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ORIGINAL ARTICLE

GASTRIC CANCER MODELING IN IMMUNODEFICIENT MICE WITH ORTHOTOPIC XENOTRANSPLANTATION

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ABSTRACT

Purpose of the study. Creation of a transplantable orthotopic PDX model of gastric cancer in Balb/c Nude immunodeficient mice using implantation and injection.
Materials and methods. Two methods, that are injection and implantation, were used to create an orthotopic PDX model of human gastric cancer. The first method involved injections of a suspension of a mechanically disaggregated patient's tumor after filtration into the gastric wall of Balb/c Nude mice. For the second method, small fragments (3 × 3 × 3 mm) of patients' tumors were implanted in the gastric wall of mice along the greater curvature with a dissection of the serous muscular layer.
Results. Control laparotomy in Balb/c Nude immunodeficient mice showed a successful engraftment of the tumor material at the 1st and 3rd procedures when using the implantation method for the creation of a PDX model of gastric cancer. The injection method was ineffective, and no models were created. The histological type of the obtained PDX models was compared to the type of the donor tumor by histological examination (hematoxylin and eosin staining). The tumor grade remained stable and did not change during xenograft passage, which showed that the obtained model was identical to the histotype of the donor tumor.
Conclusion. The presented implantation method for the model creation results in effective tumor engraftment. The developed model can be used to test the effectiveness of anticancer or antimetastatic drugs, for studying the functions of biomarkers, or in assessing the microenvironment of a gastric cancer.

Keywords: orthotopic xenotransplantation, gastric cancer, immunodeficient mice, PDX models, transplantation

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Compliance with ethical standards: all manipulations with laboratory animals were carried out in accordance with the "Rules for carrying out work using experimental animals" when performing the study. The study was approved by the Ethics Committee of the National Medical Research Centre for Oncology (Protocol No. 22/126 of 08/10/2021).

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ОРИГИНАЛЬНАЯ СТАТЬЯ

МОДЕЛИРОВАНИЕ РАКА ЖЕЛУДКА НА ИММУНОДЕФИЦИТНЫХ МЫШАХ ПУТЕМ ОРТОТОПИЧЕСКОЙ КСЕНОТРАНСПЛАНТАЦИИ

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РЕЗЮМЕ

Цель исследования. Создание перевиваемой ортотопической PDX-модели рака желудка на иммунодефицитных мышах линии Balb/c Nude при помощи имплантационного и инъекционного способов.
Материалы и методы. С целью создания ортотопической PDX-модели рака желудка человека были применены 2 способа – инъекционный и имплантационный. Первый способ заключался в инъекции суспензии механически дезагрегированной опухоли пациента после фильтрации в стенку желудка мышей линии Balb/c Nude. Для второго способа мелкие фрагменты (3 × 3 × 3 мм) опухоли пациентов были имплантированы мышам в стенку желудка по большой кривизне с рассечением серозно-мышечного слоя.
Результаты. При применении имплантационного способа получения PDX-модели рака желудка на иммунодефицитных мышах линии Balb/c Nude в ходе процедур контрольной лапаротомии, был обнаружен положительный результат приживления опухолевого материала при 1-ой и 3-ей процедурах. Инъекционный способ не дал эффективного результата – не была получена ни одна модель. Гистотип полученных PDX-моделей сравнивали с донорской опухолью и подтверждали при помощи гистологического исследования (окрашивание гематоксилином и эозином). Степень дифференцировки оставалась стабильной и не менялась в результате пассирования ксенографта, что показало идентичность полученной модели гистотипу донорской опухоли.
Заключение. Представленный имплантационный метод создания модели дает эффективный результат приживления опухоли. Полученная модель позволяет использовать её для проверки эффективности противоопухолевых или антиметастатических препаратов, возможных исследований функций биомаркеров, а также для оценки микроокружения опухоли желудка.

Ключевые слова: ортотопическая ксенотрансплантация, рак желудка, иммунодефицитные мыши, PDX-модели, трансплантация

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Соблюдение этических стандартов: при выполнении данного исследования все манипуляции с лабораторными животными проводились в соответствии с «Правилами проведения работ с использованием экспериментальных животных». Исследование одобрено этическим комитетом ФГБУ «НМИЦ онкологии» Минздрава России (протокол № 22/126 от 08.10.2021 г.).

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INTRODUCTION

Every year, gastric cancer is diagnosed for the first time in more than 1 million people. At the moment, gastric cancer remains the fourth cause of cancer mortality [1; 2]. Treatment of patients diagnosed with gastric cancer consists in the use of surgical methods, chemotherapy, immunotherapy. However, all these methods have limited effectiveness. Over a 20-year period, the proportion of patients with metastases increased from 24 to 44 %, which indicates an urgent need for an optimized approach to both treatment and diagnosis [3]. More and more efforts are being made to find effective ways to research and understand the biology and therapeutic features of gastric cancer.

One of such approaches is the use of orthotopic cancer models obtained from patients (PDX stands for: patient derived xenograft) [4; 5]. The main advantage of PDX models is that tumor cells tend to consistently repeat the features of the original tumor, and the orthotopic implantation method can better simulate the natural environment of the tumor. It has been shown that for some types of tumors, the subcutaneous model has a lower rate of engraftment than the orthotopic one [6]. Orthotopic xenografts of tumors are one of the best experimental models for representing the mechanisms of spontaneous metastasis [7; 8].

This model is an important tool for providing scientific substantiation of the relevance of new therapeutic combinations in gastric cancer. To study the growth and metastasis of gastric cancer, as well as to test the effectiveness of treatment and therapy, as well as testing new pharmacological substances, various methods of orthotopic transplantation to bestimus mice (Balb/c Nude) have been developed [9; 10]. Although several methods are used to develop an orthotopic model, the optimal way to create it has not yet been determined.

The purpose of the study was to create an orthotopic PDX model of gastric cancer in immunodeficient Balb/c Nude mice using implantation and injection methods.

MATERIALS AND METHODS

Tumor sample

The tumor samples required for orthotopic transplantation to laboratory animals were obtained from patients diagnosed with gastric cancer who were treated in 2022, from whom written permits were obtained for the use of samples for research purposes.

Laboratory animals

All procedures related to *in vivo* studies on mice were carried out in accordance with the "Guidelines for the maintenance and use of laboratory Animals" [11]. 39 female immunodeficient Balb/c

Table 1. Characteristics of tumor material donor patient, and evaluation of xenotransplantation results to immunodeficient Balb/c Nude mice

Method of xenotransplant isolation	Characteristics of tumor material donor patient						Assessment of implantation outcomes (1st generation)
	Procedure number	Donor patients sex	Method of sample isolation	TNM stage	Micromorphology	Prior therapy	Total number of transplantations / number of successful implantations
Tumor fragment implantation	1	F	Distal subtotal resection	$T_3N_2M_0$	Low-differentiated adenocarcinoma	-	7/6
Injection of tumor suspension	2						7/0
Tumor fragment implantation	3	F	Distal subtotal resection	$T_3N_1M_0$	Low-differentiated adenocarcinoma	-	7/5
Injection of tumor suspension	4						7/0

Nude mice were used to create tumor models. The animals were kept in the SPF-zone of the vivarium, in individually ventilated cages at a temperature of 21–23 °C. The mice were provided with free access to food and water.

Creating an orthotopic model

The orthotopic PDX model of gastric cancer was obtained in two ways. The first method consisted in implanting a fragment of the patient's tumor into the gastric wall of Balb/c Nude mice along the large curvature. The second method was the injection of a homogenized crushed tumor of the patient into the gastric wall of Balb/c Nude mice along the large curvature. The tumor material was obtained from two patients. General characteristics of patients and evaluation of the results of tumor engraftment to animals are presented in the table 1.

Xenograft engraftment and growth were evaluated by performing control laparotomies 20 and 40 days after implantation and injection of tumor material. Surgical manipulations were performed using injectable anesthesia for laboratory animals using veterinary drugs "Xylazine" and "Zoletil-100".

Assessment of the growth of tumor xenografts

Measurements of tumor nodes were performed during laparotomy using a caliper. The volume of tumor nodes was calculated by the formula: $V = L \times W^2 / 2$, where V is the volume of the tumor (mm^3); L, W are the linear dimensions of the tumor (mm).

Histological examination

Fragments of tumor tissue were fixed in 10 % formalin for 24 hours, then subjected to dehydration, after which they were enclosed in paraffin. After that, microsections were prepared, which were stained with hematoxylin and eosin according to the standard procedure. A donor and xenogenic tumor were subjected to histological examination.

Statistical analysis

Statistical data processing was performed using the STATISTICA 8.0 software package. The results are presented as median values [25th and 75th percentiles].

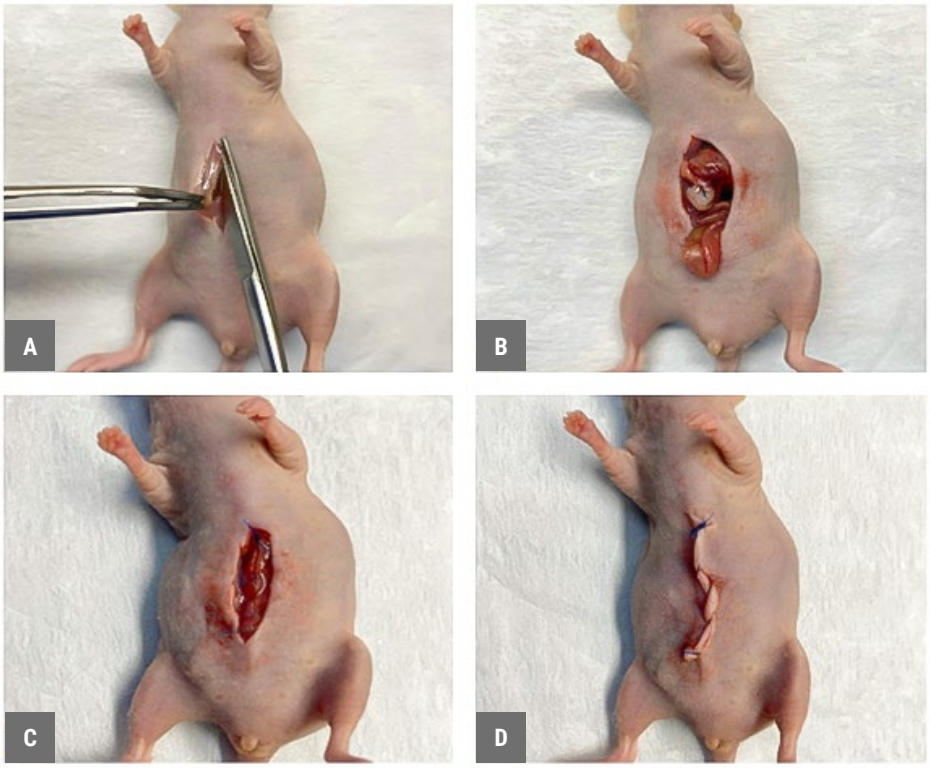

Fig. 1. Generating an orthotopic PDX model by implanting a fragment of a human gastric cancer into the body wall of the immunodeficient mice stomach. A – dissection of the skin, muscles and tissue of the abdominal wall of an immunodeficient mouse; B – implantation of a human tumor fragment into the body of the mouse stomach; C – suturing of the mouse abdominal wall tissue; D – final appearance after transplantation.

RESEARCH RESULTS

Modeling of gastric cancer on immunodeficient Balb/c Nude mice was carried out in two ways – the method of injection of a suspension of the patient's tumor cells; the method of implantation of a fragment of the patient's tumor. To create xenografts, 4 procedures were performed (2 procedures for each method). As part of one procedure, the model was created on a group of seven animals.

After surgery, the patient's tumor fragment was washed with a nutrient medium (DMEM and 1 % penicillin/streptomycin) and areas with signs of necrosis were removed.

The first method of creating a PDX model was carried out as follows. The tumor was cut into small fragments (3 × 3 × 3 mm), then the resulting fragment was implanted into the gastric wall of an immunodeficient Balb/c Nude mouse. After anesthesia of the animal with the sedative "Xylazine" at a concentration of 20 mg/kg and the general anesthesia drug "Zoletil 100" at a concentration of 50 mg/kg, layered dissection of the skin and tissue of the abdominal wall of the mouse was performed. After the expansion of the surgical wound with the help of anatomical tweezers, the stomach was isolated and the serous-muscular layer of the stomach was dissected along a large curvature. Then the resulting fragment of the donor's tumor was sewn with a ligature to the gastric wall at the site of the incision and the abdominal cavity and skin were sewn in layers (Fig. 1).

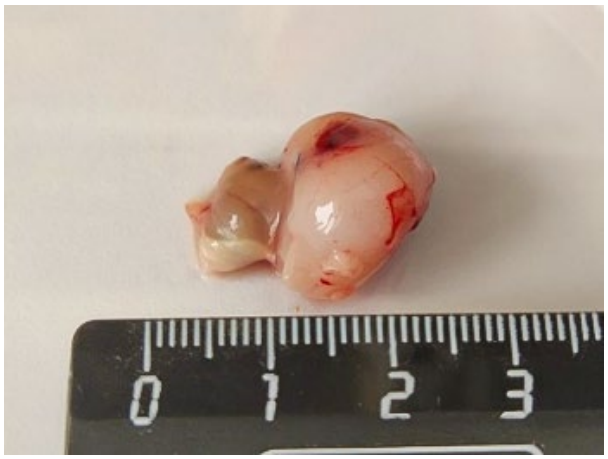

Fig. 2. Orthotopic xenograph of human gastric cancer on the gastric body of an immunodeficient mouse of the Balb/c Nude line.

When using the implantation method for obtaining a PDX model during the control laparotomy procedures, a positive result of the engraftment of tumor material was found (Fig. 2).

The tumors of the obtained PDX models retained identical histological features of the original tumors of the donor patients (Fig. 3).

The second method was as follows: the fragments of the tumor were subjected to mechanical disaggregation, then the resulting suspension was transferred to a vial, passed through a filter and typed into a syringe. After that, an injection was carried out into the corresponding part of the gastric wall of an immunodeficient mouse.

DISCUSSION

When using the injection method to obtain a PDX model, no tumor growth was detected during the control laparotomy procedures. It can be assumed

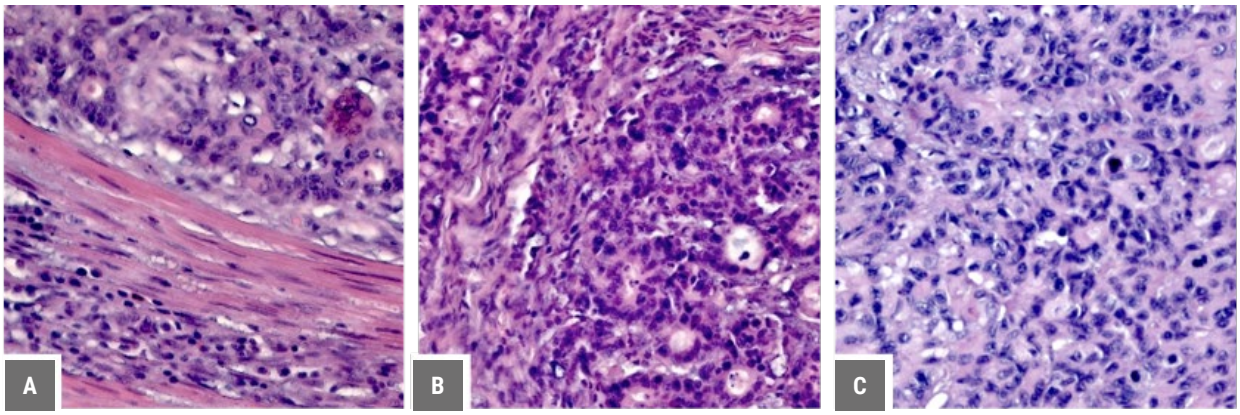

Fig. 3. Histological preparations of gastric cancer. A is the patient's tumor (donor tumor). H&E staining. × 200; B – xenograft, 1st generation; C – xenograft, 2nd generation.

that the absence of a positive result in the form of engraftment of tumor material and the formation of a tumor node was probably due to the phenomenon of anoikis – a form of apoptosis that occurs in response to loss of connection with the matrix or caused by the separation of cells from neighboring cells caused by mechanical disaggregation. Thus, the method of injecting a suspension of tumor cells is insufficiently effective due to the complexity of manipulations and lack of effectiveness, as was shown in our study and in the researches of other authors [12; 13].

Implantation procedure No. 1 showed a result with 86 % engraftment and tumor growth as a result of xenotransplantation during the first generation. The first laparotomy was performed 20 days after the operation. According to the results, the median volume of xenografts was 99.61 [70.44; 138.29]. With laparotomy performed after 40 days, the median was 221.21 [184.27; 202.17]. The second generation showed 100 % engraftment and faster tumor growth. The median volumes of xenografts were 125.56 [106.21; 168.51] and 288.61 [223.48; 344.1] 20 and 40 days after implantation, respectively (Table 2).

As a result of procedure No. 3, the growth of xenografts was observed in five of the seven animals in the group at the 1st generation. At laparotomy on day 20, according to the results of measurements, the median volume of xenografts was 67.37 [55.35; 118.59]. As a result of laparotomy on day 40, the median tumor size was 126.77 [104.76; 169.99].

The second generation was also characterized by higher growth rates. The median volumes of xenografts of the second generation were 157.71 [102.16; 172.96] and 291.5 [251.42; 346.32] 20 and 40 days after implantation, respectively (Table 2).

The tumor pathology in both PDX and patients was a low-grade adenocarcinoma of a solid type. There were no changes in the degree of differentiation as a result of xenograft passage (within the two generations obtained). The observations obtained indicate the ability of xenotransplanted tumors of early generations (1st and 2nd generations) to accurately display the morphological features of donor tumors.

CONCLUSION

Up to the date, a number of methods of orthotopic transplantation have been developed, but each of them has disadvantages that limit its widespread use. As part of our study, 2 methods of creating an orthotopic model of gastric cancer were analyzed: injection and implantation. The injection method proved to be ineffective. The implantation method yielded a result with a high level of tumor engraftment, i. e. 86 % and 100 % of the two patients' tumor materials. The presented method of creating a model allows us to transplant tumor tissue into the stomach orthotopically without additional labor, and also gives an effective result of tumor engraftment. The obtained models can potentially be used for screening and evaluation of known and new drugs.

Table 2. Volumes of orthotopic xenografts of human gastric cancer of two consecutive generations 20 and 40 days after implantation of the tumor fragment to immunodeficient Balb/c Nude mice, presented as median (M) and interquartile span (implantation procedure No. 1, 3)

Procedure No.	Generation	Laparotomy	M	25 percentile	75 percentile
Procedure 1	1	20 days	99.61	70.44	138.29
		40 days	221.21	184.27	202.17
	2	20 days	125.56	106.21	168.51
		40 days	288.61	223.48	344.1
Procedure 3	1	20 days	67.37	55.35	118.59
		40 days	126.77	104.76	169.99
	2	20 days	157.71	102.16	172.96
			291.5	251.42	346.32

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May;71(3):209–249. https://doi.org/10.3322/caac.21660
2. Kit OI, Kasatkin VF, Maksimov AYu, Mamulyan HG. Combined gastrectomy at locally-advanced gastric cancer after neoadjuvant polychemotherapy. News of higher educational institutions. The North Caucasus region. Natural sciences. 2012;(4):108–111. (In Russ.). EDN: PCIYTL
3. Bernards N, Creemers GJ, Nieuwenhuijzen G a. P, Bosscha K, Pruijt JFM, Lemmens VEPP. No improvement in median survival for patients with metastatic gastric cancer despite increased use of chemotherapy. Ann Oncol. 2013 Dec;24(12):3056–3060. https://doi.org/10.1093/annonc/mdt401
4. Corso S, Isella C, Bellomo SE, Apicella M, Durando S, Migliore C, et al. A Comprehensive PDX Gastric Cancer Collection Captures Cancer Cell-Intrinsic Transcriptional MSI Traits. Cancer Res. 2019 Nov 15;79(22):5884–5896. https://doi.org/10.1158/0008-5472.CAN-19-1166
5. Kit OI, Vashchenko LN, Dashkova IR, Kutilin DS, Maksimov AYU, Goncharova AS. Xenograft models of human breast cancer in experimental studies. Modern problems of science and education. 2019;(6):184–184. (In Russ.). EDN: OWQEZP
6. Hoffman RM. Orthotopic metastatic (MetaMouse) models for discovery and development of novel chemotherapy. Methods Mol Med. 2005;111:297–322. https://doi.org/10.1385/1-59259-889-7:297
7. Julien S, Merino-Trigo A, Lacroix L, Pocard M, Goéré D, Mariani P, et al. Characterization of a large panel of patient-derived tumor xenografts representing the clinical heterogeneity of human colorectal cancer. Clin Cancer Res. 2012 Oct 1;18(19):5314–5328. https://doi.org/10.1158/1078-0432.CCR-12-0372
8. Kiblitskaya AA, Karasev TS, Goncharova AS, Maksimov AYu. Methods for modeling tumor growth in mice in experimental studies of human gastric cancer. South Russian Journal of Cancer. 2021;2(4):26–37. (In Russ.). https://doi.org/10.37748/2686-9039-2021-2-4-4, EDN: BKTBYG
9. Busuttil RA, Liu DS, Di Costanzo N, Schröder J, Mitchell C, Boussioutas A. An orthotopic mouse model of gastric cancer invasion and metastasis. Sci Rep. 2018 Jan 16;8(1):825. https://doi.org/10.1038/s41598-017-19025-y
10. Reddavid R, Corso S, Moya-Rull D, Giordano S, Degiuli M. Patient-Derived Orthotopic Xenograft models in gastric cancer: a systematic review. Updates Surg. 2020 Dec;72(4):951–966. https://doi.org/10.1007/s13304-020-00751-4
11. Guidelines for the maintenance and use of laboratory animals. Ed. by I. V. Belozertseva, D. V. Blinova, M. S. Krasilshchikova. Eighth edition. Moscow: Limited Liability Company "IRBIS", 2017, 304 p. (In Russ.). EDN: ZRJVDJ
12. Kuwata T, Yanagihara K, Iino Y, Komatsu T, Ochiai A, Sekine S, et al. Establishment of Novel Gastric Cancer Patient-Derived Xenografts and Cell Lines: Pathological Comparison between Primary Tumor, Patient-Derived, and Cell-Line Derived Xenografts. Cells. 2019 Jun 14;8(6):585. https://doi.org/10.3390/cells8060585
13. Kang W, Maher L, Michaud M, Bae SW, Kim S, Lee HS, et al. Development of a Novel Orthotopic Gastric Cancer Mouse Model. Biol Proced Online. 2021 Jan 4;23(1):1. https://doi.org/10.1186/s12575-020-00137-1

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Goncharova A. S. – developed concept and design of the study;

Kolesnikov E. N. – performed surgical manipulations;

Maksimov A. Yu. – performed surgical manipulations, took the lead in data interpretation;

Averkin M. A. – analyzed the received data, worked out technical details of the paper;

Galina A.V. – conducted the experimental part of the study, performed technical design;

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