One of the main technical aspects that until recently limited the creation of tissue and organ transplants is the complexity of the framework selection, which would ensure adequate oxygen and nutrients transfer. This problem has been potentially solved with the development of methods for the decellularization of organs while preserving the structural and functional characteristics of their native microvascular network [1, 3, 4].

Decellularization-derived scaffolds have several advantages over other techniques. Firstly, they retain the native extracellular matrix 3D structure, which fosters cell repopulation and proper function. Secondly, they can incorporate growth factors and release important bioactive molecules upon degradation. Thirdly, they are available and easy to be obtained from humans and animals [1, 3, 5].

The aim of this study was to generate decellularized liver scaffolds from different species, analyze their morphological (architecture, level of residual cellularization and others), biochemical (molecular composition), mechanical (elasticity, durability, etc.) and immunological properties.

Methodological approaches for creating decellularized animal hepatic scaffolds have been generated, residual DNA has been determined and the bioreactor prototype for subsequent recellularization has been worked up. Primary hepatocytes, endotheliocytes and Kupffer cells cultures as well as mesenchymal stromal cells have been isolated and cultures have been established followed by cell culture cryobank creation.

Overall, such biomedical products can serve as effective models for testing pharmacological agents, also they have the potential to be used in clinical treatment as patient-specific transplants with full biocompatibility.

BIBLIOGRAPHY

1. *Badylak, S. F.* The extracellular matrix as a biologic scaffold material / S. F. Badylak, et al. // Biomaterials. – 2007. – № 28. – P. 3587–3593.

2. *Jadlowiec, C. C.* Liver transplantation: current status and challenges / C. C. Jadlowiec, et al. // World J. Gastroenterol. – 2016. – № 22. – P. 4438–4445.

3. *Oldani, G.* Chimeric liver transplantation reveals interspecific graft remodeling / G. Oldani [et al.] // J. Hepatol. July 5, 2018.

4. *Soto-Gutierrez, A.* A Whole-Organ Regenerative Medicine Approach for Liver Replacement / A. Soto-Gutierrez, et al. // Tissue Eng Part C Methods. – 2011. № 17(6). – P. 677–686.

5. *Uygun, B. E.* Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix / B. E. Uygun, et al. // Nat Med. – 2010. – № 16(7). – P. 814–820.

IDENTIFICATION OF CLONAL REARRANGERS OF GENES OF ANTIGEN-RECOGNIZING RECEPTORS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIS

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The use of modern, high-tech molecular genetics methods of diagnosis in oncohematological practice has a wide practical potential. One of the most powerful methods to evaluate the effectiveness of therapy, compare the protocols of treatment, control the preservation of remission and predict the risk of relapse, is the definition of minimal residual disease (MRD).

Keywords: acute lymphoblastic leukemia, gene rearrangement, minimal residual disease, PCR screening, heteroduplex analysis.

Discrimination between polyclonality and monoclonality remains one of the goals in the differential diagnosis between normal lymphoid population and lymphoid neoplasia.

Currently several methods are used for detection of malignant lymphoid cell monoclonality. They are: 1) flow-cytometric immunophenotyping; 2) cytogenetical definition of chromosome aberrations; 3) polymerase chain reaction (PCR) analysis of breakpoint fusion regions of leukemia-specific chromosome translocations. The attractive approach to evaluate lymphoid cell monoclonality is PCR-based analyses of specific junction region rearrangement of immunoglobulin (Ig) and T cell receptor (TCR) genes [1, 3]. A junctional diversity of TCR and IG gene loci determines the clonal variety of normal T and B lymphocytes. Lymphoid tumors originate from

a clonal expansion of cells carrying identical copies of rearranged genes, which determines the possibility of using TCR and IG genes as clone-specific markers for minimal residual disease monitoring [2, 3].

The aim of the study was to identify clonal rearrangements of the antigen-recognizing receptors of lymphocytes for use as molecular-genetic targets in monitoring acute lymphoblastic leukemia in children. Bone marrow samples of 20 patients with acute lymphoblastic leukemia were included in the study. Isolation of genomic DNA was carried out by phenol-chloroform extraction from the fraction of mononuclear cells. We performed PCR screening with pramers panel including 26 clonal Ig/TCR gene rearrangements of five genes: TCRB, TCRD, TCRG, IGH, IGK. PCR products were visualized in a 2% agarose gel. Heteroduplex analysis in non-denaturing conditions was performed to determine monoclonal and polyclonal rearrangements. Monoclonal PCR products were stored as DNA fragments of the appropriate size (250–300 nucleotide pairs). Monoclonal rearrangements were identified in all 20 patients (from 1 to 3 targets per patient). Bends of homoduplexes were cut from polyacrylamide gel, DNA eluted and sequenced with the same pair of primers used for PCR. Sequence data were processed by the SeqAnalysis 5.2 software. The alignments of the forward and reverse sequences and creation of assembled sequence were performed by the Conting Express (Vecot NTI). Identification of V- D- J-gene segments and junction was performed using the on-line IMGT web tool [2]. As a result of sequencing and analysis for all 20 DNA samples, the nucleotide sequence of all monoclonal rearrangements was determined. Patient-specific primers were selected for their junctional regions of Ig/TCR genes.

We were able to identify clonal Ig/TCR rearrangements by PCR in all 20 leukemic samples included in the study.

BIBLIOGRAPHY

1. *Campana, D.* Minimal residual disease in acute lymphoblastic leukemia / D. Campana // Hematology. American Society of Hematology.– 2013. – P. 7 –12.

2. *Meleshko, A. N.* Prognostic value of MRD-dynamics in childhood acute lymphoblastic leukemia treated according to the MB-2002/2008 protocols / A. N. Meleshko [et al.] // Leuk. Res. – 2011. – № 35. – P. 1312–1320.

3. *Meleshko, A. N.* Cloning of variable fragments of tumor immunoglobulin, assembling and expressing of human scFv protein in E.coli for anti-idiotype vaccination / A. N. Meleshko, et al. // Experimental Oncology Exp Oncol. 2013. – Vol. 35. – № 1. – P. 1–7.

OBTAINING RECULLYARIZED LIVER TRANSPLANTATS AS A PERSPECTIVE DIRECTION OF REGENERATIVE MEDICINE

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Bioengineering of tissues and organs, to date, is one of the intensively developing and promising areas of regenerative medicine. The idea of creating artificial organs for transplantation, in the light of the lack of donor organs and growth in their needs, looks very attractive. The achievements of recent years in the field of regenerative biomedicine are very impressive, but there are many unsolved scientific problems and social aspects.

Keywords: regenerative medicine, tissue engineering, decellularization, recellularization, scaffold, allogeneic cell culture.

The development of bioengineering of tissues and whole organs potentially allows solving a number of tasks of transplantology: the problem of lack of donor organs, biocompatibility, the need for lifelong application after immunosuppressive therapy. This direction of regenerative medicine has an interdisciplinary nature, and is located at the intersection of biology, medicine, cybernetics, biophysics, biochemistry, bioinformatics and exists because of their intensive development [1, 2].

One of the main problems of tissue and organ bioengineering is obtaining frames with adequate vascularization, which would ensure optimal perfusion of blood for the transport of nutrients and oxygen in the creation of complex volumetric organs. This problem was partly solved with the introduction of methods for the decularization of whole organs from corpses or animals. The use of modern protocols makes it possible to obtain decellular