**Lecture notes on the discipline "Cell differentiation"**

**Lecture 1. What is a stem cell? Asymmetric division and differentiated progeny stem**

**Stem cells, the current state of research on the problem and the main directions of their development. From theory to clinical practice**

For the first time, the term "stem cells" (SC) was used by the famous Russian scientist, histologist and embryologist A.A. Maximov in 1908 city of in his speech at the International Congress of Hematology in Berlin. In his works, he put forward the idea of ​​the origin of hematopoietic cells and part of connective tissue cells from lymphocyte-like cells [1].

Since then, the doctrine of SCs, their nature, functions and place in the life processes of humans and animals has been widely developed and replenished with a huge number of discoveries.

One of the decisive milestones on this path was the work of A.Ya. Friedenstein and his school [2-4].

It can be said without exaggeration that research on KS problems is one of the most important for theory and clinical practice. It is not by chance that in 1999 the journal Science recognized the discovery of the SC as the third most important event in biology after the deciphering of the DNA double helix and the Human Genome program [5].

It is known that biological systems, including at the organismal, tissue and cellular levels, are built on the principle of multiple insurance and compromises between maximum economy and “sufficient” efficiency of their function, which ensures their optimal integral efficiency [6].

Another example of the mechanism of checks and degrees of freedom in the choice of paths for the development of events in embryogenesis can serve as noted in the article by I.N. Saburina and V.S. Repin, antagonistic relationships of a number of genes at the stages of embryogenesis: “During gastrulation and the formation of meso-, endo- and ectoderm, there is an irreversible loss of expression of the canonical genes that control the pluripotency of the genome of true ESCs: Oct4, Nanog , FoxD3, Rex-1” [7].

One of the adaptations in the kinetics of SC division is the asymmetric distribution of cell lines in progeny cells that retain multipotency and cells that undergo differentiation and therefore drop out of the SC pool .

**The phenomenon of asymmetric stem cell division**

Asymmetric division of the SC (ADSC) is a prerequisite for the normal development of the body of animals and humans. This is one of the most ancient adaptations through which multicellular organisms are built from a zygote [8].

This mechanism is observed not only in higher organisms, but also in such simpler forms as Drosophila, etc. [9].

The construction of tissues and organs of multicellular organisms is carried out by successive cycles of cell division, some of which retain their multipotent potential, that is, remain in the pool of SCs, the other begins to differentiate and thus lose their multipotency .

Thanks to ADSC, self-renewal of SCs occurs, retaining this ability throughout life and maintaining the overall quantitative constancy and , at the same time, the balance of both the SC reserve and the replenishment of the population of differentiated cells, which is equally important for maintaining the homeostatic status of the organism [9].

Violation of this balance can lead to serious consequences. Thus, a decrease in the release of SA from the ADS process can lead to the development of tissue destruction and atrophic processes, while the overproduction of SA is fraught with the development of tumors [10-12].

Drosophila SCs were used as a classical model for studying the ADSC mechanisms .

The data of studies carried out on Drosophila testified the existence of two alternative mechanisms of ADSC: genetically determined ( cell-intrinsic ) and determined by localization in the area of ​​niches in which the influence of the microenvironment is realized.

The mechanisms by which these two variants of ADSC are carried out have been extensively investigated in the Drosophila neuroblast .

The first variant of ADSC is realized in two daughter cells, which divide asymmetrically along the apical- basal axis. At the same time , the expression of the *Numb*, *Miranda,*and *Prospero*genes responsible for ADSC is observed in the basal complex of the neuroblast . One of the daughter cells divides one more time and then turns into a ganglion differentiated cell.

As for the second variant of ADSC, in *Drosophila*an example of ADSC is male and female germline SCs (HSCs), which are controlled by external determinants. HSCs are found in a microenvironment that determines the characteristics of S.K. This complex of microenvironmental factors that determine the characteristics and behavior of SCs was named the niche by S.K. A niche creates an environment in which, thanks to the signaling system, the behavior of SCs is determined and, in particular, their expansion is limited within physiological limits [13].

At the same time, it is indicated that the ADSC mechanisms in vertebrates are more complex than in invertebrates and, therefore, may have certain peculiarities in comparison with simpler forms of organisms [13].

**On the plasticity of SC, its significance for the organism and on the mechanisms of expanding its limits**

First of all, let us note that multipotency itself is a kind of multiplication of cell types (types of cell lines) formed as a result of cell division (types of daughter cells) and , ultimately, their differentiation. So, for mesenchymal SCs (MSCs), these are bone, cartilaginous and fat cells [14].

Until recently, it was a question of certain limits of multipotency for SCs of an adult organism, and therefore, this limited multipotency was taken as the main difference between SCs in an adult organism and pluripotency of embryonic cells [15].

It was believed that, in contrast to pluripotent embryonic SCs, which are capable of giving rise to all cell types, SCs of adult mammals under normal conditions are able to differentiate only within the limits of their tissue belonging.

However, new data obtained in recent decades indicate the opposite - that in reality, the plasticity of adult SCs goes far beyond the limits of the tissues to which they belong.

The development of a fundamentally new experimental strategy based on the removal of SCs from the system of genetically determined dynamic relationships inherent in the ontogeny of multicellular organisms, including humans and animals, made it possible to reveal the possibility of reversing the phenotypic characteristics of SCs with their return to the plasticity lost with age.

Thus, evidence was obtained for the plasticity of bone marrow SCs, in particular, the ability to transdifferentiate hematopoietic bone marrow SCs and bone marrow MSCs [16].

M. Eglitis and E. Mezey in 1997 g. published data showing that the hematopoietic bone marrow cells of adult mice transplanted in the brain of these animals, gave rise to glial cell lines [17].

In another work, the ability of cells derived from the stroma of the bone marrow of an adult to produce a line of SCs differentiating towards cells with a neural phenotype expressed in the expression of mRNA , nestin , Enolase 2 and associated with the cytoplasmic microtubular system by protein 1b (MAP1b) was demonstrated. Over time, in some cases, the activation of neurogenic transcription factors, such as Engrailed-1 and Nurr1, was noted [18].

The data confirming the similar ability of human skeletogenic cell progenitors were also published by S. Schultz and P. Lucas [19].

Thus, the results of recent studies have made it possible to switch to the most effective methods in the development of protocols that solve the problem of the regeneration of nerve elements, including the use of cells obtained from muscle tissue. Moreover, it has been established that even xenogenic cellular material can be effective . The introduction of human precursor cells to mice caused the development of axonal regeneration in experimental animals with the participation of donor cells [20].

Particularly noteworthy are the data published in a number of articles on the epitheliomesenchymal plasticity of multipotent mesenchymal stromal cells in health and disease [21].

It was pointed out that the striving for phenotypic unification "cuts" the limits / scales of heterotypy and thereby hides ( cumulates ) from researchers the phenomenon of adult SC plasticity as a genetically inherent ability in them for diversification, for SC transdifferentiation associated with their histogenetic plasticity, which can certain conditions [22].

This is a strategically very important moment, because it is the possibility of revealing phenotypic heterogeneity that allows one to gain an idea of ​​the phenomenon of SC plasticity, in particular, to observe and study the mesenchymal- epithelial conversion of SC in adults.

**From theory to practice**

The introduction of new methodological concepts into practice was hampered by the emergence of data on complications associated with the systemic use of cellular material obtained from adults and propagated *in vitro*, which will be discussed below.

Nevertheless , in a number of areas, very encouraging reports have appeared, indicating the effectiveness of the use of adult SC as one of the methods for implementing the strategy of regenerative medicine. Thus, it became known about the experience of using adult SCs, brought by the authors to the level of " totepotency " in the complex treatment of Parkinson's disease [23].

The successful use of SC injections into the affected area in osteoarthritis has been reported [24].

In addition, there was evidence that the use of autologous SC bone marrow transplants increased the remission effect of breast cancer therapy in cases where other therapeutic agents were ineffective [25].

However, the use of adult SCs, especially those "artificially" brought to the level of pluripotency , is associated with serious risks. The authors point out that adult SCs that have acquired a high level of plasticity are behaviorally similar to tumor cells, including in terms of their pluripotency (and plasticity) [26].

There are some indications that the use of adult SCs may pave the way for tumorigenesis [27, 28].

A severe and fairly frequent complication of systemic implantation of allogeneic hematopoietic SC is bronchiolitis obliterans [29, 30].

**Development and implementation of the concept of SC plasticity in dentistry and maxillofacial surgery**

Perhaps, dentistry and maxillofacial surgery can be recognized as one of the well-developed areas of practical application of cell technologies [31].

First, dental tissues, and specifically their pulp, turned out to be the optimal source of SA, which is characterized primarily by the ease of obtaining the cellular material, its usefulness in terms of viability and plasticity [32].

A number of studies have shown that this source is promising by S.K. Experiments on laboratory animals confirmed the expectations of the researchers [33].

An extremely promising is the use of SK derived from pulp of deciduous teeth, the pulp is a number of teeth facing, impacted teeth, as well as the root region and the tissue of the periodontal ligaments [34, 35].

A number of studies have demonstrated the possibility of inducing high pluripotency and plasticity of SCs from the immature pulp of a human tooth (third molar or wisdom tooth) [36].

Two points attracted attention: SC obtained from dental pulp, being seeded on dense scofolds , showed a clear tendency to the formation of bone structures with organized vascular systems, that is, they practically reproduced the process of formation of mature bone tissue (but not dentin!) [ 37].

Recently, data were published on a new and more effective approach to solving the problem of cell therapy aimed at regenerating the pulp and dental tissues in general, using S.K. The strategy proposed by the authors, was used with the purpose of the saturation population SC cells dentinopoeticheskoy line, granulocyte colony stimulating factor ( Granulocyte-colony stimulating factor - G-CSF). This essentially epigenetic effect caused the activation of cell multiplication in the subpopulation of “mobilized” pulp SCs , endowed with phenotypic characteristics of pulp cells , including the ability to carry out dentinogenesis , and , moreover , resistant to apoptosis and without a tendency to genetic aberrations [38].

**Conclusion**

In historical terms, the development of the doctrine of the UK and their role in the process of life of animals and the body of man is characterized by the accumulation of basic data on the forms and manifestations of their functional activity at different stages of development of the organism and monitoring mechanisms for their implementation at the various stages of the ante-and postnatal ontogeny.

The following should be indicated as the most important events and at the same time stages on the path of the development of the theory of SC:

- 1908 - the formulation of the idea of ​​the existence of SCs - immature precursors of A.A. Maximov ( hematopoietic , according to A.A.Maksimov);

- 60s of the twentieth century - the development of a general theory of stem stem cells A.Ya. Friedenstein and his school based on bone marrow- derived material ;

- the end of the 20th century - the study of ADSC as a homeostatic mechanism [39];

- development of strategies based on epitheliomesenchymal conversion and other manifestations of plasticity of "adults" S.К. Development of ideas about SC pluripotency , including at various stages of ontogenesis, and study of the plasticity properties of adult SC, development of SC reprogramming methods [40, 41].

The theory of SC entered the 21st century with a powerful information reserve and well- developed strategies based on fairly fully substantiated theoretical concepts, which were translated into clinical practice in many directions .

In this review has been given due care required in the practical application of cellular technology, the more when it comes to interventions on genetically determined levels of functions SK.

This review is devoted to the state of research on problems with SC and the prospects for their development. And at the same time, it shows the relevance of this issue for dentistry and maxillofacial surgery.

**Lecture 2.**Genetic modification and labeling of cell lines

Currently, the problem of providing food to the growing population of the planet Earth is one of the most acute in the world. according to the data provided in the report "The State of Food Insecurity in the World", [2] in 2011-2013, 842 million people, or 12% of the world's population, could not meet their high-calorie food needs. According to experts in the field of food security, the number of people without adequate access to food is gradually decreasing. So, in 2010-2012, their number was 868 million people. [3] However, the rate of reduction in malnutrition and hunger does not allow us to speak of any significant shifts in ensuring global food security, also because it is inextricably linked to global growth in the number and growth of life expectancy of the population on planet Earth. Currently, this can be seen as another problem - the problem of overpopulation. Moreover, the need to ensure physical and economic access to sufficient safe and nutritious food for all people at any given time further exacerbates this problem.

Thus, according to the 23rd World Population Outlook, published by the United Nations, in mid-2013, the world's population was 7.2 billion people. By 2025, the world's population will exceed 8 billion. By 2050, it will reach more than 9.6 billion. And by the end of the century, according to the forecast, this figure may reach 11 billion people [4].

Nevertheless, in the modern economy one can see attempts to solve the food problem through the introduction of modern farming technologies based on the use of genetically modified plants. In order to understand whether the introduction of genetically modified organisms into the food production process solves the problem of food security, it is necessary to determine what is the essence of this innovative approach and whether it can positively affect the social and economic spheres.

Food safety and genetic modification of organisms

Changing the genotype of a plant is, perhaps, the only way to radically increase the nutritional value of its proteins and ensure the plant's resistance to pests and diseases without using strong pesticides that are ineffective and extremely environmentally harmful. Briefly, the process of changing the genotype of a plant can be described as follows: a gene is introduced into a plant, which is taken from another biological (natural) source. Another biological species (plant, insect, or, much less often, an animal) can become such a source.

Thus, a genetically modified, or transgenic , organism (GMO) is an organism, into the genome (genetic structure) of which a gene or genes of another organism is “introduced” with the help of modern technologies. The goals of such changes can be purely scientific, or applied - for using the results of GMOs, for example, in agriculture. Genetic modification is not a random process, differing in a purposeful change in the genotype of a living biological organism. In the field of food production, genetically modified organisms are only those that contain one or more transgenes .

The first stage in the development of GMOs can be considered the appearance in 1992 in the People's Republic of China of tobacco, which was genetically protected from harmful insects. 1994 can be considered the beginning of the introduction of genetically modified products, when tomatoes appeared in the United States that remained of high quality during transportation and did not deteriorate. At first, they were kept green for up to six months at a temperature of 14-16 degrees, and then ripened at room temperature.

Following this tomato variety, in 1995, the American company Monsanto launched GM soybeans into which, in order to increase its ability to resist weeds, an alien gene was introduced.

Since then, transgenic products have been actively conquering agricultural world markets, food markets, which causes massive indignation in scientific circles around the world.

It is believed that the main reason for the spread of GMOs in agriculture is the simplification of agricultural technology and, accordingly, the reduction in production costs. Producers of GM plants, as the main competitive advantage, highlight their resistance to weeds and, as a result, savings on chemicals. Because GM plant varieties are pesticide-resistant, mechanized plant maintenance is simplified. The use of GM products in animal husbandry (hormones, food additives, etc.) opens up the possibility of turning animal husbandry into an animal protein industry. All this provides a noticeable economic benefit, especially for large farms.

As practice shows, as a result of the introduction of GMOs, the time for breeding new varieties of plants has been reduced to a minimum: the emergence of a new improved version of the organism now takes 2-3 years, instead of 10 years that had to be spent during traditional crossings, I use the selection method. This saves both time and money. Transgenes that are already resistant to insect pests do not need pesticides, which require no small financial costs. The yield of genetically modified organisms is estimated to be 15-25% higher than that of ordinary biological species. It follows from this that landowners and farmers, growing GM varieties, spend several times less money than on natural (biological) plants.

Experts not only fight for the harvest, but also strive to increase the useful qualities of products. For example, in some they artificially increase the dose of vitamins and minerals, in others - the nutritional value, and in the third they try to invent new medicines. With the help of this, American scientists, for example, decided to develop a new breed of GM chickens, in which the eggs will contain substances that prevent the development of cancer cells in the body.

But the main slogan under which the global introduction of genetically modified organisms into agriculture and the food sector is to rid mankind of hunger.

In practice, the introduction of GMOs has never been the main solution to the food problems in need of countries.

According to the International Food and Agriculture Organization of the United Nations (FAO), in 2012-2014, about 805 million people suffered from chronic malnutrition, approximately one in nine people in the world does not have enough food for an active healthy life. The vast majority of these malnourished people live in developing countries, where the number of chronically hungry people in 2012-2014 was estimated at 791 million people, almost one in eight in these regions - or 13.5% of the total population suffers from chronic malnutrition. The leaders in this regard are the countries of East Asia (161.2 million people), South Asia (276.4 million people) and Sub-Saharan Africa (214.1 million people). [five]

Moreover, according to studies by the same organization, about 40% of food produced in the United States of America is never eaten by humans. In Europe, for example, about 100 million tons of food is thrown away annually. On average, in the world, almost a third of all food products produced, or 1.3 billion tons per year, is lost or misused. [6] All this is also associated with the loss of labor, water, energy, land and other resources used for food production. according to the UN, the world produces enough food to provide each person with 4,000 calories a day. In reality, only 2 thousand calories reach the consumer.

On the other hand, for example, African countries, in which the problem of hunger is very acute, refused, or rather introduced a complete ban on the import of food products with GMOs and GMO seeds into their territory. This is due to the desire to protect oneself from genetic contamination, mutation of pure biological species and the unwillingness to make oneself dependent on transnational corporations such as Monsanto , which produce an increasing number of genetically modified plants and conduct an extremely aggressive policy of introducing GMOs into the food production process. all over the world. In India, an example of the result of Monsanto's aggressive expansion to introduce GMOs into crop production is the fact that over 290,000 farmers have committed suicide over the past 20 years. This is attributed to the fact that the legalization of GMOs in India in 2002 and lobbying for the use of GMO seeds by Indian officials led to the fact that many Indian farmers went bankrupt without receiving the necessary harvest and unable to return the loans they took to buy GMOs. seeds. " Monsanto " denies all charges of involvement in the death of Indian farmers.

It is also a fact that in African countries a huge amount of food rots and disappears every year, the population of these countries in most cases has no income to buy food. Thus, the problem of food shortages is more related to the problem of ensuring sufficient incomes for the population to meet their key needs.

These reasons are fundamental in considering the problems of hunger and food security in the world.

The spread of GMOs is stimulated by their producers - transnational companies, and in this sense it is one of the features of the globalization process. A typical example is genetically modified rice containing provitamin A. An advertisement for GM rice claimed that the variety was designed to overcome the vitamin A deficiency common in Southeast Asia. However, to get the required daily intake of vitamin A, you need to eat 9 kg of this rice. The solution to the problem of vitamin A deficiency - many times cheaper and more realistic - is the widespread use of local fruits and vegetables.

The fact that high yields can be obtained “without chemicals” and without GMOs, on the basis of selection and conventional agricultural techniques, contradicts the interests of corporations that are introducing this innovative approach. They are imposing development paths on global agriculture that increase their profits (the creation of GM varieties that can withstand significant concentrations of pesticides, and the use of pesticides in greater quantities than before).

At present, biotechnology in the field of genetic engineering is extremely imperfect. For example, specialists in the field of genetics do not give any guarantee that a foreign gene will be inserted into a specific part of the gene chain of the biological organism that is being altered. The result is the so-called pleiotropic effect, i.e. the multiplicity of the influence of genes on the development of the organism, the influence of a gene on the manifestation of not one, but several signs, while the manifestation of such development is practically unpredictable and can be detected only after several generations. The result of this effect can be an uncontrolled transfer of genetically modified constructs from plants subjected to genetic modification into ordinary bacteria, which can cause the emergence of previously unknown pathogenic strains of phytoviruses that are more dangerous than their natural predecessors. [7] There are also many other risks of introducing GMOs, most of which are still not objectively assessed.

In reality, neither food security problems nor the desire to save humanity from predicted hunger are not the main arguments of GMO producers. Economic benefit is the main driver. Only those who are behind the creation of GMOs receive superprofits. The creation of each new type of GM-organism, according to expert estimates, costs about three billion dollars, and the total number of genetically modified varieties is already more than a thousand. Only large transnational corporations can do this with nature. As already mentioned, the leader of the GMO market is the American company Monsanto - it controls over 80%. Besides Monsanto , significant players are the American company DuPont, the Swiss company Sintenta and the German Bayer. It is these corporations that have huge revenues from the sale of GM seeds, GM crops, as well as chemical treatment of crops. One of the most common pesticide is glyphosate " Roundup " Company " Monsanto ", almost all of the GMO companies have artificially implanted gene for resistance to this pesticide. The company " Monsanto ", in this way promoting the sale of their pesticides, generated enormous revenues not only from the sale of GMOs, but also from the sale of means of chemical processing plants.

As a result, only a few companies are gradually taking over the global food market, already dictating their terms to global agriculture. Currently, an increasing number of farmers around the world, being subjected to tough lobbying for the interests of the above companies, are beginning to abandon traditional agricultural varieties and, in fact, become completely dependent on patented transgenic products and their accompanying pesticides.

According to statistics, Monsanto had a turnover of $ 13.5 billion in 2012 and a net profit of $ 2 billion. In fiscal 2009, Monsanto received revenue from the sale of GM seeds in the amount of 7.3 billion. dollars, which is almost 2 times higher than the amount of income of the company "DuPont" in the amount of 4 billion dollars, which is on the 2nd place in the world in terms of income from this type of activity. The sales volumes of biotechnological products of these companies are growing annually due to the growth of demand in the USA, Europe and Latin America due to the increase in acreage for GM crops. [8]

Thus, today it seems necessary to thoroughly and comprehensively study the need for introducing GMOs into the sphere of food production, an impartial assessment of the risks associated with this process, long-term experiments on the consumption of GM products and an assessment of the consequences for human health. It is necessary to make a comprehensive assessment of the economic benefits for governments and private farmers and the impact of the widespread introduction of GMOs on solving the problem of food security and hunger.

**Lecture 3.**Simple tissue culture; Integrated tissue culture

The term " culture of      cells, tissues and organs of     plants" is applied to the following aseptically grown parts of a plant: isolated   embryos, isolated organs ( root tips  , shoot meristems   , leaf  primordia , parts of young flowers and  fruits ), callus tissue , suspension culture , culture protoplasts . A set of    objects of plant origin, which   mozhnoperevesti    in   kulturuinvitro wide enough. As a   rule, most   studies are carried out with  explants of   various  organs, tissues,   and cells of seed plants.  This can be   explained by  bolshoyrolyu   in the life of mankind and gymnosperms pokryto-  rasteniy.Sredinih    most convenient objects for cultivation   yavlyayutsyadvudolnye     herbaceous plants, followed    odnodolnyetravyanistye   types and crops.  It is more difficult to create   conditions for  stable invitro crops of woody plants, especially gymnosperms.

The   objects used for the  cultivation of invitro   can also be  mosses , lichens and ferns.  Cultures of their  cells are used to study processes specific to these plants .  Multicellular algae   are difficult to maintain in their  culture , although they are undoubtedly promising as  sources of phytoproducts    for medicine, the food industry, and   other biotechnology . Biologists pay much attention to the   cultivation of microalgae    .   Currently   , there is no such higher plant, from which it is  impossible to obtain cultivated cells and tissues. However   vyraschivaemyepoverhnostnym      method on     agar      nutrient     sredekallusnye tissue and grown in a liquid nutrient medium suspenzionnyekletochnye   crops require different conditions for creating growth  vsluchae razlichnyhtaksonov.V     based cell and tissue culture method of plants    lezhitunikalnoe     property plant kletki totipotentnost.Totipotentnost - a cell's ability to implement geneticheskuyuinformatsiyu providing it differentiation and development of the   whole organism. Totipotency is   inherent in the fertilized ovum of plants and  animals . As for the differentiated cells, in animals, the type-potency is inherent only in some intestinal cells . In higher animals, with the beginning of   specialization of cells , i.e. already starting from the early stages of embryogenesis, totipotency    is not realized. In contrast to animals, in plants   in   natural conditions    , specialized cells can also exhibit   totipotency .    For example,   totipotency     in plants is realized    during   wound healing.  In this   case, on the wound surface of the plant,  as a result of unorganized cell proliferation, the development of   callus (Latin "corn") occurs .  Callus formation   can be observed  during grafting   in the places where the scion and rootstock grow together .  Callus is  initially   composed of undifferentiated cells.  Subsequently, secondary differentiation with the formation of specialized tissues and organs can take place in   it.  However,   under natural conditions, plants of a number of systematic  group-typepotency   do not show.  Due to the high   specialization of  cells, many   monocotyledonous plants have lost the ability to wound  reaction and    vegetative reproduction. Meanwhile, under   experimental conditions   in vitro when   growing fragments of tissues, organs or  cells    on artificial nutrient media, it is possible to realize the suppressed in vivo typotency . Speaking about the    significance of totipotency , it should be emphasized that  totipotency , as a  very valuable    feature of a plant cell, makes it possible to   model unique differentiations and experimentally study the phenomenon of epigenetic     heredity, and also opens up    enormous opportunities for plant genetic engineering and biotechnology. The method of plant cell and tissue culture is currently widely used   to solve a number of theoretical and practical  tasks of biology . This is due to the fact that this method has the  following advantages : • simplicity of cell models; • lack of correlative interactions and control from  other   tissues, organs, the whole organism, which allows you to  trace the state of the cell at the level of primary elementary processes in response to any impact; • the ability to quickly obtain sufficient weight in  aseptic conditions ; • growing conditions controlled by many parameters. Invitro plant    cells are a convenient model for   studying many   physiological and biochemical processes and genetics of a plant organism

**Lecture 4**Early development of mice and and humans

During the first week of development, the mammalian embryo goes through several stages. First - crushing, a lump of identical cells - blastomeres - is formed. Then - compaction , blastomeres are compressed, forming a morula. Then a cavity is formed in the middle of them and a blastocyst is formed . This is a cell ball, inside which there is a cavity with fluid and an adjacent lump of cells. The surface of the ball is called trophoblast , it will subsequently give extraembryonic membranes (including the placenta). The internal cell mass turns partly into membranes, and partly into the actual tissue of the embryo. After a blastocyst formed two cell layer, it is implanted in the wall of the uterus, where the cells begin to specialize, and further having a body axis ( antero -the rear and top-bottom).

But since all these processes take place entirely in the womb, they are rather difficult to study in the laboratory. For this, as a rule, stem cell cultures are used, from which scientists are trying to assemble (sometimes literally, layer by layer) a model of an embryo at one or another stage of development. Recently, we talked about the fact that with the help of microfluidics, it was for the first time possible to create an imitation of the postimplantation development of a mouse.

Nevertheless, all the methods of constructing artificial embryos known so far make them completely unviable. The farthest so far is the experiment of Dutch scientists who assembled a blastoid ( blastocyst- like structure) from two types of cells: embryonic stem cells and trophoblast stem cells . These embryos even managed to be implanted into the uterus, but it is impossible to study the early stages of development before implantation.

The next step in this direction to make an international team of scientists, led by Professor of the Catholic University of Murcia (Spain) and the Salk Institute (USA), Juan Carlos Ispisua Belmonte ( of Juan by Carlos Izpisúa Belmonte ). They decided to build an embryo based on expanded pluripotent stem cells . These cells are similar to blastomeres, that is, they can give rise not only to the embryo, but also to extraembryonic tissues. To get them, the researchers took embryonic stem cells and cultured them under the influence of a cocktail of signaling substances, "returning" their lost functions.

The researchers found that if you act on such pluripotent cells with substances that cause the differentiation of both the trophoblast and the inner cell mass, then full-fledged blastoids grow from them in about 15 percent of cases. But with embryonic stem cells, it was not possible to repeat the experiment - blastoids were not formed from them .

When blastomere-like cells were grown singly, they first died. Then the scientists took a line of cells resistant to puromycin and mixed it with other, unstable cells. They were grown together for some time, and then treated with puromycin, and the "helper cells" died. Thus, with the help of temporary support of other cells, it was possible to grow an embryo from a single stem cell - however, only in 2.7 percent of cases.

The researchers confirmed that their blastoids are similar to regular blastocysts : in cellular composition, staining for different markers and gene expression. They even checked that blastoids are characterized by processes characteristic of mammalian development - for example, inactivation of the X chromosome. In the inner cell mass at the beginning of development, both of its copies are active, and in trophoblast cells , as a rule, the paternal X chromosome "becomes silent" - and this is exactly what happened in most of the outer cells of the blastoid .

Then the authors of the work demonstrated that their blastoids are similar to blastocysts in vitro . For example, they isolated a culture of embryonic stem cells and showed that they can integrate into the embryos of ordinary mice and produce chimeric animals. In addition, blastoids can be cultured and their cells begin to form various postimplant tissues.

The blastoids were implanted in the mice in the uterus, and about 7 percent of them were implanted. The artificial embryos caused the formation of decidual tissue in the uterus - the precursor of the placenta - and established close contact with it, sufficient for the dye from the mother's body to enter the embryo. The embryos continued to develop until at least 7-8 days, tissue gradually grew in them, but they significantly lagged behind healthy embryos in development.

Finally, the scientists obtained blastomere-like cells from mouse somatic cells. They reprogrammed them according to the same principle as for the creation of induced pluripotent cells, and the resulting culture turned out to be similar in properties to the "improved" pluripotent cells. She also managed to grow blastoids and implant them into the uterus.

Despite the fact that the new method has not yet led to the creation of fully viable embryos, the authors of the work expect that one day it will be able to grow bioengineered embryos with its help. If this really succeeds, then we can talk about the third method of cloning. Now two are known: the transfer of the somatic nucleus into the egg and the transfer of induced pluripotent cells into a foreign embryo. If the third method of cloning works, then this will be the first "real" cloning, since the embryo will fully develop from the somatic cell of an adult organism.

**Lecture 5**Mouse pluripotent stem cells; Mouse embryonic stem cells

Perhaps the youngest trend in modern medicine can be considered cellular technologies, in which cells serve as a source of certain necessary factors, for example, tumor antigens during vaccine therapy. But the cell can be used not only as a source of any substances, but also for regenerative medicine. Stem cell technologies are of particular interest here. The ability to divide indefinitely and transform into different types of cells (the so-called pluripotency ) makes them an ideal material for transplant therapy methods. The most readily available are adult stem cells. However, the real potential of their differentiation is still poorly understood.

Human embryonic stem cells (ESCs) are extremely attractive in this respect: any type of body cells can be obtained from them. But many of the properties and cellular mechanisms associated with the presence of the so-called “ stem nature ” in a cell put it very close to a transformed, cancer cell. That is why it is so important today to study the characteristics of the embryonic cells themselves. In the eight years that have passed since the first human ESC lines were obtained, it was possible to elucidate only a small part of the mechanisms that ensure self-maintenance of undifferentiated cells or their differentiation in culture .

Until recently, the number of human ESC lines available for study was small. Currently, there are many more of them, but the methodological difficulties and high cost of working with them still limit the circle of researchers. The ethical side imposes no less restrictions on research in the field of human embryonic cells. Despite the debate about the ethics or unethicality of working with human ESCs, it is clear that the question is no longer whether to conduct research in the field of human ESCs, but how the research in this area will be carried out. Over the past two years, a large number of countries have already passed laws permitting research on human embryonic stem cells.

Embryonic stem cells are obtained from the inner cell mass of a blastocyst at the earliest stages of embryonic development, when it has not yet been implanted into the uterine wall. It is from the cells of the inner cell mass that the whole organism develops in the future. Quite often, especially in the Russian-language literature, embryonic stem cells are called cells of a postimplantation embryo of various stages of pregnancy, which in their properties are rather similar to adult stem cells. We will only talk about true embryonic stem cells originating from a blastocyst - at the stage when the embryo consists of 150-200 cells of the trophoectoderm and the inner cell mass in approximately equal proportions.

Stable lines

Stable human ESC cell lines were first obtained by the American researcher J. Thomson in 1998 [1]. This achievement was preceded by the work of M. Evans and M. Kaufman . In 1981, they showed for the first time the fundamental possibility of obtaining stable cultures of mammalian cells with the property of pluripotency . The mouse ESC lines remained in an in vitro culture in an undifferentiated state for more than a hundred doublings, and then in vivo could participate in the formation of special tissues of the animal. In 1995, Thomson and colleagues, having modified the technology for isolating murine cells, obtained a primate ESC line, and in 1998 - a human cell line.

To obtain stable lines of human ESCs, they take unclaimed human blastocysts after artificial insemination . Usually, after such a procedure, the number of blastocysts is greater than the recipient needs. They can be frozen, destroyed or, with the consent of donors, used for scientific purposes. It is best to isolate human embryonic cells 4-6 days after fertilization. First, using the pronase enzyme, the transparent membrane of the blastocyst is dissolved , and then the trophoblasts are removed by complement-dependent lysis . The inner cell mass is placed in a culture medium on a support of inactivated mouse embryonic fibroblasts, which serve as a source of growth factors. By transplanting cells, you can get a cell line capable of virtually unlimited division. Today, about 150 ESC lines have been identified in laboratories around the world. In our country, embryonic stem cells were obtained in 2003 at the Institute of Gene Biology of the Russian Academy of Sciences and at the Institute of Cytology of the Russian Academy of Sciences.

Embryonic stem cells grow in dense cell colonies on a substrate of mitotically inactivated mouse embryonic fibroblasts (Fig. 1). The success of obtaining human ESC lines is rather high when morphologically normal blastocysts with visible inner cell mass are used, almost half of which can produce karyotypically normal cells. These results are consistent with the level of embryo implantation after transplantation into recipients. Building cell mass is difficult. Mouse cells grow well after enzymatic treatment to single cells, but human cells devoid of intercellular contacts usually die. Therefore, they are divided into individual fragments of 50-500 cells (either by enzymatic treatment, or mechanically, cutting into pieces with microinstruments).

Manipulations with oocytes in vitro make it possible to obtain human ESC lines with a given genotype and, accordingly, immunologically compatible with a potential donor. Several approaches are possible here: transfer of nuclei of somatic cells, parthenogenesis, or cell fusion. The transfer of somatic cell nuclei is often not quite correctly called cloning. In the last months of 2005, a detective story unfolded around the work of scientists from Seoul National University under the leadership of W. Hwang . In 2004-2005. in the journal " Science " they published two papers describing the method of obtaining a human ESC line from the inner cell mass obtained after transplanting the nucleus of a somatic cell into an oocyte. Unfortunately, the work turned out to be a grandiose falsification, the reasons for which have not yet been clarified. It is possible that everything could have been arranged in advance by third parties for commercial or political purposes. However, these events not only did not reduce interest in the problem, but also intensified work in this direction.

Main characteristics

Like all cell cultures, embryonic stem cells need a clear characterization [2]. The simplest is an external description, but it gives very limited information about the properties of cells. Recently, it is customary to distinguish cells by surface antigens, which more fully describe one type or another. Human embryonic stem cells have surface immunological markers, for example : SSEA-3, SSEA-4 - antigenic determinates ( epitopes ) of glycolipids and TRA-1-60, TRA-1-81 - different epitopes of the same proteoglycan of the cell surface.

The presence of a set of certain markers indicates that cells belong to human ESCs, but not their ability to proliferate for a long time. It is determined by the activity of the telomerase enzyme and the length of telomeric repeats. In somatic cells with a limited number of divisions, the telomere length is small, and telomerase activity is usually very low. On the contrary, in tumor cells the enzyme activity remains very high, while the length of telomeric repeats is preserved. Embryonic stem cells have the same property.

Both immunological markers of ESCs and high telomerase activity are inherent in transformed cells, i.e. cells in which genetic changes have occurred. This means that an analysis of the karyotype is required for an accurate characterization of human ESC lines. A normal set of chromosomes and the absence of chromosomal abnormalities are signs of a normal karyotype, which, however, can be disturbed during cell culture. Thus, during long-term cultivation (after about two years), we noted a significant change in the growth rate of cells, as well as in their ability to differentiate. Karyotypic analysis showed abnormalities in chromosome 18 and a tendency towards karyotype instability. It is possible that this was the reason for the abnormal behavior of cells in culture. Subsequently, we have repeatedly found subclones of other ESC lines with various chromosomal aberrations. More recently, a publication has appeared confirming our observations. Therefore, long-term cultivation of human ESCs requires strict control of their karyotype.

Molecular-genetic mechanisms of self-maintenance

One of the remarkable properties of embryonic stem cells is their ability to maintain pluripotency in culture. On mouse cells, this is easy to verify experimentally: from one cell cultured in vitro , an entire organism can be recreated. This is how animals with a genetic "knockout" get. The essence of the technology is that in vitro genetically modified mouse embryonic cells are injected into a blastocyst , which is implanted in a pseudopregnant mouse. As a result, so-called chimeric mice are born, in which some of the cells are from the recipient's blastocyst , and some are genetically modified. If such cells enter the embryonic pathway, in the second generation it is possible to obtain an animal, all of whose cells will be the descendants of one genetically modified embryonic cell.

This technology not only allows “turning off” certain genes in strictly determined tissues, but also “turning on” defective ones, creating model systems of diseases. For obvious reasons, such a procedure is impossible with human cells. Here, to test for pluripotency , human embryonic cells are injected into immunodeficient mice. As a result, benign tumors - teratomas - are formed in animals, in which several types of formed tissues can be found [3]. However, for constant monitoring of the state of ESCs and to ensure optimal cultivation conditions, this method seems to be irrational. Here it is very important to elucidate the molecular mechanisms that determine the specificity of embryonic stem cells, namely, their ability to remain in culture in an undifferentiated state. Some mechanisms are common for mouse and human ESCs, and some are different.

The transcription factor OCT4, which manifests itself from the eight -cell stage of the mouse embryo, is involved in the self-maintenance of ESCs . It is necessary for the formation of the inner cell mass of blastocysts ( it is absent in the cells of the trophoectoderm ). The corresponding activity of the oct4 gene maintains the undifferentiated state of embryonic cells, and its increase or absence causes their transformation into endoderm and mesoderm or trophoblast cells, respectively [4]. In the cells of somatic tissues, the expression of the oct4 gene, characteristic of human ESCs, has not been found, although recently there have been reports of its low activity in stem cells of an adult organism. Even at the beginning of ESC differentiation into embryoid bodies, the activity of the oct4 gene decreases.

The homeobox transcription factor NANOG is also involved in maintaining the pluripotency of mouse and human ESCs . If the nanog gene is blocked, the embryonic cells develop into a primitive endoderm. In the absence of the growth factor LIF, the increased activity of the nanog gene provides a pluripotent state of mouse ESCs. Like the oct4 gene, the nanog gene is not manifested in the cells of somatic tissues , with the exception of the fetal brain, reproductive organs (testes and ovaries), and embryonic carcinoma cells. As human ESCs spontaneously differentiate into embryoid bodies, the nanog gene activity decreases.

In addition to genetic mechanisms, the fate of a cell is also determined by the so-called epigenetic mechanisms (i.e., changes in the functioning of genes inherited by the cell , not associated with a change in the DNA sequence). A striking example of their action is the inactivation of one of the X chromosomes in female XX cells. Epigenetic mechanisms play an essential role in the processes of early embryonic development, controlling the work of genes. Epigenetic modification of the regulatory regions of genes ensures the shutdown of their functions at subsequent stages of development. This is extremely important, since untimely or uncoordinated work of genes can lead to cell death or transformation. For example, the regulatory region of the oct4 gene is epigenetically modified in almost all cells of an adult organism. Such a change is one of the main problems in the transfer of nuclei of somatic cells of an adult organism into an oocyte. In our laboratory, it has been shown that the regulatory region of the nanog gene in the cells of an adult organism is epigenetically modified, which further complicates the transfer of nuclei.

Modern methods of analysis, such as microchips, make it possible to quickly determine the activity of several thousand genes, which creates a more accurate picture of the molecular genetic state of the cell. This is especially important for long-term cultured cells intended for therapy.

Speaking of maintaining the pluripotency of mammalian embryonic stem cells, one cannot fail to note the role of external growth factors, including fibroblasts, as a substrate-feeder (from the English feed - feeding, nutrition). The first mouse and human ESC cell lines were obtained using primary mouse embryonic fibroblasts, which provide not only better cell growth, but also their undifferentiated state. They were later replaced by an immortal cell line. However, it is believed that these cell lines cannot be used in regenerative medicine or gene therapy, since pathogenic microorganisms and viruses can be transferred from mouse cells. In addition, human cells begin to present murine antigens, which can lead to transplant rejection. Today, human foreskin fibroblasts are sometimes used for the cultivation of human ESC lines. In the literature, there are separate publications on the production of such lines and in feederless conditions.

**Lecture 6**Male reproductive system.

Male genital organs are anatomically subdivided into external - the penis and scrotum and internal - testicles, epididymis, vas deferens, prostate gland, seminal vesicles. In the area of ​​the external genital organs, receptors are concentrated that perceive erogenous stimuli (erogenous zones).

External genital organs

The penis (penis, phallus) is the external genital organ of a man, which serves for intercourse, delivery of sperm ( ejaculate ) into the woman's vagina, as well as the removal of urine from the bladder.

Distinguish between the root (base), body (trunk) and the glans penis. The trunk is formed by two cavernous and spongy bodies containing a large number of depressions (lacunae) that are easily filled with blood. The spongy body at the end of the penis ends in a cone-shaped thickening - the head of the penis. The edge of the head, covering the ends of the cavernous bodies, grows together with them, forming a thickening (corolla) along the circumference, behind which is the coronal groove. The head is covered with thin, delicate skin (foreskin) with a large number of glands that produce smegma .

The head of the penis has a large number of nerve endings, which makes it the most sensitive to touch. The shaft of the penis is also highly sensitive, especially its lower zone in the region of 2-3 cm from the head. Stimulation of the penis leads to increased erection. On the top of the head there is an opening - this is the exit of the urethra, through which both urination and the release of sperm are carried out.

The appearance of the penis, like other parts of the human body, is very individual. A straight penis is rare, often the penis in a calm state seems straight, but with an erection it bends.

The size of the penis of an adult man on average at rest is 5-10 cm, in a state of erection - 14-16 cm, that is, approximately corresponds to the size of a woman's vagina. Often, with an erection, the short penis proportionally increases more than the long one. The shape of the penis during erection and the angle of inclination are individual. An erect penis with a length of 16-18 cm is considered large, and 18-20 cm or more - giant. The diameter of such a penis, as a rule, does not exceed 3-4 cm.

The average length of the penis at birth is from 2.4 to 5.5 cm, at the beginning of puberty - 6 cm, reaching adult size occurs over the next years, up to 17 years, the penis grows actively, up to 25 years - slightly.

With sexual arousal, the penis increases in volume by 2-8 times, while becoming quite dense. The maintenance of an erection is provided by a decrease in venous outflow, which is facilitated by the contraction of special muscles that are located at the root of the penis. At the end of the excitement, the muscles relax and the blood filling the penis easily flows out, after which it decreases to its usual size and becomes soft. The head of the penis during erection always remains less elastic and more elastic than its body, which prevents trauma to the female genital organs.

In the anterior part of the body of the penis, the skin forms a fold of skin - the foreskin, which completely or partially covers the glans. The foreskin, if it completely covers the glans, usually slides back easily, exposing it. On the back of the penis, the foreskin is connected to the head by a longitudinal fold called the frenum. Between the head of the penis and the foreskin there is a slit-like (preputial) cavity, which is finally formed by the age of two. In the preputial sac, smegma usually accumulates .

With age, hair follicles become visible on the skin of the penis body, and in the future, a small amount of hair. Sometimes hair follicles with enlarged sebaceous glands are perceived by teenagers as "acne".

Smegma (preputial lubrication) is the secret of the glands of the foreskin, accumulating under its inner leaf and in the coronal groove of the penis. The main components are fats and mycobacteria. Fresh discharge is white and evenly distributed on the surface of the head, after a while they acquire a yellowish or greenish tint. Smegma acts as a lubricant that covers the head and reduces friction on the foreskin. Smegma formation increases during the period of greatest sexual activity (18 - 25 years old) and is practically absent in old age.

Prolonged stagnation of smegma in the preputial sac with phimosis, violations of personal hygiene rules contributes to the development of inflammatory and precancerous diseases of the penis. For the purpose of prevention, it is necessary to prevent stagnation of smegma , starting from early childhood, to observe the rules of hygiene of the male genital organs. Smegma , like other substances that serve as a lubricant, should be removed daily. Daily thorough washing is an absolute must. This rule also applies to men who have undergone circumcision - in them smegma can accumulate in the folds of the frenum, if it is preserved, and the coronal groove.

Usually adolescents have troubles from accumulated smegma if they disregard the rules of hygiene. They call smegma "putty" and remove it with dirty hands as it hardens. In adolescence, it is the lack of hygiene that is the most common cause of infectious diseases of the genital organs. With proper care, smegma does not pose a health hazard.

Sperm (seminal fluid, ejaculate ) - a mixture of secretion products of male genital organs secreted during ejaculation: testicles and their appendages, prostate gland, seminal vesicles, urethra. Sperm is composed of two separate parts: seminal plasma - mainly formed from the secretion of the prostate gland, secretions of the testicles, their appendages and ducts of the sperm gland, and from the formed elements (spermatozoa or primary germ cells of the testicles).

    Seminal vesicle fluid (65%)

    Prostate fluid (30%)

    Sperm (5%).

The sperm of an adult male is a sticky, viscous, mucoid, heterogeneous and opaque liquid with a characteristic odor. The taste of semen, as well as the smell, is determined by the nature of the diet and is usually slightly sweet-salty with a sour or bitter aftertaste. With frequent ejaculations, the semen becomes less sweet and the taste of bitterness increases. Within 20-30 seconds, the semen liquefies, becomes homogeneous, viscous and has an opaque whitish-gray color. Its amount is individual and can range from 1-2 to 10 ml or more. Sperm count can fluctuate depending on age, health status, the amount of fluid you drink, the frequency of ejaculation, and so on. The more often sexual or masturbatory acts are performed , the less the volume of each subsequent portion of ejaculate . A large semen volume does not mean a higher fertilizing capacity. The average semen volume, assuming ejaculation occurs at intervals of 3 days, is 3 to 5 ml.

The fertilizing ability of sperm is characterized by the number of sperm in 1 ml of sperm, which is normally 60-120 million.Motile sperm should be at least 70% of their total number; the lower limit of the norm (according to WHO) is considered to be at least 20 million. sperm in 1 ml spermogram ).

The scrotum is a musculocutaneous organ, in the cavity of which the testes, epididymis and the initial section of the spermatic cord are located, separated by a septum, to which the embryonic suture corresponds outside. The seam can be clearly visible or, conversely, almost invisible. This does not affect health in any way.

The skin of the scrotum is pigmented, covered with sparse hair, contains a large number of sweat and sebaceous glands, the secret of which has a specific odor. Placing the testicles in the scrotum creates a temperature lower for them than inside the body. The optimum temperature is considered 34- 34,5 ° C. The temperature is kept approximately constant due to the fact that the scrotum sinks lower in warm conditions and is pulled up to the body in cold conditions. The scrotum is also the male sexual sense organ (erogenous zone).

Internal genital organs

The testicles (testes, testicles ) are a paired male sex gland, the main function of which is the formation of sperm and the release of male sex hormones (testosterone) into the bloodstream. The testicles are located inside the scrotum and are usually located at different levels (usually the left below the right), and may also differ in size. The dimensions of each testicle are 4-6 cm long, 2.5-3.5 cm wide.

The testicles require special attention to comply with the rules of hygiene of the male genital organs. Testicular temperature should be 4 degrees below body temperature, as too high a temperature interferes with sperm formation. Even a one-time immersion of the testicles in hot water can impair fertility for the next six months. Sedentary men should get up and walk from time to time so that the testicles move away from the hot body.

The vas deferens (vas deferens) are the ducts that carry sperm out of the testicles. They are a continuation of the canal of the epididymis, pass through the inguinal canal, then, connecting with each other, form a single ejaculatory duct . which passes through the prostate gland and opens with an opening at the back of the urethra. The advancement of sperm along the vas deferens is carried out by their wave-like contraction, at the time of orgasm, the sperm through the common vas deferens into the urethra, and from it out or into the vagina.

The spermatic cord is a paired anatomical organ that runs from the epididymis to the place of confluence with the duct of the seminal vesicle. Its main functions are blood supply to the testicle and the excretion of semen from the epididymis to the vas deferens.

The prostate gland (prostate) is an unpaired organ of the male reproductive system that produces a secret that is part of the sperm, which is located between the bladder and the rectum. The urethra passes through the prostate gland.

The size of the prostate depends on age, the full development of iron reaches 17 years. Outside, the prostate is covered with a capsule of dense connective tissue. The glandular tissue consists of glands that open into the prostate part of the urethra through the excretory ducts. The removal of secretions from the prostatic glands is facilitated by the contraction of the smooth muscles of the gland. Massive secretion is observed during ejaculation.

The secret of the prostate (prostatic juice) is a cloudy white liquid, which is involved in the liquefaction of sperm, activates the movement of sperm. The prostate gland provides the movement of sperm along the vas deferens and ejaculation, participates in the formation of libido and orgasm.

The seminal vesicles are paired glandular formations that produce a secret that is part of the sperm. Its contents consist of a viscous protein liquid with a high content of fructose, which is a source of energy for spermatozoa and gives them great resistance.

**Lecture 7**Female reproductive system.

The female genital organs are divided into external (vulva) and internal. The internal genital organs ensure conception, the external ones participate in intercourse and are responsible for sexual sensations.

The internal genital organs include the vagina, uterus, fallopian tubes, and ovaries. To the outside - the pubis, large and small labia, clitoris, vestibule of the vagina, large glands of the vestibule of the vagina ( Bartholin's glands). The border between the external and internal genital organs is the hymen, and after the onset of sexual activity - its remains.

External genital organs

The pubis (venus tubercle, lunar mound) is the lowest part of the anterior abdominal wall of a woman, slightly elevated due to the well-developed subcutaneous fat layer. The pubic area has a pronounced hairline, which is usually darker than on the head, and in appearance is a triangle with a sharply defined upper horizontal border and apex directed downward. The labia (pudendum) are folds of skin located on both sides of the genital slit and the vestibule of the vagina. Distinguish between large and small labia

The labia majora are folds of skin, in the thickness of which there is a fat-rich fiber. The skin of the labia majora has many sebaceous and sweat glands and is covered with hair from the outside during puberty. In the lower parts of the labia majora, the Bartholin glands are located . In the absence of sexual stimulation, the labia majora are usually closed in the midline, which provides mechanical protection for the urethra and vaginal opening.

The labia minora are located between the labia majora in the form of two thin delicate pink skin folds that limit the vestibule of the vagina. They have a large number of sebaceous glands, blood vessels and nerve endings, which makes them an organ of the sexual sense. The small lips converge over the clitoris to form a fold of skin called the foreskin of the clitoris. With sexual arousal, the labia minora are saturated with blood and turn into elastic rollers, narrowing the entrance to the vagina, which increases the intensity of sexual sensations when the penis is inserted.

The clitoris is the female external genital organ located at the upper ends of the labia minora. It is a unique organ whose sole function is to concentrate and store sexual sensations. The size and appearance of the clitoris are individual. Length - about 4-5 mm, but in some women it reaches 1 cm or more. With sexual arousal, the clitoris increases in size.

The vestibule of the vagina is a slit-like space bounded laterally by the labia minora, in front by the clitoris, and behind by the posterior commissure of the labia. From above, the vestibule of the vagina is covered with the hymen or its remains. On the eve of the vagina, the external opening of the urethra opens, located between the clitoris and the entrance to the vagina. The vestibule of the vagina is sensitive to touch and at the time of sexual arousal is filled with blood, forming an elastic elastic "cuff", which is moistened by the secretion of large and small glands (vaginal lubrication) and opens the entrance to the vagina.

Bartholin's glands (large glands of the vestibule of the vagina) are located in the thickness of the labia majora at their base. The size of one gland is about 1.5-2 cm. The glands, during sexual arousal and intercourse, secrete a viscous, grayish, protein-rich liquid (vaginal fluid, lubricant).

Internal genital organs

The vagina ( vagina ) is the internal genital organ of a woman, which is involved in the process of intercourse, and during childbirth is part of the birth canal. The length of the vagina in women, on average, is 8 cm. But in some it may be longer (up to 10-12 cm) or shorter (up to 6 cm). The inside of the vagina is lined with a mucous membrane with many folds, which allows it to stretch during childbirth.

The ovaries are the female sex glands and contain over a million immature eggs since birth. The ovaries also produce the hormones estrogen and progesterone. Due to the constant cyclical change in the content of these hormones in the body, as well as the release of hormones by the pituitary gland, the maturation of the eggs occurs and their subsequent release from the ovaries. This process is repeated approximately every 28 days. The release of an egg is called ovulation. In the immediate vicinity of each ovary is a fallopian tube.

Fallopian tubes (fallopian tubes) are two hollow tubes with holes that run from the ovaries to the uterus and open at the top of the uterus. There are villi at the ends of the tubes near the ovaries. When the egg leaves the ovary, the villi, with their continuous movements, try to grab it and drive it into the tube so that it can continue its path to the uterus.

The uterus is a hollow, pear-shaped organ. It is located in the pelvic cavity. During pregnancy, the uterus enlarges as the fetus grows. The walls of the uterus are made up of layers of muscle. With the onset of contractions and during labor, the muscles of the uterus contract, the cervix stretches and opens, and the fetus is pushed into the birth canal.

The cervix is ​​the lower part of the uterus with a passageway connecting the uterine cavity and the vagina. During childbirth, the walls of the cervix become thinner, the cervical pharynx expands and takes the form of a round opening with a diameter of about 10 centimeters, due to this, it becomes possible for the fetus to exit the uterus into the vagina.

**Lecture 8**Embryological concepts; Commitment to development; Embryonic induction; Symmetry breaking

The phenomenon of regulation indicates that cells appear to be sensitive to the presence or absence of adjacent cells; usually the easiest way to explain this is that the presence of some cells does not allow others to realize in development all the potencies that they possess.

However, a different situation is possible, in which cells receive stimuli from their neighbors that induce them to normal differentiation. The phenomenon of embryonic induction belongs to such cases.

A classic example in this regard is eye lens induction in amphibians. Lenses are formed on both sides of the embryo from thickenings of the ectoderm (the so-called placodes ) covering the head, and the formation of lenses occurs soon after the contact of the placodes with the eye vesicles (the future retina and pigment epithelium of the eyeball). Lens placodes invaginate, detach from the rest of the cephalic ectoderm, and occupy their definitive position within the iris. In many amphibian species, the lens does not form until the optic vesicle comes into contact with the superficial ectoderm. Moreover, transplanting to the place of the lens-forming ectoderm ectoderm from other areas of the head can cause the cells that make normal skin epidermis, turn instead to the lens cell. Thus, under the influence of the cells of the optic bladder, ectodermal cells form a lens, regardless of whether they form it normally or not. Is this process called induction? and the cells responding to the induction stimulus are competent cells.

Similar, but much more complex induction relationships are observed in the development of the brain and spinal cord in vertebrates. And the one and the other formed from dorsal ectoderm gastrula their foundation in the form of a tube located above the chord. On either side of the neural tube are myotomes . If normal morphogenetic movements are prevented in amphibians during gastrulation, then the future ectoderm turns into an empty, collapsed sac of cells, next to which the future mesoderm will be located into the endoderm. This is most easily achieved by growing embryos in hypertonic saline solution. As a result, despite the fact that the presumptive mesoderm and endoderm are differentiated to some extent, neither the brain nor the spinal cord appears in the presumptive ectoderm. Transplant experiments also confirm that not only cells normally destined for the formation of the nervous system need an inductive stimulus from the chord and mesoderm below them, but that other cells of the ectoderm of early gastrula are able to respond to this stimulus.

The brain and spinal cord of an adult animal are so complex anatomical structures that the question naturally arises whether the inductor determines all the details of their structure. Does it contain and transmit to competent cells all the information necessary for the formation of the central nervous system? Of course, this question can only be answered in the negative. For example, a competent ectoderm can form rather complex neural structures, such as the eye, under the influence of a stimulus that clearly has nothing to do with natural. Osmotic or temperature shock and a variety of chemical stimuli can induce forebrain-like structures in the ectoderm. We do not know all the details of very complex, inductive interactions in the formation of the brain, but it seems likely that the inductor "supplies" information that determines the development of competent tissue only in the most general terms, for example, information about the development of nervous tissue in accordance with the area in which it is located: in the anterior, middle, posterior or spinal cord.

In such cases, we are naturally interested in the mechanisms of cell communication with each other. What is transferred from the tissue of the inductor to the competent tissue in the process of induction? This, of course, may be a specific organic molecule, but in principle, the stimulus does not have to be molecular in nature. It can be, for example, of an electrical nature. It is likely that induction consists in the recognition by cells of the specific character of the distribution of molecules on the surface of neighboring cells. All these assumptions have been made to explain the phenomenon of induction, and the role of each of these mechanisms can be demonstrated with a number of examples. It is well known, however, that proteins and nucleoproteins are mediators in the case of neural plate induction.

**Lecture 9**Organogenesis

At the 3rd week of development , tertiary villi are formed in the villous chorion, more precisely, at the placenta formation site. A capillary grows into each villi, and from that time on, the histotrophic type of nutrition of the embryo is replaced by a hematotrophic (more complex and effective) one.

In the construction of the placenta, not only embryonic, but also maternal tissues are involved. Chorionic villi are in direct contact with maternal blood. Thanks to this, the embryo (embryo, fetus) during the entire intrauterine development receives from the mother the nutrients it needs, oxygen, releases metabolic products, carbon dioxide.

From the 3rd week of development, the placenta performs the functions:

• food;

• breathing;

• discharge;

• synthesis of hormones necessary for the development of the fetus;

• immunosuppression (suppression of cellular immunity);

• regulation of hemostasis in the intervillous space and the fetal circulatory system, providing low-resistance blood flow.

In the early placenta there is no protective function, therefore, physical, chemical, medicinal, radiation effects easily damage the process of differentiation and specialization of cells, which can stop the life of the embryo and the development of the placenta or cause gross malformations.

A primary stripe appears on the surface of the double-layered embryonic disc, which determines the axis of symmetry, the location of the head and tail ends of the embryo, its dorsal and ventral surfaces. The determination of the polarity of the organ buds precedes the embryogenesis process and is provided by a number of organs.

At the 3rd week of development, two most important structures appear on the surface of the embryonic disc on both sides of the midline: the neural plate and somites.

A third ( mesodermal ) layer develops inside the bilayer embryo .

During the entire 3rd week of development, the primary yolk sac appears - an extraembryonic organ that provides nutrition and respiration between the mother and the embryo until the chorionic villi begin to vascularize .

By the end of the 6th week of the embryo's life, the yolk sac undergoes reverse development. Simultaneously with the yolk sac, another extraembryonic organ, the amnion, develops. After some time, a large amniotic cavity will form, into which the embryo will be immersed.

With the beginning of the 3rd week of pregnancy, the differentiation of cells into specialized organs and tissues begins - the laying of all organs. The neural tube, heart and genital gonads are laid first. On the 21st day of pregnancy, ultrasound can record the heartbeat and with a frequency of 110-130 beats / min. The formation of a neural tube (isolation of its head section), heart and first vessels are a signal for the simultaneous laying of the liver, trachea, lungs, primary intestine, pancreas, and primary kidney.

The beginning of the embryonic period (the third week of development) coincides with the beginning of the first wave of the invasion of interstitial cytotrophoblast and the formation of a new circulation - matochno- placental -plodnogo.

The period of organogenesis, which is characterized by high rates of proliferation, mitotic division, cell differentiation, protein synthesis, growth factors, requires optimal blood flow, good blood supply, low vascular resistance, which improves the fluidity of the rheological properties of blood.

At the stage of histo- and organogenesis, genes regulating the differentiation and growth of organs, spatial morphogenesis are switched on, since during this period there are directed processes of induction, migration (movement) of cell layers, specialization of some, programmed death of other cells. Some of the cells, capillaries, which were unclaimed, disappear; the tail of the embryo is eliminated. The gills are transformed into the jaw appendages; the development of the male genitals reduces the Müllerian ducts.

The embryogenesis process is strictly sequential, complex, integrative. Therefore, the termination of the development of pregnancy is explained by the general term - " embryoplacental insufficiency", which depends on many factors, but the main thing is the genetic plan of human development.

Organogenesis is the most dangerous period of development.

Its calm natural course without the influence of damaging factors is ensured by the synchronous development of the placenta and the fetus.

Violation of the integrated system mother - placenta - fetal organs can lead to severe malformations incompatible or (worse!) Compatible with the life of the fetus. A child can be born with severe external and internal malformations and die either immediately or after a long time.

The development of gonads in a male embryo begins early - from the 3rd week, simultaneously with the heart and neural tube.

The first stage of gonad formation is the migration of undifferentiated germ cells from the yolk sac to the genital ridges. There they turn into gonadoblasts , and the coelomic epithelium covering the genital ridges transforms into the germinal epithelium. Gonadoblasts , plunging into the primary germinal epithelium, are formed into the sex cords.

**Lecture 10**Cell differentiation; Regulation of gene activity; Lateral braking

Histologically, the gonads are already clearly distinguishable, but so far they represent bipotent cells capable of becoming a testicle or ovary. Their structural organization is entirely determined by signals from the SRY region, which is located on the Y chromosome. In this region of the Y chromosome, a gene called "male determination factor" (FDMF) is induced. In its presence, sustentocytes ( Sertoli cells ) are formed, secreting an anti - Müllerian factor, which suppresses the development of Müllerian ducts. The testicles of the fetus immediately produce the male sex hormone - testosterone (the second stage of development of the genital organs of the fetus).

Further differentiation of the genitals depends on testosterone. If testicular hormone is absent, the phenotype will develop exclusively in the female pattern.

At the 4th week, the embryonic disc “folds” into a cylinder, inside which an intestinal tube is formed in the longitudinal direction.

In the middle segment of the intestinal tube, a connection with the secondary yolk sac is formed.

Organogenesis begins from this stage.

The first organ of the fetus is the heart. Its contractions can be observed using ultrasound from the 22nd day after fertilization.

At week 4, neurulation occurs - the formation of the nervous system, and by the end of this week the embryo has segments of the brain and spinal cord.

The brain is divided into cerebral vesicles (anterior, middle and posterior). At the same time, the respiratory system (2 rudiments of the lungs) is formed, the primary kidney ( mes-onephros ) and the mesonephral ( Wolffian ) duct differentiate .

In addition to the heart, neural tube, genital gonads, at 4 weeks of gestation , the embryo clearly shows the rudiments of the upper and lower extremities, the bulging of the area of ​​the pulsating heart. There are 5 pairs of branchial arches. Of course, the human embryo does not need gills, but this fact is referred to the biological law of development: "Ontogenesis repeats the main stages of phylogenesis." Repetition, of course, is not complete. The openings of the gill slits soon overgrow. From the first pair of branchial pockets, the middle ear develops, from the rest - the thyroid and parathyroid glands. Eyes are formed (there are no eyelids yet, and the eyes are wide open), a nose, nasal passages.

The embryo grows and develops rapidly. From 4 weeks , the first flexion movements in lateral directions appear . The movements coincide with the increase in the head end of the neural tube. During this period of development, the future brain occupies almost half of the neural tube. The beginning of the formation of spinal nerves and nodes is traced. In a two-chambered heart, an interventricular septum and thickenings arise, from which atrioventricular (atrioventricular) valves are formed.

At 4 weeks , the rudiments of the adenohypophysis and then the hypothalamus appear in the brain .

Fifth week of development - the most intensive formation of the fetal brain. Nerve fibers are formed that go from the organs to the brain. The rectum and bladder, trachea and esophagus are isolated from each other. The genitourinary sinus is differentiated. The spine grows in length, forming the first bend. The structure of the pancreas becomes more complicated. The upper and lower limbs grow intensively, and the upper ones grow much faster. The genital ridges are differentiated, migration of the sex cells to the rudiments of the gonads is observed.

The structure of the vessels of the placenta becomes more complicated. At 5-6 weeks of development, the peak of the first wave of cytotrophoblast invasion into the walls of the spiral arteries of endomyometrial segments is noted , due to which the elastomuscular components are destroyed . The endothelium of the vessels, placenta and subplacental zone is lined with fibrinoid . This process is very complex, it is regulated by decidual cells of the endometrium, in which regulatory proteins (PAPP-A) are simultaneously produced, which enhance the processes of cytotrophoblast invasion , and TGF, which limits the proliferation and invasion of cytotrophoblast . Fibronectin , laminin, and type 4 collagen, which are synthesized by the extracellular ( extracellular ) matrix, play a regulatory role in the two opposite processes .

As a result of the first wave of cytotrophoblast invasion , blood flow increases and the blood supply to the embryo increases. It has been proven that the process of invasion is, as it were, duplicated by the internal cytotrophoblast , which penetrates through the endothelium deep into the muscle wall (intravascular invasion) and from the anchor villi, which not only tightly fix the villous tree of the placenta, but are also stem cells for the formation of interstitial cytotrophoblast .

In the first 5-12 weeks and the entire II trimester of development, the invasion of the interstitial and internal cytotrophoblast adapts the vascular system of the uterus (in the area of ​​the placental bed) to optimal blood flow in the placenta and blood supply to the rapidly developing fetus.

Sixth week of development - rapid structural separation of the brain and spinal cord continues, the structure of neurons becomes more complex, the cerebellum differentiates. The development of the brain is accompanied by the activation of DAP. The embryo at this stage of growth bends and straightens its head, makes movements to the side. The dimensions of the head prevail over the body. A person's face looms. The upper and lower extremities become distinctly different. The elbow and wrist zones have been formed, the fingers and toes are clearly distinguished. The eyes are still wide open, pigment has appeared in the cells of the retina. The auricles are formed, the thymus gland is formed. Immediately after its formation, it is populated by fetal fetal lymphocytes.

If there is no Y chromosome in the chromosome set, then the gonad develops into an ovary. Primary germ cells from the yolk sac move to the gonadal cortex (the gonadal medulla degenerates). In contrast to male germ cells, female ones undergo mitosis and meiosis, ovogonia are formed , then oocytes , which by the 20th week of development are covered with granulosa cells and turn into primordial follicles. By the 7th week of development, up to 7 million stem cells are present in the ovary, most of which undergo reverse development.

The reproductive organs of the embryo develop from different duct systems. Male - from wolf , female - from Müllerian ducts.

The male sex determination factor, located at the SRY locus of the Y chromosome, inhibits the formation of Müllerian ducts and stimulates the development of Wolffians . Under the influence of fetal testosterone , the epididymis, vas deferens and seminal vesicles are formed from the wolf ducts.

The synthesis of testosterone by the embryonic testicles is not controlled by the cells of the hypothalamus and pituitary gland, which is formed at the same time. It is induced by HCG of placental genesis.

In the absence of anti - Müllerian factor , the uterus, fallopian tubes and the upper third of the vagina are formed from the Müllerian ducts. It is interesting to emphasize that the neck of the mark and the inner layer of the myometrium are initially formed . And much later - by 20 weeks of gestation , the middle and outer layers of the myometrium are formed .

The formation of the female genital gonad and internal genital organs of the female fetus proceeds against the background of a high content of estrogens of maternal origin. And although it is believed that hormones are not as necessary for the intrauterine development of a female fetus as testosterone is for the formation of male genital organs, nevertheless, hormonal disturbances at 6-12 weeks of pregnancy can cause deviations in the formation of the fetal uterus.

It is known that the use of diethylstilbestrol , prescribed for the threat of miscarriage in the first trimester of pregnancy, caused cervical and vaginal cancer in a number of patients exposed to this effect in utero. Diethylstilbestrol does not affect the development of male fetuses . The consequences of damaging factors, including hormonal disorders, can manifest themselves only after 20-30 years.

In utero exposure to diethylstilbestrol was exposed to persons born in the period 1940-1980, whose mothers during pregnancy took this synthetic estrogen to prevent miscarriage. Subsequently, it was revealed that diethylstilbestrol causes malformations of the uterus, hypoplasia of the cervix, a violation of the shape and structure of the uterus.

The mechanism of action of synthetic estrogens is the activation of estrogen-dependent genes.

Testosterone is the main androgen synthesized by the testicle of the fetus (as in the adult male). Testosterone secretion begins at the 5th week of gestation . Testosterone has a direct stimulating effect on the Wolffian ducts, inducing the development of the epididymis, the vas deferens.

Acting on the urogenital sinus, testosterone determines the formation of the male urethra, the prostate gland, and its effect on the urogenital tubercle leads to the formation of the external male genital organs. During these periods of development, dehydrotestosterone is produced , which affects the formation of the external genital organs in the male pattern. A fetus exposed to dehydrotestosterone during this period will masculinize regardless of its genotypic or gonadal sex. Conversely, the lack of androgens will lead to the development of a female phenotype.

Dehydrotestosterone is formed from testosterone by the enzyme 5α- reductase .

Under the influence of unfavorable factors in the early stages of pregnancy (hormonal disorders), the transition of the FDMP gene to the X chromosome is possible, and then a male fetus with a female karyotype 46XX or a female fetus with a male karyotype XY develops.

The FDMP gene encodes the formation of a protein called the zinc finger protein (ZFY) and is capable of reversing sex not only in the fetus, but also in adolescence and even adulthood. A gene mutation can cause gonadal dysgenesis, sometimes gonadal dysgenesis develops even in the absence of a gene mutation. The causes of this pathology are not known, hormonal disorders, viral infections that easily penetrate the early placenta are possible. As a rule, the offspring of such women is infertile.

Until now, the reasons for the mutation of genes and their transfer to chromosomes, including " point mutations", are unknown . Gene mutations lead to structural and functional disorders in the hypothalamus, pituitary gland, adrenal glands, ovaries, causing abnormalities in the sexual differentiation of the brain (which differs in male and female fetuses), sex reversal, and a change in sexual orientation. But all this can happen many years after birth, when neither the mother nor the obstetrician remember what factors could have caused the deviation.

The sixth week of development includes the peak of cytotrophoblast invasion into the walls of the spiral arteries of the endometrial segments of the uterus and the formation of uterine-embryonic circulation.

At the seventh week of development , the limbs of the embryo change greatly. Most often, the embryo keeps the upper limbs on the chest, the lower limbs are bent at the knee joints, the embryo periodically unbends the legs or places them along the body.

The vessels of the placental bed stop responding to vasoconstrictor factors, their lumen expands, blood flow increases, and the intensity of BMD increases significantly.

Cytotrophoblast cells and giant multinucleated cells periodically accumulate in the lumen of the spiral arteries, preventing the penetration of maternal erythrocytes into the fetal bloodstream. By this time, instead of erythroblasts , erythrocytes circulate in the blood of the embryo. Cytotrophoblast cells sometimes move against the bloodstream, which indicates their extreme activity.

The embryo (with the formation of placental- embryonic circulation) grows even more intensively. In one week (from the 7th to the 8th), the embryo completely loses the somiton , turning into a fetus with the species-specific characteristics of the human body. The final kidney, adrenal glands, ureters are formed. Divided fingers and toes. The fetus periodically brings its hands to the face, its thumb touches the mouth, and sucking movements appear. The eyes are still wide open, the superciliary arches are highly developed. Sleep phases are followed by short periods of active movement. For the first time, isolated movements of individual hands are observed.

The eighth week of development is the last week of the period of embryogenesis, during which the embryo has everything to be considered a fetus.

After 8 weeks, the embryo is called the fetus.

The fetus has its own blood group, it has (or does not have) the Rh factor. In the areas of the brain, the differentiation of the first layer of the cerebral cortex occurs, although their processes are still short and the cells do not contact each other. The boundaries of the forebrain, posterior and midbrain deepen, the boundaries of the medulla oblongata are clearly traced. All brain structures are intensively supplied with blood.

The head has a rounded shape, its dimensions are still disproportionately large. It takes up almost half the length of the body.

The end of the embryonic period is characterized by complete differentiation of the brain and spinal cord, the central section and the peripheral nervous system.

The fetal behavioral responses become more complex. The fetus covers its face with its hands, tries to suck its thumb. In case of danger (artificial termination of pregnancy) - tries to evade the inserted instruments, while the movements of the fetus away from the medical curette are recorded . The fetus swallows amniotic fluid, the kidneys function, and urine accumulates in the bladder.

At 8 weeks of pregnancy, the first wave of cytotrophoblast invasion ends . All walls of the spiral arteries are lined with fibrinoid . The spiral arteries of the uterus are essentially transformed into typical uteroplacental arteries, providing a constant flow of arterial blood to the intervillous space.

Each support villus is divided into 20 new villi. Their number at 8 weeks is 3 times the number of villi of a 5-week placenta.

Stromal canals appear , oriented along the course of some villi, and multinucleated Kashchenko- Hofbauer cells circulate through them , which have the function of placental macrophages.

The growth of the mass of the placenta in the first trimester outstrips the growth of the embryo / fetus.

At 6-8 weeks of pregnancy, the most active synthesis of CG takes place, which coincides with the laying of the nuclei of the hypothalamic-pituitary region and the formation of the genital gonads. After 10 weeks of pregnancy, the level of hCG in the blood and urine decreases and remains constantly low until the end of pregnancy, increasing by 5% at 32-34 weeks of gestation. At the same time, the permeability of placental microchannels increases . With multiple pregnancies, the hormone content is higher, in proportion to the number of fetuses.

HCG has an immunosuppressive property important for pregnancy . An embryo with foreign paternal genes, in the absence of a decrease in cellular immunity, should be rejected from the mother's body as a foreign transplant. However, most often this does not happen precisely due to the suppression of the activity of the immune system. HCG provides immunological tolerance, reducing the risk of immune rejection of the fetus in the first 12 weeks of pregnancy.

In the subsequent trimesters of pregnancy, placental proteins are immunosuppressants: trophoblastic β1-glycoprotein (TBG), placental α1-microglobulin and α2-microglobulin of fertility.

At 6 weeks of pregnancy (at the peak of cytotrophoblast invasion and intensification of uterine-embryonic blood circulation), the synthesis of all hormones that ensure the growth and development of the fetus passes from the ovary to the placenta.

It should be noted that from the 6th to the 8th week of pregnancy, the synthesis of PGE2, which has a vasodilating, antiplatelet and anticoagulant effect, significantly increases . Their effect after the 8th week of gestation is so significant that blood pressure decreases by 8-12 mm Hg. Art. in the general system of maternal hemodynamics.

Thus, the period of pregnancy from the 3rd to the 8th week is the most significant and responsible.

Main events:

• embryogenesis and construction of the structure of the early placenta;

• structural organization of all organs with the inclusion of their functional activity;

• formation of the phenotype in accordance with the genotype of the fetus.

The sex of the fetus is determined by the set of chromosomes: XX - female, XY - male. However, the gonads and germ cells initially have the same organization. For the formation of the male genital gonad, not only the Y chromosome is required, but also FDMP, which suppresses the formation of female genital organs. If the Y chromosome is absent, only the female sex is formed.

The reproductive organs of the male fetus are determined by the effects of testosterone and dehydrotestosterone . Violation of hormonal relationships in the mother's body can lead to genetic errors in the development of the fetus.

**Lecture 11**Intestinal epithelium; Intestinal stem cells; In vitro culture ; The clonality of intestinal crypts

The intestinal suction epithelium of mammals is a typical single-layer columnar epithelium with a pronounced polarity of the cellular elements that form it. Differentiated, actively functioning cells make up the epithelial layer that covers the outer surface of the villi - the main structural units of the mucous membrane of the small intestine (Fig. 14, a ). They are microorganic structures with their own nervous, muscular and vascular apparatus. Exfoliation of differentiated cells that have completed their life cycle occurs at the top of the villi. The constancy of the cellular composition of the epithelial layer is provided due to the intensive multiplication of cambial crypt cells - finger-like invaginations of the epithelium into the mucous membrane. Crypt epithelial cells differ from differentiated ones in smaller size, greater basophilia of the cytoplasm, absence or weak development of morphological signs of specific differentiation.

The morphological and cytochemical differences between cambial and differentiated cells also correspond to the unequal intensity and nature of metabolic processes. They are especially clearly detected on autographs of the intestinal mucosa of laboratory rodents in experiments with precursors of protein or RNA synthesis. In the early stages after the injection of labeled amino acids, the intensity of their incorporation into the proteins of the cells of the crypt - villus system clearly predominates in the cambial cells of the crypts in comparison with the functioning cells of the villus epithelium. The intensity of protein synthesis in the latter gradually decreases from the base of the villi to their tops. In experiments with the precursor of RNA synthesis (3H-uridine), its incorporation into newly synthesized RNA occurs only in crypt cells. Thus, these experiments show that specific cytodifferentiation in the intestinal epithelium leads not only to complete blocking of autosynthetic processes and cell reproduction, but also stops reading information from the DNA of the nuclear apparatus in differentiated cells, i.e., blocks transcription. Protein synthesis, necessary for the functioning of cells, proceeds with the use of various types of RNA synthesized during the period of their reproduction.

Thus, the intestinal epithelium is a complex heterogeneous system. A monolayer epithelial layer consists of subpopulations of cells differing in their properties and significance : cambial, poorly differentiated, differentiated and ending the life cycle. These cell subpopulations are distributed on the lateral surfaces of crypts and villi and at the apical ends of the latter. Despite the differences in metabolic activity and topographic position, all subpopulations of cells represent a single system. It is represented by cells at successive stages of the life cycle.

The rates of their reproduction, the duration of existence and the intensity of death are strictly coordinated.

The main differentiated cells of the intestinal epithelium in quantitative (88% in the small intestine) and in functional terms are absorbing prismatic cells. Their feature is a pronounced polarity, manifested in the structural and functional differences between the apical and basal parts of cells. The apical cytoplasm and the outer plasma membrane form microvilli - relatively large formations, in mammals 1.4 microns long and 0.08 microns wide, occupying the entire cell surface facing the intestinal lumen. Outside, each microvillus has a plasma membrane, due to which the total suction surface of the epithelial layer is very extensive (Fig. 14.6).

Longitudinal fibrillar actin structures are clearly visible in microvilli . At the base of the microvilli, they pass into the fibrillar layer located parallel to the apical surface of the cells and containing, in addition to actin, myosin and a- actinic fibrils. One of the important properties of microvilli is their ability, as a result of slow contraction and relaxation, to change the absorbing surface of cells and the distance between adjacent microvilli. Functionally very important, their structure is a well-developed mucopolysaccharide supramembrane complex that clothe each microvillus and is secreted by the absorbing cells. Intensive renewal of the supramembrane complex was demonstrated by electron autoradiography using 3H-glucose.

The main function of the microvilli apparatus is to provide active transport - the first link in the selective flow of substances into the cytoplasm of the absorbing cell. The specialized plasma membrane has a pronounced ability for active and passive transport. In addition, enzymes are located on the surface of the membrane of microvilli that provide hydrolytic degradation of macromolecules that are not destroyed by the action of enzymes of gastric and intestinal juices. This auxiliary mechanism is called "parietal, or membrane, digestion". Its work involves mucopolysaccharides of the supramembrane complex of microvilli. They form a complex network between adjacent microvilli that does not allow large food particles, as well as bacteria, to pass through. In addition, it plays the role of a lattice into which enzymes from the intestinal cavity can be incorporated. These enzymes, together with the enzymes synthesized by the absorbing cells and built into their membranes, carry out the final stages of hydrolysis of small molecules passing through the cells.

**Lecture 12**Regeneration, wound healing and cancer

1. Healing by primary intention Wound healing is a regenerative process; it is an expression of the biological, physiological response of the body to the injury. Regeneration depends on the characteristics of the damaged tissue. The laws of regeneration are common to all tissues, that is, each tissue gives, during regeneration, cells that are homogeneous with the mother; the epithelial cover is regenerated from epithelial cells, connective tissue elements from connective tissue, etc. In the parenchymal organs, healing proceeds by scar formation. The cells of the central nervous system do not regenerate.

2. Conditions for wound healing General conditions. Young age, the absence of physiological disorders in the most important organs and systems (central nervous system, cardiovascular, hematopoietic, metabolism, etc.) contribute to the correct and timely regeneration. With exhaustion, cachexia, vitamin deficiency, diabetes, anemia, syphilis, tuberculosis, vascular sclerosis, the healing conditions worsen.

Local conditions. Localization, anatomical and physiological conditions in the wound, good blood supply to the wound site, preservation of innervation, unfavorable conditions for the development of infection are factors that accelerate regeneration. For example, wounds on the head and face heal quickly, thanks to good blood supply; if the nerve is damaged, they heal more slowly, since trophism in the wound area is disturbed, and sometimes they do not heal at all, persistent ulcers remain at the site of the scar or amputation stump. Foreign bodies, sequesters, non- absorbable sutures prevent wound healing; wound regeneration depends on the presence or absence of infection in the wound.

3. Types of wound healing If the edges of the wound are even, not bruised, closely adjacent to one another, healing is the fastest and most perfect; this type of healing is called prima intentio . If the wound is gaping or there is a cavity between the edges of the wound, healing is slower through the development of granulation tissue; this type of healing is called secondary intention ( secunda intentio ).

Primary tension in wound healing is characterized by fusion of the wound edges without microscopically visible intermediate tissue. Such healing is possible:

with full and tight contact of the wound edges, but without tissue tension;

in the absence of infection in the wound;

in the absence of a hematoma;

while maintaining the viability of the edges of the wound;

in the absence of foreign, infected bodies and foci

in the absence of necrosis.

Primary intention heals wounds after clean operations, that is, aseptic wounds, as well as wounds with damaged, uneven, bruised edges after excision of these edges within healthy tissues, that is, when, after initial surgical treatment, they are brought into a state of aseptic wound with smooth edges and they are sutured. Healing takes place within 5-7 days with the formation of a delicate scar.

Clinically, after removing the stitches, slight signs of aseptic inflammation are observed: slight edema, slight redness; scar tissue gradually loses signs of inflammation; the newly formed epidermis becomes keratinized , the developed capillary network of blood vessels becomes empty, the scar becomes dense and pale.

The formation of connective tissue (scar) is the basis of wound repair. Vascular neoplasm begins in the first hours after injury. The neoplasm of the epithelium runs parallel to the proliferation of connective tissue and endothelial elements. By the end of the day after injury, the multiplication of epithelial cells in the depths of the Malpighian layer is noted .

**Lecture 13**Molecular genetic approaches in the study of progenitor cells and retinal stem cells

Age - related macular degeneration (AMD) is a heterogeneous clinical condition in which the central retinal or macular region is affected , which leads to a persistent decrease in visual acuity [1, 2]. Damage to the macular region is characterized by one or more of the following signs: the formation of "dry" or "wet" druses, changes in the pigment epithelium of the retina (RPE), geographic atrophy of the pigment epithelium and the choriocapillary layer in the region of the central fossa of the retina ( fovea ), neovascular or exudative maculopathy [3, 4].

At the moment, there are two main forms of AMD development: slowly progressing "dry" form and rapidly progressing "wet" or exudative form, accompanied by neoplasm of blood vessels. Both forms of AMD with different rates lead to irreversible blindness with atrophy of RPE and photoreceptors [5]. The "dry" form occurs in 85% of cases, and the "wet" form - in 10-15%.

AMD is a leading cause of blindness among the elderly, especially those over 70. About 600 thousand new cases of the disease are registered worldwide every year [6]. The projected number of people with AMD will reach 196 million in 2020, increasing to 288 million by 2040 [7].

AMD pathogenesis

The exact pathophysiology of AMD is not fully understood, there are many theories for the development of this disease, but the results of ongoing research are expanding our knowledge about the disease and its underlying mechanisms. It is believed that the pathogenesis of AMD is the result of a complex multifactorial interaction of metabolic, functional, genetic and environmental factors [8].

With aging, intracellular residual bodies containing lipofuscin accumulate in RPE cells. RPE cells express waste products that are usually removed by choriocapillaries ; however, as RPE dysfunction progresses, the permeability of Bruch's membrane changes , resulting in the accumulation of extruded material (druses) between the two layers. The appearance of drusen may be single or accompanied by thickening of the collagen layers of Bruch's membrane , degeneration of collagen and elastin and its calcification . In addition, it has been noted that the thinning and thinning of the choriocapillaries in patients with AMD may contribute to the reduction and difficulty in the removal of extracellular material, which leads to the formation of drusen [9].

Drusen formation as a signal of impaired RPE function with further progression leads to the death of photoreceptors. Progressive damage to Bruch's membrane with vascular endothelial growth factor (VEGF) activation promotes the growth of abnormal vessels under the retina, which have subretinal extravasation and may bleed before regressing and forming a scar. Thus, the visual result of any form of AMD is permanent loss of central vision [10].

Several studies have examined the molecular pathway that underlies atrophy and vision loss. This pathway describes that RPE death contributes to the loss of photoreceptors and, as a consequence, gradually leads to loss of vision [11, 12].

Existing treatment

Currently available treatments include drug therapy using drugs that protect the eye tissue, strengthen the vascular wall, block oxygen free radicals or antioxidants that occur when redox processes are disturbed, which always accompany the development of retinal degeneration and are often one of the the main links of its pathogenesis [13]. Conservative therapy is effective only in the early stages of the process, its results are unstable - as a rule, it helps to suspend or slow down further loss of vision, but does not significantly improve it. New possibilities in the treatment of diseases of the posterior segment of the eye have appeared with the use of laser treatment, combined methods combining drug, revascularizing and metabolic effects on the affected tissues [14].

The use of anti-VEGF drugs for the treatment of patients with “wet” AMD can be considered a therapeutic breakthrough; over the past decade, this therapy has become the “gold standard” in the treatment of neovascular AMD [15].

However, none of the currently existing treatment methods can completely cope with a disease such as AMD. This suggests that AMD at the moment can be considered an incurable disease. An alternative or additional method of pathogenetically grounded treatment of AMD can be such a direction of regenerative medicine as cell therapy.

Regenerative medicine

Rapid progress in the field of regenerative medicine opens up new possibilities for the treatment of serious diseases and disorders, as well as incurable diseases. Large-scale efforts to develop such a treatment as cell replacement therapy have already begun to be successfully implemented in diseases such as diabetes mellitus, parkinsonism, Alzheimer's disease, multiple sclerosis, and cardiac diseases [16, 17].

In the human body, tissue renewal and restoration depends on somatic stem cells, and eye tissue is no exception [18, 19]. Stem cells have the intrinsic ability to proliferate indefinitely, and by definition they are capable of differentiating into virtually any type of cell. Stem cells are broadly classified into (a) totipotent stem cells that differentiate into embryonic and extraembryonic tissues, (b) pluripotent stem cells that form embryonic tissues (ectoderm, endoderm, and mesoderm), and (c) multipotent stem cells that differentiate into limited the number of cell types (eg, mesenchymal stem cells) [20].

The organ of vision has an immune privilege in comparison with other organs in relation to the development and use of this direction, since the immune response in the tissues of the eye is reduced due to the mechanisms of immunological tolerance, and in the anterior segment of the eye it is carried out according to a specific type of "immune deviation associated with the anterior chamber ". This makes it possible to use in cell therapy not only autologous , but also allogeneic cells that survive for a relatively long time in the recipient's body without immune rejection [21].

Early experimental studies have shown that stem cells are highly compatible with the retina and are capable of adapting to Müllerian , amacrine , bipolar, horizontal and glial cells and photoreceptors [22, 23]. For example, several studies have demonstrated that subretinal transplantation of green fluorescent protein positive cells - precursors of the retina into degenerative retinal recipients leads to the migration of transplanted cells into the outer nuclear layer, to differentiation into immunohistochemically identifiable cells of rod photoreceptors and to an improvement in pupillary light responses [24].

Mesenchymal stem cells

Adult stem cells such as mesenchymal stem cells (MSCs) are one of the promising cell types with a high potential for regenerative properties in various diseases. Bone marrow, adipose tissue, dental pulp, peripheral blood, umbilical cord blood, and fetal liver and lungs are well known from many sources of MSCs. Adipose tissue-derived stem cells are of particular interest to scientists [25].

Initial studies demonstrating the isolation of progenitor cells from the tissues of the adult eye and the successful transplantation of these stem cells into the degenerating retina aroused wide interest among ophthalmologists and became the impetus for the development of this direction [26].

There are studies on the effectiveness of stem cells associated with the stromal-vascular fraction of adipose tissue in the treatment of "dry" AMD. In the course of the study, stem cells together with platelets were injected into the suprachoroidal space - after 6 months. a significant improvement in visual acuity, an increase in the sensitivity of the retina, as well as changes in optical coherence tomography data were obtained [27].

The latest advances in the technology of three-dimensional (3D) bioprinting cannot fail to attract attention - using a special printer, building blocks-bioindicators can be applied and layered. MSCs have been proposed in this 3D bioprinting technology for retinal reconstruction [28, 29].

Embryonic stem cells

Pluripotent embryonic stem cells (ESCs), possessing a constitutive ability to differentiate into all cell types and an extensive migration potential, are ideal candidates for the treatment of diseases of the human retina. The results of the latest experimental work prove their unique properties.

Scientists from China have published the results of their study, during which they developed a line of clinical human ESCs that differentiated into retinal pigment epithelial cells. A clinical study was initiated in 3 patients with wet AMD to study the safety and tolerability of transplantation. A suspension of these cells was injected into the subfoveal pocket after removal of the neovascularized choroidal membrane . Patients were followed up for 12 months with no side effects from transplantation. Anatomical data indicate the emergence of a new cell layer, similar to RPE, in the previously damaged area, and visual and physiological testing showed limited functional improvement [30].

In March, the London Blindness Project, carried out by University of London researchers in conjunction with Moorfields Eye Hospital , announced the results of a trial in which 2 patients with AMD were treated with a bioengineered patch containing retinal cells derived from human ESCs [31]. The patch using coaxial stem cells differentiated into RPE, a monolayer of cells that forms the interface between the retina and the circulatory system, damaged in people with AMD. Scientists were able to replace a patch of damaged epithelium with healthy cells by surgically attaching a patch to the base of the retina. Both recipients tolerated the procedure well (as in a similar study in Japan) [32]. However, unlike the Japanese study, both participants in the London Project study reported improved vision.

The search for ways to facilitate the movement of cells into the desired position, engraftment and retention of the transplanted cells in the desired tissues to realize the full regenerative potential provided by stem cells, increase the survival and viability of the transplanted cells has become the basis for many studies, including such properties of stem cells that are able to facilitate immunosuppressive therapy after their clinical allotransplantation [33].

Induced pluripotent stem cells

Programming differentiated somatic cells by forced expression of specific transcription factors can induce the transformation of somatic cells into ESC-like cells with pluripotent qualities [34, 35].

Several studies have been performed demonstrating the transformation of induced pluripotent stem cells (iPSCs) into RPE-like cells with their own pigmentation, the ability to tight junctions, the expression of a specific RPE protein, and the properties of adhesion and organic functioning with the outer segments of photoreceptors [36].

IPSCs from patients with retinitis pigmentosa have been used as a cellular platform for screening drugs that could reduce the harmful effects of rhodopsin point mutations [37]. And in recent studies, iPSCs were differentiated into mature retinal ganglion cells capable of transmitting action potentials [38, 39].

Domestic achievements of regenerative medicine

The attention of Russian scientists was attracted by the eye 's own progenitor cells found in the limbal region [40]. Successful results of constructing a biokeratoprosthetic complex from limbal mesenchymal multipotent stem cells in an in vitro experiment give grounds to consider the proposed design suitable for further experiments in vivo [41].

Currently, a technology for creating spheroids has been proposed, aimed at the development of unique reparative cell modules that support the functional potential of unique limbal cells and microtissues for the treatment of various pathologies of both the anterior and posterior segments of the eye [42].

Corneal epithelial cells, which have adequate plasticity and are able to be cultured in vitro , as well as are highly promising as a model for research and a source of cells for bioartificial cornea , are also a promising source for regenerative medicine [43]. An effective method has been developed for isolating the posterior epithelium (endothelium) of corneal cells [44, 45].

3D-cell cultures of multipotent mesenchymal stromal cells and RPEs have been considered as an effective method of treatment providing safe and long-term neuroprotection in the treatment of neurodegenerative diseases of the eye organ [46, 47].

A successful technology for the cultivation of MSCs with magnetic particles for subretinal administration was developed [48].

In the work of the employees of the department of traumatology and reconstructive surgery of Helmholtz ”of the Ministry of Health of Russia, the safety and effectiveness of intravitreal , retrobulbar and suprachoroidal transplantation of neural stem / progenitor cells under experimental conditions have been proved . Based on the results of complex clinical, electroretinographic and histological studies, the authors showed that the suprachoroidal administration of neuronal and mesenchymal stem cells in the developed doses has a neuroprotective effect, which is most pronounced for the function of photoreceptors and Müller cells after modeling laser damage to the retina of rabbits and retinal ischemia. In the long term, stem cell transplantation helps to accelerate the recovery of retinal function.

Conclusion

An analysis of the scientific literature, which provides the results of studies aimed at improving the technologies of cell therapy and regenerative medicine, unfortunately, indicates that the development of domestic regenerative medicine in ophthalmological practice lags behind international analogues, which is mainly due to the lack of the necessary legislative foundation. Successful and effective research on stem cells, as well as the rapid increase in the prevalence of incurable diseases, indicate the need to eliminate this problem.

**Lecture 14**Animal models for assessing the contribution of stem cells to liver development

In the last 20 years , preclinical studies using various in vivo models have played an important role in understanding human hematopoiesis [1-6]. Hematopoietic stem cells (HSCs) are key players in hematopoiesis . A small population of HSCs is able to give rise to the offspring of cells with high proliferative activity, which in turn can differentiate into millions of mature lymphohemopoietic cells, which has been proven in experimental and clinical studies on bone marrow transplantation [7-11].

Initially, these properties of HSCs were shown in experiments on transplantation in mice, where in order to demonstrate the restoration of populations of lymphoid and myeloid lines in a recipient, genetic markers were used that were absent in the donor animal [12-17]. The possibility of using retrovirus- labeled HSCs made it possible to conduct a more detailed analysis of their engraftment and proliferation after transplantation. As a result of these studies, an idea has emerged about the possible behavior of donor HSCs in the recipient [18, 19].

By analogy with mice, human HSCs were studied, where bone marrow with different genetic labels [20] or autogenous cells transduced with retroviral vectors were used for selective detection . However, these studies were retrospective due to the limited number of informative clinical data and low rates of retroviral transduction [21-23]. In addition to in vivo models, the development of in vitro studies using primitive human HSCs has facilitated the study of hematopoiesis . Models that used long-term cultivation of bone marrow cultures demonstrated the ability of primitive HSCs to differentiate into cells of various myeloid and lymphoid lines [24-26]. One of the studies described the ability of LT-CIC cells (LT-CIC - stem cells that provide hematopoiesis in a long-term culture of bone marrow) to maintain the population of hematopoietic progenitor cells in culture for up to 100 days . [26]. In the experiments of SJ Szilvassy et al . (1990) demonstrated a directly proportional relationship between the amount of LT-CIC and the ability of HSCs to self-renewal during serial transplantation [27, 28]. The correlation between clinical transplantation and in vitro models of human HSC research has significantly expanded the possibilities of studying hematopoiesis .

It is also known that transplanted cells or organs from one species to another ( xenotransplants ) can be maintained in a macroorganism if they are introduced before the animal develops a competent immune system [29–31]. The transplanted organ or cells serve to "train" the developing immune system, creating tolerance for heterologous cells.

There are a number of methods for induction of tolerance in developing mice, sheep and pigs that are effective in the study of organ transplantation [1-3].

Human cell lines, tumors and organs transplanted into immunodeficient mice function for a long time due to the provision of an appropriate microenvironment in which the transplanted cells or tissues can take root or undergo expansion for a long time without being rejected by the host animal organism. Many strains of mice have been described with naturally developing mutations or with targeted gene damage that are important for normal immune function [32, 33]. For transplanted human HSCs in preclinical studies, the most commonly used models are naturally developing recessive mutations of severe combined immunodeficiency ( scid ), beige ( bg ), nude ( nu ), and X-linked immunodeficiency ( xid ), which turned out to be the most useful in research ...

Thus, we see how various experimental models for studying the biological properties of cells and their interaction with each other are able to complement each other and serve the development of science as a whole. In this article, we will try to briefly describe the various experimental models that have contributed to the understanding of hematopoiesis .

Sheep xenotransplantation model

The gestational period in a sheep is approximately 160 days . In the interval between 50 and 60 days of gestation , the hematopoietic system of the sheep fetus develops rapidly. This development precedes the onset of the immune system between 67 and 77 days of gestation . ED Zanjani's group (1996, 1997) showed that transplantation of human HSCs between 50 and 60 days of gestation can facilitate the engraftment and existence of human cells in the fetus before the immune system becomes active and cannot induce tolerance to human antigens [1 -3]. Thus, understanding the combination of increased engraftment and tolerance would provide prolonged graft survival.

The first description of long-term engraftment of human HSC in a sheep fetus was published by AW Flake et al . in 1986 [34]. Human fetal liver cells were selected for the first experiments because the fetal liver does not contain any T-lymphocytes that can lead to graft-versus-host disease (GVHD). The cells were transplanted into 65 day old sheep fetuses. In a large series of born animals studied over 10 years, approximately 70% of human HSCs were found in the peripheral blood and bone marrow [35-38]. The number of human cells averaged about 5% and was represented by all hematopoietic lines , including T cells. Human cells were identified up to 4 years of age of the animal [35-38]. To assess self-renewal of bone marrow cells from primary animals, HSCs were transplanted into secondary pre- immune fetuses. Human cells were found in approximately one third of the recipients, which demonstrates self-renewal of the original engrafted cells [37, 38]. Moreover, the proportion of human cells in chimeric sheep could be increased by injection of recombinant human cytokines. Thus, injection of a combination of interleukin-3 (IL-3) and granulocyte- macrophage colony-stimulating factor (GM-CSF) or human stem cell factor (SCF) increased the number of identified human cells in the peripheral blood or bone marrow of a sheep by five or two times , respectively [39, 40].

Human fetal liver cells are not used as a source of HSC for clinical transplantation. For this reason, ED Zanjani et al . (1990, 1993, 1995) determined whether adult human bone marrow cells or umbilical cord blood cells, which are widely used as a source of HSC in the clinic, can take root in a sheep fetus [41-43]. Approximately 50% of sheep transplanted with human bone marrow or umbilical cord blood cells, the graft engrafted, providing the proportion of human cells the same (bone marrow) or more (umbilical cord blood) as in recipients of human fetal liver cells [41-43]. Unfortunately, over 80% of these recipients developed signs of GVHD, which made it difficult to track long-term results [41-44]. Bone marrow or cord blood transplantation with T-cell depletion prevented GVHD, but was associated with a lower engraftment rate and a lower survival rate [43, 44]. It has also been shown that co-injection of human stromal cells of bone marrow origin with human CD34 + cells of human bone marrow after depletion of T cells improves long-term engraftment [45]. These results are almost identical to those observed with bone marrow cell transplants in the clinic, and confirm the adequacy of the sheep fetus model for the analysis of human HSC.

A research group led by ED Zanjani in 1993, studying human HSCs, raised a fundamental question regarding their phenotype [36]. Most of the protocols for enrichment of human HSCs are based on the knowledge that they express the CD34 antigen and are characterized by the absence of markers of mature myeloid or lymphoid cells, such as Lin . Lin - / CD34 + / HLA-DR - human bone marrow cells are capable of long-term engraftment in sheep fetuses and the production of mature offspring of all lines, in contrast to Lin - / CD34 + / HLA-DR + cells, which do not take root [44]. CD38 is expressed by colony- forming cells but is probably not characteristic of HSCs. To confirm this hypothesis, Lin - / CD34 + / CD38-- and Lin - / CD34 + / CD38 + - bone marrow cells were transplanted into sheep fetuses. Long-term engraftment was observed in animals that received cells of the first population, while after intrauterine administration of Un - / CD34 + / CD38 + cells, transient, short-term engraftment was observed [44]. In a similar work, it was shown that CD133, together with CD34, can be used as a marker for HSC [46]. Studies in immunodeficient mice injected with human HSCs have shown that CD34 cells can also take root and generate mature human HSCs [47]. When CD34 + - and CD34 - cells of human bone marrow or peripheral blood after treatment with cytokines were injected into sheep fetuses, both populations engrafted and gave rise to human HSCs, confirming the initial observations [48, 49].

Self-renewal of HSCs and maintenance of primitive progenitor cells in sheep chimeras have been investigated in other series of experiments. In particular, sheep fetuses were transplanted with Lm - / CD34 + - human bone marrow cells differing in the expression of Thy-1 [50, 51]. Interestingly, engraftment was observed only in animals that received CD34 + / Lin - / Thy-1 + cells, while engraftment was not shown in the case of Thy -1 ^ -cells. After 3 months. in sheep that received CD34 + / Lin - / Thy-1 + cells, the number of primitive CD34 + cells that could export the dye Rhodamine 123 (Rho10), as well as the number of CAFC ( cobblestone area-forming cells ) were determined . By the number of CAFC can predict the number of primitive hematopoietic kletok- preceding -nits with properties corresponding GCW. The frequency of CAFC among CD34 + Rho1ow cells and in chimeric sheep was similar to the frequency of CAFC among CD34 + Rho1ow cells in the original transplant [51]. These studies have demonstrated that the initial population of CD34 + / Lin - / Thy-1 ^ cells contains primitive HSCs capable of engraftment and expansion in the sheep body [50, 51].

In studies on mice, a high level of expression of the c- kit receptor was characteristic of HSCs [17, 18]. Human CD34 + bone marrow

cells that express c- kit on high (c- kithigh ) and low (c- kitlow ) levels or not expressed at all (c- kit -), fruits injected sheep. An insignificant level of engraftment was determined in animals that received CD34 + / c- ki ^ - pets [52]. Engraftment was initially observed at a similar level in animals transplanted with human CD34 + / c - kithigh - and CD34 + / ck / tl0w-pets. However, after 3 months. only in the case of the introduction of cells with a low level of c- kit expression in the organism of the recipient animals were human cells determined [42]. These data suggest that the primitive human cells responsible for engraftment and expansion in the body of a sheep fetus were CD34 + / ck / tl0w-kettles [52].

The sheep fetus has also been used as a model for the study of human genetic diseases such as X-linked severe combined immunodeficiency (X-SCID), which is characterized by a deficiency of T-lymphocytes and non-functional B-lymphocytes, which in turn leads to a lack of humoral and cyto- toxic immune response. X-SCID is caused by a mutation in the gene for the y-receptor IL-2, which encodes the common y-chain (yb) of the receptors IL-2, IL-4, IL-7, IL-9, and IL-15 [53]. To determine if the ovine fetus could serve as a digestible model for the study of X-SCID therapy, cytokine-mobilized CD34 + peripheral blood cells from patients with X-SCID were transplanted into ovine fetuses. As with the X-SCID patients, human mustache-myeloid cells and B-cells were found in sheep chimeras, but T-lymphocytes were absent. Transduction of retrovirus containing the y-receptor gene IL-2 to X-SCID cells of patients prior to transplantation led to the formation of chimera sheep with a large number of us + T-lymphocytes [54], which coincided with the results of a clinical study of X-SCID gene therapy [ 55, 56]. However, 2 out of 11 patients in the clinical study subsequently developed T-cell leukemia [57], while in the experiment - in six sheep chimeras - no side effects were noted for 10 months. observations [54].

Mouse models

Although the sheep fetus meets all the criteria for use as a model of human hematopoiesis , it is unacceptable to most research laboratories. The cost of keeping large animals, combined with a 100-fold increase in the amount of cytokines required to produce the largest number of human cells, imposes significant economic constraints on this model. In addition, transplanting human cells into a sheep fetus requires delicate and complex surgical skills that few people possess. Many scientific groups have worked to create an animal model of human hematopoiesis that summarizes the benefits exhibited by the sheep fetus with the cost-effectiveness of a small animal model. These efforts have focused on mice with genetically determined immunodeficiencies [32, 33].

BNX mice are homozygous for the three mutations causing immunodeficiency: mutation allele beige ( bg , bezhevyyL mutation allele nude ( nu , bald) and X-linked immunodeficiency ( xid ).

Mice homozygous for the bg mutation have skin hypopigmentation and form giant intracellular granules, leading to incorrect sorting of lysosomal proteins. The immune function in these mice is impaired primarily due to a defect in the activity of natural killer cells (NK cells). The effects of the bg mutation are similar to those in human Chédiak-Higashi syndrome (CHS). Genes containing bg and CHS mutations have been isolated [58, 59]. Murine and human genes are highly homologous , are expressed ubiquitously, and encode a protein that resembles statmin , a protein involved in microtubule polymerization [58, 59].

Mice homozygous for the nu mutation are bald and lack thymus; the latter leads to severe suppression of the immune system [60]. The gene, which is mutated in nu mice and rats, is a member of the winged helix or fork head families of transcription factors [61, 62]. The xid mutation causes a mild immune defect associated with the loss of some B cell functions in homozygous females or hemizygous male mice [63]. The xid mutation is a point mutation in the Bruton's tyrosine kinase or Btk gene [64, 65].

The combination of xid and nu mutations causes an immunodeficiency that is more severe than with each mutation alone. The deficiency of NK cells caused by the bg mutation makes bg / bg , nu / nu , xid / - (known as BNX) mice almost completely immunodeficient .

The original description of the successful transplantation and maintenance of primitive human hematopoietic cells in a mouse model was made by S. Kamel-Reid and JE Dick (1988), who injected human bone marrow cells into sublethally irradiated BNX mice [66]. The recipient mice contained low concentrations (<1%) of human cells in the bone marrow and spleen, which could be determined by detecting human repetitive DNA in these organs. In addition, human colony- forming cells from the same tissues could be identified by selectively growing them in a culture medium supplemented with human cytokines IL-3 and GM-CSF. Since these cytokines do not support the growth of murine colony- forming cells, it was possible to stimulate a small number of human progenitor cells for 8 weeks . after transplantation [66].

Building on this initial success, other research groups have focused on the problem of increasing low levels of human cells engrafting in BNX mice. Thus, JA Nolta et al . (1994) suggested that the murine microenvironment may be “inhospitable” for human HSCs and put forward a hypothesis according to which the simultaneous administration of human stromal cells with HSCs , providing the production of IL-3 (a species-specific cytokine that is important for the maintenance and differentiation of human HSCs) , can lead to the formation of optimal niches for HSC [67]. Co-transplantation of HSC and processed bone marrow stroma improved the rate of engraftment of human cells by 6% [67]. Human hematopoietic progenitor cells were isolated from the spleen and bone marrow of the recipient mouse up to 9 months. after transplantation. Since processed stromal cells do not persist as long as hematopoietic progenitor cells, the authors concluded that the introduction of stromal cells facilitates engraftment and proliferation of human HSCs [67]. In the same study, the authors showed that the cells responsible for long-term engraftment expressed CD34 [67].

In BNX mice transplanted with bone marrow CD34 + cells, production of human T-lymphocytes, progenitor cells, and mature cells of most myeloid types was observed, but there was no production of B-lymphocytes [68].

Since the full range of human HSC types cannot be fully identified in most transplanted BNX mice, definitive analysis of human HSCs cannot be made. However, the potential for differentiation into lymphoid and myeloid cells is a property associated with most primitive hematopoietic progenitor cells. To clarify whether any cells that have taken root in the BNX mouse are capable of giving rise to both lymphoid and myeloid cells, human CD34 + umbilical cord blood cells transfected with retroviruses with the neomycin resistance ( neo ) gene were transplanted into irradiated BNX mice along with processed stromal cells. After transplantation of human hemo-poetry colonies containing neo-provirus was isolated from the recipient animals. We also separated human CD33 + myeloid and CD3 + lymphoid cells from murine cells by FACS sorting and cultured myeloid and T lymphocyte colonies separately . DNA was isolated from colonies, and the presence of a provirus was confirmed by polymerase chain reaction (PCR) [69]. If Mi- eloidnye progenitor cells and T-lymphocytes containing the neo-provirus , came from one of progenitor cells, the gene is a retrovirus had to be built into the same place in the genome of each cell type. The insertion site of the provirus was established using PCR, where the long terminal sequence of the provirus was inserted into the cellular DNA [70]. During the analysis of the results obtained, it was revealed that in all progenitor cells the provirus had the same localization. These studies provided the first and most significant evidence for the presence, among human cells that take root in BNX mice, cells that have properties associated with HSCs [69].

Despite initial success with BNX mice, the low rate of human cell engraftment has led many groups to investigate the switch to using SCID mice as human cell recipients. The scid mutation is a deletion of the gene for DNA-dependent protein kinase (DNA-PK) [71]. Mice homozygous for the scid mutation lack functional B and T lymphocytes due to defects in V (D) J recombination and repair of double-stranded breaks, making SCID mice deficient in both cellular and humoral immune functions. T. Lapidot et al . (1992) demonstrated that the engraftment of human cells injected with CB.17 SCID mice was higher compared to the engraftment observed in BNX recipients. Also, improved engraftment and proliferation of human cells can be obtained in SCID mice injected with human SCF and PIXY 321 fusion molecules . Human cells persisted in mouse bone marrow for 8-10 weeks , i.e. the time during which the animals were usually withdrawn from the experiment [72].

Despite the fact that homozygous scid / scid mice are deficient in humoral and cellular immune functions, their NK cells function normally. Thanks to the careful work of L. Shultz et al . (1995) found that a strain of nonobese diabetes (NOD) mice was NK-deficient [73]. The scid mutation was transferred to the NOD base by repeated backcrossing. The resulting line, designated NOD-SCID, is "more immunodeficient " than any other line carrying the scid mutation due to a deficiency in NK cell activity [73]. It has been shown that multipotent mesenchymal stromal cells (MMSCs) from NOD-SCID mice support the engraftment of human HSCs better than MMSCs from mice of other immunodeficient lines [74]. Compared to CB.17 SCID or other SCID lines, the engraftment rate of human HSCs in NOD-SCID mice averaged 10–30%, depending on the number and source of injected cells [75–77]. Approximately 30% of homozygous NOD-SCID mice are prone to developing thymomas , which have been shown to result from endogenous invasion of the retrovirus Emv-30 [78]. Careful backcrossing led to the development of an additional line of NOD-SCID mice, in which the frequency of thymoma was significantly reduced [79]. Residual immune function in NOD-SCID mice is further compromised by backcrossing the allele with P2-microglobulin dropout and NOD-SCID background. NOD-SCID P2-0 mice maintain the highest level of transplanted human cells [79].

The human cells found in NOD-SCID mice after transplantation were primarily of the B cell line (CD19 +) with a small number of myeloid cells (CD14 + and CD11b +). Occasionally, a small number of human T lymphocytes can be identified in the rudimentary thymus of NOD-SCID mice. However, mature erythroid cells and erythroid progenitor cells cannot be reliably identified in NOD-SCID mice transplanted with normal bone marrow CD34 + cells [80].

As with BNX mice, the inability to maintain all hematopoietic lines complicates the analysis of human HSC activity in NOD-SCID mice. In this regard, the cells that restore the lymphocyte population in SCID mice are usually described as SCID- repopulating cells (SRC) [5, 80]. Similar to human HSCs, SRCs are part of the Lin - / CD34 + population of human umbilical cord blood cells, bone marrow, and mobilized peripheral blood. Also, many authors found that the Lin - / CD34 + / CD38-- subpopulation of cells contains a large number of SRCs [81–83]. Recently, it has been shown that human HSCs in NOD-SCID mice are derived from two populations of CD34 + cells. The "first wave" of cells comes from short-lived progenitors; The “second wave” that overlaps the first is derived from long-lived progenitor cells [84 ]. These data suggested that the first and second waves of human HSCs may reflect the progeny of CD34 + / CD38 + and CD34 + / CD38 - cells, respectively.

Also, NOD-SCID mice showed signs of human primitive hematopoietic progenitor cells that did not express CD34. This phenomenon was described when CD34 cells were injected into NOD-SCID mice. Human hematopoietic progenitor cells and mature cells were formed in quantities that could compete with those formed during transplantation of CD34 + cells [47]. Many of the cells in NOD-SCID recipients who underwent CD34 transplantation expressed CD34, thereby indicating that CD34 cells may give rise to CD34 + cells in NOD-SCID and BNX mice [47, 85].

Similar to BNX mice, clonal human hematopoiesis has been shown in NOD-SCID virally transfected human cell recipients . NC Josephson et al . (2002) investigated multiple clones of CD19 + -B-lymphocytes and exposed populations of individual myeloid cells obtained from NOD-SCID mice, which were transplanted with cells transfected with foamy- virus [86]. In contrast to BNX mice, where all inclusions in lymphoid cell lines had partners among myeloid cells, in NOD-SCID mice, less than 20% of proviral inclusions had partners in B- lymphocyte clones and myeloid cells [87]. Thus, these data confirmed that human cells present in NOD-SCID mice at 6-10 weeks . after transplantation, come from different populations of short-lived and long-lived SRCs.

NOD-SCID mice are used as models for the study of human hematopoietic diseases. A series of studies have shown that bone marrow cells from patients with lymphoid and myeloid leukemias transplanted into SCID mice are involved in the development of leukemias [86-91]. There are also several examples of the use of NOD-SCID mice as a model for the study of inherited human hematopoietic diseases. Thus, the bone marrow of patients with β-thalassemia has overt erythroid hyperplasia caused by ineffective red cell production. When NOD-SCID mice transplanted bone marrow CD34 + -cells obtained from patients with p-thalassemia erythroid hyperplasia was usually sufficient to overcome the inefficient production of erythroid cells in Ms - votnogo -retsipienta [75]. NOD-SCID-P2-0 mice injected with CD34 + cells from a patient with X-linked chronic granulomatous disease (gp91phox deficiency) produced human neutrophils with a defect in oxidase activity. Transplantation of X-CDG CD34 + cells with transduction of a lentivirus containing the gp91phox gene restored the oxidase activity of human neutrophils [92]. Thus, NOD-SCID mice can serve as a model for the development of treatments for human hematopoietic diseases.

Humanized SCID Mice

To optimize the hematopoietic microenvironment of human cells in immunodeficient mice, a humanized SCID- hu mouse strain was developed . Initially, fragments of the human fetal thymus were implanted under the mouse SCID kidney capsule, followed by injection of human CD34 + cells. The result was the production of circulating human T lymphocytes and myeloid cells in a relatively short period [93]. The SCID- hu Thy / Liv model represents an improvement to this system in which both the human fetal thymus and fetal liver are transplanted under the SCID mouse kidney capsule. In most of these animals, human cells and T cells of the lymphocytic and myeloid lines are detected in the peripheral blood of the animals in the period from 6 to 12 months. [94]. The study of transplanted organs revealed the presence of human hematopoietic granulocyte macrophage colony -forming cells (CFU-GM) and primitive human erythroid burst- forming units (BFU-E) [94]. In addition, these animals contained a full complement of differentiated human T lymphocytes, including CD3 + / CD4 + CD8 +, CD3 + / CD4- CD8 + and CD3 + / CD4 + /

CD8 cells [95]. Usually, human T cells in immunodeficient mice cause severe lethal GVHD [96]. However, human thymus transplants in SCID- hu Thy / Liv mice have been shown to contain murine dendritic cells, which explains both tolerance and long-term production of human T-lymphocytes without GVHD [97].

The final improvement of the SCID- hu line is the SCID- hu bone model. In this model, fragments of human fetal bones are transplanted subcutaneously with SCID mice. Bone fragments vascularize within 4-6 weeks , providing a “human” hematopoietic microenvironment in which a small number of human myeloid progenitors and B cells can be maintained for 12 weeks . and more [98]. SCID mice with fragments of human fetal bones, thymus and spleen (BTS) are known as SCID- hu BTS mice. SCID- hu BTS mice can maintain human HSCs of all strains for 36 weeks . and more [99]. Moreover, bone fragments can be injected directly with purified hematopoietic cells, which may subsequently affect their ability to restore population and proliferate. The SCID- hu BTS model is used to determine the phenotype of most primitive human HSCs. CD34 + / Lin - / Thy-1 + cells injected into human bone fragment in SCID- hu BTS mice were enriched in cells that generate hematopoietic progenitor cells and mature cells

**Lecture 15**Hematopoietic stem cells: identification, characterization and analyzes

Hematopoietic stem cell transplantation (HSC) is a rapidly developing technology that has the potential to provide a cure for malignant blood diseases (leukemias, lymphomas , myelomas) and other hematological diseases (for example, primary immunodeficiency, aplastic anemia, myelodysplasia ). HSC transplants are also sometimes used for solid tumors (for example, some germ cell tumors) that respond to chemotherapy.

HSC transplantation promotes healing by:

    Bone marrow recovery after myeloablative cancer killing

    Replacement of abnormal bone marrow with normal bone marrow in benign hematological diseases

BSC transplantation can be autogenous or allogeneic. Stem cells can be collected from

    Bone marrow

    peripheral blood

    Umbilical cord blood

Peripheral blood has largely replaced the bone marrow as a source of stem cells, especially in HSC autotransplantation, since it is easier to harvest stem cells, and the recovery of neutrophils and platelets is faster. Umbilical cord blood HSC transplantation is often used only in children, because there are too few stem cells for an adult. A potential future source of stem cells are pluripotent stem cells (certain cells taken from adults and reprogrammed to act as stem cells).

There are no contraindications for autogenous HSC transplantation.

Relative contraindications to allogeneic HSC transplantation include age over 50, previous HSC transplantation (HSCT), and severe comorbidities.

Allogeneic HSCT is limited mainly by the lack of tissue-compatible donors. The ideal donor is an HLA-identical sibling, followed by an HLA-matched sibling. Therefore, related HLA-incompatible HSC donors or unrelated compatible donors (found in international registries) are often used. However, the rate of long-term complication-free survival may be lower compared to HSC transplants from HLA-identical related donors.

The technology of using HSCs isolated from umbilical cord blood is in the stage of development, it is possible that HLA compatibility does not play a decisive role in it.

Methodology

For isolation of bone marrow stem cells, 700–1,500 ml (maximum 15 ml / kg) of bone marrow are aspirated from the posterior iliac crest of the donor; local or general anesthesia is used.

To isolate stem cells from peripheral blood, the donor is injected with recombinant growth factors (granulocyte- colony-stimulating factor or granulocyte-macrophage- colony - stimulating factor) to stimulate proliferation and mobilization of stem cells, followed by standard apheresis after 4–6 days. Then, to identify and isolate stem cells, fluorescence-activated cell sorting is performed.

Stem cells are injected over 1–2 hours using a large-diameter central venous catheter.

Conditioning modes

To prevent transplant rejection, prior to allogeneic HSCT for cancer, the recipient is first given a conditioning regimen [for example , myeloablative regimen, such as cyclophosphamide 60 mg / kg / day IV for 2 days with total full- dose whole-body irradiation, or busulfan 1 mg / kg orally 4 times a day for 4 days and cyclophosphamide without general radiation] to induce remission and suppress the immune system.

Similar conditioning regimens are used in allogeneic HSCT, even if not indicated for this malignant disease, to reduce the incidence of rejection and relapse.

Such conditioning regimens are not used prior to autogenous HSCT in cancer; instead, cancer-specific drugs are used.

A non-myeloablative immunosuppressive conditioning regimen (eg, cyclophosphamide , thymus irradiation, antithymocyte globulin [ATG], and / or cyclosporine ) may reduce the risk of illness and death and is useful in elderly patients, patients with underlying medical conditions, and those susceptible to graft-versus-tumor reactions (eg, with multiple myeloma).

After transplant

After transplantation, the recipient receives colony-stimulating factors to reduce the duration of post-transplant leukopenia, a prophylactic course of drugs to protect against infections, and with allogeneic TCSC - a prophylactic course of immunosuppressants lasting up to 6 months (usually methotrexate and cyclosporine ) to prevent a reaction from donor T-lymphocytes to to HLA molecules of the recipient (graft versus host disease). If the patient does not have a fever, broad-spectrum antibiotics are usually avoided.

Graft engraftment usually occurs 10–20 days after HSCT (earlier in the case of stem cell transplantation from peripheral blood) and is determined by the absolute neutrophil count> 500 × 106 per liter.

Complications

Complications after stem cell transplantation can occur in the early period (less than 100 days after transplantation) or in the late period. After allogeneic HSCT, the risk of infection increases.

Early complications

Serious early complications include:

    Impaired engraftment

    Rejection

    Acute graft versus host disease (GVHD)

Engraftment failure and rejection occurs in <5 % of patients and is manifested by persistent pancytopenia or an irreversible decrease in the number of blood cells. Treatment is carried out with glucocorticoids for several weeks.

Acute GVHD is observed in recipients with allogeneic KSC transplantation (in 40% of patients who received cells from HLA-compatible siblings, and in 80% from unrelated donors). In this condition, fever, rash, hepatitis with hyperbilirubinemia , vomiting, diarrhea, abdominal pain (with possible development of intestinal obstruction), and weight loss are noted.

Risk factors for acute GVHD include:

    Inconsistency by sex and the HLA antigen system

    Unrelated donor

    Elderly age of the recipient and / or donor

    Pre-sensitization of the donor

    Inadequate prevention of GVHD

Diagnosis of acute GVHD is based on history, physical examination, and liver function tests. Treatment: methylprednisolone 2 mg / kg intravenously once a day, increasing to 10 mg / kg if there is no response within 5 days.

Late complications

Serious late complications include:

    Chronic GVHD

    Recurrence of the disease

Chronic GVHD can occur on its own, develop from acute GVHD, or appear after acute GVHD resolves. Chronic GVHD usually begins after 4–7 months. after HSCT (the period can vary from 2 months to 2 years). Chronic GVHD is observed in recipients with allogeneic HSCT (35-50% of recipients who received transplants from HLA-compatible related donors, 60-70% from unrelated donors).

Chronic GVHD primarily affects the skin (eg, lichenoid rash, scleroderma) and mucous membranes (eg, keratoconjunctivitis dry , periodontitis, orogenital lichenoid reactions), as well as the gastrointestinal tract and liver. The main characteristic is immunodeficiency; obliterating bronchiolitis may also develop , similar to those that develop with lung transplantation. Ultimately, GVHD is fatal in 20-40% of patients who develop it.

Treatment for GVHD that affects the skin and mucous membranes is optional; for more severe conditions, treatment is similar to that for acute GVHD. Using monoclonal antibodies or mechanical separation, T-lymphocyte depletion in the allogeneic donor graft is depleted, which reduces the incidence and severity of GVHD, but it also reduces the graft-versus-tumor response, which can enhance cell proliferation, improve engraftment, and reduce disease recurrence. The relapse rate with allogeneic HSCs is higher due to the lack of graft versus tumor response and because circulating tumor cells can be transplanted. Ex vivo examines tumor cells isolated before autogenous transplantation.

In patients without chronic GVHD, all immunosuppressants may be discontinued after 6 months. after HSCT; thus, late complications are rare in this patient population.

Forecast

The prognosis after HSC transplantation varies depending on the indication and the procedure performed.

In general, disease recurrence occurs in

    40-75% of autogenous HSC transplant recipients

    10-40% of allogeneic HSC transplant recipients

Successful (without bone marrow cancer) rates are

    30-40% of patients with recurrent lymphoma sensitive to chemotherapy

    20-50% of patients with acute leukemia in remission

Compared with chemotherapy alone, HSC transplantation improves survival in patients with multiple myeloma. The success rate is lower in patients with more advanced disease or reactive solid cancers (eg, fetal cell tumors). Recurrence rates are reduced in patients with graft-versus-host disease (GVHD), but in general mortality is increased if GVHD is severe.

An intensive conditioning regimen, effective prophylaxis of GVHD, cyclosporine- based treatment, and good supportive care (eg, antibiotics, if necessary, prophylaxis of herpes virus and CMV infection) increase long-term survival after HSCT without disease recurrence.