REVIEW

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EXPERIMENTAL CANCER RAT MODELS

Yu. D. VINNICHUK✉, O. M. PLATONOV, O. O. GRYSHCHUK, S. V. KOMISARENKO

Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv;
✉e-mail: vinnichukju@gmail.com

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Experimental rat models are widely used in cancer research. This is facilitated by the diversity of existing inbred animal lines and their relatively low cost. The purpose of this review was to analyze and systematize the publications 2000-2024 selected in PubMed and in national author databases on various cancer rat models. The advantages, disadvantages, and prospects of using these models in the study of different aspects of cancer pathology are discussed. The information will help researchers choos an adequate experimental rat model to study the mechanisms of cancer development and the possibility of its treatment.

K e y w o r d s: experimental cancer models, malignant tumor, rats, carcinogenesis, in vivo, data analysis.

Over the last 100 years, oncopathology has risen to be the second most frequent cause of mortality; the first place belongs to cardiovascular disease. Despite substantial scientific achievements in clarifying the malignant growth origin and development, cancer incidence rate and lethality keep growing. According to the GLOBOCAN database (Global Cancer Observatory), in 2022, there were registered in the world – over 19.0 million cancer cases and over 9.0 million deaths. In Europe, there were 4.5 million cases, of which 2.0 million were fatal [1]. According to the WHO, before 2030, cancer lethality will grow by 45% compared to 2007 [2]. Considering how fast the cancer spreads through the body and how hard they are to diagnose early and treat, oncological diseases can be rated as some of the most dangerous for people with the most pressing problems in medicine.

The results of multifaceted fundamental and clinical studies, unfortunately, cannot yet break through the current 50% barrier to efficient treatment for patients with oncology. Moreover, cancer is growing ever younger and more resistant to therapy. Unsolved problems include the mechanisms of metastasizing, the cancer ability to avoid detection by the immune system, the input of the microenvironment in predicting the therapy efficiency and the progression of the illness [3].

Malignant tumors are characterized by low differentiation of cells, fast growth and infiltration of the surrounding tissue, and metastases. Currently, there are over 200 types of tumors. They differ in their tissue of origin, histological type, aggressiveness, growth rate, metastasizing ability, responsiveness to therapy and prognosis. A malignant tumor is a complex tissue which includes also stromal elements such as immune cells, fibroblasts, endothelial cells, pericytes, neuronal cells, and the intercellular matrix. Complex interactions between the cells and non-cellular components of the microenvironment doubtlessly influence the initiation, progression, and endpoint [4]. Thus, it is important to have adequate experimental models for cancer research to understand the cancer progression and develop efficient prophylaxis and treatment methods. Analyzing and classifying existing modern rat models used in experimental oncology are the main objectives of this review.

Animal models in cancer study

In laboratory animals, there are numerous cancer models which, to some extent, reflect the development and progression of the disease in humans and provide opportunities to research various aspects of carcinogenesis impossible to efficiently study in the human body [5-8]. In particular, to rep-

licate the process of tumor development under pathophysiological conditions, ensure the genomic heterogeneity of human cancers, and study the complex tumor microenvironment, which includes immunocompetent and stromal cells [5, 9]. Animal models allow for initiating tumor growth in a more physiologically relevant setting [5], reflect human cancer genomic heterogeneity, and create an environment that includes a multitude of immune and stromal cell populations in the tumor microenvironment [9].

Not only do experimental animal models have significant advantages in modeling the origin and studying the mechanisms of malignant transformation; they are used to evaluate the effects of various treatments – an important method of cancer research for which there is no substitute. Although there has been much progress in treating solid tumors and oncological diseases of the hematopoietic system due to some well-known therapeutic strategies (such as immune checkpoints inhibitors, ICI, and the chimeric antigen receptor T-cell immunotherapy, CAR-T), the problems of a growth's initiation and progression, proliferation mechanisms, angiogenesis, metastasizing, recurrences and treatment resistance remain controversial and require further research [10].

Models with the use of transgenic animals (GEM) that have a mutation in some individual gene (overexpression or knock-out) allow studying the role of oncogenes, suppressor genes, marker proteins, and signaling pathways in the initiation, progression and metastasizing of a growth. However, GEMs tend to have low metastasizing frequencies. Also, the results are difficult to analyze because of significant variability in how long a cancer can appear and metastasize [11, 12].

Experimental models of cancer are initiated in different animals – fruit flies, fishes (*Danio rerio*, *Xiphophorus* sp.), mice, rats, hamsters, dogs, pigs, and horses [13-16]. Compared to other animals, mammals (especially mice and rats) are the most similar to people. Short reproduction cycles significantly improve the experimental studies' efficiency. Sequencing of the murine genome showed 99% of genes shared with humans, with homology reaching 78.5% [17]. Pig models are closer to humans in genetics, anatomy, organ size, and pathological manifestations [17]. Large animals also have a longer lifespan, making it possible to observe the process longer and sample the blood and tissues more than once [18, 19]. However, experimental models should not only be as close to human diseases as possible; to be scalable, they also need to be easy to use, reproducible, and economical [20].

Most often, studies of cancer development use the mouse or rat models. Recent studies of the rat genome showed that the rat has some experimental advantages over the mouse; its immune system is closer to human, the structure of chromosomes facilitates the study of the genome, organ sizes allow lengthwise analysis of tumor growth, and the biological samples of same tumors are large enough for a multiplex molecular analysis of the tumor and the host. Thus, experimental models of tumor growth in rats make them promising for cancer research [5, 21].

Animal cancer models (Fig.) include transplants (syngeneic, allogeneic, and xenografts) and autochthonous (spontaneous and induced by carcinogens (chemical, viral, or other), and genetically engineered. Depending on the way the cells are administered, they can be orthotopic (transplanted

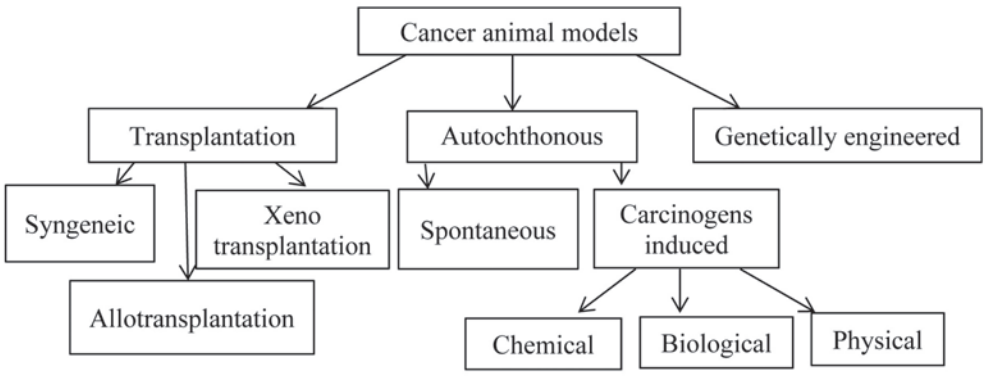

Fig. Main existing cancer animal models and ways of cancer induction

into the same organ or tissue from which the tumor originates), heterogeneous (subcutaneously), and metastatic (into a blood vessel).

General classification and main characteristics of existing cancer animal models

Autochthonous models

For the autochthonous models, orthotopic localization and natural morphology are typical (all human cancers are autochthonous). Such models allow studying the role of signaling pathways, genes, marker proteins and stem cells in the origin of malignant growth. Although an autochthonous model most closely imitates the neoplasia's development in the human body, such malignancies tend to have a long period of latency (human tumors are also characterized by long-term latency, but these animal model requires long-term observation and may be limited by the lifespan of experimental animals) and highly variable growth rates.

Spontaneous autochthonous models. They are used to study cancers that arise naturally, without the interference of extreme factors. The tumors develop similarly to human cancers. Another advantage is the substantial time it takes for them to grow, allowing for a comprehensive approach to treatment and study of the genetic factors influence. It does, however, prolong the experiments and make them more expensive. These models require a large number of animals, and the frequent heterogeneity and individual features of such tumors complicate obtaining tumor-bearing animals with uniform growth in bulk [10].

Carcinogen-induced autochthonous models. Methods of cancer induction include exogenous physical, chemical, and biological factors. Various types of cancer can be induced by bacterial and viral infections (biological factors) [22]. The most important known carcinogenic infectious agents worldwide are Helicobacter pylori, human papillomavirus, hepatitis B virus, hepatitis C virus, Epstein-Barr virus [23], human immunodeficiency virus (HIV), human T-cell leukemia virus (HTLV-I) and Merkel cell polyomavirus (MCPyV) [22, 24].

Autochtonous models of tumors induced by biological agents. Tumor-related viruses are known to be involved in the initiation and progression of certain tumors. Epstein-Barr virus (EBV, human herpesvirus 4, HHV-4) and Kaposi sarcoma-associated herpesvirus (KSHV, human herpesvirus 8; HHV-8) are human gammaherpesviruses that have oncogenic properties [25]. HV-8 is associated with several distinct lymphoproliferative disorders: primary effusion lymphoma, multicentric Castleman disease (MCD), MCD-associated plasmablastic lymphoma and HHV-8$^+$, EBV$^+$ germinotropic lymphoproliferative disorder [26]. The viruses associated with the greatest number of cancer cases are human papillomaviruses (HPVs), which cause cervical cancer and several other epithelial malignancies. Chronic infection with hepatitis viruses HBV and HCV is responsible for the majority of hepatocellular cancer. These oncoviruses include various classes of DNA and RNA viruses and induce cancer by a variety of mechanisms [22, 27]. The use of virus-induced carcinogenesis and experimental animal models of cancer is essential in understanding the mechanisms of cancer development to advance prevention, diagnosis, and treatment methods [28].

While human oncoviruses are necessary for cancer development, they are rarely fully oncogenic on their own. Viral-induced cancers typically arise in the context of persistent infections and can develop many years to decades after the initial infection. The immune system can either hinder or protect against these cancers; some virus-associated cancers increase with immunosuppression, while others emerge in the context of chronic inflammation. Thus, in the process of multistep carcinogenesis, viral infection provides additional cofactors such as immunosuppression, chronic inflammation or mutagens action – that are necessary for malignant transformation. Despite their prevalence and significance for public health, as well as their potential for immunoprophylaxis and targeted therapies, understanding and managing virus-induced cancers remains challenging. This is due to limited animal models, the diverse nature of viral-induced cancers, the variety of viruses involved, and the complex interactions between viruses and host cells leading to cancer development [29].

The animal model should be susceptible to the pathogen under study to obtain meaningful data and key elements of pathogenesis should also be reflected when compared to humans. Well-designed small animal models for HIV, hepatitis viruses and tuberculosis require, additionally, a thorough understanding of the similarities and differences in the immune responses between humans and small animals and should incorporate that knowledge into the goals of the study [30].

Most research has been conducted using mouse models of virus-associated cancer, because the laboratory mouse (*Mus musculus*) is one of the most effective *in vivo* experimental systems for modeling human viral infections and cancer. However, mice are not naturally susceptible to some known oncogenic viruses. Many murine models have been developed to explore various aspects of virus-associated carcinogenesis to address this limitation. These models include tumors resulting from xenografts of human tissues and cells (both cancerous and virus-infected) and genetically engineered mice that are susceptible to viral infections and associated cancer [31]. Humanized mouse models (models with functional human genes, cells or tissues) are used for lympho- and hepatotropic infections, such as HIV and HBV and HIV/*Mycobacterium tuberculosis* (Mtb) co-infections. Non-humanized animal models, such as C57BL6 and C3HeB/FeJ mice, guinea pigs, and rabbits, can only accommodate Mtb infections and not HIV or HBV/HCV [30].

Information about rat virus-induced models of cancer is very limited. Investigated cytotoxic properties of immunocompetent cells on *in vivo* growth of viral-induced Moloney sarcoma and Gross virus-induced lymphoma (Gross leukemia virus, causing lymphoid leukemia in mice and rats) in rat [32, 33].

Autochtonous models of tumors induced by physical carcinogens. Physical carcinogens include various agents: electromagnetic radiations of different kinds, low and high temperatures, mechanical traumas, and solid and gel materials. X-rays or radioactive materials, UV rays from sunlight, and asbestos fibers are examples of physical carcinogens. Radiation alters the genome of cells, UV light changes the structure of amino acids, and asbestos fibers interfere with chromosome alignment during cellular division, ultimately causing uneven numbers of chromosomes in the daughter cells. These changes in gene expression may serve as potential mechanisms underlying the development of neoplasia [34].

Chemical carcinogens-induced autochthonous model. Chemical carcinogens (the most common: nitroso compounds, hydrazines, heterocyclic/aromatic amines) with a high frequency cause the formation of tumors in certain organs. However, the process is characterized by a long latent period, and continued observation makes it difficult to metastasis study.

Animal biochemistry of carcinogen-induced tumor development is similar to human, which allows studying how carcinogens act on the body, their threshold and toxic doses, and how the microenvironment affects the process. Such models are relatively simple, fast, and highly reproducible. However, these models metastasize only rarely and some kinds of cancer often have long latent periods and greatly vary in growth rates [35].

Transplanted tumors

Transplanted tumor models are obtained by administering cancer cells from one organism into another. If the donor and the recipient belong to the same species, this is called allotransplantation, otherwise – xenotransplantation or xenograft. In a syngeneic model, cancer cells are transplanted into immunocompetent animals of the same line; for xenotransplantation, human cancer cells are mostly transplanted in immunodeficient animals to avoid immune reactions and transplant rejection. Patient-derived xenograft (PDX) models preserve the genetic and histopathological features of the patient's malignant tumor and are most often used to study and select a target of cancer drugs and evaluate their efficiency. However, such a model has limitations due to the differences between species [36].

Orthotopic implantation of tumors, i.e., implantation into the same organ or tissue from which tumor originated in the patient, is based on Paget's principle that tumor growth is favorable when based in "congenial soil" [37]. Compared to the subcutaneous space, the orthotopic location can provide the appropriate microenvironment to model tumor invasiveness and study the metastatic cascade realistically. Wang W. and Sordat B. were the first to describe the technique of orthotopic implantation in 1982 [38]. They injected colon cancer cells into the cecum of nude mice and saw that the tumors displayed primary and secondary features of invasiveness: they infiltrated layers of the colonic wall, formed peritoneal deposits, and disseminated to mesenteric lymphatics. Since then, orthotopic models have been developed for gastric cancer [39, 40], pancreatic cancer, lung cancer [41], bladder cancer [42], breast cancer [43, 44], head and neck cancer [45].

Such methods as intra-cardiac intravenous, splenic, and foot-pad injections of cancer cells have been attempted to model the metastatic cascade, but it is generally accepted that animal models using these approaches are not physiologic [46]. These experimental animal models of metastasis only demonstrate the last few steps of metastasizing: the entry

of tumor cells into the circulation, arrest in capillary beds, extravasation, survival, and proliferation in secondary sites [47]. While these injected tumor cells reach many organs, just the presence of a viable tumor cell within an organ will not guarantee metastasis development [48]. Except for intra-cardiac injections that can form widespread metastases, these various approaches usually form metastases at limited sites, such as the liver for splenic injection or the lung for intravenous injections. They normally require the use of a sequentially selected metastatic population of a given cell line to improve efficacy, often overestimating the malignant phenotype [49].

Autochthonous cancer rat models

Spontaneous cancer rat models

Spontaneous cancers of the mammary gland and liver are fairly common in inbred rats. Spontaneous mammary cancers occur in different rat lines, e.g., Wistar (obtained in 1907 by the Wistar Institute, USA), SD (derived from the Wistar line in 1925), F344 (an inbred rat line based on the Fischer strain). Thus, such benign tumors as mammary fibroadenomas and adenomas are found in 21.3 and 16.9% of SD females and 12.9 and 9.5% of Wistar, respectively; mammary cancers occur in 10.1% of SD females and 3.4% of Wistar. In F344 rats, mammary fibroadenomas are found in 41.2% of cases [11]. Chronic hepatitis and acute hepatocellular carcinoma are found in 60% of one-year-old LEC rats (Long-Evans Cinnamon, inbred rats described by Joseph A. Long and Herbert M. Evans, University of California, USA), while 40% of these animals die of acute liver failure at 3-4 months. Such hereditary hepatitis and carcinoma are explained by copper over-accumulation in the liver and the production of significant amounts of hydroxyl radicals leading to oxidative stress which is one of the mechanisms of hepatocellular carcinoma development in humans [50]. Therefore, LEC rats are widely used to recreate the hepatocellular carcinoma.

Induced cancer models

Rat models of chemically induced cancer. Chemical carcinogens are the most widely used because they have such an important feature as organ specificity; they offer models to evaluate carcinogenesis in virtually any given organ (Table 1). When evaluating and selecting such chemicals, knowing whether the carcinogenesis involves gene mutations is crucial. If a chemical induces gene mutations in the target organ and so causes cancer, it is classified as a genotoxic carcinogen without any threshold or safe dose. It is then prohibited for use as a food additive, pesticide or veterinary medicine. Suppose a dose of the chemical does not induce mutations in the target organ despite being carcinogenic. In that case, it is classified as a non-genotoxic carcinogen with a threshold/safe dose and can be used at concentrations below the threshold [51].

According to the results of animal investigations, probable human carcinogens include nitrosamines (compounds that induce gastrointestinal tumors). High concentrations of nitrates and herbicides, nitrates and amines lead to the production of nitrosamines which are especially active at reduced stomach acid secretion [52].

Dimethylnitrosamines (DMN) and diethylnitrosamines (DEN) are compounds that can induce various tumors in mice and rats, mostly tumors of the liver and lungs. Nitrosamines are activated by the CYP450 enzymes and turn into strong alkylating agents which produce DNA adducts leading to tumors [11]. Besides DEN, which induces hepatic neoplasia, one can use 2-acetylaminofluorene (2-AAF)

Table 1. Autochthonous rat cancer models, induced by chemical agent

Chemical drugs	Type of cancer
Nitro group	
Dimethylnitrosamine	Liver cancer
Diethylnitrosamine	Liver cancer
Methylbenznitrosamine	Esophageal cancer
Methylnitrosamine-1-3-pyridinebutanol	Lung cancer
Nitroso-N-methylurea	Breast cancer
Hydrazines	
Dimethylhydrazine	Colon cancer
Polycyclic aromatic carbohydrates	
Dimethylbenzanthracene	Breast cancer
Aromatic amines	
O-toluidine	Kidney cancer*
Mycotoxins	
Aflatoxin B1 produced by *Aspergillus sp.*	Liver cancer*
Alkaloids	
Aristolochic acid (natural *Aristolochia* alkaloids)	Bladder cancer*

Note. *Highly sensitive transgenic rats

as an inducer which causes cholestasis and fibrosis in Wistar rats (the system consists of a single dose of the genotoxic carcinogen DEN, short-term dietary exposure to 2-AAF, sufficient to suppress the growth of virtually all normal hepatocytes, and partial hepatectomy to trigger the growth of DEN-altered hepatocytes not suppressed by 2-AAF). These carcinogens permit to efficiently and simply determine subclinical hepatic cancer [53].

Methylbenzylnitrosamine (NMBA) is another important carcinogenic nitrosamine. Nowadays, NMBA is the most efficient esophagus tumor inducer in rats. Thus, 100% of SD rats given 0.5 mg/kg NMBA thrice a week for five weeks or once a week for 15 weeks had esophagus cancer at 200 weeks [11].

(Methylnitrosamine)-1-(3-pyridine)-1-butanone (NNK) is one of the main chemical carcinogens in cigarette smoke (in humans, smoking is known to raise stomach cancer risk fourfold). The substance can also efficiently induce lung cancer in rats, mice, and hamsters [11].

Another highly toxic substance with carcinogenic, mutagenic and teratogenic properties is N-nitroso-N-methylurea. It induces mammary gland cancer in Sprague-Dawley rats. Such tumors reflect the heterogeneity of mutational profiles and positive expression of the estrogen receptor (the prevailing subtype in patients) and have similar mechanisms of immune response avoidance seen in malignant growth of the human mammary gland [5].

To initiate mammary tumors, one can also use 7,12-dimethylbenz(a)anthracene (DMBA), a polycyclic aromatic hydrocarbon with a pronounced carcinogenic and immune-suppressing effect. Female 7-weeks-old SD rats were fed 80 mg/kg DMBA in 0.5 ml corn oil once by gavage; 12 weeks later, all of them had mammary cancer [11]. The pathophysiology of DMBA-induced ovarian cancer in rats resembles in many ways that of human ovarian cancer [54]. Dimethylhydrazine is another indirect carcinogen (DMH undergoes metabolic activation in the liver, with its intermediates entering the bile and being transported to the intestines. Metabolically active DMH then modifies the DNA, histones, and DNA-binding proteins of target cells) and initiates colorectal cancer dose-dependently if administered subcutaneously or intraperitoneally [55]. This model is widely used to evaluate histological features and biochemical processes of tumor development since it is morphologically similar to human colorectal cancer [56], the incidence of which is also gradually growing [57].

Other substances, such as alkaloids and aromatic amines, are powerful human carcinogens and also initiate cancers in various organs of transgenic rats. Aflatoxin B1 affects the liver, o-toluidine acts on the kidneys, and the aristolochic acid on the bladder [51].

Some anticancer drugs also possess carcinogenic properties. Thus, tamoxifen has been for many years widely used for adjuvant therapy for breast cancer. However, it causes endometrial cancer in women. Given that tamoxifen has estrogen-like effects on certain tissues, such as the human uterus, there is concern that it could promote endometrial cancers in women undergoing long-term tamoxifen therapy. Observations from some randomized trials of adjuvant tamoxifen therapy suggest a small but real increased risk of endometrial cancer in these women. Additionally, tamoxifen has been shown to induce hepatic cancers in rats (though not in humans) via alpha-hydroxitamoxifen-induced mutagenesis, further activated by sulfotransferase, leading to the formation of DNA adducts. *In vivo* studies of transgenic Big Blue and gpt delta rats showed that tamoxifen induces mutations of the lacI, cII, gpt and Spi genes in the liver [51, 58].

Rat models of cancer induced by physical agents. Ionizing radiation is one of the few well-characterized etiologic factors of cancer. The study of carcinogenesis in animal model after irradiation has many advantages in providing information about radiation-associated human cancer risk. Thus, they are the only measure for estimating radiation-associated cancer risk when epidemiologic evidence is lacking (animal experiments are essential to estimate the carcinogenic effect of neutron radiation and nuclear fuel materials); since randomized human studies are impossible, animal experiments provide complementary information where epidemiologic studies suffer from biases and confounding factors; animal models are usually well defined with respect to genetic and environmental conditions, which is important for the epidemiological observations interpretation [59, 60].

Animal experiments can be performed under standardized and controlled conditions and are also very important to assess the quantitative cancer risk of exposure to radiations of different quality and to obtain information on the dose-response relationships for carcinogenesis [61] because often radiation-

induced acute injury is the main reason for the suspension of radiotherapy and unsuccessful treatment of cancer [62].

Rodent models provide a variety of cancer types and can be used for numerous types of studies, including assays of putative oncogenic agents, genetic alterations, pathogenesis and specific treatment. Rats and mice have been widely used to estimate the radiation risk and tumorigenic effects of radiation, extrapolating the findings to humans. However, the risk of inducing neoplastic late effects after total-body irradiation with relatively high doses has been demonstrated for larger animals, such as monkeys and dogs [61].

It is known today that human tissues with a high sensitivity for cancer induction include the bone marrow, the lung, the thyroid and the breast in women [61].

Studies have shown radiation-induced genotoxicity and apoptosis in rat bone marrow cells, which can also be observed in patients receiving radiation therapy [63]. In rats, models of partial body irradiation sparing part of one hind leg (leg-out PBI) have been developed to simultaneously expose multiple organs to high doses of ionizing radiation without inducing hematological [64, 65]. These models can be used in drug research to reduce the side effects caused by radiotherapy in cancer patients or the delayed effects of radiological attacks or nuclear accidents.

A radiation-induced lung damage rat model was created by high focal radiation (40, 60, or 90 Gy) administered to a 5-mm diameter area in the right lung in Wistar rats. All rats that received the 90 Gy radiation treatment developed acute radiation-induced pneumonitis and late-occurring radiation-induced lung fibrosis [66].

Currently, among radiation-associated cancers, thyroid cancer is a focus of special attention in populations exposed to ionizing radiation. Particularly, children and adolescent atomic (A)-bomb survivors and residents of the contaminated areas after the Chornobyl power plant accident have higher rates of thyroid cancer than the general population. Thyroid carcinogenesis was induced in seven-week-old male Wistar Kyoto rats (WKY/Izm) with body weight ranging from 173 to 211 g whose were locally irradiated at their anterior necks with 0.1, 1 or 4 Gy X-rays at a dose rate of 0.5531 Gy/min. Thyroid cancers were histologically confirmed in the irradiated rat and increased in a time- and dose-dependent manner and reached the highest rate of 33% at 16 months after irradiation with 4 Gy [67]. This model was used for detecting radiation-specific molecules expressed during radiation-induced rat thyroid carcinogenesis. Age-dependent effect on radiation-induced thyroid cancer was evaluated in rats irradiated with 8 Gy X-rays in 4-week-old, 4-month-old and 7-month-old, 18 months after irradiation [68]. Exposure to carcinogens early in life was assessed using Eker rats as a kidney tumor model. F1 hybrids of male Eker (Tsc2 mutant) and female F344 rats were whole-body irradiated with 0.5 or 2 Gy of 137Cs gamma irradiation at a dose rate of 0.7 on gestation days 15 and 19, and on postnatal days 5, 20, and 49, which led to the development of kidneys adenoma and adenocarcinoma in animals irradiated in perinatal age [69].

Rodent models play an important role in understanding the natural history, mechanism, and modifying factors of breast cancer development because ionizing radiation is a potent mammary gland carcinogen. Female ACI rats at 8 weeks of age were exposed to a single dose of 3 Gy of X-rays one week later (90 kVp, 5 mA). This model of carcinogenesis is utilized for analysis of the molecular and epigenetic changes induced in mammary gland tissue upon exposure to ionizing radiation (a loss of genomic cytosine methylation in the exposed mammary tissue, increased levels of phosphorylated extracellular signal-regulated kinase (p-ERK1/2), phosphorylated AKT kinase (p-AKT), cyclin D1 and proliferating cells nuclear antigen (PCNA) proteins, significant induction of apoptosis in the exposed tissue 6 hours after irradiation) [69].

A malignant neoplasm of bone tissue – osteogenic sarcoma was induced in Sprague-Dawley inbred strain of the rats using seven serial injections of 32P-orthophosphate. This tumor is a model for the study of hormone responsiveness for tumor growth and differentiation [70, 71].

Radiation-induced skin cancer has been studied in both mice and rats, although the majority of such studies have focused on the rat model because the rat is significantly more sensitive to skin tumor induction. Multiple repeated radiation exposures are generally required for tumors to develop in mouse skin, while a single high dose (>10 Gy) is capable of inducing tumors in rat skin. Rats are also often used to generate animal models of radiation-induced brain injury. Neuroinflammation, epigenetic and histopathological changes, cell apoptosis, impaired cell

proliferation and differentiation, and other radiation-triggered events can then be observed at the molecular, cellular, and tissue levels [72].

Syngeneic transplant cancer rat models

Syngeneic transplants of tumor cells from one animal to another of the same line can also be widely used for experimental research on malignant growth (Table 2).

Among such tumors, the Walker-256 mammary carcinosarcoma is one of the most widely used to study cancer since it is specific to rats, grows rapidly, and is highly aggressive, invasive, and highly capable of metastasizing. Its development causes anorexia-cachexia syndrome, which includes weight loss, intense degradation of fat and muscle tissues, immunosuppression, anemia, and other physiological changes that can cause the animal to die within a few weeks [73-76].

The carcinosarcoma was derived from a spontaneous mammary adenocarcinoma of an outbred rat [77]. The Walker-256 strain's cell population is currently known to include two subpopulations. One is estrogen-independent, sensitive to dibutyryl-cAMP, and tumorigenic only for females and neutered males. The other is insensitive to hormones and dibutyryl-cAMP and transplanted even for sexually mature males. Estrogen receptor content on the Walker-256 cells does not exceed its average content in the cells of non-reproductive organs [78]. Given the morphological features and progression of the disease, the tumor is seen as an experimental model for human breast cancer which allows recreating the initiation, promotion and progression in a brief period of 12-16 days [79].

Another widely used rat model is Guerin's adenocarcinoma, isolated from an outbred white rat's uterus by Guerin P. and Guerin M. in 1934. Histologically, it is a low-differentiated cancer that rarely forms glandular-like structures. Tumor grafting is 50 to 90%, spontaneous resorption is not observed. The animals live on average 30-40 days after the transplant. The cancer is believed to affect rats regardless of strain or sex. However, most studies use female rats (often sexually immature) since the tumor grows in them more often and faster than in males [80].

Guerin's carcinoma is used to model endometrial cancer; it is a classical rat model of a low-differentiated, non-metastasizing, solid adenocarcinoma of the uterus body, rapidly progressing and highly fatal [81].

Another transplanted solid tumor is sarcoma-45, obtained in Professor L. O. Zilber's laboratory in 1947 by transplanting a fibrosarcoma originating from a rat's hypodermis after an injection of 9,10-dimethyl-1,2-benzanthracene. Morphologically, it develops from connective tissue cells. Under standard subcutaneous transplanting to rats of any gender, the tumor grows as a solitary rounded node and does not metastasize. In outbred rats, it is grafted successfully in almost 100 % of cases; the average lifespan of the tumor-bearing animals is 25-35 days [82].

An interesting rat model of liver cancer is Zajdela's ascitic hepatoma. It was induced at the end of the 1950s by 4-dimethyl-aminoazobenzene;

Table 2. Syngeneic transplantable models of cancer in rats

Type of tumor	Origin	Experimental model
Walker's carcinosarcoma (Walker-256)	Spontaneous tumor of mammary in rat	Breast cancer
Guerin's adenocarcinoma	Spontaneous tumor of corpus uteri in rat	Endometrial cancer
Sarcoma-45	Fibrosarcoma, induced by 9-10-dimethyl-1,2-benzathracene	Malignant diseases of connective tissue
Zajdela's ascitic hepatoma	Spontaneous ascites form	Liver cancer
Pliss's lymphosarcoma	Induced by 3-3-dichlorobenzidine	Lymphosarcoma (malignant neoplasm that develops from lymphoid tissue)
Myelomonocytic leukemia L5222	Induced by ethyl nitrosurea	Leukemia

the ascitic form arose spontaneously as cancer progressed: "islands" of cells pass into the abdominal cavity creating the ascitic form which is a stage of tumor cells dissemination through the body, e.g., of the metastatic cascade. A specific feature of this tumor is its high frequency of metastasizing into the paratracheal (cervical) lymph nodes: as early as five days after rats are intraperitoneally injected with hepatoma ascites, metastases are observed in the lymph nodes in 90-100% of cases. The high frequency of ascite accumulation and the possibility of obtaininig large amounts of cancer cells without stromal elements make it a promising model for studying mechanisms of tumor progression and metastasis [83].

The C6 glioma cell line is the most similar to human gliomas by its genetic profile, invasion character and spread, and is one of the most widely used transplantable cell lines in rats [84]. Another strain is the orthotopic rat glioblastoma 101.8; histobiologically, it resembles malignant human gliomas and is an anaplastic glioma in which the astrocyte glia, oligodendroglia and ependyma simultaneously become malign [85]. The tumor rapidly proliferates and grows invasively. The model's reliability and reproducibility have been proved [60]. One should keep in mind that the glioma is transplanted by opening the rat's skull and inoculating the cells intracranially [85-88], which requires special expertise, equipment, an aseptic regime, and special conditions during the surgery followed by post-operational observation and care.

Pliss's lymphosarcoma is a model for lymphoid tissue cancer. It was developed in 1958 by subcutaneous transplantation of a tumor obtained in a rat that had been administered with 3,3-dichlorobenzidine. The tumor grows rapidly and aggressively, tends to invade the surrounding tissues, penetrates the retroperitoneal space, metastasizes hematogenously, and becomes necrotic; it has decreased sensitivity to cytostatics.

Spontaneous leukemias in rats are rare. However, there are many models induced by chemicals (methylcholanthrene, ethylnitrosourea, dimethyl benzanthracene) and ionizing radiation. Acute myelomonocytic leukemia 5222 that developed 326 days after a three-month-old female BD IX rat was given a single intravenous injection of ethylnitrosourea was described in 1967. This leukemia model is transplanted to BD IX rats and used in preclinical studies to test various preparations' therapeutic efficiency. The BN acute myelocytic leukemia (BNML) modeled in Brown Norway rats can also be used in the preclinical efficiency tests of the chemo- and radiation therapy. Another model was created in a female BN rat using 9,10-dimethyl-1,2-benzanthracene; it is similar to human acute myeloid leukosis. It was used to study minimal residual disease (a condition in which so few leukemia cells remain in the marrow that they are no longer identifiable by microscopy). In particular, it was used to study the kinetics, development of drug resistance, disease progression during remission, and the efficiency of cytostatics, radiation, and other means of eliminating residual leukemia cells [89].

Xenograft transplant cancer rat models

Xenograft models are created by transplanting human cells or tissues to animal recipients that almost always have immune deficiency to avoid rejection. The xenografts preserve the primary malignant tumor's histological, immunological, and biological features, including the gene expression profile. Such models have big advantages for screening, evaluation of safety and efficiency of new drugs, biomarker development and evaluation of other therapeutic effects. Most human tumors can be grafted this way; the difference in the cancer nodes' formation periods is not large, and the tumors develop equally fast. However, such models have limitations because of immune response and tumor microenvironment or some other, more nuanced species-specific differences. Besides that, the immunodeficient recipient requires special aseptic conditions, which is expensive [10]. Lines of tumor cells can also be grafted; this is a simpler and easier approach, yet just like *in vitro*, such models cannot fully reflect every patient's unique characteristics.

There are numerous oncological studies using nude immunodeficient mice that lack a thymus (and thus are not protected by the T-cells' immune defense) and mice with severe combined immunodeficiency disease (SCID). SCID mice's B- and T-cells do not fully develop because of a point mutation in the Prkdc gene (DNA-activated protein kinase catalytic subunit). There are also NOD/SCID mice that have significant defects of the innate and adaptive immunity (low activity of natural killer cells, loss of function of the T- and B-cells), a stable and widely used model for grafting human hematopoietic stem cells (HSC) and solid tumors. From the NOD/SCID strain, there were derived the NSG and NOG mice

that have mutations in the interleukin-2 gene (IL-2Ry); currently, they have the most pronounced immunodeficiency and are used to study antitumor effects of the CAR-T therapy and immune checkpoint inhibitors [10].

Compared to mice, rats are bigger and grafting various tissues and organs is easier. Nude rats without a thymus resemble nude mice by the absence of functionally mature T-cells and are used to study tumor growth mechanisms. However, athymic rats of some lines strongly reject allograft lymphocytes or bone marrow cells because of alloreactive natural killer cells. Thus, nude rats (and mice) models are not suitable for lymphoma and leukemia transplants. In such rats, dendritic cells are also present; they take part in innate immune reactions if mature T-cells are absent. The rats eventually develop T-like cells expressing CD3 and T-cell receptors (TCR) [90].

F344/Jcl rats were obtained by CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats). They have severe combined immunodeficiency with a knockout gene of the γ-chain of the interleukin-2 receptor (Il2rg) single or together with recombination activating gene 2 (Rag2). The immunodeficiency phenotype was pre-confirmed by severe thymic hypoplasia of both the sKO rats (Il2rg knockouts) and the dKO rats (Il2rg/Rag2 double knockouts) [91]. Such an experimental model can be used in different oncology and regenerative medicine branches.

Genetically engineered/transgenic models

Over the last years, significant amounts of data have been obtained, proving that mutations are one of the main causes of tumor development. Gene therapy for cancer patients is promising, yet its efficiency is frequently limited due to eventual drug resistance. Thus, understanding the mechanisms of the causal mutations underlying malignization is of crucial importance. When tumors are induced in transgenic animals, primary mechanisms of tumor development and immune response can be studied. However, the limited availability of experimental animals creates a challenge for conducting rapid, high-throughput research [10]. Currently, over 30 lines of various transgenic rats have been used in neuroscience, endocrinology and cancer research. Transgenic rats are highly sensitive to carcinogens, have a high frequency of spontaneous growths and thus are good models for screening new therapeutic approaches and to trace carcinogenesis mechanisms and the carcinogenic activity of various substances [51]. Commonly, in the knockout cancer model, the tumor-suppressor gene is inactivated or replaced via site-specific homological recombination between the cell DNA and the target sequence in the vector. Transgenic mice have been used to study tumorigenesis since the middle 1980s [10], and the first knockout rat was obtained in 2009 through the ZFN technique (zinc-finger nucleases) [92, 93]. In 2010, another method was proposed based on embryonic stem cells [94]. Recently, knockout rats have been generated using effector nuclease systems such as TALENs (transcription activator-like effector nucleases) and CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/Cas-based RNA-guided DNA endonucleases). In addition to the ease of generating synthetic gRNAs, a key advantage of the CRISPR/Cas system is the ability to target multiple genes simultaneously using multiple gRNAs. Thus, the CRISPR/Cas system offers sophisticated and flexible gene-targeting tools for creating suitable animal models of human diseases [51, 95].

Table 3. Transgenic rats in cancer research

Rat strains	Susceptibility to disease
Hras128	Breast cancer, esophagus cancer, bladder cancer. Highly susceptible to chemical-induced carcinogenesis.
TRAP	Prostate cancer. Males demonstrate atypical epithelial cell proliferation in the prostate from 4 weeks of age and develop prostate carcinomas at 100 % incidence before they are 15 weeks old.
Cx32ΔTg	Liver cancer. The gap junctional intercellular communications were disrupted in the liver and highly susceptible to chemical-induced hepatocarcinogenesis.
Hras250 Kras327	Pancreatic cancer. Targeted activation of a human oncogenic-ras transgene in rat pancreas induces pancreatic ductal adenocarcinomas

Most commonly, studies employ the following transgenic rats (Table 3).

Hras128 rats have the human c-Ha-*ras* proto-oncogene. They are sensitive to mammary carcinogens such as the N-nitroso-N-methylurea, DMBA, 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (females) and esophagus and bladder tumors (males) induced by n-nitrosomethylbenzylamine [96].

TRAP are probasin-SV40 T antigen transgenic rats (T antigen under probasin control). Probasin is a gene specific to the prostate used as a marker for prostate differentiation and investigation of androgens' action. Thus, these rats are sensitive to prostate carcinoma that develops in 100% of cases in all lobes of the gland before the animals are 15 weeks old. The TRAP model has been employed in studies of tumor recurrences independent of androgens and in chemoprevention research [51].

Cx32ΔTg (**Connexin 32** dominant-negative transgenic) rats express the dominant mutant Connexin 32 which is a cell-cell contact protein and the main protein of the liver's gap junctions. In the transgenic animals, normal connexins' localization is disrupted, and gap junction capacity is noticeably decreased. Studying chemical carcinogenesis (using dimethylnitrosamin) in Cx32ΔTg rats showed that the disruption of cell-cell communication in the gap junctions contributes to the appearance and progression of liver cancer [97].

Hras250 and Kras327 – transgenic rats carrying a mutated H- or K-ras gene controlled by Cre/loxP activation express the human-activated RAS oncogene which is regulated by the Cre/lox system (Cre-Lox recombination is a site-specific recombinase technique for deletion, insertion, translocation and inversion in some DNA sites. The Cre/loxP system was isolated from the bacteriophage P1, is a member of the tyrosine kinases, and is comprised of two short DNA sequences: LoxP (locus of crossing-over) and the recombinase gene Cre). The rats with the H- or K-ras mutated gene controlled by Cre/loxP activation develop ductal adenocarcinoma of the pancreas histopathologically similar to human cancer and so are used to identify the early biomarkers [98].

Discussion

Cancer rat models, whether established or still in progress, are of great importance for detailed studies of the disease, understanding of its progression specifics, and molecular mechanisms of initiation, progression, and metastasizing. They also enable the improvement and development of novel treatments. Clinical studies alone cannot solve these problems. Finding the most appropriate model allows us to select exactly the one that would most fit the task at hand, the studied clinical type, and the duration constraints, providing a way for further productive study of the disease.

The advantages and disadvantages of some cancer rat models are presented in Table 4.

Animal cancer models play a crucial role in cancer research, providing insights into the mechanisms of disease development and progression, as well as aiding in the development of new therapeutic drugs. These models serve as a vital bridge between basic and clinical research [99].

Experimental models with clinically predictive properties switch candidate drugs to phase 2 clinical trials with confidence from the pre-clinical phase. Therefore, most cancer animal model research focuses on the pre-clinical evaluation of the efficacy of biological and chemical agents. However, more than 90% of drugs that successfully pass pre-clinical studies on such models as monolayer cell lines culture or syngeneic and xenograft models are ineffective in humans [100].

However, replicating the complex biological processes of the human body in animal models remains challenging. Despite extensive efforts to create new and improved pre-clinical animal models, significant physiological and pathological differences persist. These differences can lead to inaccurate results when translating experimental findings to human biology. Additionally, ethical concerns surround animal experimentation. The 3R principle – Reduction, Refinement, and Replacement – was first proposed by Russell W. and Burch R. in 1959 [101] and is now an internationally recognized standard. This principle has enabled scientific advancements in alternative methods and significantly reduced the number of laboratory animals used. Together, these improvements ensure the humane treatment of research subjects, mitigating harm while supporting scientifically sound results [99].

Rat models, as well as other animal cancer models, are limited in their ability to mimic the extremely complex process of human carcinogenesis, physiology and progression. Therefore, the safety and efficacy identified in animal studies are generally not translated to human trials. Three essential criteria are suggested to be evaluated to

Table 4. Advantages and limitations of some rat cancer models

Model	Advantage	Disadvantage
Autochthonous (spontaneous and carcinogen-induced)	It allows us to study the role of signaling pathways, genes, marker proteins, stem cells in tumor formation; the mechanism of action of carcinogens, their threshold and toxic doses, the influence of the microenvironment. Different carcinogens cause the formation of tumors in certain organs, which makes it possible to choose a model of carcinogenesis of almost any localization. Physical carcinogens – the possibility of assessment of the nature of dose-response relationships, determination of the relative biological effectiveness of radiations of different qualities, and effects of fractionation or protraction of the dose on tumor development. Experiments can be performed under standardized and controlled conditions. Putative oncogenic agents, genetic alterations, pathogenesis and specific treatment. Chemical carcinogens – different chemical agents induce the development of tumors in relevant organs (organ specificity). It allows us to study the action mechanisms of threshold and toxic doses of carcinogens.	Low, variable growth rate, long latent period, requiring long-term observation. As a rule, they do not metastasize. A large number of animals is necessary for statistically significant results. Chemically-induced tumors have a long latent period and rarely metastasize.
Genetically engineered (GEM)	Tumors have histological similarities with human tumors. They allow the study of signaling pathways, oncogenes, and suppressor genes. Allow to investigate a specific molecular target for anticancer drugs. Preventive therapy is allowed.	They do not possess the entire molecular genetic spectrum of human tumors. Large, individual fluctuations in the duration of the latent period, tumor growth, and metastasis. Low frequency of metastasis. The genetic background of an animal line affects the expression of target genes. The complexity of processing the received data. Low correlation of anticancer drug test results with clinical practice. Long time of research.

achieve the best result. Face validity is the first criteria that describe the biological similarity between the human disease and the animal model; the second is target validity which the target agents should have a similar function in the model as in the clinical aspect; and the third is predictive validity which demonstrates that clinically effective therapeutic agents show a similar effect in the disease model [102]. For the successful application of cancer models, it is also suggested not to reproduce the human disease with

Table 4. (Continuation)

Syngeneic/ transplanted	Cells are transplanted into immunocompetent animals, contributing to the tumor's natural development and microenvironment. It allows us to study the process of metastasis. Testing of targeted drugs, including immunotherapeutic ones is possible. Orthotopic transplantation contributes to the fastest growth of the tumor and its metastasis; the microenvironment of the tumor corresponds to that in the human body; response to anticancer drugs is highly correlated with the effectiveness of therapy in clinical practice. Heterotypic x-ray imaging allows to assess tumor growth and the effect of therapy without the use of X-ray imaging methods. Metastatic transplantation makes it possible to assess the mechanisms of spontaneous metastasis, the localization of metastases, their number, and the antitumor effect of drugs.	Rodent tumors do not express the genes and marker proteins of human tumors, which limits the study of anticancer drugs. The short lifespan of the animals makes it difficult to assess the long-term results of the treatment. Orthotopic transplantation requires a surgical operation. Subcutaneous transplantation requires a large number of cells for tumor engraftment. Metastatic transplantation excludes the study of the early stages of carcinogenesis; primary tumors and metastases can show different sensitivity to drugs.
Patient-derived transplanted xenograft	Transplantation of cell lines of human tumors makes it possible to study the mechanisms of carcinogenesis, mutations, oncogenes, suppressor genes, biomarkers, and the effect of drugs. Tumor cells obtained from patients preserve the genetic and biological characteristics of the patient's tumor, which allows for the development of individual therapy protocols.	Transplantation is performed in immunodeficient animals, which requires sterile conditions. This makes it impossible to study the effect of immunomodulators. A large number of cell passages increases the likelihood of their geno- and phenotypic variability compared to the primary tumor, which requires constant molecular genetic control. The latent period of tumor development during PDX-transplantation is 2-8 weeks, which slows down the results of drug sensitivity assessment for a specific patient; it is impossible to assess the long-term results of treatment.

all its complexities in the animal. Instead, the model evaluates particular aspects of the disease. When using a model, it is crucial to ensure that the chosen model meets the purpose of the investigation [103]. Thus, autochthonous models enable the study of carcinogen action mechanisms, as well as the roles of signaling pathways, genes, and marker proteins. In contrast, transplanted models are instrumental in testing targeted drugs, including immunotherapeutics. The co-clinical quantitative imaging projects of the Animal Models and Co-clinical Trials Working Group network, organized by the National Cancer Institute, aim to attempt to use human-equivalent doses of therapeutic drugs on the orthotopic tumor models or tumors grown at cancer type-specific sites of metastases, immunocompetent hosts for murine models, and gender-appropriate murine hosts [104].

In recent years, some aspects of cancer models have been modified to improve the translational value. One of these approaches was to create humanized animal models, which mimic the human immune system (HIS) and are mainly used to create patient-derived xenograft models for cancer studies [105]. The HIS-humanized rat model has a relatively long lifespan (~3.5 years), facilitating extended research and offering physiological similarities to humans, such as heart rate and drug metabolism, providing a closer mimicry of human biology [106]. Patient-derived xenografts and genetically engineered models are considered to be the models that best represent human disease and have high translational value [104]. PDX evaluation showed significant heterogeneity and faithfully recapitulated the characteristics of their parental tumors on the microscopic, genetic and functional levels and in most cases, there was a significant correlation between the response to chemotherapy in PDX models and patients [103].

In the evaluation of cancer, the microenvironment is also of particular importance. Transplant cancer models, especially PDXs, provide the tumor microenvironment's structure, which includes stromal cells composed of tumor endothelial cells and cancer-associated fibroblasts, tumor-associated macrophages that have a significant effect on cancer progression and metastasis. These cells produce extra cellular matrix, a network of proteoglycans such as laminin, collagen, and fibronectin that regulates cellular polarization, intracellular signaling and migration, creating flexible, stable, supportive structures for different tissues and are essential in the distribution of drugs in tumor tissue [107].

Before human clinical trials and the development of anticancer agents, clinical trials using PDX models (PCT) are important for clinical decision-making. PCT is referred to as "phase II type clinical trial-like models". In 2015, Gao et al. designed a high-throughput *in vivo* drug screening method, "1×1×1", which intended one animal per model per treatment using a large number of PDX models (it was established ~1.000 patient-derived tumor xenograft models with a diverse set of driver mutations, with these PDXs, was performed in vivo compound screens using a 1×1×1 experimental design (PDX clinical trial or PCT) to assess the population responses to 62 treatments across six indications and demonstrated both the reproducibility and the clinical translatability of this approach by identifying associations between a genotype and drug response, and established mechanisms of resistance). However, two or three animals per model per treatment (2×1×1 or 3×1×1) PDX clinical trials because of more representative of generalizable drug response have recently become more common [108].

Conclusions. The provided information will promote the development of research in the field of oncology and will contribute to: a balanced approach to choosing an adequate experimental animal (especially rat) cancer model; gaining new data regarding the mechanisms of cancer development; predicting the course, clinical manifestations, and severity of the oncological process; optimizing existing, introducing/proposing new methods for diagnosis and treatment of malignant diseases. Induced cancer rat models allow us to study the role of the mechanism of action of carcinogens, their threshold and toxic doses and the influence of the microenvironment. GEMs allow studying signaling pathways, oncogenes, and tumor suppressor genes. Transplanted tumor rat models give an opportunity to observe the tumor's development and microenvironment, metastasis and investigate anticancer drugs, including immunotherapeutic. PDX and GEM are considered to be the models that best represent human disease and have high translational value.

The article contains no results obtained by any of the authors in a study involving humans or animals.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ЕКСПЕРИМЕНТАЛЬНІ МОДЕЛІ КАНЦЕРОГЕНЕЗУ НА ЩУРАХ

Ю. Д. Вінничук✉*, О. М. Платонов, О. О. Грищук, С. В. Комісаренко*

Інститут біохімії ім. О. В. Палладіна НАН України, Київ;
✉e-mail: vinnichukju@gmail.com

Експериментальні моделі на щурах широко використовуються в онкологічних дослідженнях. Цьому сприяє різноманітність існуючих інбредних ліній тварин і їх відносно низька вар-

тість. Цей огляд має на меті проаналізувати та систематизувати літературу щодо моделей раку у щурів. Публікації були відібрані в PubMed (https://pubmed.ncbi.nlm.nih.gov/) та в національних авторських базах даних, виданих у 2000-2024 роках. Обговорюються переваги, недоліки та перспективи використання цих моделей у вивченні різних аспектів онкологічної патології. Отримана інформація допоможе вченим підібрати адекватну експериментальну модель на щурах для вивчення механізмів розвитку раку та можливості його лікування.

Ключові слова: експериментальні моделі раку, злоякісні пухлини, щури, канцерогенез, *in vivo*, аналіз даних.

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