Dental Forum, Irkutsk, October 22-24, 2024 Irkutsk: "Irkutsk Scientific Center for Surgery and Traumatology"; 2024: 99-103 (In Russ.).]

6. Yashnova N.B. Pinelis Yu.I., Dutova A.A. Mikrobnyj sostav parodontal'nogo karmana pri hronicheskom generalizovannom parodontite [Microbial composition of the periodontal pocket in chronic generalized periodontitis]. Aktual'nye problemy mediciny [Actual problems of medicine. 2024; 47 (1): 89-98 (In Russ.).] DOI: 10.52575/2687-0940-2024-47-1-89-98.

7. Batih, I. Prevalence of maxillofacial anomalies and their relation to periodontal diseases in young people. Medical Science. 2024; 28 (143): 1-6. DOI: 10.54905/dissi.v28i143.e3ms3256.

8. Bankin I.K., Proshina V. P., Fedechkin V. V., et al. Influence of gum recession in periodontitis on oral health: causes, consequences, diagnosis, and surgical treatment methods. WEEK OF RUSSIAN SCIENCE (WERUS-2024), 6-19 апреля 2024 года. Saratov, 2024. P. 574-575.

9. Kydenko V, Budyansky S, Senyshyn R, Kydenko V. A modern view of etiopathogenesis of periodontal diseases. Annali d'Italia. 2024; 56: 64-67. DOI: 10.5281/zenodo.12550393. EDN LZRKBR.

10. Patil R.T., Dhadse P.V., Salian Sh. S., Punse S. D. Role of Oxidative Stress in Periodontal Diseases .Cureus. 2024. DOI: 10.7759/cureus.60779. EDN KFROLS.

11. Şahin, T. Investigation of the relationships between peri-implant diseases, periodontal diseases, and conditions: a cross-sectional study. Peer J. 2024; 12:163-186. DOI: 10.7717/peerj.18663.

12. Verma K, Singh A, Verma K. Biomarkers in periodontal health and diseases. IP International Journal of Periodontology and Implantology. 2024; 9 (2): 64-67. DOI: 10.18231/j.ijpi.2024.014. EDN ZXNDLY.

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# THE INFLUENCE OF TUMOR SAMPLING TOPOLOGY ON THE EFFECTIVENESS OF THE XENOTRANSPLANTATION PROCEDURE IN THE CREATION OF A PDX MODEL OF GASTRIC CANCER

As indicated in the extant literature, numerous research groups encounter challenges in creating PDX models of gastric cancer, which is associated with a low level of engraftment of tumor samples. Consequently, in the pursuit of enhancing and refining the conventional implantation technique, we have recognized the significance of the tumor sample collection site. It was hypothesized that cells located on the tumor periphery and forming the invasion front may have a more pronounced potential for malignant growth and, consequently, the ability to grow as xenografts. To this end, ten gastric tumor samples were obtained from each patient: five from the tumor edges and five more from areas more than 5 mm from the visible tumor edge. All samples were implanted subcutaneously on the right side of Balb/C Nude mice. A subsequent analysis of the results indicated that, upon the collection of biological material concurrent with the capture of the visible tumor edge, the formation of tumor nodules occurred in three out of five recipient animals. Consequently, the xenotransplantation efficiency in this instance was determined to be 84%. Conversely, when tissue samples were obtained from areas distant from the tumor edge, the formation of tumor nodules in recipient animals was observed in only one procedure out of five. Consequently, the xenotransplantation efficiency was 20%. The histological examination conducted as part of this study revealed that the heterotopic PDXs accurately reproduced the histotype of the corresponding donor tumors, confirming their adenocarcinoma status. We also conducted an IHC study, which demonstrated that the level of Ki-67 expression in the tumor edges forming the invasion front was significantly higher, with an average value of 70 [60; 80]%. Conversely, in samples extracted from non-marginal regions of the same tumor, Ki-67 expression levels were notably lower, with an average of 15% [5; 25%] ($p<0.05$). The obtained data suggest that the higher proliferative potential characterizing the marginal areas of tumors may contribute to more effective xenotransplantation of such tumor samples compared to samples obtained from areas of the tumor remote from the visible edge and characterized by a lower proliferative potential.

**Keywords:** gastric cancer, heterotopic model, PDX models, mouse models

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**Introduction.** Gastric cancer (GC) is an oncological disease that ranks fifth in prevalence and is also the fourth cause of cancer death worldwide [7]. The etiology of this disease is multifaceted. Its development is influenced by a multitude of both intrinsic and extrinsic factors, including, but not limited to, genetic susceptibility, infectious diseases, and dietary habits [1,13]. Patients with early-stage gastric cancer undergo surgical resection followed by chemotherapy, and the postoperative 5-year survival rate can reach 90% [1]. However, the detection rate of gastric cancer at the first stage is low, which is associated with the absence of pronounced clinical signs. Therefore, the majority of patients (>70%) are diagnosed with the disease at late stages [10]. Despite considerable progress in radiation, immune, and chemotherapy treatments, surgical resection remains the only radical method for treating gastric cancer [2,11]. Consequently, there is

an imperative for further research to enhance our comprehension of the underlying pathobiology of gastric cancer and to formulate targeted therapeutic interventions. In such studies, the preliminary selection of therapeutic candidates at the preclinical stage plays a pivotal role. In the context of experimental studies on anticancer therapy, in vivo tumor models are instrumental in facilitating the progression of a promising drug candidate to clinical trials [12]. Currently, leading pharmaceutical companies and research organizations increasingly use the practice of using PDX (patient-derived xenograft) models in preclinical trials, which are obtained by implanting tumor fragments obtained from patients into animals. Animal models are extremely important for understanding the biological behavior of tumors and the molecular mechanism of carcinogenesis and evaluating the effectiveness of drugs [5].

However, the variability of the engraftment rate of tumor material obtained from patients and, accordingly, the development of PDX models of gastric cancer in immunodeficient mice ranges from satisfactory to extremely low [8]. This characteristic of PDX models imposes limitations on their broad application in the implementation of individualized approaches to gastric cancer therapy. Consequently, there is a pressing need to prioritize the investigation of methodological approaches that have the potential to enhance the efficacy of xenotransplantation. [9].

In this regard, the aim of our study is to investigate the influence of tumor sample collection topology on the effectiveness of the xenotransplantation procedure when creating a heterotopic PDX model of gastric cancer in Balb/c Nude mice.

**Materials and methods.** *Animals and their maintenance.* In the course of the experiment, female Balb/c Nude mice aged 8-10 weeks, with an average weight of 24-27 g, were used. The animals were obtained from the in-house breeding vivarium of the Testing Laboratory Center of the National Medical Research Center of Oncology of the Ministry of Health of the Russian Federation and were kept in individual ventilated cages, food and water were provided without restrictions. All manipulations carried out within the framework of the study were carried out in accordance with the ethical principles established by the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETSN 123, Strasbourg, March 18, 1986). The study protocol was approved by the local bioethics committee of the National Medical Research Centre for Oncology of the Ministry of Health of the Russian Federation.

*Tumor samples.* Gastric cancer samples were obtained from 5 patients undergoing treatment at the "National Medical Research Centre for Oncology" of the Ministry of Health of the Russian Federation. Written informed consent for the transfer of biological material was obtained from all patients.

*Anesthesia.* The surgical manipulations performed on the animals in this experiment were carried out using intramuscular injection anesthesia of the veterinary drugs "Xyla" (20 mg/kg) and "Zolelil-100" (50 mg/kg).

*Technique of creating a heterotopic (subcutaneous) model of gastric cancer using tumor samples obtained from patients.* Consequently, surgical intervention was undertaken to obtain tumor samples from patients diagnosed with gastric cancer. The tumor material was subsequently transferred to the vivarium for a 15-minute period in a DMEM nutrient medium containing 10% gentamicin. The tumor material obtained from the patients was then implanted heterotopically (subcutaneously) into the right side of the mice. Upon reaching the requisite depth of anesthesia, the skin was dissected on the right side of the animals, followed by the introduction of sterile closed blunt scissors into the subcutaneous space to separate the peritoneum from the skin to create a subcutaneous pocket. The tumor material was then meticulously implanted without causing injury to the abdominal cavity. The surgical wound was then closed using an interrupted suture. All surgical interventions were performed under sterile conditions.

*Assessment of tumor node growth.* The linear dimensions of tumor nodes were measured using a caliper weekly, starting from the 14th day after implantation of tumor material into immunodeficient mice. The volume of tumor nodes was calculated using the formula:

$$V=LW2/2,$$

where L, W are the linear dimensions of the tumor.

*Euthanasia.* The euthanasia procedure entailed the execution of the animal by decapitation, followed by the collection of the tumor nodes at the conclusion of the observation period.

*Histological and immunohistochemical (IHC) examination.* The material obtained was fixed in 10% formalin for 24 hours, then embedded in paraffin. Sections were made using a rotary microtome, and were then deparaffinized according to the standard protocol. The hematoxylin and eosin staining was carried out in accordance with the established technique. IHC staining of tumor tissues was performed automatically in a BenchMark ULTRA Ventana immunohistostainer. The following antibodies were used: Ki-67 (clone SP6), Cell Marque, in a dilution of 1:200. To perform expression analysis, the proportion of cells with stained nuclei (percentage of the total number of tumor cells) was calculated in at least 10 fields of view.

*Statistical analysis.* Statistical data processing was performed using the Statistica 10 software package (StatSoft, USA). The results obtained are presented as a median and 25th and 75th percentiles; the Mann-Whitney test was used for comparison; the significance of differences was considered significant at $p<0.05$.

**Research results and their discussion.** According to the literature, many research groups face difficulties in creating PDX models of gastric cancer, which is associated with a low rate of engraftment of tumor samples. In one study, it was demonstrated that the rate of successful engraftment was 28.12% [4], in another study, PDX models of gastric cancer were successfully created in 24.2% [3].

Therefore, in the context of searching for a way to improve and optimize the traditional implantation method, we considered the importance of the tumor sampling zone. We assumed that cells located on the tumor periphery and forming the invasion front may have a more pronounced potential for malignant growth and, therefore, the ability to grow as xenografts.

In this regard, gastric cancer samples from each patient, obtained from the marginal areas of the tumor (n=5) and from areas more than 5 mm away from the visible edge of the tumor (n=5), were implanted subcutaneously into the right flank of Balb/C Nude mice. Tumor samples from the central areas were not used to create PDX, since these areas usually contained necrotic areas. The tumor was considered to have successfully engrafted if the volume of the tumor node reached at least 60 $mm^3$. Observation was performed for 3 months, if no growth of tumor nodes was observed during this time, the xenotransplantation procedure was considered ineffective.

The results are presented in Table.

Analysis of these results showed that when selecting biological material with the capture of the visible edge of the tumor, 3 out of 5 procedures led to the

**Results of heterotopic implantation of gastric cancer samples into Balb/C Nude mice**

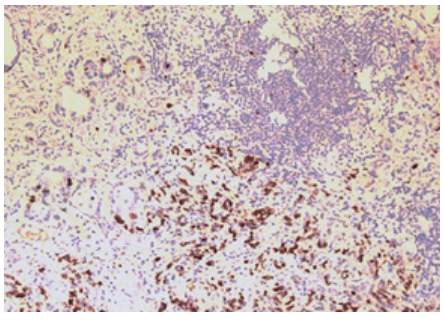
| Code xenograft procedures | Method of obtaining material | Evaluation of implantation results using samples distant from the visible edge (1st generation) | Evaluation of implantation results using samples, capturing the visible edge of the tumor (1st generation) |
|---|---|---|---|
| PDX-GC-01 | Дистальная субтотальная резекция | 0/5 | 3/5 |
| PDX-GC-02 | | 3/5 | 4/5 |
| PDX-GC-03 | | 0/5 | 0/5 |
| PDX-GC-04 | | 0/5 | 3/5 |
| PDX-GC-05 | | 0/5 | 0/5 |

formation of tumor nodes in recipient animals and the efficiency of xenotransplantation with this approach was 84%, which exceeded the results obtained when using tissue fragments from areas located further from the edge of the tumor, where only 1 out of 5 procedures led to the formation of tumor nodes in recipient animals, respectively, the efficiency of xenotransplantation was 20%.

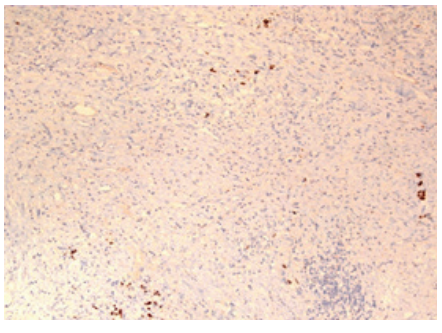
The importance of the topology of tumor samples was also demonstrated in the work of Bastola S. et al. – in the PDX glioblastoma model it was shown that cells originating from the edges of the tumor demonstrated a higher capacity for infiltrative growth in contrast to cells in the central areas of the tumor sample [6].

The histological examination performed within the framework of this work showed that heterotopic PDX reproduced the histotype of the corresponding donor tumors and were adenocarcinomas.We also conducted an IHC study of the marginal areas and central samples of patients' tumors. In the course of the work, it was found that the marginal and central samples had differences in the level of Ki-67 expression (Figure).

A



B



Immunohistochemical staining of cancer samples for Ki-67 human stomach, magnification×100. A – marginal central areas of the tumor; B – areas of the tumor, far from the edge

The results of our study showed that the level of Ki-67 expression in the tumor margins forming the invasion front was significantly higher and amounted to 70 [60; 80] %. At the same time, in samples taken from non-marginal areas of the same tumor, Ki-67 expression was significantly lower and amounted to 15 [5; 25]% ($p<0.05$).The obtained data allow us to suggest that, probably, the higher proliferative potential characterizing the marginal areas of tumors may contribute to more effective xenotransplantation of such tumor samples, compared to samples obtained from areas of the tumor remote from the visible edge and characterized by a lower proliferative potential. Based on data from scientific papers and the results of our own research, We have gained insight into the importance of tumor sample topography for the effectiveness of xenograft engraftment.

**Conclusion.** The results of the work provided the foundation for a proposed method to enhance the outcomes of xenotransplantation in the development of a heterotopic PDX model of gastric cancer. This method is predicated on the utilization of samples obtained from the marginal areas of the tumor. This novel method facilitates the augmentation of the efficiency of the conventional method of implantation. The incorporation of this approach into research protocols promises to enhance the precision and adaptability of experimental tumor models. Notably, this methodology fosters conditions conducive to a personalized approach in anti-cancer research, which has the potential to further enhance treatment outcomes for patients with gastric cancer.

*The authors declare that they have no conflict of interest.*

## References


1. Kit O.I., Derizhanova I.S., Karnaukhov N.S., et al. Osobennosti ekspressii somatostatinovyh receptorov v karcinomah zheludka v zavisimosti ot doli nejroendokrinnogo komponenta [Features of the expression of somatostatin receptors in gastric carcinomas depending on the proportion of the neuroendocrine component]. Voprosy onkologii [Issues of oncology. 2021; 67 (5): 683-686 (In Russ.).] DOI 10.37469/0507-3758-2021-67-5-675-682.

2. Kiblitskaya A.A., Karasev T.S., Goncharova A.S., et al. Puti modelirovaniya opuholevogo rosta u myshej v eksperimental'nyh issledovaniyah raka zheludka cheloveka [Pathways for Modeling Tumor Growth in Mice in Experimental Studies of Human Gastric Cancer]. Yuzhno-Rossijskij onkologicheskij zhurnal [South-Russian Journal of Oncology. 2021; 2 (4): 26-37 (In Russ.).] DOI: 10.37748/2686-9039-2021-2-4-4.

3. Choi YY, Lee JE, Kim H., et al. Establishment and characterization of patient-derived xenografts as paraclinical models for gastric cancer. Scientific reports. 2016; 6 (1): 22172. DOI:10.1038/srep22172

4. Wang H, Lu J, Tang J, et al. Establishment of patient-derived gastric cancer xenografts: a useful tool for preclinical evaluation of targeted therapies involving alterations in HER-2, MET and FGFR2 signaling pathways. BMC cancer. 2017; 17: 1-11. DOI: 10.1186/s12885-017-3177-9

5. Sexton RE, Al Hallak MN, Diab M, et al. Gastric cancer: a comprehensive review of current and future treatment strategies . Cancer and Metastasis Reviews. 2020; 39: 1179-1203. DOI: 10.1007/s10555-020-09925-3

6. Bastola S, Pavlyukov MS, Yamashita D, et al. Glioma-initiating cells at tumor edge gain signals from tumor core cells to promote their malignancy. Nature communications. 2020; 11 (1): 4660 DOI: 10.1038/s41467-020-18189-y

7. Guan WL, He Y., Xu RH Gastric cancer treatment: recent progress and future perspectives. Journal of hematology & oncology. 2023; 16 (1): 57. DOI:10.1186/s13045-023-01451-3

8. Ughetto S, Migliore C, Pietrantonio F, et al. Personalized therapeutic strategies in HER2-driven gastric cancer / Gastric Cancer. 2021; 24: 897-912. DOI:10.1007/s10120-021-01165-w

9. Aschero R, Castillo-Ecija H, Baulenas-Farres M, et al. Prognostic value of xenograft engraftment in patients with metastatic high-risk neuroblastoma. Pediatric Blood & Cancer. 2023. P. e30318. DOI:10.1002/pbc.30318

10. Song Z, Wu Y, Yang J, et al. Progress in the treatment of advanced gastric cancer. Tumor Biology. 2017; 39 (7): 1010428317714626. DOI:10.1177/1010428317714626

11. Tan Z. Recent advances in the surgical treatment of advanced gastric cancer: a review // Medical science monitor: international medical journal of experimental and clinical research. 2019; 25: 3537. DOI: 10.12659/MSM.916475

12. Li Z, Wang J, Wang Z, et al. Towards an optimal model for gastric cancer peritoneal metastasis: current challenges and future directions. EBioMedicine. 2023; 92. DOI: 10.1016/j.ebiom.2023.104601

13. Yasuda T., Wang YA. Gastric cancer immunosuppressive microenvironment heterogeneity: implications for therapeutic development. Trends in Cancer. 2024. DOI: 10.1016/j.trecan.2024.03.008