specialized diets, genetic manipulations and environmental influences.

In the first half of the twentieth century, diet-induced models of atherosclerosis were used, mainly on rabbits. It has been shown that a high cholesterol diet (HCD), as well as a diet high in animal protein, lead to atherosclerosis and hypercholesterolemia.

From the 1950s to the 1970s, various diets capable of inducing hyperlipidemia were developed and tested on rats and rabbits. Studies of diet-induced atherosclerosis have made a fundamental contribution to understanding the pathogenesis of this condition.

In the 1970s and 1980s, laboratory mice began to be actively used for the study of atherosclerosis. Studies on plasma lipoprotein metabolism in the 1980s, combined with the advent of transgenesis technologies in the 1990s, led to the emergence of mouse knockout lines such as ApoE $^{-/-}$, Ldlr $^{-/-}$, PCSK9 $^{-/-}$. In addition, in 2002, the genome of mice of the C57BL/6 line, which is relatively sensitive to modeling of metabolic disorders through diet, was sequenced.

It has been shown that models of atherosclerosis in mice generally do not demonstrate instability of atherosclerotic plaque with subsequent thrombosis, which are most often factors associated with clinically significant acute cardiovascular episodes [49]. The etiopathogenesis of the formation of unstable atherosclerotic plaque includes the presence of risk factors for the development of cardiovascular diseases, which induce endothelial dysfunction and increase vascular permeability, leading to infiltration of lipids and enhancing the adhesion and trans-migration of monocytes. In the intima of the vessels, monocytes differentiate into macrophages and absorb altered lipids, turning into foam cells. Simultaneously, at this stage, vascular smooth muscle cells migrate to the intima, where they synthesize extracellular matrix and contribute to the formation of a fibrous capsule. As the plaque progresses, the number of smooth muscle cells decreases, foam cells undergo apoptosis, releasing active metalloproteinases that destroy the capsule, increasing the likelihood of plaque rupture. The immune system takes an active part in this process and plays a key role in the destabilization of plaques [50].

In addition, unlike humans, mice rarely develop atherosclerosis in the coronary arteries, but atherosclerosis in the aortic root easily develops. Thus, the distribution of tissue lesions in mice and humans is not identical.

At the moment, *Danio Rerio* fish are of great interest as a biological test system for the study of atherosclerosis and dyslipidemic disorders, which is reflected in the statistical analysis of publications covering the corresponding models [24, 51]. *Danio Rerio* is anatomically similar to more highly organized vertebrates. Pathological processes in tissues can be studied and extrapolated for a wide range of cardiovascular and metabolic diseases in humans [52].

Genetic modifications of Danio fish also originate in the 1980s. In the 2000s, the number of published studies using *Danio* fish began to increase, the first genetically modified model of hyperlipidemia Apoc2$^{-/-}$ was obtained, and soon the LDLR$^{-/-}$ hypercholesterolemia model too.

Directed deletion of the Apoc2 gene is one of the vectors for further development of dyslipidemia models in Danio fish. People deprived of Apoc2 have familial chylomicronemia, characterized by elevated triglyceride levels and recurrent episodes of pancreatin. Mutant *Danio Rerio* show distinctive signs of human Apoc2 deficiency: decreased plasma lipase activity and severe hypertriglyceridemia. Visualization of the vascular network in Apoc2 $^{-/-}$ mutant fish reveals the accumulation of lipids and lipid-loaded macrophages, which is a sign of the formation of atherosclerotic plaques. This model of dyslipidemia may be particularly useful when studying the stages of extravasation, oxidation and absorption of LDL by macrophages of vascular walls [53]. Zebrafish mutated by LDLR are a biological model for studying hypercholesterolemia and lipid accumulation in blood vessels, which is equivalent to the early stage of human atherosclerosis. This model is characterized by the development of moderate hypercholesterolemia with normal nutrition. However, a short-term 5-day feeding of LDLR-deficient fish larvae with a high cholesterol diet (HCD) leads to exacerbation of hypercholesterolemia and the accumulation and deposition of lipids in vessels [54].

Despite the fundamental differences in the physiology of metabolism in poikilothermic and homoiothermic species, it is *Danio Rerio* that makes it possible to reproduce some of those pathological processes that cannot be studied using rodent modeling, which is why they are gaining popularity as a valuable model for studying lipid metabolism and related disorders [55].

It is known that orthologists of key factors regulating lipid metabolism, such as microsomal triglyceride transfer protein (MTTP), acyl CoA synthetase (ACS) and apolipoprotein C2 (APOC2), are known to be expressed in danio fish just as in mammals [56, 57]. At the same time, unlike rodents, danio fish have a cholesterol ester transfer protein (CETP), since the CETP orthologist is preserved in their genome. Thus, as in humans, cholesterol esters in danio fish deviate from "good" HDL to "bad" LDL, thereby increasing susceptibility to atherogenic events [58].

In addition, Danio Rerio is a suitable model for the study of autoinflammatory disorders, which are known to be a significant risk factor for the development of atherosclerotic changes and cardiovascular complications, which can play a role even with low levels of LDL in the blood [59].

Chronic inflammation, through the activation of the NLRP3 inflammosome-IL-1ß signaling pathway, is an important factor in the development of atherosclerotic cardiovascular diseases (ASCVD), being triggered by the accumulation of intracellular cholesterol in cells [60].

The pyrin domain of the NLR family contained in the 3 (NLRP3) inflammasome is one of the most fully characterized in humans and other mammals. Molecular and functional identification of the NLRP3 homologue (*Dr* NLRP3) was performed on the Danio Rerio model. Due to the *Dr* NLRP3 knockdown in zebrafish larvae and the generation of the *Dr* ASC $^{-/-}$knockout, it was possible to characterize the function of *Dr* NLRP3 inflammasome in antibacterial immunity *in vivo*.

Thus, both laboratory mice and danio fish are of interest in the study of atherosclerosis and dyslipidemic disorders as model organisms. The development of the field of biomodeling of pathological processes using experimental model systems and the use of genome editing technologies makes it possible to expand the range of available genetic and combined models of atherosclerosis and dyslipidemic disorders.

MODERN GENETIC TECHNOLOGIES FOR THE STUDY OF NEUROGENESIS AND NEURODEGENERATIVE DISEASES

The development process of the nervous system (neurogenesis) is a complex multi-stage process of formation (specialization) of nerve cells that form compartments of the nervous system (central and peripheral compartments) [61]. Of course, understanding the molecular and cellular mechanisms of development of the nervous system is necessary to decipher the functioning of the brain and its plasticity in humans. The most widely used methods of studying neurogenesis include genetic tracing methods that allow us to trace the fate of embryonic cells and tissue-specific knockout (nokin) methods that reveal the role of a single gene or an ensemble of genes in the neurodevelopment or specialization of individual neurons or glia. Tracking the hierarchy of cells in development is a process aimed at identifying offspring that originate from a single progenitor cell (stem cell or progenitor, blast cells). Tracing can be implemented by various strategies based on genetically modified organisms, using genetic markers, transfected viral vectors or DNA structures, and with the help of cell sequencing [62].

Tracing progenitor cells to the state of their ultimate specialization and determining their fate in an adult body became possible thanks to the development of some important genetic tools that have made it possible to permanently label cells. In particular, recombination systems Cre-loxP [63] and Flp-FRT

[64] are the most used systems in the study of cellular tracing. Transgenic lines of mice that are susceptible to inducible Cre recombination under the control of specific promoters can cause expression of a fluorescent reporter (fluorescent protein) for determining the fate of neural predecessors in vivo [65]. This was achieved by administering low doses of tamoxifen, and, depending on the line of interest, transgenic mice encoding various fluorescent proteins under the control of specific promoters were created, showing special advantages and disadvantages [66].

A slightly more complicated version of monocolor (one tracer) genetic tracing is a method of multicolor cell marking (Brainbow technology) [67]. Brainbow transgenic mice undergo stochastic recombination of up to four fluorophores, which are controlled by the Cre-loxP system, resulting in multicolored mosaics where individual cells can be easily identified. Modified versions of this methodology are still being developed, and they provide vivid images that allow extremely detailed visualization of the morphology of individual marked cells. This is a very effective method for mapping cells, but not for tracing the origin. However, this technology has had a huge impact on the field of origin tracking, which has led to a new wave of methods in which a stochastic combination of fluorophores is used to create unique barcodes of neuronal stem cells that can be inherited by all their descendants. This method was originally developed for mice, although the combinatorial use of fluorescent proteins was redesigned to map the fate of *Drosophila melanogaster* (Flybow, d-Brainbow, Raeppli) and zebra fish (Zebrabow) [16].

One of the most modern approaches to the effective transformation of cells, embryos or tissues has become the method of transgenesis with the help of recombinant lentiviruses encoding various fluorescent proteins that are being created to contribute to multicolor mosaics for tracing the origin [68]. These recombinant DNA constructions can be transfected into the cells of interest and make it possible to trace all the descendants of individual cells (StarTrack, iON, CLONE) [69-71]. New advances in microscopy have led to progress in tracking multicolored lines [72], expanding the possibilities of tracking lines in any organism and allowing the study of cell heterogeneity.

Genetic technologies have become important not only for studying the mechanisms of neurogenesis, but also for studying the causes of neurodegenerative diseases. In

contrast to the unsuccessful search for new and effective treatment methods, the understanding of the pathogenetic mechanisms underlying the main neurodegenerative conditions has advanced significantly. The mechanisms governing the pathological aggregation of key proteins, the nature and processes of neuronal damage associated with the formation of protein aggregates are modern areas of study of these neuropathologies.

Due to the significant similarity in the phenotype of genetic and sporadic forms of neurodegenerative diseases (for example, Alzheimer's and Parkinson's disease, frontotemporal dementia or amyotrophic lateral sclerosis), genetically modified animal models carrying human genes, which, as it turned out, mutated in familial cases of neurodegeneration, were created to study the mechanism of occurrence and progression of such pathologies.

To date, a database has been compiled that provides information on selected models of neurodegenerative diseases in rodents, including Alzheimer's, Parkinson's and lateral amyotrophic sclerosis. By summarizing, visualizing and constantly updating the available data on the characteristics of model systems, the goal of this database is to help researchers study, compare and identify models that can speed up their research (https://www.alzforum.org/research-models).

Currently, great efforts are being made to characterize animal models of Alzheimer's disease to better understand the pathophysiology of the disease, as well as to identify models suitable for research on potential therapeutics. At this stage, 210 animal models are described, which are used in this area of research and are mainly needed for the study of amyloid plaques (21 models), Neurofibrillary tangles (10), loss of neuron bodies (15), gliosis (27), loss of synapses (16 models), long-term potentiation and long-term depression (16 models) and cognitive impairment (5xFAD (B6SJL), 3xTg, A7 APP transgenic, Abca7*A1527G/APOE4/Trem2*R47H, APOE2 Knock-In, floxed (CureAlz), APOE3 Knock-In, floxed (CureAlz), APOE4 Knock-In (JAX), APP751SL/ PS1 KI). Progress in research and development of treatments for Parkinson's disease depends on reliable preclinical models, including rodent models. To date, 20 rodent models have been described that study the problems of neuronal loss, dopamine hunger, α-synuclein inclusion, neuroinflammation, mitochondrial malfunctions, motor and non-motor disorders (α-synuclein KO Mouse, Pink1 KO Rat, Thy1-αsyn "Line 61" Mouse, Parkin KO, DJ-1 KO Rat). In the case of amyotrophic lateral sclerosis, there are about forty rodent models reproducing various aspects of the disease, such as motor disorders or degeneration of motor neurons. However, no model recreates all aspects of human disease perfectly.

Indeed, basic clinical studies convincingly prove and confirm with their data that experimental animal models are extremely useful for the analysis of pathogenetic mechanisms and selection of tools for neuropharmacology, the purpose of which is to influence the initial mechanisms of disease development.

ORGANOID MODELS

In recent years, due to the development of molecular methods in research, modeling of human diseases on laboratory animals often raises additional questions about pathogenetic mechanisms. Even proteins encoded by orthologs will not necessarily have completely identical functions in organisms of different biological species. The use of humanized animals also cannot always give an answer to all questions, modification will not always affect the entire body.

Two-dimensional cell cultures have been used for quite a long time to solve problems related to the study of the toxicity of substances and the reactivity of individual cells. Classical two-dimensional cell cultures, however, have a number of limitations that do not allow this technique to be used as a universal model for personalized medicine. The transcriptomic profile of cells in the culture monolayer may undergo noticeable changes, which entails possible changes in their properties and sensitivity to influences. In addition, the absence of molecular signals from cells of other populations also affects the properties of cells in the culture. This problem is partially solved by cocultivating different types of cells in one culture (macrophages and fibroblasts, endothelium and tumor cells, mesenchymal stem cells and melanocytes, etc.) [73-75].

Organoid models are three-dimensional cell culture systems that, to a greater extent than two-dimensional ones, allow modeling both normal physiological processes and pathological conditions. They can be created from embryonic stem cells, induced pluripotent stem cells, as well as cells of adult organisms, including tumor cells. They are relatively inexpensive systems capable of self-renewal and allow modeling a variety of processes by influencing them with various biologically active molecules, physical factors, and microorganisms. At the same time, the organoid itself will "respond" to the effect by cellular signal cascades, characteristic of the organ/tissue that it models. Thus, cerebral organoids generate alpha rhythms, which are characteristic of the brain of newborns [76].

Organoids obtained from patients' neoplasms demonstrate the same molecular characteristics as the "maternal" tumor, which allows in vitro observation of genetic changes in their cells, determination of sensitivity to different types of chemotherapeutic drugs, and

the possibility of metastasis of a particular neoplasm with a high degree of probability [77, 78].

In organoid systems, it is relatively easy to edit the genome, which can be useful both in studying the pathogenesis of individual diseases and for testing certain therapeutic approaches [79, 80].

Organoid transplantation is also an interesting area. Transplantation of both tumoroids and normal tissues is possible [81]. Experiments are also being carried out on the transplantation of human organoids to laboratory animals.

CONCLUSION

Thus, the use of transgenic animals and tissue cultures allows a much more detailed approach to the treatment of patients, choosing the most appropriate strategies. It is also possible to study in vivo the molecular and cellular foundations of the disease and therapeutic approaches with an assessment of potential risk factors. The use of three-dimensional cell cultures of the patient makes it possible to most accurately determine the molecular features of the course of the disease in a particular person and select an individual treatment program most accurately.

Conflict of interest

The authors declare no conflict of interest.

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