Creation, working principles, development of applied and fundamental scientific activities of the Collection of Cell Cultures of Vertebrates

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Abstract

The review presents the history of the creation of the "Collection of Cell Cultures of Vertebrate" (CCCV), which has been in operation for over 40 years. The working principles, comprising seven points and covering both the practical and scientific activities of the CCCV, are discussed. Part of the review is aimed at describing the amount of hands-on work associated with service delivery to CCCV's users representing various institutions in the Russian Federation. The quantitative indicators presented are evidence of the active practical activity of the CCCV. Another part of the review is dedicated to the CCCV's many years of scientific work. It consists of a description of the work in 6 scientific areas throughout the lifetime of the CCCV. In conclusion, scientific and information activities of the CCCV, and participation in various State programs are indicated.

Keywords: cell cultures, mesenchymal stem cells, matrix metalloproteinases, replicative senescence, karyotype, actin cytoskeleton

The development of the most important and promising basic and applied research in the field of molecular and cellular biology is inextricably related to the widespread use of human, animal, and plant cell cultures.

Cells in culture are subject to a high degree of hereditary variability over the course of long-term culture under changing environmental conditions. At the same time, in most cases, during routine culture, it is impossible to determine what specific properties of the cells have changed. Therefore, when working with cell cultures, the risk of increasing genetic instability must be avoided. Successful preservation of the original or directionally changed characteristics of cell lines, as well as obtaining reproducible experimental results, is achieved by observing strictly maintained conditions for cultivation and cryopreservation of cell cultures. The maintenance of cell cultures with initial cell properties and the monitoring of their condition is carried out by the national collections of various countries.

Creation of the Collection of Cell Cultures of Vertebrate (CCCV)

The Collection of Cell Cultures was established in our country in the late 1970s. At the level of the State Committee of the Council of Ministers of the USSR for Science and Technology and the Presidium of the USSR Academy of Sciences on May 29, 1978, a decision was made to create the USSR-wide Collection of Cell Cultures by organizing in one collection several separate collections of human, animal, and plant cells already existing in individual institutes. The founder of the USSR-wide (Russian) Collection of Cell Cultures (RCCC) is was an emeritus scientist of the Russian Federation, professor George Petrovich Pinaev (1929–2013),

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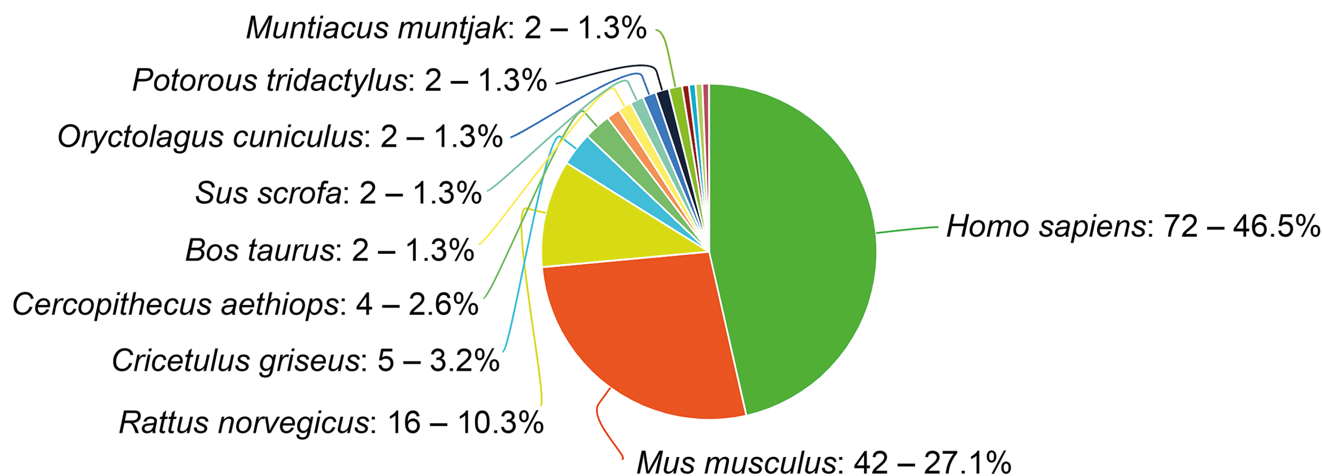


Fig. 1. Cell lines derived from various animals and stored in the Collection of Cell Culture of Vertebrate. Names of animals, number of lines and percentage are indicated. Slices of less than 1 % are not shown.

who until the last days of his life was the coordinator of RCCC's activities. Decree No. 24/25/13 of 13 February 1981 approved the work program for the creation of the USSR-wide Collection of Cell Cultures. The Institute of Cytology of the Russian Academy of Sciences (INC RAS) was identified as the lead institution on the project. The Central Bank of the USSR-wide RCCC has approved the CCCV located within INC RAS. The USSR-wide Collection included 9 specialized collections of human, animal, and plant cell cultures (Pinaev, Poljanskaya, Sakuta and Bogdanova, 1999; Pinaev and Poljanskaya, 2010). In recent years, the RCCC as a coordinated organization has ceased to exist. The Collections which compose it have turned towards independent work. Until 1995, the director of the CCCV was Irina I. Fridlyanskaya, Ph.D., who laid the groundwork for the CCCV together with G. P. Pinaev. Since 1995, G. G. Poljanskaya, who holds an Advanced Doctorate (Doctor of Science) in Biological Sciences, has served as the head of the CCCV.

At the end of 2021, the CCCV had 155 cell lines listed in the cell culture catalog (Fig. 1). The current version of the annually updated CCCV's cell line Catalog is presented in an electronic form on the INC RAS Shared Research Facility website (supported by a grant from the Ministry of Science and Higher Education of the Russian Federation, Agreement No. 075-15-2021-683): www.incras-ckp.ru.

The cell lines in the collection include immortalized cells with an endless lifespan and non-immortalized (diploid) lines with a finite lifespan. These two types of lines differ significantly in their properties. Numerous publications provide a thorough explanation of the characteristics of these lines as well as the procedures by which immortalized cell lines are created (Hayflick, 1965; Matsumura, Zerrudo and Hayflick, 1979; Duncan and Reddel, 1997; Poljanskaya 2008, 2014). Additionally, the CCCV funds contain 674 copyright cell lines and hybridomas that were deposited in connection with the patent process. The collection funds comprise about 27,500 cryotubes with collection cellular material and 7514 cryotubes with deposited cellular material stored in liquid nitrogen in a cryocomplex. Since 1987, the CCCV has been an official depositing organization on the basis of a joint decision of the Commission on Cell Cultures of the Interdepartmental Scientific and Technical Council on Problems of Physicochemical Biology and Biotechnology of the State Committee for Science and Technology of the USSR, the USSR Academy of Sciences, and the USSR-wide Research Institute for State Patent Examination on the legal protection of cell lines representing interest for the national economy. The basis for the development of deposit rules was the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure (Budapest, April 28, 1977).

The guiding principles of the CCCV

The working principles and tasks of the CCCV are basically the same as those of any research collection of cell cultures (Pinaev, 2008; Pinaev and Poljanskaya, 2010):

1. Creation, continuous maintenance and development of collection funds by collecting, breeding, certification, and storage of human and animal cell lines;
2. Development of unified requirements for the quality of collection material: unified passports, methods of analysis, storage, and control of cell lines, in accordance with international requirements;
3. Improvement of methods for collecting and working with cell lines based on many years of scientific research devoted to the study of the influence of cultivation conditions, cryopreservation, and contamination on the genetic variability of cell lines;

derivation and characterization of new cell lines and hybridomas; constant expansion and deepening of fundamental research on cell biology in culture.
4. Creation of cell culture information databases;
5. Deposition of author's cell lines and hybridomas in connection with the patenting procedure;
6. Providing samples of standard and thoroughly characterized cellular material for fundamental and applied biological, medical, and agricultural research;
7. Production of methodological guidelines; provision of scientific and methodological support to employees of the nation's scientific institutions regarding techniques for growing and analyzing cell lines.

It should be emphasized that starting from the year 2017, the main work of the CCCV is carried out in accordance with the developed standard operating procedures (SOPs). The following SOPs have been developed: SOP No. 1 — "Maintaining storage units and preparing cell samples for issuance to institutions of the Russian Federation"; SOP No. 2 — "Quality control of items of storage"; SOP No. 3 — "Methods for expanding collections. Derivation and characterization of non-immortalized (diploid) cell lines"; SOP No. 4 — "Karyological analysis of collection cell lines."

According to paragraph 1 of the guiding principles, in connection with the maintenance of the CCCV's funds, the screening of the samples laid down in the cryocomplex is carried out annually. The purpose of this work is to replenish cell samples of those lines, the number of samples of which has significantly decreased due to the issuance of them to users, as well as with the replacement of samples, the cryopreservation period of which is more than 10–15 years. Upon receipt of new samples, microbiological control is carried out and the viability of cell populations is determined, which cannot be less than 75 %.

Let us take a closer look at the CCCV's operational activities, which are mostly described in paragraphs 5–7. Quantitative indicators for the delivery of services serve as a confirmation of the CCCV's ongoing practical work (Table 1). It should be underlined that a statement of the topic for which the cell material will be used is one of the requirements of the formal application for the issuing of samples of cell lines. In addition, the guidelines for delivering the aforementioned services state that when publishing an article, the CCCV must be cited as the source of the cellular material. In connection with these requirements, there is information on the approximate number of publications in which collection cell material was used. So, for 2 years (2020–2021) 66 articles were published. Based on the analysis of received applications for 2 years, it can be concluded that collection cell lines have been used for both fundamental research and biotechnological work. So, in 2020, 117 applications were submitted, including 14 for biotechnological research, which is 12 %. In 2021, 175 applications were submitted, including 32 for biotechnological research, which is 18.5 %. Thus, there is a trend towards an increase in both the total number of applications and the number of applications for biotechnological research.

Table 1. Quantitative indicators of the CCCV's services for 2019–2021

Service name	Number of services rendered
Issuance of cell samples	675
Deposition of cell lines	13
Consultations	4

Duplicate storage and growing cells on demand are 2 more services that were implemented in 2022 and are now available to consumers from the Collection. Accordingly, SOP No. 5 "Duplicate Storage" was developed to perform the service of duplicate storage. But so far, unlike with the services presented in paragraphs 5–7, there is no data on the number of users ordering these services. As a result, numerous institutions in the Russian Federation frequently request the CCCV's services to carry out both fundamental and biotechnological research.

A detailed description of the activities of the CCCV is available on the website at www.incras-ckp.ru. This website includes a catalog of cell lines as well as a thorough description of the services offered by the CCCV and the procedures for users accessing them.

Each collection cell line has a passport, which presents its main characteristics in accordance with the international requirements for collection lines (Fig. 2). The passport is the key document that specifies the procedures to follow while working with cell cultures, as well as how to accept them into collection funds and determine their status as a collection line.

Passport of the collection cell line

Line name: given as an abbreviation using the Latin alphabet

Origin: indicates the species of the donor; the organ from which the cells were isolated; if necessary, the disease of the donor, or the method of cell transformation; a link to the publication which describes the main characteristics of the line.

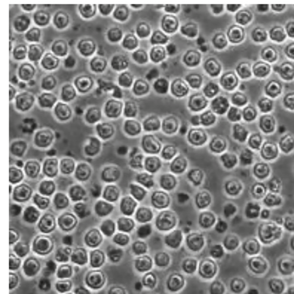
Morphology: a photo is included along with a brief description of morphology.

Cultivation method: suspension, semi-suspension, monolayer.

Cultivation conditions: standard conditions for cell growth are 37 °C, 5 % CO2, 90 % relative humidity. For

HL-60

Origin: human, peripheral blood, promyelocytic leukemia.
Nature 1977. 270: 347-349; Blood 1979. 54: 713-733; Cytology (Russ.) 1992. 34: 123.
Atlas of chromosomes of human and animal cell lines,
S.E. Mamaeva, 2002. Moscow, Scientific world.



Morphology: lymphoblast-like
Mode of cultivation: suspension
Conditions for cultivation: medium - RPMI 1640 (Initial growth is sometimes by using Iscove's DMEM)
serum - FBS 20%
subculture procedure - split ratio 1:2, optimal population density 1.0-5.0x10^5 cells/cm^2
cryoconservation - growth medium, 5%DMSO, 3.0-5.0x10^6 cells/ml in ampule
Viability after cryoconservation: 80% (0 passage, dye trypan blue)
Sterility: tests for bacteria, fungi and mycoplasma were negative
Species: karyological and isoenzymological (LDH, G6PD) analysis
Karyology: 2n=46 , variability in the range between 43-47 chromosomes, modal number of chromosomes 45, number of markers - 7 (differential dye), double minute chromosomes, number of polyploid cells 3%.

DNA profile (STR):

Locus		
Amelogenin:	X,	X
CSF1PO:	13,	14
D13S317:	8,	11
D16S539:	11,	11
D5S818:	12,	12
D7S820:	11,	12
THO1:	7,	8
TPOX:	8,	11
vWA:	16,	16

Plating efficiency: the cells cannot be plated.
Tumorigenicity: tumorigenic in nude mice
Other properties: virus susceptibility: HIV-1, HTLV-1.
Isoenzymes G6PD, B; PGM1,1; PGM3,1; ES D,1; Me-2,1; AK 1,1; GLO-1,1.
Erythrocyte rosette tests: E, 4%; EA, 17%; EAC, 1%.
Applications: differentiation, pharmacodynamics, Tumorigenicity:
Collections: : ATCC CCL 240; ECACC 88112501; DSM ACC 3; ICLC HTL 95010; SPBIC.

Fig. 2. An example of a cell line passport.

mammalian cells, these parameters are taken by default and are not specified in the passport. If other conditions are required, they are specified in the passport separately.

Medium: The name of the culture medium.

Serum: type of donor and percentage in growth medium. The main components of the growth medium are culture medium and fetal bovine serum (horse serum for some cells).

Other components of the medium: are indicated only if it is necessary to add additives to the main composition of the growth medium.

Subculturing procedure: indicate the method of detachment of cells from the substrate, the need for enzymes, as well as the multiplicity of subculturing and/or the inoculum dose.

Cryopreservation: features of the protective environment for cryopreservation; the amount of cryoprotectant, expressed as a percentage of the total cell suspension volume; the optimal concentration of cells. Standard conditions for cryopreservation (temperature decrease by 1 °C per minute to −70 °C) and further storage in liquid nitrogen at a temperature of −196 °C are not indicated. The most commonly used cryoprotectant is dimethyl sulfoxide (DMSO), the amount of which should be no more than 10 % in the growth medium prepared for cell cryopreservation. If the conditions are different from the standard, they are prescribed additionally.

Viability after cryopreservation: the ratio of living cells to the total number, expressed as a percentage. Dead cells are determined by the inclusion of Trypan blue at passage 0 after decryopreservation.

Contamination control: the results of checking the cell line for the absence of mycoplasma, bacteria, and fungi. Since cell lines are grown in collections without the use of antibiotics, determining the presence of contamination is crucial. The presence of fungi and bacteria is determined by conventional and widely used microbiological methods (Pinaev et al., 2012).

The greatest danger to cell cultures is represented by mycoplasmas. As a rule, the sources of mycoplasma contamination are the researchers themselves, components of growth media, and laboratory equipment. To date, about 30 types of mycoplasmas have been identified in cell cultures. In almost 95 % of cases, contamination occurs with following 6 types: *Mycoplasma arginini*, *M. fermentans*, *M. hominis*, *M. hyorinis*, *M. orale*, *Acholeplasma laidlawii.* Since mycoplasma do not have a noticeable detrimental impact on the cell in small amounts, it is exceedingly challenging to identify their presence at the early stages of contamination. However, some traits could change, especially the transcriptional profile of eukaryotic cells. Significant cytopathic effects, including chromatin condensation, inhibition of culture growth, cytogenetic alterations, and disruption of cell metabolism, are brought on by an increase in contamination. Mycoplasmal contamination cannot currently be determined by a single universal indicator. The use of a combination of two or three techniques is advised for the accurate identification of mycoplasma in cell cultures. The main method for detecting mycoplasmas is microbiological cultivation: inoculation on selective nutrient media; another recommended method is cell staining with fluorochromes, such as Hoechst 33258, DAPI, or olivomycin; the third method is PCR analysis (Borkhsenius, Chernova, Chernov, and Vonsky, 2002; Efremova, 2008; Uphoff and Drexler, 2013; Borkhsenius, Chernova, Chernov and Vishnyakov, 2016). The difficulty of determining mycoplasmal contamination using a single method is related to both the limitations of each method and the unique characteristics of mycoplasma (Uphoff, Gignac and Drexler, 1992; Garner, Hubbold and Chakraborti, 2000; Dvorakova, Valicek, and Reichelova, 2005; Uphoff and Drexler, 2013).

The fact that only four of the six types of mycoplasma-contaminated cells grow effectively on selected nutritional media restricts the use of only one way of microbiological seeding, which theoretically makes it feasible to identify even one colony-forming unit (CFU) of mycoplasma in culture. It has also been found that mycoplasma under various unfavorable conditions can go into an uncultivated state (Romanova and Ginzburg, 1993).

Using fluorochromes to stain mycoplasma is a technique for rapid diagnosis. The method's drawback is the difficulty of evaluation due to the potential for artifacts from the fluorescence of cell nuclei fragments and background luminescence during cultivation on an antibiotic-containing medium. As a result, the method calls for extensive operator expertise.

PCR analysis using the Universal Mycoplasma Detection Kit (ATCC, USA), which includes universal primers specific for the 16S rRNA coding region in the genome of many representatives of the Mollicutes class, makes it possible to identify more than 60 types of contaminants in cell cultures of the genera Mycoplasma, Acholeplasma, Ureaplasma and Spiroplasma. However, this method has a certain resolution (Young, Sung, Stacey, and Masters, 2010). According to different authors, using this method, mycoplasma in cell lines can be detected at a titer of 10^4–10^8 CFU. The sensitivity of the method, depending on the quality of the test sample and the specificity of the primers, can give both false positive and false negative results (McGarrity and Carson, 1982; Pruckler, Pruckler, and Ades, 1995; Young, Sung, Stacey and Masters, 2010). Therefore, the CCCV's employees always simultaneously use several methods for detecting mycoplasma in cell lines.

Control of species identity: species origin must be confirmed by one of the 3 following methods: 1. Isoenzyme analysis based on different electrophoretic mobility of certain enzymes in different taxonomic groups of animals and humans. The isoenzymes lactate dehydrogenase and glucose 6 phosphate dehydrogenase are used for testing (Margulis, 1988); 2. PCR analysis with species-specific primers; 3. Karyological analysis using different differential stains: on G-discs; C-discs, and to identify nucleolar organizers (Zakharov, Benyush, Kuleshov, and Baranovskaya, 1982; Mamaeva, 1988, 2002).

Kariology: indicate the normal diploid number of chromosomes of the donor; the limits of variability in the number of chromosomes; the modal number of chromosomes; the number of polyploids; the number of marker chromosomes, if any. The methods used are routine and differential staining.

DNA profile (STR-short tandem repeats): Each human cell line is authenticated using molecular genetic analysis of short tandem repeats situated at specific loci of a given cell line in order to validate the conformity of a given line with the source of its production and the absence of contamination by other lines. This study is outsourced in the CCCV. We send cell line samples to a company engaged in similar research (GORDIZ LLC, Moscow). This characteristic was introduced by foreign cell culture collections and is mandatory for human collection lines, subject to the publication of experimental results in most peer-reviewed foreign scientific journals.

Cloning efficiency: The ratio of the number of colonies grown to the number of cells seeded, expressed as a percentage, under conditions of very rare seeding or seeding of a single cell in a plate well.

Other characteristics: The following specific features important for the certification of cells are described in this section: sensitivity to viruses; growth characteristics; tumorigenicity; the presence of biochemical and genetic markers, etc. The basics of cell line certification are set out in a number of publications (Freshney, 1987; Hay et al., 1994).

Scope of application: indicate in which areas of fundamental and applied science this cell line is useful.

Collections: a list of national and foreign collections where this cell line can be purchased.

Scientific activities of the CCCV

For 45 years, the Collection has been conducting diverse scientific research on the biology of cells in culture. The three directions described below refer to the abovementioned third guiding principle of the CCCV's work.

1. Preparation and characterization of hybridomas. The expansion of the CCCV's contents has always been determined by the demands of fundamental research and practical problems of public health. In the 1980s employees of the CCCV derived and characterized several hybridomas producing monoclonal antibodies under the leadership and with the active participation of Irina I. Fridlyanskaya. Monoclonal antibodies have become a new generation of immunodiagnostic and immunotherapeutic drugs that have significantly expanded the possibilities of fundamental and applied biomedical research (Fridlyanskaya, 1988). During 1987–1990, 4 copyright certificates were received confirming the receipt of new hybridomas.

2. Study of the influence of cultivation conditions, cryopreservation and contamination on the karyotypic variability of cell lines. Compared to the whole body, the *in vitro* cell population has unique properties as an autonomous system. Its survival requires the formation of a balanced karyotypic structure which characterizes the stabilization stage of the cell line (Mamaeva, 1996). Cell populations *in vitro* are dynamic systems and, having lost multistage organismal control, are largely affected by external factors that contribute, in particular, to increased karyotypic variability. For many continuous cell lines, the establishment of a balanced karyotypic structure is associated with the presence in the karyotype of permanent marker chromosomes rearranged in comparison with the donor. For such lines, the most adequate way to establish a new gene balance under varying cultivation conditions is to make structural and quantitative changes in the composition of marker chromosomes. In addition to "marker" continuous cell lines, there are "marker-free" continuous cell lines. Scientific research conducted at the CCCV is devoted to identifying patterns of structural and quantitative karyotypic variability in continuous (immortalized), predominantly "marker-free" cell lines, with long-term cultivation under different conditions. The discovery of the fact that immortalized cell lines devoid of marker chromosomes are unique biological systems with distinctive karyotypic characteristics has made a substantial contribution to the understanding of fundamental cell biology concerns. Overall, the karyotypic research that was conducted allowed us to propose a theory concerning the presence of two methods for adapting cell lines to *in vitro* circumstances: (i) formation of dicentrics (telomeric associations) and changes in the regulation of gene expression; (ii) stabilization of a certain cytogenetic structure in the population based on the ratio of different structural variants of the karyotype (the number of homologous chromosomes of each morphological type). As a result, a condition known as karyotypic homeostasis is achieved in the cell population *in vitro*. The conducted cytogenetic studies also have practical significance, associated with the need for periodic cytogenetic control in cell cultures. Thus, the data concerning the recovery processes in cells after decryopreservation, associated with repair and replication, indicate the instability of the cell culture immediately after thawing, which must be taken into account when setting up experiments. It is necessary to strive for a minimum temporary contact of cells with DMSO after decryopreservation due to the established cyto- and genotoxicity of the drug. The transfer of cells to another medium, the quality, and quantity of serum can change the karyotypic structure of the cell population during long-term cultivation, which can lead to changes in other cellular characteristics studied both in fundamental and applied works. It is necessary to periodically check the presence of mycoplasma in culture, because, as the period of contamination increases, significant karyotypic changes may occur that do not appear in the early stages (Poljanskaya, Abramyan and Glebov, 1981; Poljanskaya and Efremova, 1994, 2010; Poljanskaya, 2000; Poljanskaya, Goryachaya and Pinaev 2002, 2003, 2007, 2008; Poljanskaya and Vakhtin, 2003; Poljanskaya and Koltsova, 2013).

3. Derivation and characterization of new lines of human embryonic stem cells. Since the beginning of the 21st century, much attention has been paid in the CCCV to the development and characterization of human stem cell lines, promising for their use both in fundamental studies of cell biology in culture, and in diagnostics and biomedical technologies. First of all, this affected the production of human embryonic stem cell (ESC) lines. ESC lines are a unique experimental model for fundamental research in various areas of cell and molecular biology, as well as for applied research in the field of regenerative medicine, pharmacology, and toxicology. The first continuous human ESC lines were obtained in the USA (Thomson et al., 1998). Currently, several hundred lines of human ESCs exist in different countries of the world. We have also derived several human ESC lines (Koltsova et al., 2011, 2012; Lifantseva et al., 2011; Koltsova, Yakovleva and Poljanskaya, 2016). The main characteristics of these lines are as follows: 1) unlimited cell proliferation, significantly exceeding 60 doublings of the cell population (Hayflick number) in the absence of a replicative senescence; 2) maintenance of

high telomerase activity, which ensures a stable telomere length necessary to maintain proliferative activity; 3) the presence of a normal karyotype; 4) expression of specific surface embryonic antigens SSEA-3, SSEA-4, TRA-1-60, TRA-1-81; expression of transcription factors Oct-4 and Nanog; 5) the ability for undirected differentiation into derivatives of the three germ layers and into the line of germ cells *in vitro* and *in vivo*. Human ESCs are indeed an adequate model for multilateral fundamental research.

However, a number of challenges need to be resolved before ESCs may be used in regenerative medicine. In particular, these obstacles include the following. Despite the addition of certain growth factors to culture medium, there is heterogeneity in cell cultures associated with a wide range of expressed genes belonging to different branches of differentiation. In addition, ESC cultures contain terminally differentiated cells, multipotent precursors, and undifferentiated cells all in one. Another problem is the appearance of teratomas and their probable transformation into cancerous tumors. The next problem is immune rejection of human ESC derivatives that have MHC (major histocompatibility complex) gene expression, although weaker than that of adult cells. Additionally, it should be underlined that, in contrast to most other cells, the cultivation of ESCs has unique requirements. For successful proliferation of ESCs, it is necessary to cultivate them on a layer of feeder cells. Fibroblasts of different origin act as feeder cells. Feeder cells express factors that promote the growth of ESCs. As a result of such cultivation, there are 2 dynamic living systems, each of which depends on the cultivation conditions. Thus, the conditions created for ESCs are much more variable than for other cells cultivated on a stable substrate, which can enhance the variability of ESC cell lines during long-term cultivation. Moreover, any allogeneic feeder does not eliminate the risk of infections for ESCs (Martin, Muotri, Gage, and Varki, 2005; Stojkovic et al., 2005; Skottman, Dilber, and Hovatta, 2006; Choo et al., 2008; Chen et al., 2009; Kubikova et al., 2009; Fu et al., 2010). An important task associated with the use of these cells for biomedical purposes is the development of feeder-free culture systems. Various substrates developed for the cultivation of human ESCs have been analyzed in detail at the CCCV. According to the data taken into account, a single, ideal feeder-free cultivation system has not yet been discovered. Thus, each system has disadvantages: 1) the risk of contamination (use of animal ingredients); 2) the high cost of experiments, which complicates the production of large volumes of biomaterial (serum-free media, recombinant ECM proteins and peptides); 3) the necessity of screening a variety of artificial substrates to determine the best option (synthetic substrates); 4) variation in the quantitative composition of ECM (substrate from feeder cells). It is possible that, based on the genetic uniqueness of each ESC cell line, in addition to optimizing the cultivation conditions as a whole, an individual approach is required, associated with the use of some methodological modifications (Koltsova et al., 2012).

ESCs have a high level of genomic instability compared to other stem cells. It is crucial that some genetic alterations in ESCs are adaptable. These adaptive karyotypic changes, the frequency of which increases significantly with long-term cultivation, on the one hand, contribute to the constant self-renewal of ESCs, and, on the other hand, contribute to malignancy. The potential for adaptive modifications in ESCs to transition to differentiated derivatives, as well as the occurrence of genomic changes under the influence of altered cultivation circumstances associated with directed differentiation, both facilitate genomic instability in differentiated derivatives (Poljanskaya, 2014).

Currently, the ethical and biosafety issues of using human ESCs for regenerative medicine are widely discussed. Thus, the use of human ESCs in regenerative medicine is associated with the destruction of the early embryo, which creates ethical problems for their biomedical use. The development of methods for the production and analysis of human induced pluripotent stem cells (iPSCs), similar in properties to ESCs, has removed the ethical problem associated with the destruction of early human embryos. But, nevertheless, for both types of stem cells, there remain problems of long-term biosafety of their use in regenerative medicine, associated with the likelihood of tumor transformation during *in vivo* transplantation. The high cost of deriving lines of these stem cells is also indicated (Wu et al., 2016; Rohban and Pieber, 2017; Volarevic et al., 2018). Currently, one interesting application of human iPSCs for understanding disease mechanisms and medication trials involves obtaining them from individuals with different disorders.

Below are descriptions of the CCCV's other ongoing research projects

4. Derivation and characterization of new lines of human mesenchymal stem cells. Having derived and characterized several ESC lines from different donors, including lines created under feeder and non-feeder conditions, we moved on to the generation and characterization of various human mesenchymal stem cell (MSC) lines which are more stable. Currently available data show that the incidence of instability in MSCs is substantially lower than in ESCs (Poljanskaya, 2014). MSCs, after certain testing, can be used relatively safely in cell therapy and pharmaceutical research (Kita et al., 2010; Zhang et al., 2012; Leyva-Leyva et al., 2013; Ferro, Spelat and Baheney, 2014; Sharma, Venkatesan, Prakhya, and Bhonde, 2014). Because they are non-immortalized, diploid cell populations, human MSC lines provide an easy way to examine biological

processes both in a healthy organism and under pathological conditions. The utilization of MSCs from various origins in biomedical research on a variety of disorders is now growing significantly (Adak et al., 2021; Albu et al., 2021; Taei et al., 2021; Xiao et al., 2021; Wangler et al., 2021; Zhang et al., 2021).

To date, the CCCV has received and characterized 16 lines of MSCs of different origin, of which 11 are placed in an annually updated catalog. Sources of MSCs can be conditionally divided into postnatal (adult), embryonic and extra-embryonic. The list of received lines is given in Table 2.

Table 2. List of human mesenchymal stem cell lines stored in the CCCV

Sources	Name	Origin of MSCs
Adult MSCs	DF-1	Skin of the eyelids a 37-year-old female donor
	DF-2	Skin of the eyelids a 45-year-old female donor
	DF-3	Skin of the eyelids a 53-year-old female donor
	FRSN	Foreskin of 3-year-old children
	FRSN-1	Foreskin of 2.5-year-old children
	MSC-DP	Milk tooth pulp
	MSC-GING	Gums of a 37-year-old female donor
	ADH-MSC	Epicardial adipose tissue derived during coronary artery bypass grafting
Embryonic MSCs	FetMSC	Bone marrow of a 5–6-week-old embryo
	M-FetMSC	Limb muscle of a 5–6-week-old embryo
	SC5-MSC	ESC line (SC5)
	SC6-MSC	ESC line (SC6)
Extra-embryonic MSCs	MSCWJ-1	Wharton's jelly of the umbilical cord
	MSCWJ-2	Wharton's jelly of the umbilical cord
	MSC-PL-1	Placenta
	MSC-PL-2	Placenta

The status of MSCs obtained from any source is defined by a number of mandatory features, in accordance with the standards of the International Society for Cell Therapy: adhesion to cultural plastic; active proliferation; expression of a certain panel of surface antigens or markers (CD44, CD73, CD90, CD105, and HLA-ABC), and the absence of expression of antigens uncharacteristic of MSCs (CD34 and HLA-DR); the ability to differentiate in the osteogenic, chondrogenic, and adipogenic directions; a normal human karyotype according to established criteria (Dominici et al., 2006; Sensebé et al., 2010).

Understanding the mechanisms of biological processes in the cell and expanding the applications of MSCs in regenerative medicine require a comparative study of the characteristics of human MSCs, which are crucial in maintaining the status of MSCs, as well as other characteristics responsible for the most significant cellular processes. The importance of these studies is associated with the peculiarities of the interaction of cells with their unique microenvironment, characteristic of a particular tissue. The microenvironment regulates proliferation, survival, migration, aging, differentiation potential, and other cellular functions through intercellular interactions and various bioactive molecules (Cox, Krauss, Balis and Dancis, 1972; Hooper and Subak-Sharpe, 1981; Sharovskaya, Lagarkova, Kiselev and Chilakhyan, 2009; Gattazzo, Urciuolo and Bonaldo, 2014; Choi et al., 2015; Darnell et al., 2018; Niedernhofer et al., 2018; Nimiritsky et al., 2018).

The microenvironment is constantly impacted by environmental, genetic, and epigenetic variables. Thus, the origin or source of MSCs may determine their functional characteristics. A comparative analysis of the characteristics of MSCs isolated from different sources indicates quantitative differences between the lines in terms of the most important characteristics. In particular, interline differences were found in differentiation potential, in growth characteristics, in the character of replicative senescence (RS), and in karyotypic instability (Stanko, Kaiserova, Altanerova and Altaner, 2014; Li et al., 2018; Poljanskaya, 2018; Jin et at., 2019; Musorina et al., 2019; Koltsova et al., 2020; Semenova et al., 2021; Shin et al., 2021; Tai et al., 2021; Yigitbilek et al., 2021). The reasons for the observed differences may be: 1) epigenetic factors associated with the culture conditions or with the microenvironment in which these cells existed before they were placed in vitro conditions; 2) genetic factors associated both with genetic differences between donors and with the initial genetic predisposition of specific MSC lines to cytogenetic instability (Poljanskaya, 2018).

Particular focus should be placed on RS and the cytogenetic stability of MSCs. RS in human MSCs, like in any diploid non-immortalized lines, occurring during long-term cultivation of cell populations, is a complex dynamic process induced by genetic and epigenetic factors. It is marked by numerous notable alterations in cellular characteristics, in particular: a decrease or cessation of proliferation due to the accumulation and arrest of proliferating cells in the G1 phase of the cell cycle; morphological changes, expressed in an increase in size and the formation of cells with a flattened morphology; increased activity of the RS-associated SA-β-galactosidase enzyme; decreased cell motility or cell migration; in-

creased expression of tumor suppressor genes; shortening of telomeres; a decrease in differentiation potential; accumulation of reactive oxygen species, contributing to the loss of cellular homeostasis; a decrease in the activity of antioxidants; changes in the composition of the secretome, including the secretion of pro-inflammatory cytokines, proteases, and other factors that together form SASP (senescence associated secretory phenotype). The RS process in MSCs begins at early passages and gradually increases during cultivation, entering the active stage (Wagner et al., 2008; Geissler et al., 2012; Redaelli et al., 2012; Bertolo et al., 2015; Savickienė et al., 2016; Turinetto, Vitale and Giachino, 2016; Danisovic et al., 2017; Koltsova et al., 2017; Koltsova et. al., 2019; Alessio et al., 2018; Poljanskaya, 2018; Truong, Bui and Van Pham, 2018 ; Yu et al., 2018; Musorina et al., 2019; Bobkov et al., 2020; Bobkov and Poljanskaya, 2020; Ratushnyy, Ezdakova and Buravkova, 2020).

It should be underlined that there are various stages involved in the cultivation of MSCs. The initial stage starts at passage 0 and ends at passages 5–6. This time period may be characterized by cytogenetic changes. Thus, after the isolation of a cell population from a tissue fragment and the loss of multistage organismal control at this stage, cells adapt to in vitro conditions at early passages, which is apparently a stressful situation for them. At this stage, karyotypic instability may increase, which gradually normalizes by passages 5–6 (Stultz et al., 2016). Chromosomal abnormalities detected at passages 6–8 may reflect incomplete normalization of karyotypic instability. It is also feasible that the *in vivo* cell population still contains a few aberrant cells in tiny numbers, with the likelihood that this number will rise with further *in vitro* cultivation (Wang et al., 2005). As a result, there are two categories of cytogenetic disorders that can be used to describe the entire RS process: (1) those that have as their source changes that are initially occurring *in vivo* or at an early *in vitro* stage, and (2) those that are appear during the RS phase. It is known that RS is associated with a decrease in DNA repair due to a decrease in the expression of the corresponding genes (Niedernhofer et al., 2018; Yu et al., 2018). A consequence of a decrease in reparative processes in DNA is, in particular, a violation of cytogenetic stability, which is the most important factor determining the nature of many cellular processes. The International Society for Cell Therapy and the Cell Products Working Group recommend that cell cultures with at least 10 % clonal chromosomal rearrangements (a rearrangement occurring in 2 or more cells of a minimum 20 cells by chromosome differential G-staining) be excluded from workflow as abnormal lines (Meisner and Johnson, 2008; Shaffer, Slovak, and Campbell, 2009; Barkholt et al., 2013). It should be emphasized that this recommendation does not apply to the quantitative analysis of non-clonal rearrangements (rearrangement occurring in one cell). However, one should pay attention to such lines, because there are examples of the transformation of non-clonal disorders into clonal chromosomal rearrangements. In the CCCV's findings, non-clonal rearrangement transforms into a clonal rearrangement that is repeated in different cells when either the number of cells analyzed (DF-1 line) or the length of cultivation (ADH-MSC line) increases, providing indirect evidence of the continued rise in karyotypic instability (Krylova et al., 2016; Musorina et al., 2019). Long-term cultivation may result in an increase in non-clonal karyotypic abnormalities, which could lead to intermediate stages of carcinogenesis (Borgonovo et al., 2014).

It should be emphasized that the cytogenetic analysis of different human MSCs indicates that the emerging karyotypic changes, as a rule, are not adaptively beneficial for the cell population under *in vitro* conditions and do not contribute to its immortalization (Tarte et al., 2010; Redaelli et al., 2012; Zaman, Makpol, Sathapan and Chua, 2014; Kim et al., 2015; Koltsova et al., 2017; Koltsova et al., 2019; Koltsova et al., 2020; Musorina et al., 2019). Chromosomal abnormalities that appeared at early passages behave differently during long-term cultivation. Thus, negative selection may be to blame for the increase in some abnormalities and the disappearance or decline in others (Redaelli et al., 2012; Kim et al., 2015; Koltsova et al., 2017; Krylova et al., 2017; Nikitina et al., 2018). Apparently, the fate of cytogenetic disorders during long-term cultivation is determined by the degree of adaptability of a particular chromosomal change.

Thus, human MSCs are the most important object for fundamental research and, as noted above, for biomedical applications. It should be emphasized, however, that there are data suggesting the possibility of their influence on tumor growth and metastasis due to undesirable differentiation that occurs due to MSC plasticity during transplants, despite the numerous positive effects when administering MSCs to patients for the treatment of certain diseases. In particular, undesirable differentiation of transplanted MSCs can contribute to the suppression of the antitumor immune response and the creation of new blood vessels, which can promote tumor growth and metastasis. Given this, it is necessary to focus research in preclinical trials on long-term monitoring of MSCs used in animal models (Volarevic et al., 2018). This point of view is in line with our justification, which was outlined above and in greater detail earlier (Poljanskaya, 2018), according to which a tendency toward genetic instability must be observed over a lengthy period of time, particularly during long-term culture. This topic is the subject of current research, which is intended to expand by the derivation and characterization of new MSC lines, and population analysis of a number of MSC lines.

5. Comparative characteristics of the activity of matrix metalloproteinases and the content of ECM proteins

during differentiation of different MSCs. Currently, extensive research is underway, focusing on the identification of factors involved in the regulation and control of the specific properties of MSCs, allowing their use in regenerative medicine. One of the areas of research into the functional features of MSCs is elucidation of the role of matrix metalloproteinases (MMPs) in the processes of their differentiation. MMPs are a family of Ca- and Zn-dependent endopeptidases that regulate the activity of many biological molecules by cleaving or blocking them. MMPs affect such fundamental cellular processes as proliferation, apoptosis, differentiation, etc. MMPs are involved in tissue remodeling and organ development, specifically modulating signaling pathways through interaction with substrates of different nature, as well as by rearranging the extracellular matrix (ECM) (Page-McCaw, Ewald and Werb, 2007; Kessenbrock, Plaks and Werb, 2010). ECM is subject to constant renewal due to the processes of synthesis and degradation. MMPs, which can cleave all ECM proteins, are responsible for these processes' major functions. MMPs are synthesized by different types of cells — fibroblasts, keratinocytes, phagocytes, lymphocytes, and transformed cells. All members of the MMP family share common features: they share common amino acid sequences, are synthesized as inactive proenzymes, and include zinc as a cofactor. Based on substrate specificity, MMPs are grouped into subfamilies: collagenases, gelatinases, stromelysins, matrilysins, and membrane-type MMPs (Nagase and Woessner, 1999).

The active participation of MMPs in the regulation of MSC differentiation in different directions has been convincingly analyzed in a number of works (Mannello, Tonti, Bagnara and Papa, 2006; Ghajar et al., 2010; Schneider et al., 2010; Polacek et al., 2011; Sillat et al., 2012; Ould-Yahoui et al., 2013; Sassoli et al., 2014; Tratwal et al., 2015). These studies were conducted on MSCs obtained from various tissues and donors. We are unaware of data on the comparison of MMP activity during the differentiation of MSCs derived from the same donor, but from different tissues. Previously, in the CCCV we derived 2 lines of MSCs from the bone marrow and from the limb rudiment of an early human embryo. Despite the confirmation of the MSC status for both lines, a number of differences were found between them related to growth characteristics and differentiation potential. The results obtained suggest the influence of different microenvironments in which the cells were in the body before they were transferred to culture (Krylova et al., 2014). In this regard, for the first time, a comparative analysis of MMP activity in MSCs derived from the same donor, but from different tissues, was carried out.

Simultaneously with the solution of this problem, a comparative study of the dynamics of MMP activity during the differentiation of these lines in 2D and 3D conditions was carried out. Currently, cellular spheroids derived from MSCs of various origins are widely studied. The conditions for 3D cultivation of MSCs are much closer to the physiological conditions of tissues in the body than monolayer cultivation. It is possible to study natural cellular processes like proliferation, differentiation, and apoptosis in the absence of exogenous (artificial) matrices because intercellular interactions and cell-ECM interactions are enhanced in cellular spheroids (Baraniak and McDevitt, 2012). Cellular spheroids of MSCs have an enhanced anti-inflammatory effect, increased differentiation potential, and increased expression of cytokines and pluripotent genes. They are therefore a promising subject for research into the fundamentals of organogenesis as well as for application in regenerative medicine (Alimperti et al., 2014; Bogdanova-Jatniece, Berzins, and Kozlovska, 2014; Guo, Zhou, Wang and Wu, 2014; Li et al., 2015).

In the CCCV, we derived cell spheroids from monolayer MSC lines FetMSC and M-FetMSC. Comparative analysis of the characteristics of these lines during 2-dimensional (2D) cultivation in a monolayer and 3-dimensional (3D) in spheroids confirms the status of MSCs for cell spheroids and indicates a partial expansion of their differentiation potential compared to monolayer cultures (Krylova, Musorina, Zenin and Poljanskaya, 2015).

Considering that MMPs are the most important regulators of cellular processes, in particular, differentiation, the aim of that study was to conduct a comparative analysis of MMP expression in 2 lines of MSCs isolated from different tissues of the same genetic individual, when cultivated in two ways: in a monolayer (2D) and in cellular spheroids (3D). As a result, the ability of MSC lines isolated from the bone marrow (FetMSC) and the limb bud (M-FetMSC) of an early human embryo and cell spheroids derived from them to differentiate in the adipogenic and osteogenic directions was confirmed. The presence of MMP-9, MMP-2, and MMP-1 activities during these differentiations in both lines was shown. A comparative analysis of the dynamics of the activities of these metalloproteinases during adipogenic and osteogenic differentiation showed interline differences, as well as differences between monolayer cultures (2D) and cell spheroids (3D).

In particular, a correlation was shown between the level of adipogenic differentiation and the level of MMP-9 and MMP-2 activity in both lines during 2D and 3D cultivation (in the FetMSC line, these indicators are higher than in the M-FetMSC line). The low level of adipogenic differentiation in the line M-FetMSC (2D) corresponds to a reduced activity of MMP-9 and MMP-2, and an increase in the level of differentiation (3D) leads to a significant increase in the activity of both proteinases. An opposite effect was found when analyzing the activity of MMP-1. Thus, a low level of adipogenic differentiation in the line

M-FetMSC (2D) corresponds to an increased activity of MMP-1, and an increase in the level of adipogenic differentiation (3D) leads to a decrease in MMP-1. The findings imply that MMPs have an impact on MSC differentiation in both positive and negative ways, which is related to the mechanism by which MMPs affect differentiation processes. The observed differences in MMP activity between FetMSC and M-FetMSC lines throughout differentiation can be attributed to the influence of various starting microenvironments as well as various ECM characteristics in 2D and 3D cultures (Voronkina et al., 2016).

In the CCCV we examined the dynamics of the activities of various MMPs, the expression of chondrogenesis markers, and some ECM components during chondrogenic differentiation of the MSCWJ-1 cell line isolated from Wharton's jelly of the human umbilical cord under 2D and 3D conditions in order to study the mechanisms of MSC differentiation. The main conclusion was that a more active process of chondrogenesis takes place in cell spheroids (3D) than in a monolayer (2D) culture (Voronkina et al., 2018).

Further, the analysis of MMP activities was carried out in the CCCV in connection with RS of different cell lines. Available research on this topic is currently fragmented and in a state of accumulation of results (Bobkov and Poljanskaya, 2020). Recently, we have carried out for the first time a detailed comparative study of the dynamics of MMP activity and the content of ECM proteins in 3 lines of human MSCs derived from different sources: from Wharton's jelly of the umbilical cord (MSCWJ-1), from the skin of the eyelids (DF-2), and from epicardial adipose tissue (ADH-MSC), during long-term cultivation, including RS (Voronkina et al., 2020). Changes were observed at late passages compared to early passages as a consequence of the study of the concentration of ECM proteins (type I collagen and fibronectin), as well as the activities of MMP-1, -2 and -9, in the process of RS. The results obtained indicate both the differences between the lines in the activity of one MMP and the differences in the same line of different MMPs. In general, in the process of RS, differences were found between the 3 lines in the nature of changes in the content of type 1 collagen, fibronectin, and MMP activities. Thus, MSC's RS, in addition to the factors listed above, can also be characterized by changes in the ECM composition and MMP activities. The varied microenvironment which the cells were placed in before being transferred to culture appears to be the cause of the subsequent interline variations. This is indirectly confirmed by a previous study performed in the CCCV which shows interline differences in the activity of different MMPs during osteogenic and adipogenic differentiation of two cell lines (FetMSC and M-FetMSC) obtained from the bone marrow and limb muscle of one donor, a 5–6-week-old embryo (Voronkina et al., 2016).

Another evidence in favor of the role of the microenvironment in the observed interline differences may be the results obtained in the ADH-MSC line. It is important to note that the ADH-MSC cell line significantly differed from the other two lines in terms of the RS rate, the content of ECM proteins, and MMP activities. Perhaps the reason for this discrepancy is that the cells were obtained from a donor with a heart disease during coronary artery bypass grafting, i.e., there was not only a different microenvironment due to different localization of the isolated cells, but also an unhealthy microenvironment in which they were even before being transferred to the *in vitro* state.

Further research plans are related to a comparative analysis of the dynamics of MMP activity during long-term cultivation, including the active stage of RS, simultaneously with adipogenic differentiation in newly derived MSC cell lines isolated from different regions of the same organ. This could be done from an extraembryonic organ — the placenta. It is known that the human placenta has a complex cellular composition. It is possible that different areas of the placenta have physiological features. Although these lines hold the status of MSCs, prior comparative investigation of their features revealed that during long-term cultivation, considerable interline differences, including the stage of active RS, were revealed (Koltsova et al., 2020).

6. Actin cytoskeleton reorganization during replicative senescence of human MSCs. The recent tremendous growth of biomedical research using human MSCs of various origins was underlined above. In this context, it is necessary to intensify basic research on the many characteristics of these cells at various stages of their lives, particularly the RS stage.

Despite the fact that the study of RS in connection with various cellular processes is widely represented by researchers from different countries, work in this direction does not stop. In particular, the reorganization of the structure of the actin cytoskeleton during RS is one of the poorly studied characteristics of human MSCs. Given that the cytoskeleton is involved in many crucial cellular functions, particularly cell motility (migration), the relevance of such studies is crucial. Changing the nature of cell migration is one of the hallmarks of RS. Cell migration occurs in close contact with the ECM, on which the cells are spread, and depends on the organization of the actin cytoskeleton. ECM consists of various proteins synthesized by the cells themselves and is one of the most important regulators of cellular processes, representing the microenvironment in which cells exist. Cell migration is actively involved in biomedical processes, exerting a trophic effect through the secretion of bioactive factors that change the microenvironment of damaged cells and, thereby, contribute to the improvement of tissue repair (Bobkov and Poljanskaya, 2020).

Currently, research on the molecular mechanisms of cytoskeleton reorganization during long-term cultivation, including RS, conducted in various human and animal cell types, is fragmented and at the stage of accumulating experimental results (Larsen, Tremblay and Yamada, 2003; Le Clainche and Carlier, 2008; Wang and Jang, 2009; Geissler et al., 2012; Özcan et al., 2016; Turinetto, Vitale and Giachino, 2016; Moujaber et al., 2019).

Since 2019, the CCCV has begun systematic molecular studies on the reorganization of the actin cytoskeleton during RS in human MSC lines isolated from various sources. Techniques were created to investigate how the actin cytoskeleton changed as RS progressed in human MSC lines. It is known that small GTPases of the Rho family, including RhoA, Rac1, and Cdc42, are components of signaling pathways responsible for the most important cellular functions, such as rearrangement of the cytoskeleton, regulation of cell motility, regulation of transcription of reactive oxygen species, and many others (Raftopoulou and Hall, 2004; Tkach, Bock, and Berezin, 2005; Narumiya, Tanji, and Ishizaki, 2009; Spiering and Hodgson, 2011; Kim et al., 2018). Studies on the small GTPase RhoA's role as one of the primary elements in the actin cytoskeleton's remodeling have been conducted in the CCCV. In one of such studies, immunofluorescence techniques and confocal image analysis of cells were applied. These techniques included estimate of the local connected fractal dimension coefficient for actin cytoskeleton structure assessment (Bobkov et al., 2020).

Priority results were obtained in the CCCV on the study of cell motility and organization of the contractile apparatus of MSC cells derived from Wharton's jelly of the human umbilical cord (MSCWJ-1) during RS. Simultaneously, the colocalization dynamics of F-actin and actin-binding proteins (myosin-9, alpha-actinin-4, RhoA) were studied. The results show that nuclear-cytoplasmic redistribution of RhoA occurs during RS, with the maximum nuclear localization of RhoA at passage 15. At this time point, the colocalization of myosin-9/F-actin and alpha-actinin-4/F-actin decreases. Using an automated *in vivo* confocal cytometry system, it was found in cell line MSCWJ-1 that changes in cytoskeletal organization correlate with cell motility characteristics. According to quantitative measurement of MSCWJ-1's cell motility, the rate of cell movement decreased from 9 to 36 passages. The studied factors (cytoskeleton structure and cell motility) indicate that the RS process consists of 3 stages. The first stage lasts from decryopreservation to passage 15, inclusive, and is characterized by the accumulation of actin-binding proteins in the form of aggregates that are not part of the cytoskeleton structure; accumulation of nuclear RhoA; an increase in tortuosity during cell movement. The second stage lasts from passage 15 to 28, inclusive, and is characterized by: an increase in the structural integrity of the actin cytoskeleton; the release of RhoA and alpha-actinin-4 from the nucleus; reducing the tortuosity of the path of cell movement. The third stage lasts from passage 28 to 36 and is marked by a slight decrease in the structural integrity of the actin cytoskeleton; almost complete absence of nuclear localization of RhoA; a decrease in the speed of cell movement (Bobkov et al., 2020).

Further work was continued on the analysis of the nuclear-cytoplasmic redistribution of the small GTPase RhoA during RS in two human MSC lines: DF-2 (eyelid skin of an adult donor); ADH-MSC (epicardial adipose tissue of an adult donor). It should be emphasized that the ADH-MSC line, unlike the previous lines, was isolated during coronary artery bypass grafting, i.e., from an unhealthy donor and an unhealthy organ (heart). Priority findings showed that a wave-like redistribution of GTPase RhoA between the nucleus and cytoplasm takes place during the RS process in the DF-2 line. But in the stage of active RS, there is a significant decrease in RhoA in the nucleus and accumulation in the cytoplasm. These data are consistent with the results obtained earlier on the MSCWJ-1 line (Bobkov, Polyanskaya, Musorina and Poljanskaya, 2022). It was also shown that in the process of RS in the ADH-MSC line, the actin-binding protein alpha-actinin-4 and signaling protein RhoA are redistributed from the cytoplasm to the cell nucleus, which is accompanied by changes in the organization of the actin cytoskeleton. Between passages 8 and 11, the actin cytoskeleton partially disassembled, and by passage 17, i.e., the period of active RS, the actin cytoskeleton is fully reassembled. Results comparing the aberrant ADH-MSC line to normal lines (MSCWJ-1 and DF-2) reveal that RhoA intracellular distribution during RS follows the opposite pattern. Apparently, the observed differences are associated with the microenvironment in which the cells were located prior to the isolation of lines. It should be noted that other parameters were changed in the ADH-MSC line as compared to MSCs from healthy sources. Thus, there is a change in MMP activity, premature RS, during which karyotypic heterogeneity increases, including the appearance of clonal chromosomal rearrangements (Musorina et al., 2019; Goncharova et al., 2021). According to other studies, MSC lines obtained from patients with different disorders differ in a variety of ways from MSC from healthy donors (Cardenes et al., 2018; Huang et al., 2019; Costa et al., 2021). Two lines of MSCs from different origins—DF-2 isolated from the skin of an adult donor's eyelids and FetMSC isolated from the bone marrow of an early embryo — are currently being studied to determine how the actin cytoskeleton and cell motility are affected by the activator and inhibitor of the small GTPase RhoA during the process of RS.

Summarizing the findings of the CCCV's scientific activity during the course of its 45-year existence, we can

say that the organization maintained a high level of scientific activity over the whole-time frame. Work has been done in six areas over the course of its existence: 1) deriving and characterizing hybridomas; 2) studying the effects of cultivation conditions, cryopreservation and contamination on the karyotypic variability of cell lines; 3) deriving and characterizing new lines of human embryonic stem cells; 4) deriving and characterizing new lines of human mesenchymal stem cells; 5) comparative study of the activity of matrix metalloproteinases and the content of ECM proteins during differentiation and RS of different MSCs; 6) studying the reorganization of the cytoskeleton during RS of human MSCs. Research work on the last three topics is ongoing. The results obtained on all 6 topics are important both for deepening fundamental knowledge in the field of cell biology and for their use in applied biomedical technologies. It should be noted that in scientific activity, the Collection relies not only on its own employees, but also employs specialists from other institutions. Thus, cytogeneticists from the Laboratory of Morphology INC RAS Tatiana K. Yakovlevaand Victoria I. Turilovamade a huge contribution to the scientific development of the Collection.

Information and publishing activities of the CCCV

After the demise of the RCCC, the CCCV, along with other collections of the All-Russian (previously USSR-wide) collection of cell cultures, engages in active informational operations. As a result of the joint efforts of different collections, an information database was created on cell lines available in the collection funds. In 1991, the first catalog of cell lines of the USSR-wide Collection of Cell Cultures was published. This catalog contained a list of cell cultures with indication of their individual properties and information about the source of receipt (Pinaev, editor, 1991). A comprehensive catalog of all the cell lines that make up the RCCC collections was created in 1999 under the initiative of George P. Pinaev (Pinaev, Poljanskaya, Sakuta and Bogdanova, 1999). The catalog contained all passports of cell lines, in accordance with international requirements. The catalog was published in Russian and English. It was republished in 2004 due to the high demand for the information it contains.

Since 1986, under the auspices of the Institute of Cytology of the Russian Academy of Sciences, the annual "Cell Cultures Collection Information Bulletin" (ISSN 2077-6055) has been published. The Collection contains experimental papers and reviews on the problems of cell biology. A total of 35 issues have been published. Since the beginning of the 1990s, the editorial board included: George P. Pinaev, Margarita S. Bogdanova (editor), Galina G. Poljanskaya. In recent years, Anna M. Koltsova has been included in the editorial board. Since 2020, due to the reorganization of the INC RAS, the publication of the Bulletin has been discontinued. Numerous educational and methodological digests have been published over the years of the USSR-wide (Russian) collection of cell cultures existence that help with theoretical training and mastering the abilities of practical work with cell cultures (Troshin, editor, 1984; Pinaev, editor, 1988; Mamaeva, 2002; Pinaev, Bogdanova, editors, 2008; Pinaev et al., 2012; Poljanskaya and Musorina, 2018; Poljanskaya et al., 2019).

Participation of the CCCV in scientific societies and scientific programs

Over the years, the USSR-wide (Russian) Cell Culture Collection was a member of the European Tissue Culture Society (ETCS), the World Federation of Culture Collections, and the European Culture Collections Organization (ECCO). Information about the Collection funds is presented in the International Catalogs of Cell Lines (Parodi, Aresu, Mannielo and Romano, 1993; Hay et al., 1996).

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Therefore, the CCCV is the most significant institution supporting the growth of basic and practical biomedical research in the Russian Federation.

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