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REVIEW

ABOUT EXPANDING OPTIONS FOR USING BALB/C NUDE MICE FOR EXPERIMENTAL STUDY OF HUMAN MALIGNANT TUMORS *IN VIVO*

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ABSTRACT

The article has a problematic scripting nature. At the present stage, in addition to objective factors that make it difficult to create adequate experimental models of human oncogenesis, there is a significant backlog of domestic science in the development of this direction. This reduces the availability for Russian specialists of humanized immunodeficient animals corresponding to the level of research tasks. Based on the analysis of literature data, we discuss approaches that can expand the use of a widely available immunodeficiency animal model-BALB/c nude mice. The possibility of using human mesenchymal stem cells that are not rejected by BALB/C Nude mice for local humanization of immunodeficient animals and improving the structural and functional characteristics of xenografts is considered. The possibility of obtaining xenografts of human glioblasts supported in the body of immunocompetent BALB/c mice after serial passages of organotypic tumor spheroids in the brain of BALB/c nude mice is analyzed.

Keywords:
xenografts of human malignant tumors, BALB/c nude mice, humanization methods, mesenchymal stem cells, immunocompetent animals, sibs

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ОБЗОР

О РАСШИРЕНИИ ВАРИАНТОВ ИСПОЛЬЗОВАНИЯ МЫШЕЙ BALB/C NUDE ДЛЯ ЭКСПЕРИМЕНТАЛЬНОГО ИЗУЧЕНИЯ ЗЛОКАЧЕСТВЕННЫХ ОПУХОЛЕЙ ЧЕЛОВЕКА *IN VIVO*

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РЕЗЮМЕ

Статья имеет проблемный, постановочный характер. На современном этапе, помимо объективных факторов, затрудняющих создание адекватных экспериментальных моделей человеческого онкогенеза, имеет место значительное отставание отечественной науки в разработке данного направления. Это снижает доступность для российских специалистов гуманизированных иммунодефицитных животных, соответствующих уровню исследовательских задач. В работе на основе анализа сведений литературы обсуждаются подходы, которые могут расширить варианты использования широкодоступной иммунодефицитной животной модели — мышей BALB/c nude. Рассматривается возможность использования мезенхимальных стволовых клеток человека, не отторгаемых мышами BALB/c nude, для локальной гуманизации иммунодефицитных животных и улучшения структурно-функциональных характеристик ксенографтов. Анализируется возможность получения ксенографтов человеческих глиобластом, поддерживаемых в организме иммунокомпетентных мышей BALB/c после серийных пассажей органотипических сфероидов опухоли в головном мозге мышей BALB/c nude.

Ключевые слова:
ксенографты злокачественных опухолей человека, мыши BALB/c nude, методы гуманизации, мезенхимальные стволовые клетки, иммунокомпетентные животные, сибсы

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The goal of the scientific direction for creating effective models of human tumors is to reproduce human oncogenesis and related systemic changes as completely as possible in the body of experimental animals. This model is designed to provide an objective assessment of the effectiveness of anti-cancer drugs and technologies in relation to specific patients, as well as to clarify the mechanisms of development of human malignant tumors [1, 2]. At the present stage, the most promising models of the "avatar" type are considered to be immunodeficient animals that are transplanted with human hematopoietic stem cells (HSCs) and biopsy material obtained directly from cancer patients (patient derived xenograft, PDX) [1–3]. At the same time, there are a number of problems that make it difficult to reproduce the malignant process and the main part of the human immune and hematopoietic systems in the body of such animals [1, 2, 4].

First, a significant restriction on the use of humanized animals is the development of the graft-versus-host disease reaction, which occurs at different times depending on the type of human cells that are used for humanization, and inevitably leads to the death of animals. Secondly, the modern development of humanization methods allows us to recreate only some parts of the human immune system, and they are only partially reproduced. More complete restoration of populations of various blood cells in most cases requires the inclusion of transgenesis methods and additional research, which significantly reduces the availability of such animals.

In addition to objective factors that make it difficult to create adequate experimental models of human oncogenesis, there is a historical situation of a significant backlog of Russian science in the development of this direction. In Russia, there is no industry for the production of various versions of humanized immunodeficient animal models for fundamental and clinical medicine, which is already established in the United States, Western Europe, and China [1–3]. Thus, a serious problem is the high cost of animals belonging to the modern popular immunodeficiency lines NSG (NOD/SCID gamma mouse) and BRG (BALB/c Rag2), which are produced mainly in foreign laboratories [2]. At the same time it's well known, that immunodeficient BALB/c nude mice the most available to Russian researchers are not suitable for the main humanization procedures involving the introduction of either mature human peripheral blood mononuclears (h-PBL) or human hematopoietic stem cells (h-HSCs) [1, 2].

In our opinion, in this situation, in parallel with the development in accordance with foreign standards, we should also develop other approaches that allow us to obtain scientific and practical results based on available animal models. In this regard, it seems appropriate to carry out exploratory research in two directions. The first direction involves identifying effective modes of coimplantation of BALB/c nude xenografts of malignant tumors and human mesenchymal stem cells (MSCS) in mice to approximate the growth characteristics of transplants in the body of immunodeficient animals to the parameters of the original malignant process. The second direction may be related to the use of BALB/c nude mice to produce human glioblastoma xenografts that aren't rejected by their immunocompetent heterozygous sibs.

About the possibility of implantation of xenografts of human malignant tumors and human mesenchymal stem cells (MSCs) in mice BALB/c nude

When experimentally using xenografts of human malignant tumors, the key question is whether their structural, kinetic, invasive and metastatic characteristics correspond to the parameters of the initial malignant process [1–4]. It is known that cells of the immune system actively participate in the development of tumors, exerting an inhibitory or, on the contrary, stimulating effect on malignant cells, depending on specific systemic and local changes in the tumor-bearing organism [5–7]. In addition to the modifying action of the immune system cells, the achieve-

ment of such compliance is largely determined by the adequate xenograft microenvironment, which may strongly influence on the development of the tumor and its sensitivity to therapeutic effects [4, 5, 8]. This is why orthotopic PDX transplantation, which involves the transfer of biopsy material to areas similar to the loci of the original tumor development, has undoubted advantages over heterotopic subcutaneous transplantation [1, 2, 4]. Thus, in the case of orthotopic transplantation, PDX growth is supported by cells that are heterologous to human tissues, but functionally similar to them, and their constellations of the animal body located in the peritumoral zone. At the same time, orthotopic PDX transplantation cannot fully provide similarity to the malignant process in the human body [4, 5, 8].

Meanwhile, the results of a number of studies indicate the prospects of using human mesenchymal stem cells (MSCs) to overcome significant differences between the growth of primary tumors and their transplants in the body of immunodeficient animals, mainly NOD/SCID mice. As you know, MSC are multipotent stromal cells that are localized in different organs and tissues (cord blood, bone marrow, adipose tissue, dental pulp, placenta, etc.), which have the ability to differentiate into varied types of cells (osteocytes, chondrocytes, adipocytes, etc.) and migrate to the area of the tumor or the focus of inflammation [11, 12]. At the same time, it is assumed that MSCs can be differentiated directly in the tumor zone. It is known about the immunoregulatory effects of human MSCs in NSG mice [13]. Of great importance is the question of the interaction of human MSC and malignant cells, about which there is conflicting information. Thus, the inhibitory effect of human MSCs on the growth of orthotopically transplanted xenografts of the U87MG line cells was shown [14]. At the same time, accumulated data indicate the key role of MSCs in tumor progression due to their ability to facilitate epithelial-mesenchymal transition and increase tumor metastatic potential by their interaction with tumor cells [11, 15]. The ability of MSC to enhance regenerative processes also indirectly indicates the tumor-stimulating potential of these cells [16, 17]. The diverse effects of human MSCs on xenograft growth, obviously are depended on the difference in the types of interaction between human MSC and tumor cells — direct intercellular interaction or indirect modulation through the release of cytokines and other biologically active factors [18].

Recently, however, the view of MSCs as tumor-stimulating factor has begun to dominate. Thus, the results of a meta-analysis and a systematic review of a number of studies published in 2018 indicate that the introduction of MSCs contributes to an increase in the number of metastases and the frequency of metastasis by 1.25–2.0 times compared to the control parameters [19]. Very impressive results were obtained in an earlier period by American researchers from the University of Salt Lake City [20]. It was shown that orthotopic transplantation of breast tumors of major molecular subtypes directly from patients to NOD/SCID mice, accompanied by implantation of human MSCs supports a significant number of characteristics of the original tumors. The authors used fresh tissue fragments from primary tumors or samples of metastatic breast cancer cells collected immediately after surgery or ascitic fluid drainage from 42 different patients. At the same time, xenografts of the tumor were propagated by serial transplantation without any stages of in vitro cultivation, which eliminated the problem of selective adaptation to the conditions of cultivation. As a result, new models of breast tumor growth and metastasis in the form of transplantable tumors obtained directly from patients were proposed. The grafted material largely reflected the diversity of breast cancer forms and preserved critical features of parent tumors, including histological features, metastasis sites, clinical markers, gene expression profiles, number of DNA copies, and estrogen dependence for ER+tumors. At the same time, the combined administration of human MSCs with the tumor material maintained the stability of the properties of the original tumors and accelerated

the proliferation of malignant cells, stimulating angiogenesis. Moreover, the survival rate of the obtained xenografts had a prognostic value by clearly correlation with the patient's lifespan [20].

In our opinion, the above information suggests the prospects of using human MSCs to improve the growth of xenografts of human malignant tumors in BALB/c nude mice. Unlike h-PBLs and h-HSCs human MSCs are not rejected by these immunodeficient animals [12, 16], so the introduction of such cells can be considered as a kind of local humanization of BALB/c nude mice. At the same time, attention should be paid to a number of conditions and strategies, the significance of which for optimizing the growth of xenografts using human MSC should be subjected to a conscientious study. In our opinion, first of all, it is the use of PDX, rather than immortalized cell cultures, as well as the search for effective modes of administration of human MSCs, that may be especially important in the case of subcutaneous xenograft transplantation.

About the possibility of using BALB/c nude mice to produce human glioblastoma xenografts that are not rejected by their immunocompetent heterozygous sibs

The second direction of research, which may also be promising, is related to the use of immunocompetent animals. This circumstance seems to us very important due to the fundamental impossibility of comprehensive reproduction of human oncogenesis and, especially, human immune and hematopoietic systems in the body of immunodeficient laboratory animals [1, 4. 8]. In this regard, it is of great importance to create immunocompetent animal models that can support the growth of xenografts of human malignant tumors. In this case, we are talking about the feasibility of reproducing and further developing studies on BALB/c nude mice and their immunocompetent sibs that were previously carried out by researchers at the University of Bergen (Norway) on nude rats and immunocompetent heterozygous animals of the same brood [8].

The aim of this work was to create a model of human infiltrative glioblastoma growing in immunocompetent animals. The choice of an animal model with a complete immune system was due to the low translational significance of the results obtained on immunodeficient animals. The objects of the research were nude and immunocompetent Rowett rats of both sexes at the age of 8–12 weeks. The biopsy material obtained from patients during neurosurgical interventions was initially cultured as organotypic spheroids in accordance with a previously developed procedure [21]. The study of spheroids involved light and electron microscopy revealed morphological features similar to those of the original tumor tissue. They differed from the features of spheroids obtained from permanent cell cultures. The spheroids contained vessels, connective tissue, and macrophages, showing a marked similarity to the structure of the original tumor. Flow cytometry with an assessment of the cell cycle in the samples revealed the same ploidy and the same number of proliferating cells in the spheroids as in the original tumor. For transplantation, spheroids were selected that did not show a decrease in size after 80 days of cultivation. Spheroids with a diameter of 400 microns were implanted in the right hemisphere of the cerebral cortex to a depth of 2.5 mm. the Growth of spheroids in the brain of animals was evaluated using MRI.

It was shown that human glioblastoma xenografts in the form of organotypic spheroids serially passed in the brains of Rowett nude rats later can develop in the brain of their immunocompetent sibs, in contrast to spheroids obtained directly from the biopsy material of patients. In the case of engraftment in immunocompetent rats, growth of xenotransplants was observed in the absence of leukocyte infiltration of the tumor bed, just as it occurred in nude animals. Graft rejection was associated with massive infiltration of the tumor bed by white blood cells, mainly ED1 + microglia/macrophage cells, CD4 + T-helper cells, and CD8 + effector T-cells, and also correlated with elevated levels of pro-inflammatory cytokines IL-1β, IL-18, and TNF-α in serum. It was noted that the adaptation of the human tumor

to the brain of an immunocompetent animal occurred after several cycles of passivation in the brain of nude rats and was characterized by a pronounced weakening of the infiltration of the tumor by microglia cells. In addition, there was a decrease in tumor production of those chemokines that contributed to the migration of white blood cells and their penetration into the Central nervous system. Thus, during serial passaging in the brain of nude rats, human glioblastoma cells acquired the ability to avoid and/or suppress host immune responses and subsequently take root in immunocompetent rats without signs of an inflammatory response.

Currently, it's not possible to characterize the mechanisms that provide the tolerance of immunocompetent heterozygous Rowett rats to human glioblastoma cells. The authors assumed that the development of tolerance is associated with a sufficiently high content of regulatory immune cells [22]. There is also an important question about the therapeutic context of this phenomenon — whether a human tumor that develops in animals with a complete immune system can retain its structural and functional features and sensitivity to the action of antitumor agents. If the response is positive, this model can provide significant progress in the development of effective personalized antitumor treatment. Thus, it seems appropriate to carry out the similar researches on BALB/c nude mice and their heterozygous sibs. If the result obtained in Rowett rats is reproduced on mice, further research should be carried out to determine whether the characteristics of xenografts supported by immunocompetent animals correspond to the indicators of the malignant process in the human brain. In our opinion, in the latter case, experimental and clinical studies should include a comparative analysis of neuronal-glial relations [23], as well as changes in the immune system of the brain [24] with the growth of xenografts and original tumors, both as in cases of coincidence as at mismatch of their structural and functional characteristics and sensitivity to the action of tested antitumor agents.

CONCLUSIONS

We assume that the successful realisation of the suggested research directions, one of which is related to the local humanization of immunodeficient BALB/c mice with human mesenchymal stem cells, and the other — with the use of immunocompetent BALB/C nude mouse sibs to ensure the growth of xenografts of human glial tumors in the absence of immune deficiency, can make a significant contribution to the development of informative experimental models of the malignant process in humans.

Authors contribution:
Zhukova G.V. – concept, literature search, text writing.
Shikhlyarova A.I. – participation in the development of the concept.
Sagakyants A.B. – scientific editing.
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