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REVIEW



# METHODS FOR MODELING TUMOR GROWTH IN MICE IN EXPERIMENTAL STUDIES OF HUMAN GASTRIC CANCER

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## ABSTRACT

Gastric cancer (GC) is a group of malignant tumors originating from the gastric mucosa cells. The highest incidence of GC is recorded in Japan, China and Russia, and the lowest one in the USA and New Zealand. Extensive molecular genetic research of GC has revealed its heterogeneity associated with the genomic instability of the tumor and the complexity of its phenotype due to simultaneous changes in several oncogenes and suppressors. This was the basis for the creation of the GC classification by molecular subtypes. The creation of a realistic preclinical model is essential for translational GC studies. Cancer cell lines and xenografts derived from them are among the most common preclinical models. They are easy to generate, but they also have limitations, since these models cannot sufficiently reproduce the unique characteristics of each cancer patient. Patient-derived xenografts (PDX) are currently the best model for testing targets and predictors of response to therapy. PDX models are created by transplanting surgically resected human tumors into immunodeficient mice. These models maintain morphological similarity and replicate the molecular characteristics of parental tumors providing an indispensable tool for assessing anticancer drug response. Statistical data from preclinical studies with PDX models can significantly save the time and resources required for clinical trials. Transgenic and knockout mouse models are also widely used in scientific laboratories in order to study specific genetic pathways of oncogenesis and develop experimental therapy for GC. This review discusses the molecular classifications of GC and experimental murine models that reproduce cancer in situ and are a universal platform for preclinical research in experimental oncology.

**Keywords:**
gastric cancer, molecular subtypes, PDX model, orthotopic xenograft, genetically modified models, targeted therapy

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ОБЗОР

# ПУТИ МОДЕЛИРОВАНИЯ ОПУХОЛЕВОГО РОСТА У МЫШЕЙ В ЭКСПЕРИМЕНТАЛЬНЫХ ИССЛЕДОВАНИЯХ РАКА ЖЕЛУДКА ЧЕЛОВЕКА

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**РЕЗЮМЕ**

Рак желудка (РЖ) – группа злокачественных опухолей, происходящих из клеток слизистой оболочки желудка. Самый высокий уровень заболеваемости РЖ регистрируется в Японии, Китае и России, низкий – в США и Новой Зеландии. Обширные молекулярно-генетические исследования рака желудка выявили его гетерогенность, что связано с геномной нестабильностью опухоли и сложностью её фенотипа за счет одновременных изменений в нескольких онкогенах и супрессорах. Это явилось основанием для создания классификации по молекулярным подтипам. Создание реалистичной доклинической модели имеет важное значение для трансляционных исследований рака желудка. Раковые клеточные линии и полученные из них ксенотрансплантаты – одни из самых распространенных доклинических моделей. Но, несмотря на легкость генерации, они имеют и ограничения, поскольку эти модели не могут в достаточной степени воспроизводить уникальные особенности каждого больного раком. Ксенотрансплантаты, полученные от пациентов (Patient-derived xenograft; PDX), в настоящее время являются лучшей моделью для проверки мишеней и предикторов ответа на терапию. PDX-модели создаются путем трансплантации хирургически резецированных опухолей человека иммунодефицитным мышам. Эти модели поддерживают морфологическое сходство и повторяют молекулярные характеристики исходных опухолей, таким образом, являясь незаменимым инструментом для оценки противоопухолевого лекарственного ответа. Статистические данные, полученные в ходе доклинических исследований с использованием PDX-моделей, помогают значительно сэкономить время и ресурсы, необходимые для клинических испытаний. Также с целью изучения специфических генетических путей онкогенеза и разработки экспериментальной терапии рака желудка в научных лабораториях широко применяют трансгенные и нокаутные мышиные модели. В данном обзоре обсуждаются молекулярные классификации РЖ и экспериментальные модели мышей, которые воспроизводят рак *in situ* и являются универсальной платформой для доклинических исследований в экспериментальной онкологии.

**Ключевые слова:**
рак желудка, молекулярные подтипы, PDX-модель, ортотопический ксенотрансплантат, генно-модифицированные модели, таргетная терапия

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Gastric cancer (GC) is the third most common cause of cancer death worldwide. Unfortunately, approximately 90 % of cases of the disease are diagnosed at late stages, which leads to unsatisfactory treatment results. The development of GC, along with hereditary factors, is influenced by environmental factors and the presence of H. pylori [1]. Nitroso compounds, smoking, and improper nutrition significantly increase the risk of GC. The pathological processes leading to GC include: atrophic gastritis, intestinal metaplasia and dysplasia. Resection in gastric ulcer disease increases the risk of adenocarcinoma by 2 times [2]. To date, there is a need to improve our understanding of the pathogenesis of GC and to create more effective and less toxic therapeutic drugs, which can only be achieved by using adequate preclinical models in the experiment. The search for literary sources was carried out using the databases Web of Science, Scopus, Pubmed and CyberLeninka by keywords: gastric cancer, mouse models, PDX, target therapy, transgenic and gene knockout mice, stomach cancer, mouse models, targeted therapy, transgenic and knockout mice.

## Morphological classification of GC

According to the International Histological Classification (WHO 2010), GC can be divided into the following histological types: adenocarcinomas (papillary, tubular highly/moderately differentiated, low-differentiated, mucinous, ring-shaped cell), glandular cell carcinoma, squamous cell carcinoma, carcinosarcoma; choriocarcinomas; undifferentiated cancer [3].

The International TNM Classification of Stomach Cancer was revised in 2017 (8th edition) The American Joint Committee on Cancer (AJCC). This classification is applicable only for a confirmed diagnosis of gastric carcinoma. According to the TNM system, carcinoma is classified according to the following criteria: T – primary tumor (the degree of invasion of the tumor into the stomach wall and neighboring structures is estimated); N – regional lymph nodes (the number of regional lymph nodes affected by metastases is estimated); M – distant metastases (distant metastases and tumor cells in ascitic fluid are evaluated). There is a classification of gastric cancer according to Lauren (1965), according to which tumors can be represented by intestinal, diffuse and mixed types [4]. Stomach cancer is also divided into types according to the degree of differentiation and localization of the tumor. The proximal (cardiac) and distal varieties of GC differ markedly in epidemiology, etiology and pathogenesis, while the cardiac form of stomach cancer is similar in many ways to esophageal tumors [5].

## Molecular genetic classifications of GC

The prognosis for the diagnosis of gastric cancer depends on the following indicators: the degree of invasion, the presence of metastases in the lymph nodes, the histological type of tumor and the stage of the process. But even for "twin" tumors with a similar size, stage and histotype, the prognosis can vary greatly. This may be due to the fact that standard morpho-histological criteria cannot fully predict the course of the disease. The Cancer Genome Atlas (TCGA) project performed a full-scale study of 295 tumor tissue samples from patients with stomach cancer using sequencing and bioinformatics methods. As a result, four molecular subtypes of gastric cancer were described: microsatellite instability (MSI), Epstein-Barr virus (EBV), chromosomal instability (CIN) and tumors with genomic stability (GS) [6]. The Asian Cancer Research Group (ACRG) has established another classification based on the transcriptomic tumor profile. The mRNA expression level of 300 patients' tumors was analyzed, on the basis of which 4 subtypes were identified: microsatellite instability (MSI), microsatellite stability with signs of transition from epithelium to mesenchyma (MSS/EMT), microsatellite stability with activity of tumor suppressor p53 (MSS/TP53) and microsatellite stability with loss of activity of suppressor p53 (MSS/TP53-). The Singapore-Duke research team analyzed gene expression in 248 gastric tumors and identified 3 subtypes (proliferative, metabolic and mesenchymal) [7].

The listed molecular classifications of gastric cancer describe the main pathways of the pathogenesis of this disease and facilitate the search for biomarkers and targets for targeted therapy in each tumor. Table 1 shows the main characteristics of all molecular subtypes of gastric cancer according to four classifications. Each subtype is characterized by specific gene mutations and changes in signaling pathways, as well as prognosis and response to chemotherapy.

One of the primary tasks is to study the molecular mechanisms underlying the pathogenesis of gastric cancer in order to develop new treatments that can improve patient survival. A number of chemically and genetically modified mouse models of stomach cancer have provided a significant insight into the contribution of genetic and environmental factors to the onset and progression of the disease.

**Induced mouse models of stomach cancer**

N-nitroso compounds (N-nitro-N-nitrosoguanidine (MNNG) and N-nitroso, N-methyl-N-nitrosourea (MNU)) – chemical carcinogens, the addition of which to drinking water induces gastric adenocarcinoma in rodents. The use of MNNG leads to adenomatous tumors only in the glandular epithelium of the stomach. At the same time, MNNG and MNNG cause adenocarcinomas in the mucosa of the antrum and rarely in the normal mucosa of the fundus [8; 9]. Butylated hydroxyanisole (BHA), an antioxidant used in food preservatives, when introduced into the diet of rodents for 2 years caused an increase in hyperplasia and papillomas in the cardiac part of the stomach [10]. ethylenedibromide (EDB), a soil, grain fumigant, was also used as a carcinogen, which, when administered to rats and mice, promoted oncogenesis in the cardiac part of the stomach [11].

After the World Health Organization (WHO) declared H. pylori one of the class I carcinogens, it became urgent to develop a correct animal model in vivo in order to study the pathogenesis mechanisms of this pathogen H. felis, a close relative of H. pylori isolated from the stomach of a cat, colonizes the gastric mucosa of a mouse and forms gastric lymphomas with somewhat similar patterns of human disease. Although these models provided the initial, very important experimental data, they did not completely simulate the process of human infection with H. pylori. Several strains of H. pylori adapted to mice with different genotypic combinations (SS1 and AM1) have been reported in the literature [12]. Most of the research is currently focused on the preventive effect of H. pylori eradication. It has been shown that eradication of helicobacter in mice can be useful for the prevention of stomach cancer, even if it is carried out relatively late in the natural history of the disease [13].

In 6–16 % of cases, stomach cancer worldwide is associated with Epstein-Barr virus (EBV) and is characterized by unique morpho-phenotypic features. In order to study the mechanisms of EBV-induced gastric cancer (EBV-GC), researchers have developed models of EBV engraftment using infected epithelial cell lines. The obtained xenographs showed moderately differentiated carcinomas without the formation of glands and areas of necrosis [14].

**Genetically modified mouse models with GC**

The appearance of genetically modified mouse models is due to gene transfer technologies and allows us to investigate the significance of various specific genetic pathways in oncogenesis: abnormal expression of growth factors and cytokines, mutations in oncogenes loci and tumor suppressor genes. A transgenic mouse is an animal whose genome contains an artificially introduced foreign gene (transgen), it is used most often to study the consequences of overexpression of genes. In a knockout mouse, certain genes are removed from the body or rendered inoperable. Transgenic and knockout mice infected with Helicobacter and treated with carcinogens develop precancerous and cancerous lesions and are used in the study of gene function and the development of experimental therapy [15].

The INS-GAS transgenic mouse contains two exons of the human gastrin gene that encode a progastrin precursor under the control of an insulin promoter. This model was used to study the effect of gastrin on the development of stomach cancer. The INS-GAS mice showed an increase in the maximum secretion of gastric acid and an increase in the number of parietal cells. At the age of 20 months, metaplasia, dysplasia and stomach cancer were observed in INS-GAS mice [16]. Infection of INS-GAS mice with H. felis or H. pylori led to accelerated carcinogenesis (7 months after infection) [17].

Gastrin-knockout mice (Gastrin knockout mice, GAS-/-) lack gastric acid secretion and, as a result, the architecture of the stomach is changed and the number of parietal cells is reduced. At the age of 12 months, these mice develop spontaneous tumors of the antrum of the stomach associated with excessive bacterial growth and inflammation [18].

To investigate the Wnt pathway in gastric carcinogenesis, K19-WNT1 transgenic mice were created that express Wnt1 in the gastric mucosa. K19 Mice-

Table 1. Classifications of molecular subtypes of human gastric cancer

| GS molecular classification TCGA (The Cancer Genome Atlas) | | | | |
|---|---|---|---|---|
| Molecular s/ type | Molecular characteristics | Localization | Sex/ age/ prognosis/ therapy | Type according to the classification of Lauren |
| MSI | • Hypermethylation of the MLH1 promoter<br>• High mutation rate in PIK3CA, KRAS/ KRAS, JAK2, ERBB3, ERBB2, and EGFR genes<br>• Changes in the two main genes of the class 1 histocompatibility complex (B2M and HLA-B) | | Women/ elderly/ better prognosis/ lower frequency of metastasis into lymph nodes | Intestinal type |
| EBV | • DNA hypermethylation (CDKN2A promoter hypermethylation<br>• Mutations in the PIK3CA, ARID1A genes and the B-cell lymphoma 6 corepressor gene<br>• Amplification of PD-L1/2 and JAK2<br>• Activation of immune cell signaling pathways | The bottom and body of the stomach | Males/ the worst prognosis | |
| CIN | • Activation of receptor tyrosine kinase signaling pathways (RTK)/RAS<br>• Amplification of MET, EGFR, HER2 and FGFR2high mutation rate of TP53<br>• Changes in tumor suppressor genes SMAD4 and APC | The area of the gastro-esophageal junction and the gastric cardia | The greatest benefit of adjuvant chemotherapy | Intestinal type |
| GS | • Mutations in CDH1 and RHO-family GTPase (RAS) genes<br>• Activation of angiogenesis and cell adhesion (E-cadherin)<br>• CLDN18/ARHGAP gene fusion | | Young age/best prognosis/ least benefit from adjuvant chemotherapy | Diffuse type |
| GC molecular classification ACRG (Asian Cancer Research Group) | | | | |
| Molecular s/ type | Molecular characteristics | Localization | Prognosis/recurrence rate/diagnosis | Type according to the classification of Lauren |
| MSI | • Mutations in the genes of the KRAS, ALK, ARID1A and PI3K pathways | Antral part of the stomach | The best prognosis/ lowest recurrence rate/ is diagnosed in the early stages (I-II) | Intestinal type |
| MSS/ EMT | • Loss of PDX1 expression<br>• Reduction in the number of mutations compared to other subtypes | | The worst prognosis/ highest recurrence rate/ is diagnosed in the late stages | Diffuse type |
| MSS/ TP53 | • Associated with EBV infection<br>• Mutations in APC, ARID1A, KRAS, PIK3CA and SMAD4 genes | | | |
| MSS/ TP53- | • High mutation rate of TP53<br>• Amplification of HER2, EGFR, cyclin E1 (CCNE1), CCND1, MDM2, Robo2, GATA6 and MYC | | | |

Wnt1 were crossed with K19-C2ME transgenic mice to study the effect of Wnt and PGE2 on gastric carcinogenesis [19].

Cyclin-dependent kinase (CDK) inhibitor p27Kip1 plays an important role in the regulation of the cell cycle and is associated with many malignant neoplasms. p27Kip1 knockout mice develop mild gastric hyperplasia, occasional foci of moderate metaplasia and atypia or dysplasia of low degree. After infection with H. pylori, these mice have intestinal metaplasia, high-grade intraepithelial neoplasia of the stomach, polypoid adenomas and sometimes in situ carcinoma or intramucosal carcinoma. Thus, a mouse with p27Kip1 deficiency is a useful model for studying the pathogenesis of H. pylori in gastric carcinogenesis and for testing eradication and chemoprophylaxis strategies [20].

IL-1β-transgenic mice have in their genome an alien human interleukin-1ß gene, the increased production of which leads to the risk of H. pylori-induced hypochlorhydria and stomach cancer. Under conditions of H felis infection, these mice show accelerated development of gastric inflammation and carcinoma compared to control mice. There is also a decrease in the recruitment of macrophages and neutrophils in H. pylori infection and a decrease in the activation of NF-KB [21].

Transgenic COX-2 mice obtained on a C57BL/6 genetic background expressing full-sized human

Table 1. Classifications of molecular subtypes of human gastric cancer

| Molecular classification of gastric cancer Singapore-Duke | | | |
|---|---|---|---|
| Molecular s/type | Molecular characteristics | Therapy sensitivity | Type according to the classification of Lauren |
| Proliferative | • Increased expression of cell cycle genes<br>• Frequent mutations of the TP53 gene<br>• DNA hypomethylation<br>• Activation of E2F, MYC and RAS genes | | Intestinal type |
| Metabolic | • Increased regulation of metabolism and digestion genes<br>• Hyperactivation of antispasmodics-polypeptide-expressing pathway of metaplasia | Sensitive to 5-fluorouracil | |
| Mesenchymal | • Increased expression of cell adhesion-related genes with extracellular matrix-receptor interaction, focal adhesion and activation of EMT and cancer stem cell pathways<br>• Changes in p53, TGFβ, VEGF, NF-κB, mTOR and Shh signaling pathways | Sensitive to PI3K/AKT/mTOR inhibitors | Diffuse type |
| Classification of internal subtypes of gastric cancer (Tan, et al.) | | | |
| Molecular s/type | Molecular characteristics | Prognosis/sensitivity to therapy | Type according to the classification of Lauren |
| G-INT | • High expression of carbohydrate and protein metabolism genes (FUT2) and cell adhesion (LGALS4, CDH17) | A successful forecast/Sensitive to 5-FU and oxaliplatin | Intestinal type |
| G-DIF | • High expression of cell proliferation (AURKB) and fatty acid metabolism (ELOVL5) genes | Poor prognosis/Sensitive to cisplatin | Diffuse type |

COX-2 cDNA showed an increased incidence of MNU-induced gastric cancer [22].

Mutations of the K-ras gene are detected in diffuse (6 %) and intestinal (18 %) gastric cancers. K-ras transgenic mice with systemic activation of K-ras are characterized by changes in gastric cellular homeostasis, depletion of parietal cells, increased levels of inflammatory response factor (COX-2), stem cell marker (DCAMKL1, CD44), activated MAPK pathway, as well as hyperproliferation of the squamous epithelium in the gastric cardia and metaplasia in the glandular stomach, reminiscent of preneoplastic changes that occur during gastric carcinogenesis in a person. This suggests that mutant K-ras signaling modulates important molecular events in initiated gastric carcinogenesis [23].

Tff1-knockout mice –TFF1-/-) are mice in which the shamrock tumor suppressor gene factor 1 has been knocked out. TFF1 expression is often lost in gastric carcinomas and leads to activation of the β-catenin and AKT-GSK3ß signaling pathway. Homozygous mutant Tff1 (TFF1-/-) mice develop anthropiloric adenoma and even multifocal carcinomas, which is consistent with increased indicators of inflammation of Tff1+/- mice used for studies of gene heterozygosity and transcript regulation [24].

## Xenogenic models of GC

The preclinical phase of gastric cancer research should include in vivo models that accurately simulate the clinical situation in the human body. To promote the concept of precision medicine, xenogenic models of gastric cancer have been developed, capable of reproducing the histological and genomic features of a patient's tumor and predicting the reaction to the antitumor drugs under study. In the creation of experimental models of stomach cancer, special thymus-free Bald/nude mice with a mutation in the Foxn1 gene are used [25]. Deficiency of T-lymphocytes significantly weakens the immunity of mice, which contributes to the engraftment, growth and metastasis of tumor cells in xenografts after implantation [26]. However, intact innate immunity and high NK cell activity may limit the rate of engraftment of most primary solid tumors. Also in experimental oncology, mice with severe combined immunodeficiency in T and B lymphocytes (SCID mice), mice with severe immunodeficiency and diabetes (NOD-SCID mice) and mice with the absence of mature T, B and NK cells, dysfunction of macrophages and dendritic cells and reduced activity of the complement system (NOG mice) are used [27].

*Xenografts derived from cancer cell lines (cell-line-derived xenografts; CDX)* are frequently used model systems in the field of studying the genetics of gastric cancer. However, such models have a number of limitations: the inability to reproduce intra-tumor heterogeneity and microenvironment, weak predictive ability to assess the effectiveness of drug treatment, highly aggressive cell lines and their susceptibility to genetic changes due to prolonged in vitro cultivation [28].

*Xenografts obtained from a patient (patient-derived xenografts – PDX)* are currently the best preclinical model of GC for testing targets and predictors of response to therapy. Modern procedures for creating PDX models include both heterotopic (subcutaneous) and orthotopic methods of transplantation. Heterotopic xenografts are obtained by implanting human tumor tissue or cells into a mouse area unrelated to the original tumor site, usually subcutaneously in the lateral or dorsal region or subrenally. The obtained models are morphologically and biochemically similar to the primary tumors of donors, but they have a number of limitations, such as abnormal microenvironment and pseudocapsule. In a large-scale study by Takeshi Kuwata et al. Out of 232 primary tumors of patients with diagnosed gastric adenocarcinoma, 35 PDX models and 32 CDX models were created. Most PDX tumors showed histologically consistent morphology with primary tumors, and more than half of CDX had histologically confirmed inconsistency with primary tumors. PDX, whose donors had lesions with lymph node metastases, had a higher rate of engraftment. In more than half of the cases, lymphoproliferative lesions obtained from B-lymphocytes were observed at the site of engraftment of the donor's tissue [29]. Also in this study, it was shown that none of the subcutaneous PDX and CDX models developed matastatic lesions in mice. In Hernandez MC, et al. the possibility of creating subcutaneous PDX models from biopsy samples of patients with unresectable or metastatic disease in clinical settings has been shown [30].

To study the mechanisms of tumor metastasis, orthotopic mouse models are used, which are created by transplanting fragments of patient material into the organs of tumor origin to

immunodeficient mice. The technique of creating an orthotopic xenograft has been improved from the "stitching" method to the "sticking" method. Illert B, et al. In 2003, a technique was described for creating an orthotopic xenograft of GC, in which the serous membrane of the anterior wall of the mouse stomach was removed with a scalpel and 2–3 fragments of the donor tumor were sewn with non-absorbable sutures. Primary tumor growth was observed in 90 % of mice and metastases spread to the liver (70 %), lungs (10 %) and lymph nodes (10 %) [31]. Jones-Bolin and his research group developed a technique for creating orthotopic xenografts by stitching two fragments of a 2 × 2 $mm^3$ donor tumor with the dorsal side of the mouse stomach in the middle part using 2–3 nodes. The tumor grew in more than 90 % of the animals, and metastases developed in the liver (40 %), lymph nodes (40 %) and the surface of the peritoneum (60 %) [32]. In 2009, a group of scientists from Germany proposed a method for creating a PDX model by fixing a fragment of a donor tumor in a tissue pocket of the stomach with one drop of tissue glue. The pocket was made either in the submucosa of the distal stomach or in the cardia. Orthotopic tumor growth was observed in 100 % of cases, metastases spread to the lungs, pancreas, liver, intestines and kidneys [33]. In the studies described above, animals were slaughtered if the tumor increased in diameter to 10 mm or the general condition worsened. Li, et al. a method for generating a PDX model by "gluing" a tumor fragment into a tissue pouch made in the middle of the large curvature of the recipient mouse's stomach was published. 100 % tumor engraftment was observed, metastases after necropsy were found in lymph nodes (79 %), liver (91.5 %), kidneys (62.5 %) [34]. In a study by Busuttil, et al. three gastric cancer cell lines (MKN45, AGS, MKN28) were taken, 50 microliters of cancer cell suspension and Matrigel were inoculated into the subserous layer of the antrum of the stomach. Successful engraftment was observed in 76 % of cases, metastases were found in the thoracic and abdominal regions. As a result of the analysis of the above-mentioned works, it can be concluded that the rate of engraftment of the donor tumor and the spread of metastases do not depend on the place of implantation of the sample into the stomach.

## The use of PDX models of gastric cancer in the development of molecular targeted therapy

The lack of standard chemotherapy strategies and the low overall survival rate create a need for a treatment method with more specific anticancer efficacy and low non-selective toxicity and resistance. As a result of molecular genetic studies of gastric cancer, special attention was focused on understanding the mechanisms underlying targeted therapy of advanced cancer. Targeted inhibitors effectively regulate the work of signaling pathways involved in tumor growth processes, which ensures better specificity and selectivity of anticancer therapy. PDX models are an important tool for screening patients who may benefit from targeted therapy.

The epidermal growth factor receptor (EGFR) should be considered as a target for targeted therapy of gastric cancer in the first place. It is a transmembrane glycoprotein with tyrosine kinase activity. Also, the family of epidermal growth factor receptors is represented by its other types: HER2, HER3 and HER4. These receptors are involved in the activation of signaling pathways that promote proliferation, differentiation, cell invasion and suppression of apoptosis. Therefore, it is expected that drugs targeting EGFR and HER2 will improve the therapeutic effectiveness of the treatment of stomach cancer. An example is the drug Cetuximab, which is a monoclonal antibody and specifically binds to the extracellular domain of EGFR. In a Chinese study by Wang X, et al. It was found that the number of copies of the EGFR gene is a prognostic biomarker of the effectiveness of cetuximab in the PDX model of gastric cancer [35]. A monoclonal antibody that inhibits the formation of ligand-dependent heterodimers of the HER2 receptor with other representatives of the family – pertuzumab in combination with trastuzumab, capecitabine and cisplatin – demonstrate pronounced antiproliferative and antitumor activity on xenographic models of gastric cancer with HER2 overexpression. Trastuzumab, in turn, is a humanized monoclonal antibody that binds to the HER2 receptor to eliminate or reduce the activity of the receptor [36]. Preclinical studies have shown that pertuzumab in combination with trastuzumab enhances the antitumor effect in the HER2-positive xenograft model of gastric cancer [37].

Studies have found that the dysregulation of the MET signaling pathway occurs in gastric cancer, which correlates with poor clinical outcomes and drug resistance. At the same time, the drug volitinib (EGFR inhibitor) demonstrates strong antitumor activity in PDX models with overexpression of MET and pMET by inhibiting the PI3K/mTOR pathway. In addition, the efficacy of two EGFR monoclonal antibodies (BK011 and cetuximab) was evaluated on five PDX models with different levels of EGFR expression or amplification. Both BK011 and cetuximab induced complete regression of the PDX model with EGFR amplification [38].

Recent studies have shown that monotherapy with afatinib, a selective irreversible inhibitor of protein kinase receptors of the ErbB family, led to regression of HER2-amplified GC by prolonging inhibition of HER3 and EGFR, which was superior to trastuzumab monotherapy. In Zuhua Chen et al. PDX models of GC with EGFR amplification, EGFR overexpression, or HER2 amplification have been shown to be treatable with afatinib. Afatinib is a pan-HER inhibitor; Therefore, further studies are needed to determine whether afatinib is effective in patients with changes in the EGRF family [39].

Lapatinib is a dual inhibitor of tyrosine kinase receptors type 1 and type 2 (ErbB1 and ErbB2). PDX models with high microvessel density are more sensitive to apatinib compared to other models with low CD31 expression [40].

Violation of the regulation of the cell cycle in GC occurs quite often, while amplification of the CCNE1, CCND1 and CDK4 genes is observed/6. In this case, cyclin-dependent kinase inhibitors can be used. CDK1/2/9 inhibitor AZD5438 was shown to have significant tumor inhibition in two PDX models with a high CCNE1 copy number [41].

Thus, PDX are universal models for evaluating potential targeted molecules and serve as a screening tool for patients for targeted therapy.

## CONCLUSION

As precision medicine develops, molecular-oriented therapeutic strategies should be individualized for cancer patients. When creating preclinical models, it is important to evaluate the differentiation and classification of xenografts according to Loren, since changes in these characteristics can lead to a shift in the genetic and histopathological parameters of xenografts in relation to the primary tumor. The reason for such changes is the high heterogeneity of stomach cancer. Mouse models are an important experimental platform (tool) for studying the molecular mechanisms of the occurrence and development of gastric cancer, as well as for screening and testing the effectiveness of new targeted drugs that target tumor cells with virtually no damaging effect on normal tissues. Further study of the molecular features of the pathogenesis of GC and the use of xenogenic, "avatar", mouse models to predict the reaction to the studied drugs in patients' tumors should make a significant contribution to the development of translational medicine.

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