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Variants of creating heterotopic and orthotopic PDX models of human colorectal cancer

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ABSTRACT

Aim. To create heterotopic and orthotopic patient-derived xenograft (PDX) models of colorectal cancer (CRC) by transplantation of patient's tumor samples into immunodeficient BALB / c Nude mice.

Materials and methods. The study was performed on 15 female BALB / c Nude mice aged 6–8 weeks weighing 21–25 g. All animals underwent transplantation of the tumor material obtained from CRC patients into the following sites: heterotopic transplantation (under the skin of the thigh and into the omentum), orthotopic transplantation (into the descending and ascending colon and into the cecum). Weight and general condition of the animals and the size of the tumor nodule had been monitored for 80 days. The success of each model was assessed by the degree of engraftment, the dynamics of tumor growth, and the reproducibility of histopathologic characteristics. At the end of the experiment, the animals were euthanized by cervical dislocation.

Results. 100% survival of the animals and similar tumor growth dynamics in the xenograft models were observed throughout the experiment. The analysis of histologic specimens obtained from the xenografts and patient's tumor showed their correspondence to moderately differentiated intestinal adenocarcinoma. The main advantages and disadvantages of different variants of PDX models were described.

Conclusion. Heterotopic and orthotopic PDX models reproduce the morpho-histologic characteristics of human tumors and demonstrate stable growth dynamics. Therefore, they are a suitable tool for the development, testing, and validation of potential anticancer drugs.

Keywords: PDX model, xenograft, colorectal cancer, adenocarcinoma, orthotopic transplantation, heterotopic transplantation, BALB / c Nude

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Conformity with the principles of ethics. The patients signed an informed consent to transfer of the biological material. The study was approved by the Bioethics Committee at the National Medical Research Center of Oncology (Protocol No. 6/84 of 30.06.2020).

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Варианты создания гетеротопических и ортотопических PDX-моделей колоректального рака человека

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РЕЗЮМЕ

Цель. Создание гетеротопических и ортотопических моделей ксенографтов колоректального рака (КРР), полученных от пациентов (Patient-derived xenograft, PDX-модель), путем трансплантации образцов опухоли пациента иммунодефицитным мышам линии Balb/c nude.

Материалы и методы. Проведено исследование на 15 самках мышей линии Balb/c nude, возраст 6–8 нед, масса тела 21–25 г. Всем животным проведена трансплантация опухолевого материала, взятого от пациентов с КРР, в следующие сайты: гетеротопические (под кожу бедра, в сальник); ортотопические (в нисходящий и восходящий отделы толстой кишки, в слепую кишку). В течение 80 сут у животных контролировали следующие параметры: массу тела, общее состояние, объем опухолевого узла. Успешность каждой из моделей оценивали по степени приживления, динамике опухолевого роста и воспроизводимости гистопатологических характеристик. По завершению эксперимента животным выполнена эвтаназия методом цервикальной дислокации.

Результаты. На протяжении всего эксперимента наблюдалась 100%-я выживаемость животных и схожая динамика роста ксенографтов. Анализ гистологических препаратов ксенографтов и опухоли пациентов показал их соответствие умеренно дифференцированной аденокарциноме кишки. Описаны основные преимущества и недостатки создания различных вариантов PDX-моделей.

Заключение. Гетеротопические и ортотопические PDX-модели воспроизводят морфогистологические признаки человеческой опухоли и обладают устойчивой динамикой роста, следовательно, являются подходящим инструментом для разработки, тестирования и валидации потенциальных лекарственных препаратов против рака.

Ключевые слова: PDX-модель, ксенографт, колоректальный рак, аденокарцинома, ортотопическая трансплантация, гетеротопическая трансплантация, Balb/c nude

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Соответствие принципам этики. Пациенты подписали информированное согласие на передачу биологического материала. Исследование одобрено комиссией по биоэтике ФГБУ «НМИЦ онкологии» Минздрава России (протокол № 6/84 от 30.06.2020).

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INTRODUCTION

Colorectal cancer (CRR) is the fourth most common cancer worldwide with high mortality rates at advanced stages [1, 2]. 85% of colon tumors occur sporadically, and 15% are associated with hereditary predisposition [3, 4].

The choice of the correct strategy for colon cancer treatment largely depends on the anatomical location of the tumor [5]. Right-sided colon cancer (proximal

tumor location) and left-sided colon cancer (distal tumor location) exhibit different molecular characteristics and histology and, accordingly, show different therapeutic responses. Therefore, effective medical therapy should be developed taking into account all the differences between these tumors [6].

Histologic and morphological characteristics of right-sided and left-sided colon tumors are determined by their different embryonic origin. The cecum, appendix, ascending colon, hepatic flexure, and proximal two-thirds of the transverse colon develop from the midgut, while the distal third of the transverse colon, splenic flexure, descending colon, sigmoid colon, and rectum develop from the hindgut. Right-sided tumors are represented by sessile serrated adenomas or mucinous adenocarcinomas with polyp morphology, while left-sided tumors include tubulovillous and conventional adenocarcinomas [7].

Genomic heterogeneity of these tumors is due to the fact that right-sided CRC displays high microsatellite instability (MSI-high), while left-sided CRC is associated with high chromosomal instability (CIN-high) [8]. Adjuvant chemotherapy (5-fluorouracil (5-FU)) and targeted anti-epidermal growth factor receptor (EGFR) therapy are the most effective strategies for patients with left-sided CRC. Patients with right-sided CRC respond much better to immunotherapy due to high antigen load in these tumors [9].

It is advisable to study the effectiveness of anticancer drugs using preclinical models that fully reflect all clinically significant characteristics of the original human tumor. Cancer cell lines and their xenografts are used for screening tests of drugs *in vitro* and *in vivo*. However, growing neoplastic cells in an artificial environment leads to a change in genetic, transcriptomic, and histologic parameters of the tumor due to its adaptation to the altered conditions [10]. Disadvantages of cancer cell lines are reduced tumor heterogeneity, their belonging mainly to highly aggressive malignant tumors, and low predictive value in the clinical practice [10, 11].

To more accurately reproduce the biological characteristics of a donor human tumor, patient-derived xenograft (PDX) models were created. PDX models are created by transplanting malignant tumors from patients into immunodeficient mice [12]. The fragments of the patient's surgical material retain intercellular interactions, so these models contain not only malignant cells, but also components of the tumor microenvironment [13–16]. Many recent studies have described the successful creation of PDX models for various cancers, such as colorectal cancer [17], breast cancer [18], kidney cancer [19], stomach cancer [20], and non-small cell lung cancer [21].

In PDX models, tumor fragments are transplanted into a recipient mouse either heterotopically or orthotopically. Heterotopic xenografts are created by implanting a tumor fragment into a site not associated with the site of the original tumor, usually subcutaneously [14, 22]. In orthotopic transplantation, the patient's tumor tissue is transplanted into the corresponding organ. Subcutaneous transplantation of PDX models rarely causes metastasis in mice and does not reproduce the organ-specific tumor microenvironment [14]. Since orthotopic transplantation reproduces a natural environment in which the tumor developed in humans, it is considered the highest priority for testing highly selective targeted drugs [22]. At the same time, sites for orthotopic transplantation of the surgical material differ depending on the experiment objectives. The use of this model system takes into account not only an organ specific for a tumor, but also left-sided and right-sided location in case of CRC.

The aim of this study was to create five variants of PDX models of CRC by transplantation of patient's tumor samples into immunodeficient BALB / c Nude mice, as well as to analyze the advantages and disadvantages of each model.

MATERIALS AND METHODS

Human tumor material was transplanted into 15 female BALB /c Nude mice aged 6–8 weeks, weighing 21–25 g. The mice were maintained in an SPF vivarium at 22–24 °C and relative humidity of 60%, with a day / night light regime, in mechanically ventilated cages with sterilized food, water, and bedding. All surgical manipulations in the experiment were performed in compliance with the rules for the use of laboratory animals. The mice were observed for 80 days, then the animals were euthanized.

Tumor samples were obtained from CRC patients receiving treatment at the National Medical Research Center of Oncology from October, 2020 to January, 2021. All patients gave their written informed consent to transfer of the biological material. The donor tumor material for orthotopic transplantation into the ascending colon and cecum was obtained from mucinous colorectal adenocarcinoma, while a tumor fragment for transplantation into the descending colon was obtained from the patient with conventional intestinal adenocarcinoma. For heterotopic transplantation, the

material was also obtained from conventional intestinal adenocarcinoma.

The volume of subcutaneous tumor nodules was measured according to the Shrek's formula for an ellipsoid:

$$V = a \times b \times c \times \pi / 6$$

where V is the tumor volume (mm3), and a, b, and c are the maximum ellipsoid diameters in three planes (mm).

The mice were injected with Xyla at the concentration of 20 mg / ml as premedication and Zoletil at the concentration of 22.57 mg / ml as the general anesthetic. Immediately after excision, the human tumor tissue was placed in 5% gentamicin dissolved in Hanks' balanced salt solution. Immediately before the transplantation, tumor pieces of approximately 3 mm x 3 mm x 3 mm were excised with scissors removing all necrotic tissues. The time from the tumor material resection to the transplantation into the large intestine of a mouse did not exceed 40 minutes. Immediately before the surgery, the mouse skin in the dissection area was treated with a povidone – iodine 10% solution. The skin and peritoneum were closed using a 4-0 Prolene suture; the fragments of the patients' tumors were sutured to the intestinal wall and omentum using 5-0 Prolene ligation.

Heterotopic transplantation of the tumor samples under the skin of the thigh. Tumor fragments were transplanted subcutaneously, dorsally, into the posterior thigh, since this site facilitates tumor monitoring. An incision was made in the thigh using scissors. A fragment of the donor tumor was placed in a pocket made using blunt dissection. The surgery was completed by suturing the skin at the incision site.

Heterotopic transplantation of the tumor samples into the omentum. Transrectal access to the omentum was provided by layer-by-layer dissection of the skin and peritoneum along the rectus abdominis muscle at a distance of 2.5 cm from the xiphoid process. A section of the large intestine suspended by the omentum was placed into the surgical wound. A tumor fragment was sutured to the omentum. The intestine was placed back in the abdominal cavity, and the peritoneum and skin were sutured in layers using the glover's suture.

Orthotopic transplantation of the tumor samples into the cecum. Layer-by-layer dissection of the skin and peritoneum was performed along the midline of the body. The incision was started 20 mm from the end of the xiphoid process and ended 5 mm from the urethra. The cecum was exposed by passing it in the surgical wound. A purse-string serous – muscular suture was applied without tightening the ligature on the end of the cecum at a distance of 5 mm from the edge. Then, the serosa and the muscular layer of the cecum were dissected, the end of the cecum was invaginated creating a pocket, and a fragment of the human tumor was placed into the pocket. The ligature was sequentially tightened, the cecum was placed in the abdominal cavity, and the peritoneum and skin of the mouse were sutured in layers.

Orthotopic transplantation of the tumor samples into the ascending colon. Similarly to the method described earlier, the cecum and the ascending colon were passed into the surgical wound, and the serosa and the muscular layer of the intestinal wall were dissected longitudinally. Next, a tumor fragment was sutured above the dissection site. The intestine was placed back in the abdominal cavity, and the peritoneum and skin were sutured in layers using the glover's suture.

Orthotopic transplantation of the tumor samples into the descending colon. A 10-mm long incision was made in the skin and peritoneum with scissors at a distance of 7 mm from the base of the tail, retreating 3 mm from the spine to the right. Then, the rectum was exposed using anatomical forceps. Longitudinal dissection of the intestinal wall was performed at a distance of 5 mm from the site where the descending colon connects to the rectum. The human colon tumor tissue was ligated to the mouse colon. The peritoneum and skin of the mouse were sutured in layers.

Weight and general condition of the animals and the volume of the tumor nodules were monitored for 80 days after the surgery. The degree of engraftment and tumor growth in orthotopic and heterotopic xenograft models were assessed by control laparotomy on day 40, 60, and 80 after the transplantation. Successful xenotransplantation was evidenced by tumor growth to 1–1.5 cm3. The size of the tumor nodules was measured using a caliper.

After necropsy, fragments of the tumor material were fixed in 10% formalin for 24 hours, and at the processing stage, they were dehydrated and impregnated with paraffin. Then, the paraffin blocks and sections were prepared, mounted on glass slides, and stained with hematoxylin and eosin. Light microscopy was used for a histologic examination of the donor human tumor and heterotopic and orthotopic xenograft models. The Aperio Scan Scope XT slide scanner was used to obtain images of entire areas at 200x magnification.

A statistical analysis of the data was performed using the STATISTICA 10 software package. To assess

the significance of differences, the Mann – Whitney test was applied.

RESULTS AND DISCUSSION

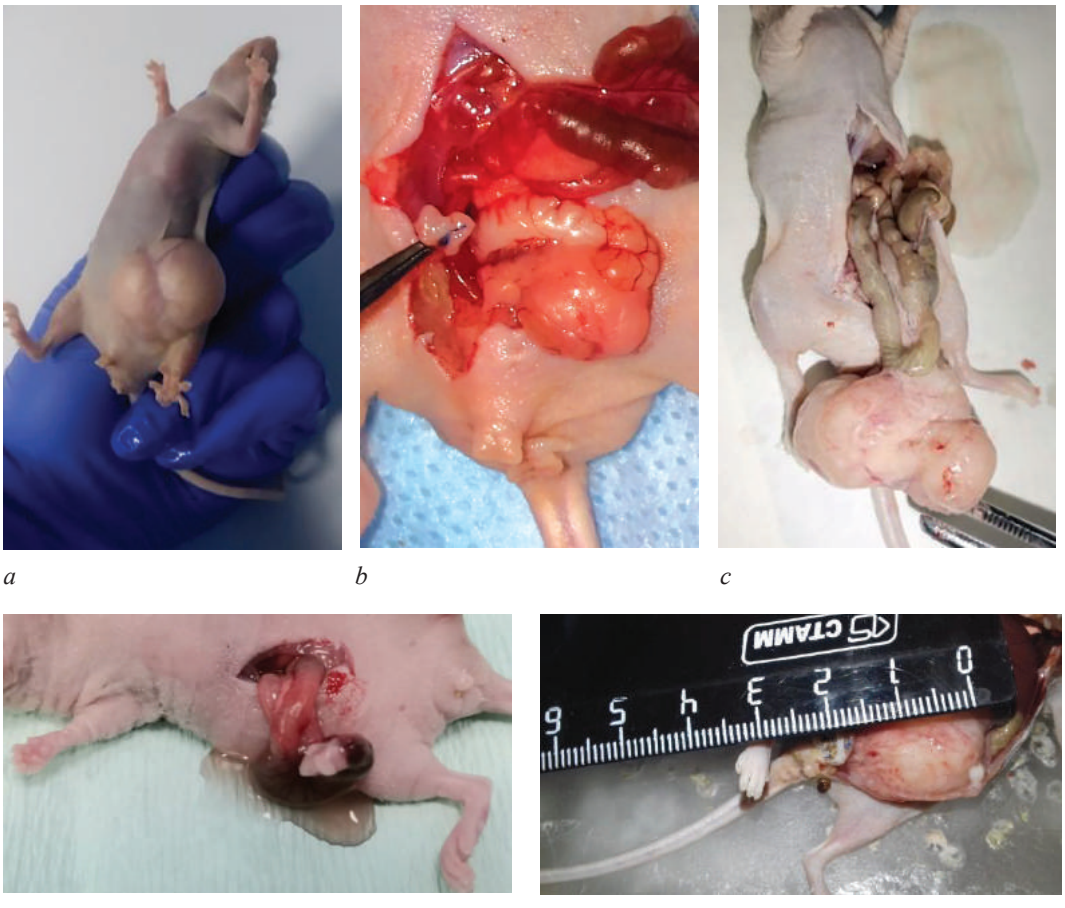
During the experiment, we transplanted the tumor material into immunodeficient mice to the following sites: under the skin and into the omentum (heterotopic transplantation), to the descending and ascending colons and to the cecum (orthotopic transplantation) (Fig.1). Depending on the site of transplantation, the animals were divided into 5 groups (3 females in each group). Throughout the experiment, the survival of the animals was 100%, and their condition was satisfactory. Growth of the engrafted PDX tumors was observed throughout the experiment.

12 of 15 heterotopic and orthotopic transplantation procedures resulted in creating a PDX model, i.e. the overall engraftment rate was 80%. Figure 2 demonstrates the growth dynamics of the tumor nodules in the heterotopic and orthotopic PDX models of human CRC. The statistical analysis using the Mann – Whitney test showed that the volumes of the tumor nodules in all 5 experimental groups of animals on day 40, 60, and 80 of the experiment differed significantly from the baseline volume of the transplanted donor tumor fragment. Therefore, a statistically significant increase in the heterotopic and orthotopic xenograft models was observed throughout the experiment.

A histologic analysis showed similar characteristics between different PDX models of CRC and original donor tumors. Tumor differentiation, necrosis, and stroma were assessed for each xenograft model and in 3–5 different samples of the original patient tumor. Microimages of the histologic preparations of the developed PDX models are shown in Fig.3.

Control laparotomy of the orthotopic xenograft model (transplantation into the ascending colon) on day 80 demonstrated liver metastasis (Fig. 4).

The table summarizes the main advantages and disadvantages observed when creating and using different variants of PDX models in preclinical studies.



a *b* *c*

d *e*

Fig. 1. Xenotransplantation of the patient's tumor material into immunodeficient mice using various sites: heterotopic transplantation (day 80 after the surgery): *a* – under the skin of the thigh, *e* – into the omentum; orthotopic transplantation (day 20 after the surgery): *b* – into the descending colon; *d* – into the cecum; *c* – orthotopic transplantation (day 80 after the surgery) into the ascending colon

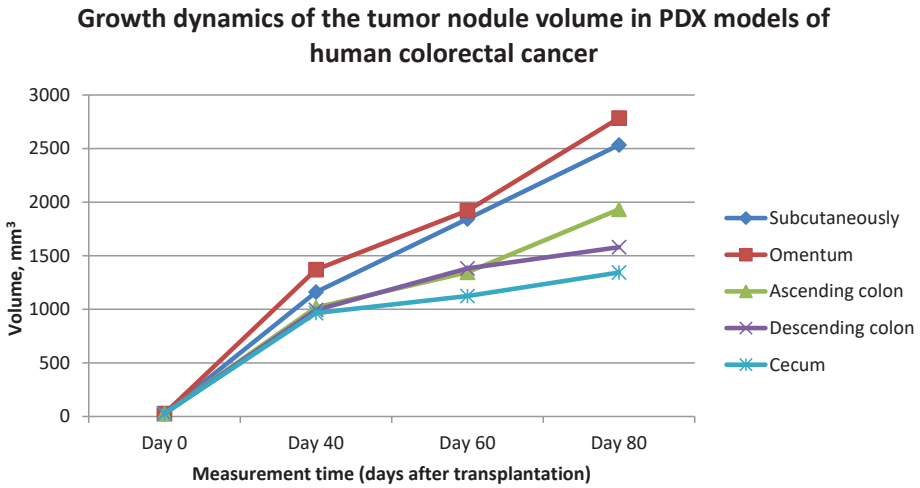


Fig. 2. Growth dynamics of tumor nodule volume in PDX models of human colorectal cancer, mm^3

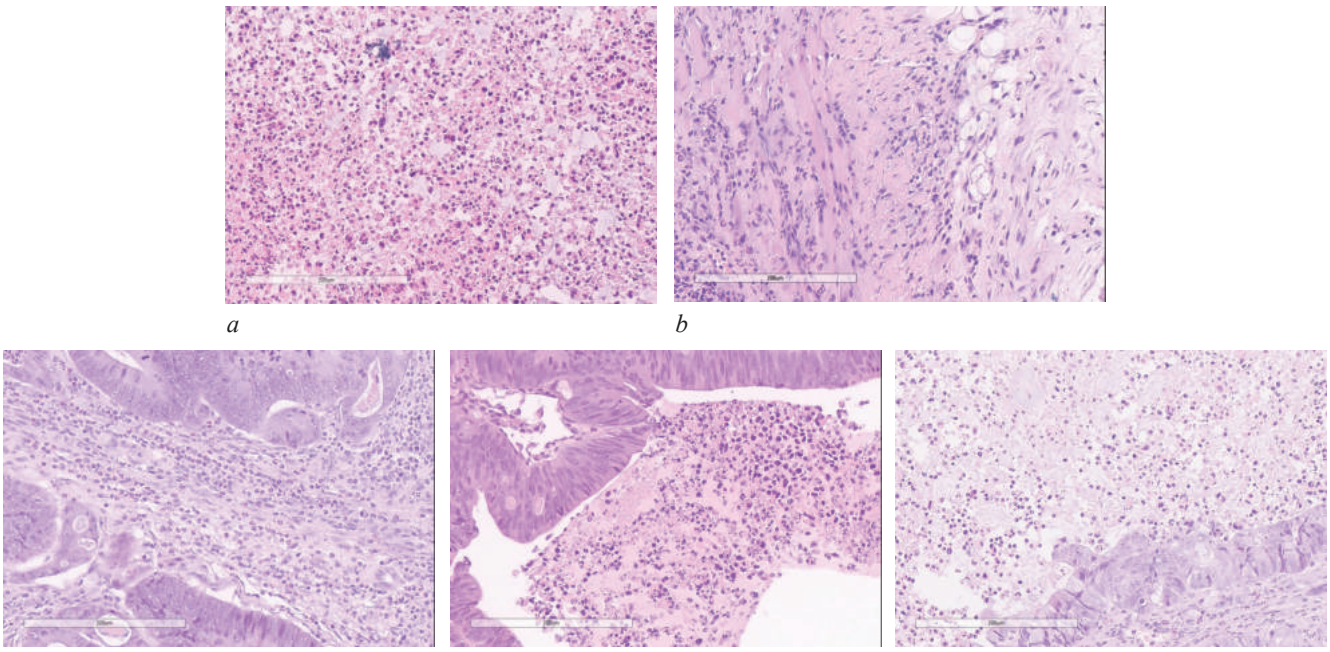


Fig. 3. Histologic preparations of the tumor material. Heterotopic transplantation of tumor xenograft model: *a* – under the skin, *b* – into the omentum, *c* – into the descending colon, *d* – into the ascending colon, *e* – into the caecum. Stained with hematoxylin and eosin, x200

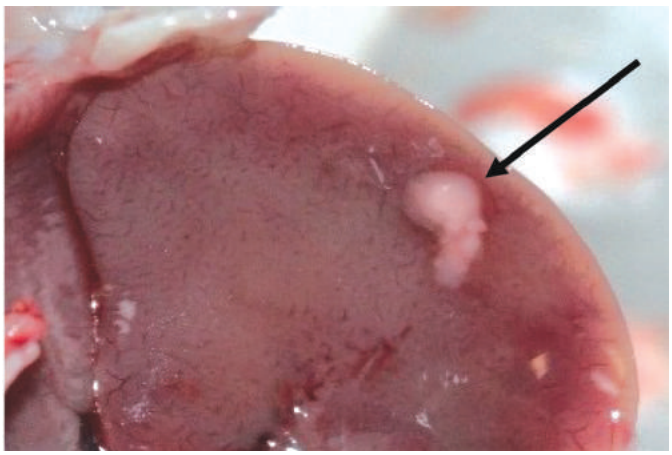


Fig. 4. Liver metastasis (marked with an arrow) during the control laparotomy of the orthotopic xenograft model on day 80 after the transplantation

Table

Advantages and disadvantages of creating different variants of PDX models of colorectal cancer

Type of PDX model transplantation	Characteristics of the transplantation site	Model advantages	Model disadvantages
Heterotopic	Created by transplanting a fragment of a human colon tumor under the skin of the thigh of an immunodeficient mouse	Simple and fast surgery. Easy to visually assess the dynamics of tumor growth. A wide variety of reproducible human tumors. Less traumatizing for the animal.	Replacement of tissue-specific tumor stroma. Microenvironment is not specific for the tumor. No metastasis.
	Created by transplanting a fragment of a human colon tumor into the omentum of an immunodeficient mouse	Good blood supply ensuring good trophism and engraftment	Microenvironment is not specific for the tumor. Complicated surgery. Complicated assessment of the tumor growth dynamics.
Orthotopic	Created by transplanting a fragment of a human colon tumor (mucinous adenocarcinoma) into the ascending colon and cecum of an immunodeficient mouse; created by transplanting a fragment of a human colon tumor (typical adenocarcinoma) into the descending colon of an immunodeficient mouse	Specific intraorgan microenvironment. Suitable for studying metastasis.	Complicated surgery. Complicated assessment of the tumor growth dynamics.

Heterotopic transplantation requires less time and efforts. The surgery is less traumatizing for the experimental animal. In our experiment, this transplantation type demonstrated 100% survival rate. Subcutaneous transplantation allows for easy monitoring of the tumor growth dynamics by measuring the tumor size with a caliper. The most significant disadvantage is a non-specific organ microenvironment resulting from tumor transplantation into a site not typical of the original patient's tumor.

Orthotopic transplantation into the intestinal wall requires significant technical skills and is quite traumatizing for the animal; therefore, this model has a limited capacity and reproducibility. It is worth noting that, when creating a xenograft model for the descending colon, it was still impossible to palpate the tumor and visually assess the results on days 40–80 of the experiment. During the control laparotomy, the access to the xenograft was complicated due to the descending colon location near the lumbosacral region of the spine.

The main advantage of orthotopic transplantation is a specific transplantation site inside the organ, which makes it possible to reproduce the features of the tumor microenvironment, thereby preserving the main molecular genetic signaling pathways.

The use of orthotopic and intraperitoneal xenograft models in studies on the effectiveness of drugs also requires certain technical facilities, since an assessment of tumor growth dynamics requires such imaging methods as magnetic resonance imaging (MRI) and computed tomography (CT).

Despite the required technical facilities, orthotopic PDX models can also be used as spontaneous metastasis models to evaluate the metastatic potential of a tumor and the effectiveness of anti-metastatic drugs, which is an important aspect in fundamental studies on biology of oncogenesis. It is important to take into account that rapid growth of an inoculated tumor orthotopically transplanted into the intestine of an immunodeficient mouse can lead to intestinal obstruction and death of the animal and, consequently, to forced termination of the experiment.

The experiment showed that the choice of a transplantation method in creating PDX models depends on many factors, such as the aim and objectives of the experiment, the quality and biological characteristics of the donor tumor material and model organism, technical facilities of the laboratory, staff training, funding, time frame, etc.

CONCLUSION

The study allows to conclude that, regardless of the site of donor tumor material transplantation into an immunodeficient mouse, xenograft models show a high engraftment rate and largely reproduce the morphological and histologic characteristics of the original human tumor. This determines the possible use of PDX models in searching for biomarkers and assessing the effectiveness of new anticancer drugs.

REFERENCES

1. Fedorov V.E., Podelyakin K.A. Epidemiologic aspects of colorectal cancer (review). *Meditsinskiy Almanakh – Medical Almanac.* 2017;4(49):145–148 (in Russ.).

2. Bray F., Ferlay J., Soerjomataram I., Siegel R.L., Torre L.A., Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin*. 2018;68(6):394–424. DOI: 10.3322/caac.21492.
3. Stastna M., Janeckova L., Hrckulak D., Kriz V., Korinek V. Human colorectal cancer from the perspective of mouse models. *Genes.* 2019;10(788):33. DOI: 10.3390/genes10100788.
4. Gevorkyan Y.A., Kolesnikov V.E., Soldatkina N.V., Kharagezov D.A., Dashkov A.V., Kaymakchi D.O. et al. Minimally invasive surgery in treatment of patients with metastatic colorectal cancer. *Yuzhno – Rossiyskiy Onkologicheskiy Zhurnal – South Russian Journal of Cancer.* 2020;1(2):22–27 (in Russ.). DOI: 10.37748/2687-0533-2020-1-2-3.
5. Kit O.I., Gevorkyan Y.A., Soldatkina N.V., Kolesnikov V.E., Kharagezov D.A. Combined laparoscopic surgery in metastatic colorectal cancer. Koloproktologiya – *Coloproctology.* 2015;4(54):19–23 (in Russ.).
6. Imyanitov E.N. Clinical and molecular aspects of colorectal cancer: etiopathogenesis, prevention, individualization of treatment. *Prakticheskaya Onkologiya – Practical Oncology.* 2005;6(22):65–70 (in Russ.).
7. Baran B., Ozupek N.M., Tetika N.Y., Acarb E., Bekcioglua O., Baskina Y. Difference between left-sided and right-sided colorectal cancer: a focused review of literature. *Gastroenterology Res.* 2018;11(4):264–273. DOI: 10.14740/gr1062w.
8. Luisetto M., Ahmadabadi B.N., Nili-Ahmadabadi H., Rafa A.Y., Hamid G.A., Mashori G.R. et al. Comparison of risk factors and molecular analysis of right-sided colon and left sided colon cancer. *Adv. Can. Res. & Clinical Imag.* 2019;2(2):27. DOI: 10.33552/ACRCI.2019.02.000533.
9. Kwak H.D., Ju J.K. Immunological differences between right-sided and leftsided colorectal cancers: a comparison of embryologic midgut and hindgut. *Ann. Coloproctol*. 2019;35(6):342–346. DOI: 10.3393/ac.2019.03.17.1.
10. Wilding J.L., Bodmer W.F. Cancer cell lines for drug discovery and development. *Cancer Res.* 2014;74(9):2377–2384. DOI: 10.1158/0008-5472.CAN-13-2971.
11. Srivastava P., Kumar M., Nayak P.K. Role of patient derived cell lines and xenograft in cancer research. *The Pharmstudent*. 2016;27:40–48.
12. Cho S.-Y. Patient-derived xenografts as compatible models for precision oncology. *Laboratory Animal Research.* 2020;36:14. DOI: 10.1186/s42826-020-00045-1.
13. Williams S.A., Anderson W.C., Santaguida M.T., Dylla S.J. Patient-derived xenografts, the cancer stem cell paradigm, and cancer pathobiology in the 21st century. *Laboratory Investigation*. 2013;93(9):970–982. DOI: 10.1038/labinvest.2013.92.
14. Hidalgo M., Amant F., Biankin A.V., Budinská E., Byrne A.T., Caldas C. et al. Patient-derived xenograft models: an emerging platform for translational cancer research. *Cancer Discov.* 2014;4(9) 998–1013. DOI: 10.1158/2159-8290.
15. DeRose Yo.S., Wang G., Lin Y.-C.., Bernard P.S., Buys S.S., Ebbert M.T.W. et al. Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. *Nature Medicine*. 2011;17(11):1514–1520. DOI: 10.1038/nm.2454.
16. Inoue A., Deem A.K., Kopetz S., Heffernan T.P., Draetta G.F., Carugo A. Current and Future Horizons of Patient-Derived Xenograft Models in Colorectal Cancer Translational Research. *Cancers*. 2019;11(9):1321. DOI: 10.3390/cancers11091321.
17. Linnebacher M., Maletzki C., Ostwald C., Klier U., Krohn M., Klar E., Prall F. Cryopreservation of human colorectal carcinomas prior to xenografting. *BMC Cancer*. 2010;10:362. DOI: 10.1186/1471-2407-10-362.
18. Marangoni E., Vincent-Salomon A., Auger N., Degeorges A., Assayag F., de Cremoux P. et al. A new model of patient tumor-derived breast cancer xenografts for preclinical assays. *Clinical Cancer Research*. 2007;13(13):3989–3998. DOI: 10.1158/1078-0432.CCR-07-0078.
19. Sivanand S., Peña-Llopis S., Zhao H., Kucejova B., Spence P., Pavia-Jimenez A. et al. A validated tumorgraft model reveals activity of dovitinib against renal cell carcinoma. *Science Translational Medicine.* 2012;4(137):137ra75. DOI: 10.1126/scitranslmed.3003643.
20. Zhu Y., Tian T., Li Z., Tang Z., Wang L., Wu J. at al. Establishment and characterization of patient-derived tumor xenograft using gastroscopic biopsies in gastric cancer. *Scientific Reports.* 2015;5:8542. DOI: 10.1038/srep08542.
21. Xu Y., Zhang F., Pan X., Wang G., Zhu L., Zhang J. et al. Xenograft tumors derived from malignant pleural efusion of the patients with non-small-cell lung cancer as models to explore drug resistance. *Cancer Commun.* 2018;38(1):19. DOI: 10.1186/s40880-018-0284-1.
22. Jung J., Sook H., Chang S.S. The generation and application of patient-derived xenograft model for cancer research. *Cancer Res. Treat.* 2018;50(1):1–10. DOI: 10.4143/crt.2017.307.

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