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Traditional Approaches and Innovative Strategies in Laboratory Animal Models for Cancer Research: A Comprehensive Review

Kanser Araştırmalarında Laboratuvar Hayvanı Modellerinde Geleneksel Yaklaşımlar ve Yenilikçi Stratejiler: Kapsamlı Bir İnceleme

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ABSTRACT

Cancer remains one of the foremost challenges in medical research, necessitating diverse and sophisticated models to understand its complexity and develop effective treatments. This review explores the evolution and utility of experimental cancer models, highlighting their pivotal role in bridging the gap between basic research and clinical application. From the traditional use of xenografts, which provide a direct avenue for studying tumor growth and drug response in a living organism, to the innovative approaches of genetically engineered mouse models (GEMMs) that replicate human cancer's genetic and phenotypic traits, each model offers unique insights into cancer biology. Recent advances have introduced organoid models, offering a three-dimensional perspective that closely mimics the tumor's microenvironment, and computational models, which leverage patient-specific data to predict disease progression and treatment outcomes. These models enhance our understanding of cancer's molecular drivers, facilitate the development of targeted therapies, and underscore the importance of personalized medicine in oncology. Despite the diversity and potential of these experimental models, challenges remain, including the replication of the tumor's complexity and the integration of immune system interactions. Future research is directed toward refining these models, improving their predictive accuracy, and combining their strengths to offer a holistic view of cancer biology and treatment.

Keywords: Cancer experimental research; xenograft; genetically engineered mouse models; organoid model; computational models; personalized medicine; tumor microenvironment.

ÖZ

Kanser, tıbbi araştırmalarda önemli bir zorluk olarak kalmaya devam etmektedir ve karmaşıklığını anlamak ve etkili tedaviler geliştirmek için çeşitli ve karmaşık modellere ihtiyaç duyulmaktadır. Bu derlemenin amacı, deneysel kanser modellerinin evrimini ve faydasını incelemek ve temel araştırma ile klinik uygulama arasındaki boşluğu kapatmada önemli rol oynadıklarını vurgulamaktır. Tümör büyümesini ve ilaç yanıtını bir canlı organizmada çalışmak için doğrudan bir yol sağlayan ksenograftların geleneksel kullanımından, insan kanserinin genetik ve fenotipik özelliklerini kopyalayan genetiği değiştirilmiş fare modellerinin (genetically engineered mouse models, GEMMs) yenilikçi yaklaşımlarına kadar, her model kanser biyolojisine benzersiz bir bakış sunmaktadır. Son dönemdeki ilerlemeler, tümörün mikroçevresini yakından taklit eden üç boyutlu bir perspektif sunan organoid modellerini ve hastalık ilerlemesini ve tedavi sonuçlarını tahmin etmek için hastaya özgü verileri kullanan hesaplama modellerini tanıtmıştır. Bu modeller, kanserin moleküler etkenlerinin anlaşılmasına yardımcı olmakta, hedefe yönelik tedavilerin geliştirilmesini kolaylaştırmakta ve onkolojide kişiselleştirilmiş tıbbın önemini vurgulamaktadır. Bu deneysel modellerin çeşitliliği ve potansiyeline rağmen, tümörün karmaşıklığının kopyalanması ve bağışıklık sistemi etkileşimlerinin entegrasyonu gibi zorluklar devam etmektedir. Gelecekteki araştırmalar, tahmin doğruluklarının artırılmasına ve güçlü yanlarının birleştirilmesine odaklanarak kanser biyolojisi ve tedavisine bütünsel bir bakış açısı sunmak için bu modellerin iyileştirilmesine yöneliktir.

Anahtar kelimeler: Kanser deneysel araştırma; ksenograft; genetiği değiştirilmiş fare modelleri; organoid model; hesaplamalı modeller; kişiselleştirilmiş tıp; tümör mikroçevre.

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INTRODUCTION

In cancer research, in vitro cell cultures are frequently utilized to reveal the biological behaviors of cancer cells (1). However, studies conducted in cell cultures fall short in addressing the impacts of cancer on human metabolism. On the contrary, examining the local or systemic effects of metabolism, the immune system response, angiogenesis, and the effects of drugs to be used in the treatment of cancer, the microenvironment, and other systems can only be accomplished through animal models. The type of animal to be used and the cancer models to be established have a wide spectrum. Since the biology of each animal to be used is different from one another, each subject can also be considered as a control group in its own right. For this reason, the number of animals to be used is kept to a minimum.

Mouse models, which can mimic many of the significant characteristics of human tumors, are generally very suitable models for cancer. In cancers developed in mice, mechanisms similar to those in humans are observed in terms of tumor behaviors such as metastasis development or treatment response (2). Thanks to these small laboratory animal models, the in vivo functions of cancer can be provided based on quantitative data on both normal and tumor tissue. In this way, the natural progression of the disease and the effectiveness of treatment are monitored.

Creating cancer models in laboratory animals has certain advantages and disadvantages. To discuss the general advantages; the costs of frequently used small-sized animals, such as mice or rats, are low and they are easy to maintain. They reproduce and grow quickly, and their life span can be monitored. Manipulations during the experiment are easy. The complete genetic sequence and characterization of the mouse are possible, and manipulation of the genome is relatively easy. The physiology of mice is similar to that of humans, thus enabling studies on drug pharmacodynamics and pharmacokinetics. In contrast, the small size of tumors in these animals can cause limitations in procedures such as imaging methods. Their high metabolic rates can affect the absorption and elimination of drugs and molecules.

The general ethical approach is to conduct experiments in cell environments if possible, and in laboratory animals if not, while working with the minimum possible number of animals in regard to animal rights. Various biodistribution methods make it possible to conduct an experiment with as few animals as possible. Another critical issue is whether the developed model accurately reflects the disease in humans, highlighting the importance of selecting the appropriate model.

Cancer biology varies from organ to organ and individual to individual, making it impossible to address all cancers with a single model. Therefore, different models must be developed for each type of cancer. These models will have various advantages and disadvantages, so it is crucial to select suitable models for confirming the thesis. This review aimed to explore the evolution and utility of experimental cancer models, highlighting their pivotal role in bridging the gap between basic research and clinical application.

XENOGRAFT MODELS

This model involves the transplantation of cancer cells or tissues from humans into experimental animals. According to some researchers, these models are considered a step beyond tissue culture, described as "animal culture." Nude mice are generally used in these models. These mice are immunodeficient animals with defective thymic epithelial cells and a limited number of functional T and B cells. Their lack of fur makes it easy to visualize or measure tumors subcutaneously. Tumor implantation can be done intraperitoneally, subcutaneously, intravenously, intrathecally, into tissue, or into the tissue from which the tumor originates. Xenograft models are simple and frequently used models. Tumor cells are usually transplanted into a region of the origin organ, providing a suitable microenvironment for the growth and development of the tumor cells. Since xenograft models use human-origin tumor cells, they cannot be used to assess the initial stages of the disease. The requirement for immunodeficient animals means that aspects such as the immune system's effect on cancer development and treatment response are overlooked. The creation of immunosuppressed models necessitates the isolation of these animals, thus xenograft models come with disadvantages such as production difficulty and high costs (3). These models are ideal for testing new and personalized cancer therapeutics. These models are divided into two categories based on how the tumor sample from humans is obtained.

Orthotopic Xenograft Models

The impact of the microenvironment on tumor biology has long been a subject of focus. In cancer treatment, researching the effects, toxicities of drugs, or the response of the cancerous organ to treatment necessitates the replication of the microenvironment in animal models. These models are created by the percutaneous implantation of a human cancer tissue sample into the animal. The implantation is done in the organ from which the tumor originates, thus creating the most appropriate microenvironment. This model is quite effective for developing personalized treatments (4). However, there is a high possibility of the human tumor cells being rejected by the host. To eliminate this possibility, animals with suppressed immune systems are used, which means the response of the immune system to the tumor tissue is ignored.

Xenograft Models with Circulating Tumor Cells

In these models, instead of taking a tissue sample from cancer patients, a blood sample is obtained. With the sample, circulating tumor cells or circulating cancer DNA is acquired and transferred to an immunodeficient animal. The formation of these models does not require an invasive procedure to obtain tumor samples, thus reducing the potential for harming the patient. Simple blood sampling allows for samples to be taken from the patient at different times for replication. This way, the evaluation of the tumor in humans at various developmental stages is possible (5).

SYNGENEIC MODELS

These models are created by transferring immunologically compatible cancer cells to immunologically compatible animals. They can be applied among beings of the same species and with the same genetics. The creation of these models is quite challenging, costly, and limited. A general advantage of these models is the low probability of immunological rejection of the injected cells by the experimental animal's immune system due to compatibility. This allows for more detailed studies related to the microenvironment compared to other models.

TRANSGENIC MODELS

In some experimental models, genetic modifications have been made to animals to induce spontaneous neoplastic growth. In these models, genes that initiate neoplasia are transferred into the pronucleus of the animal zygote DNA by microinjection. These genetically modified models allow for the investigation of the effects of genetic abnormalities on cancer development and progression. In transgenic models, the transferred genes can be passed on to the offspring. From the initial stage of the tumor, all development processes can be monitored. These models provide the opportunity to examine the pathogenesis of the disease within a natural stroma and in the presence of a natural immune system. They can be used to assess the effect of hormones on the disease and the response to chemotherapeutic agents.

GENETICALLY MODIFIED MODELS

These models use genetically modified animals designed to mimic many pathophysiological characteristics related to human cancers. With these models, chains of molecular events causing cancer can be examined and replicated as needed. For this purpose, methods such as physically removing a gene from the genome (knock-out), blocking its function with various agents, or physically adding a gene into the genome (knock-in) can be used (6,7).

MODELS INDUCED BY CARCINOGENIC AGENTS

Cancer models in experimental animals can be created using physical, biological, or chemical agents. Chemically induced animal models are those created using environmental factors effective in carcinogenesis in the human population (8).

VARIOUS EXPERIMENTAL CANCER MODELS

Leukemia Models

From 1955 to 1970, many experiments induced leukemia by administering 3-methylcholanthrene intraperitoneally at 20 mg/kg to DBA-2 mice. However, the use of these models has declined over time. One reason is that the leukemia induced in experimental mice by these substances is not phenotypically completely compatible with human leukemia. Another reason is that disease development occurs in only a small number of animals after a long delay when exposed to the carcinogen. Whole-body irradiation with high doses of gamma radiation or X-rays at once, or prolonged low doses, can be used to induce leukemia or lymphoma development in mice (9). Murine leukemia virus (MuLV) is a virus frequently used to induce leukemia (10).

Lung Cancer Models

To create a xenograft lung cancer model in experimental animals, tumor cells in suspension can be inoculated into the right bronchial stump of the right lung under anesthesia. The optimal number of tumor cells to be transplanted ranges between 106-107, and the time required for tumor development varies from 1 to 8 weeks (11). Cell lines such as A549, H1975, HCC4006, and HCC827 can be used to create lung adenocarcinoma. For large cell carcinoma, NCI-H460, and for squamous cell carcinoma, NCI-H226 cell lines are utilized (12).

Thyroid Cancer Models

Studies related to thyroid cancer are frequently conducted using xenograft models, where tumor cells are injected into immunodeficient mice. These models include cell-derived xenograft models, patient-derived xenograft (PDX) models, and genetically modified models (13). In cell-derived thyroid cancer xenograft models, tumor cells developed from cell lines such as 8505C, TPC-1, and FTC133 are transplanted subcutaneously, orthotopically, or metastatically into the animal (13). These models allow for the assessment of cancer cell properties such as invasion, metastasis, or angiogenesis. Instead of subcutaneous injection, delivering 30,000 thyroid cancer cells intravenously or intraventricularly to the mouse can rapidly create metastasis models in bone and lungs (14). However, models created using immunosuppressive mice lack microenvironment effects such as tumor stroma relations and the impact of the immune system on the tumor.

Patient-derived thyroid cancer xenograft models are created by transplanting tissue or cells from human tumors into immunosuppressed mice, creating a stroma-based tumor environment. These models often use NOD/Shi-scid/IL-2Rγnull and NOD.Cg-PrkdcscidIl-2rgtm1Wjl/SzJ mice (15). Models that establish the microenvironment are useful for researching new cancer drugs. Preclinical studies of drugs like Obatoclax, LOXO-292, Sorafenib, Lenvatinib, PLX51107, PD0325901, and Cabozantinib for thyroid cancer treatment have been conducted using these models (13). Genetically modified animal models are increasingly used in thyroid cancer research to investigate the roles of gene mutations, amplifications, deletions, and translocations in tumor etiopathogenesis.

Papillary Thyroid Cancer Models

BRAFV600E mutations are observed in a significant portion of human papillary thyroid cancer (PTC) cases and are indicators of aggressive tumor behavior and poor prognosis. Several mouse models have been created with BRAF activation, with the simplest being transgenic models targeting the BRAFV600E gene using the bovine thyroglobulin (Tg) promoter (16). Using this method, more than 90% of the animals can develop PTC within 12 weeks.

RET-PTC1 tumor models are generated by delivering the RET-PTC1 transgene to the animal using the bovine (Tg) promoter. In these animals, malignant thyroid overgrowth develops from the 18th day of the embryo (17). RET-PTC3 tumor models, like other transgenic models, result in thyroid hypercellularity in 69% of the animals within 3 months, creating solid PTC formations similar to those in humans (18). About 10% of PTCs have RAS mutations, and mouse models have been developed by transgenically activating Ras gene isoforms in the thyroid gland (19).

Follicular Thyroid Cancer Models

Models developed to induce follicular thyroid cancer using single transgenes like Ras-Rap1, PPFP, PTEN knockout, Prkar1a knockout, thyroid hormone receptor β, and phospho-inositide-3-kinase activation have not successfully induced thyroid carcinogenesis. However, DUAL-HIT models combining some of these genes have achieved a higher rate of carcinogenesis. Notably, combining PTEN knockout with the PPFP model has led to the development of an aggressive phenotype of follicular thyroid cancer within 5 months (20).

Medullary Thyroid Cancer Models

In this model, excessive expression of the p25 gene activates Cdk5 kinase, leading to phosphorylation and inactivation of the Rb gene, resulting in the development of medullary thyroid cancer in the animal within 16 weeks (21).

Anaplastic Thyroid Cancer Models

Deletion of the PTEN gene and inactivation of the P53 gene in mice leads to the development of undifferentiated thyroid cancer within 9 months from existing follicular hyperplasia in 75% of the animals (22).

Breast Cancer Models

Chemically Induced Breast Cancer Models

N-methyl-N-nitrosourea (MNU) directly causes DNA alkylation, disrupting DNA synthesis and repair. Intraperitoneal, subcutaneous, or intravenous injections in 4-7 week-old rats can lead to the development of ER+/PR+ tumors (23). Studies have reported obtaining breast cancer with single or double doses of 50-70 mg/kg.

2-amino-1-methyl-6-phenylimidazo [4,5-B] pyridine (PHIP) is a heterocyclic amine containing methanol and dimethyl sulfoxide (DMSO), found especially in fried foods, meat, fish, or cigarettes. It affects the colon, prostate, and breast tissues. Experimentally, breast cancer models can be created by administering 80-100 mg/kg PHIP through gavage four times a week for two weeks (24).

3-methylcholanthrene, a polycyclic aromatic hydrocarbon compound, can induce breast cancer in rats after being given 3-6 times through gavage at 10 mg/kg over 44-52 days (25).

7,12-dimethylbenz(a)anthracene (DMBA) is a polycyclic hydrocarbon, and its single-dose application can lead to breast cancer. DMBA is typically mixed with sesame oil, olive oil, or directly into the stomach. Cancer develops approximately 40 weeks after administering 50 mg/kg DMBA in olive oil through gavage to rats. BALB/c 53-P hemizygote mice can develop breast cancer 3-7 weeks after being given 1 mg/kg DMBA in flaxseed oil for six weeks (26).

Breast Cancer Model with Tumor Cell Xenografts

4T1 breast cancer cells, first isolated by Fred Miller and colleagues, are transplantable cancer cells that can grow in BALB/c hemizygote mice and tissue culture. Breast cancers developed with this model possess highly tumorigenic characteristics and unlike other tumor models, can metastasize from the primary breast tissue to distant organs such as lymph nodes, blood, liver, brain, lungs, and bones, thus resembling human breast cancer in its properties (27).

Radiation-Induced Breast Cancer Model

Breast tissue is sensitive to radiation. Calaf and Hei (28) demonstrated that 30 cGy of radiation can lead to tumor development in thymus-less mice.

Transgenic Breast Cancer Models

Mouse mammary tumor virus (MMTV) is among the primary promoters used in transgenic breast tumors in mice. This virus enters the mammary tissue with lymphocytes, infects the mammary gland epithelial cells, and thus initiates tumorigenesis (24).

Hepatocellular Carcinoma (HCC) Models

Experimental animals often utilize chemically induced models, genetically engineered models, and transplantation models to create HCC.

Chemically Induced HCC Models

Diethylnitrosamine (DEN) is the most commonly used genotoxic chemical in HCC models. Besides the liver, these substances can also induce cancer development in the gastrointestinal system. A single intraperitoneal dose of 5-25 μg/g DEN in 12-15 day-old B6C3F1 mice can induce HCC. In older mice, hepatocarcinogenesis can only be initiated with a co-carcinogen such as 2-AAF, phenobarbital, N-nitrosomorpholine, or carbon tetrachloride (CCL4) (29,30).

Carbon tetrachloride is a potent hepatotoxin that works in two ways: it directly increases oxidative damage in hepatocytes and disrupts cell membrane integrity, leading to inflammation. The inflammation causes Kupffer cells and stellate cells to secrete cytokines and chemokines. Liver damage resulting from these processes continues with tumorigenesis. Typically, liver fibrosis development is observed 4-6 weeks after two weekly intraperitoneal injections of 0.5-2 mL/kg (CCL4). After this process, some animals only show fibrosis and cirrhosis, while a significant portion develops HCC. The combined use of CCL4 and DEN often results in HCC development (30,31).

Thioacetamide (TAA) is well-known for creating liver fibrosis models in rodents. Administering 100-200 mg/kg TAA intraperitoneally three days a week for 3-4 weeks can create this model. Also, adding 200 μg/L TAA to drinking water for 6-18 weeks induces liver fibrosis (32,33).

Diet-Induced HCC Carcinogenesis Model

A cancer model can be created by adding methionine to a diet deficient in choline. Diets deficient in choline and L-amino acids can also induce HCC. A diet deficient in choline and L-amino acids can lead to 100% cancer development in rats and mice within 52 weeks (30).

Alcohol-Induced HCC Carcinogenesis Model

HCC can be induced in 16-week-old experimental mice by administering alcohol for seven weeks followed by DEN injection.

Oncogenic, Transgenic Mice in HCC

Downregulation of glycine N-methyl-transferase (GNMT) is common in human HCC. In mice, GNMT knockout (GNMT-/-) transgenic models can create chronic hepatitis, fatty liver, and HCC models, developing multiple HCC lesions up to 5 mm in size within 16 months. These models are often used to identify biomarkers for early diagnosis of hepatocarcinogenesis (34).

Cholangiocellular Carcinoma (CCA) Models

Chemically-Induced CCA Models

Administering the *O. viverrini* parasite intragastrically to mice and adding 0.0025% dimethylnitrosamine (DMN) to the drinking water after four weeks can induce CCA in 100% of the animals. Additionally, administering DMN to Syrian hamsters with biliary duct ligation can induce CCA in 40% of the animals (35).

Male albino rats given 0.03% TAA in their drinking water for eight months develop cystadenomas, and after 12 months of TAA treatment, 100% of the subjects develop CCA. The significant advantage of TAA animal models is the initiation of carcinogenesis without surgical procedures, though these models are primarily limited to rats (36).

In rats, chronic use of high doses (8 mg/kg) of Furan for 15 months can lead to 98% CCA development (37).

Cholestatic CCA Models

This model is achieved by ligating the left medial bile duct (LMBDL), leading to cholestasis. Following ligation, adding DMN to the treatment can induce CCA development in 40% of the subjects after 40 weeks. In another model, chronic cholestasis is induced by LMBDL following bi-weekly intraperitoneal injections of DEN. One week after LMBDL, mice are given DEN again in corn oil via oral gavage. 28 weeks after these applications, 50% of the subjects develop CCA (35).

Xenotransplant CCA Models

Heterotopic CCA models can be created by injecting human tumor cells or tissue subcutaneously into the flank area of immunodeficient mice. These ectopic CCA models allow for drug studies. However, the main disadvantages of these models are the ectopic location of the tumor ignoring microenvironment factors, and the effects of the immune system due to the use of immunodeficient mice (1).

Gastric Cancer Models

Chemically Induced Gastric Cancer Models

Various chemical carcinogens are utilized to explore the mechanisms of gastric cancer development. Researchers have particularly focused on N-nitroso compounds produced by anaerobic bacteria in the stomach after the intake of nitrates and nitrites, considered significant inducers of human cancer. N-methyl-N-nitro-N-nitrosoguanidine (MNNG) is the first nitrosamine shown by researchers to induce stomach tumors in rats. Subjects exposed to 400 ppm MNNG in drinking water for 50 weeks develop stomach adenocarcinoma in 63% of cases (38).

In a model developed with MNU, BALB/c mice receiving weekly intragastric intubation of 0.5 mg MNU develop squamous cell carcinoma in the fore-stomach, with most subjects dying from this squamous cell carcinoma. However, if the fore-stomach is surgically removed before MNU treatment, well-differentiated stomach adenocarcinoma develops in 100% of the subjects within 40 weeks (39).

Gastric Cancer Model Induced by Helicobacter Infection

Due to the key role of *H. pylori* infection in the etiology and pathogenesis of gastric cancer, researchers have developed gastric cancer models with Helicobacter species. Gastric cancer development can be induced in wild ferrets infected with *H. mustelae* and exposed to a single dose of 100 mg/kg MNNG (39).

Gastric Cancer Model with Gastrin Knockout Mice

Many laboratories have reported that gastrin knockout (GASKO or GAS-/-) mice are susceptible to stomach cancer. Hypergastrinemic mice (INS-GAS) develop corpus cancers, while GAS-/- mice develop antral stomach cancers (40).

Gastric Cancer Model with TFF1 Knockout Mice

In humans and mice, TFF1 (pS2) proteins are normally expressed in the epithelial cells of the stomach mucosa. This protein is abnormally expressed in various diseases and cancers of the gastrointestinal tract. To elucidate the function of TFF1, researchers have created TFF1-/- mice by disrupting the TFF1 gene through homologous recombination. These mice, lacking TFF1 expression, display markedly elongated stomach folds and hyperplastic gastric epithelial development, with 30% developing multifocal intraepithelial or intramucosal carcinomas (41). Additionally, there are many other models used for gastric cancer development.

Colorectal Cancer (CRC) Models

Chemically Induced CRC Models

For the induction of sporadic CRC in animal models, direct inducers like azoxymethane (AOM) and indirect carcinogenesis inducer 1,2-dimethylhydrazine (DMH) are used. DMH is a specific pro-carcinogen agent for the colon, activated in the liver and transported to the intestine with bile. It promotes the production of free radicals causing oxidative damage to the DNA of colon and liver cells. In male Wistar albino rats, the subcutaneous injection of 20 mg/kg DMH once a week for 12 weeks leads to the development of colon adenocarcinomas after an average of 8 months (44). AOM is an active metabolite of DMH, primarily affecting organs like the liver, lungs, and colon, with lesion occurrence proportional to exposure time and administered dose (42).

CRC Model with Enema

This model involves the induction of transient colitis in nude mice using a 3% dextran sulfate sodium (DSS) enema, followed by the transanal transplantation of human colon cancer cells (LS174T), leading to CRC development. Two weeks later, a 95% tumor development rate can be observed in the rectums, although significant metastasis may not be observed (43,44).

Acetic Acid-Induced CRC Model

Irritation of the rectum with a 4% acetic acid solution for two minutes, followed by washing of the distal rectum with 6 ml phosphate-buffered saline, disrupts the epithelial cell layer of the distal rectal mucosa. After these procedures, a CRC cell line (CT-26) or human CRC cell line (HCT-116) can be transanally transplanted to induce CRC (43).

Transanal Low-Dose Electrocoagulation Technique CRC Model

In immunodeficient and nude mice, CRC can be developed by transplanting human (LS-174T and HT-29) and murine (CRL-2638 and CRL-2639) colon cancer cell lines transanally after transanal low-dose mucosal electrocoagulation. This technique results in CRC development in 87.5% of mice (43,45).

Genetically Modified Animals in CRC Models

In the development of CRC, tumor suppressor genes such as APC, DCC, p53, and MCC; oncogenes like K-ras, SRC, and C-myc; DNA repair genes including hMSH2, hMSH6, hMLH1, hPMS1, hPMS2, as well as DC44 and COX-2 genes play roles. Numerous genetically modified animal models have been developed from these genes involved in CRC development (43,46). APCmin animals are genetically modified animals with a mutation in the APC gene. "Min" stands for multiple intestinal neoplasia. Similar to familial adenomatous polyposis cases, APCmin animals develop colorectal adenomas but die within 120 days. Although the autosomal dominant mutation is lethal for APCmin homozygote animals, heterozygote animals develop tumors in the large and small intestines within 60 days (47). p53 gene knockout animals rarely develop colorectal tumors. However, the combination of APCmin and p53 knockout mutations leads to an abnormal increase in crypt numbers compared to APCmin animals. Similarly, administering AOM to APCmin and p53 knockout animals can also lead to CRC development (43,48).

Metastasis Models

In experimental metastasis models, tumor cells are directly applied to the systemic circulation of immunodeficient

animals. Depending on the application site of the vessel, metastasis develops. Injections into the mouse tail vein result in lung or spleen metastasis; application into the portal vein leads to liver metastasis; injection into the carotid results in brain metastasis; direct injection into the tibia or femur causes bone metastasis; intracardiac application leads to bone and bone marrow metastasis. The general advantages of these applications are their rapid development and the elucidation of the biology of metastasis. However, they do not provide information about the early stages and initial phases of metastasis.

Experimental metastasis models are crucial tools for understanding the complex processes involved in the spread of cancer from a primary tumor to distant organs, a hallmark feature of cancer progression. These models are designed to mimic the dissemination of tumor cells through the body, allowing researchers to study the mechanisms underlying metastasis, evaluate the metastatic potential of different cancer cell lines, and test the efficacy of anti-metastatic therapies (49-51).

Injection Models for Studying Metastasis

1. ***Tail Vein Injection:*** This method involves injecting tumor cells into the tail vein of immunodeficient mice, leading to the development of lung and potentially spleen metastases. It simulates the hematogenous spread of cancer cells and is commonly used to study lung metastasis mechanisms.
2. ***Portal Vein Injection:*** By injecting tumor cells into the portal vein, researchers can specifically target liver metastasis. This model is particularly relevant for cancers known to metastasize to the liver, such as CRC.
3. ***Carotid Injection:*** Injection into the carotid artery allows for the modeling of brain metastasis. This approach is used to study cancers that have a propensity to spread to the brain, including lung, breast, and melanoma.
4. ***Direct Bone Injection:*** Tumor cells can be directly injected into the tibia or femur to create models of bone metastasis. This method is crucial for studying bone-tropic cancers, such as prostate and breast cancers, and understanding the bone microenvironment's role in cancer metastasis.
5. ***Intracardiac Injection:*** This technique involves injecting tumor cells into the left ventricle of the heart, leading to widespread dissemination of cancer cells and metastasis to bone and bone marrow, among other sites. It is used to study the metastatic spread to various organs simultaneously.

Advantages of Experimental Metastasis Models

- ***Rapid Development:*** These models allow for the quick establishment of metastases, enabling timely evaluation of therapeutic interventions.
- ***Biological Insight:*** They provide valuable insights into the biological processes of metastasis, including tumor cell intravasation, circulation, extravasation, and colonization of new tissues.

Limitations

- ***Lack of Early Stage Insights:*** While invaluable for studying late-stage metastasis, these models do not adequately represent the initial steps of the metastatic process, such as local invasion and the early interactions between tumor cells and the microenvironment of the primary site.
- ***Artificial Circumstances:*** The direct injection of tumor cells into circulation or specific organs may bypass important natural barriers and interactions that occur in spontaneous metastasis.

PATIENT-DERIVED XENOGRAFT (PDX) MODELS

Xenografts, derived from the Greek term "Xenos" meaning foreign, are sourced from one organism and transplanted into another. These grafts, which include organs, tissues, or living cells, are predominantly implanted in immunocompetent mice for research purposes. Within cancer research, xenografts play a crucial role in addressing fundamental questions by employing animal models that closely mimic tumor progression observed in human patients (52).

Models incorporating primary carcinoma tissues sourced directly from a patient's tumor are established with minimal passage numbers, specifically fewer than ten transfers from human patients, to maintain the integrity of the original tumor characteristics. These include cellular heterogeneity, clinical biomolecular markers, malignant genetic and phenotypic expressions, tumor architecture, and vascular structure (52,53). The rationale behind developing PDX models lies in the anticipation that they will enhance preclinical evaluation, offering predictive insights into the molecular biology of cancer-relevant to human conditions and patient responsiveness to therapy (54). PDX models have proven beneficial for examining cancer metastasis, drug resistance, personalized medicine approaches, and the preclinical discovery and testing of novel anticancer drugs (55).

Primary or metastatic tumors are harvested via surgical or biopsy techniques and conserved as intact tissue structures (54). This extraction method enables the gradual growth of tumor specimens in immune-deficient mice, marking a pivotal shift towards utilizing patient-derived tumor tissue xenograft models in the exploration of anticancer drugs and therapeutic strategies (56).

The most common implantation site in mice is subcutaneous (on the dorsal side), although orthotopic implantation -transplanting into the same organ as the original tumor- serves as a viable alternative for organs like the pancreas, brain, oral cavity, ovary, and breast. Efforts to implant tumors at the renal capsular site have been made to increase engraftment rates, offering the advantage of preserving tumor histology relative to the primary sample across successive xenograft generations and retaining original genetic and phenotypic traits (54,57).

Additionally, experimental metastasis models employ controlled quantities of tumor cells for metastasis induction. These models require a comparatively short duration for metastasis development, allowing for subsequent identification of metastatic sites (58).

The advancement of cancer drug development faces challenges due to the lack of preclinical cancer models that accurately replicate the clinical evaluation of significant new compounds in human patients. These challenges are being addressed through the use of patient-derived tumor xenografts in immunocompetent mice (preclinical models) such as nude mice, severe combined immunodeficiency mice (SCID), nonobese diabetic (NOD)-SCID gamma mice, recombination-activating gene (Rag), and NOD rag gamma mice (52).

An essential aspect of extensive preclinical studies using PDX models is their capability to prioritize potential clinical indications and contribute to the identification of potential drug efficacy biomarkers. In CRC, studies indicate that PDX models with Kirsten rat sarcoma (KRAS)-mutant do not respond to cetuximab, making KRAS wild-type status a well-documented biomarker for this therapy in preclinical research. Similar observations apply to non-small-cell lung cancer. PDX models also serve as adaptable tools for modeling resistance in clinical treatment protocols, notably in ovarian cancer where exposure to cisplatin initiates resistance to the drug in platinum-sensitive models, mirroring clinical outcomes. This model is utilized to explore new therapeutic agents for use in platinum-resistant patients (59). Breast cancer PDX models effectively recapitulate various tumor biology aspects, making them ideal for translational research endeavors (60).

However, PDXs also have limitations, including differing tumor microenvironments and the inability to undergo genetic modifications or incorporate the immune system due to their development in immunodeficient mice. Consequently, they do not fully represent the host immune system's role. Additionally, they are not suitable for testing immunomodulatory approaches in cancer prevention, lack feasibility for high-throughput drug screening, and do not support biobanking due to genetic heterogeneity and epigenomic instability (61).

GENETICALLY ENGINEERED MOUSE MODELS (GEMMs) FOR CANCER RESEARCH

The inception of genetically engineered mouse models (GEMMs) arose from the necessity to bridge the genetic gap between xenografts and the human tumor's genetic makeup. Recent technological advancements have enabled researchers to precisely modify the mouse genome to either conditionally or permanently alter the expression of crucial genes responsible for tumor development. GEMMs serve as pivotal tools in oncology for delineating molecular pathways, allowing for the manipulation of the genome to mimic the loss or gain of function in oncogenes or tumor suppressor genes. This manipulation directly correlates with tumor phenotype manifestations, thereby validating significant genes as therapeutic targets (52,62,63).

GEMMs have been employed for over two decades in cancer research, primarily due to the mouse's genome sharing a 99% similarity with humans. This, coupled with the extensive molecular toolbox available and their small size, makes mice an economical choice for large-scale studies. Transgenic mice models provide an effective platform for preclinical safety assessments and screening, offering insights into gene functions linked to human diseases and potential treatments (52,64).

Various types of GEMMs have been utilized in chemoprevention research. The initial models, or oncomice, facilitated direct evaluation of specific gene functions in tumor genesis. Subsequent generations, such as those with targeted deletions of the Rb1 and Trp53 genes, displayed a spectrum of cancer phenotypes. More sophisticated models include Cre-inducible gene targeting and Tet-regulatable systems, allowing for precise control over gene expression and the modeling of human cancer with high fidelity. These models include gain-of-function tumor virus models and RNAi gene silencing for loss of function, providing a dynamic approach to studying gene expression levels (62,65,66).

The suitability of GEMMs for human disease research and their predictive value for cancer prevention is paramount. For instance, GEMMs of colon cancer are instrumental in examining chemopreventive drugs' effects on tumors originating from genetic mutations. Similarly, GEMMs for mammary cancer have demonstrated the potential of specific drugs to halt the progression to invasive carcinoma. Nutritional interventions targeting different molecular pathways have shown promise in prostate cancer models, aiming to create more predictive models for human preventive measures (63).

Despite their advantages, GEMMs are not without limitations. They often focus on a limited number of genes, not fully representing the complex heterogeneity of human tumors. The development of GEMMs is time-consuming and expensive, with variable and slow tumor evolution compared to human cancers. Critics argue that the relevance of GEMMs to human cancer is unproven, while proponents believe that more appropriately designed experimental conditions could enhance translational research from GEMMs to human cancer. Essential evaluation criteria for GEMMs include pathological assessment, disease progression, tumor microenvironment, molecular pathways, and environmental factors (62,67,68).

Specific mutations in Kras and P53 in lung cancer GEMMs have shed light on the NFκB pathway's role in tumor development, offering potential therapeutic targets. In HCC, GEMMs have highlighted the importance of genetic diversity in understanding tumor subtypes, providing a platform for bench-to-bedside research, especially with systems allowing for the controlled overexpression of genes like MYC, relevant to human carcinomas (69,70).

ORGANOID MODELS IN CANCER RESEARCH

Over the past decade, the advent of organoid technology has transformed the landscape of primary and clinical research in cancer. Organoids, essentially miniaturized versions of human organs and tissues, accurately replicate the functional attributes and architecture of specific organs. Developed from cancer patient-derived tumor cells placed in a tailored extracellular matrix and specific culture media, cancer organoids offer a dynamic model system (71). These organoids enable detailed molecular and cellular studies, supporting the investigation into cancer's origins and paving the way for new cancer stratification methods for both conventional and targeted treatments through early genetic, transcriptomic, and biochemical profiling (52).

Organoids, embedded within a matrix, are cultures of primary epithelial cells proliferating under the influence of Wnt signaling and mitogens. Stem cells from tissues, when embedded into a three-dimensional matrix, form self-sustaining organoid structures (72). Patient-derived organoids (PDOs) not only mirror the primary tumor's structure but also preserve the genetic and expression

profiles, including copy number alterations (CNAs), transcriptional patterns, and mutation profiles. Despite their detailed representation of various cancers such as HCC, breast, pancreatic, and prostate cancers, PDOs typically lack vital components like immune cells, blood vessels, and stromal cells, posing a challenge for their application in cancer immunotherapy (73).

The relationship between cancer and infectious agents, like *S. enterica* in gallbladder cancer or *H. pylori* in gastric cancer, can be elucidated through co-culturing organoids with these pathogens. This approach has highlighted the significant role of chronic *H. pylori* infection in gastric cancer development, with microinjection of *H. pylori* triggering robust inflammatory responses in the gastric epithelium (73,74).

Organoids derived from healthy organs, through genome sequencing of their clonal cultures, facilitate the analysis of organ-specific mutation spectrums and intratumor heterogeneity by developing clonal cultures from different tumor areas. This genetic stability allows for the examination of mutagenic processes over an extended period (63). Comparative studies of lesions from the same individual through organoid cultures help in understanding tumor evolution, with sequencing confirming common origins and shared driver mutations among organoids from primary and metastatic lesions, indicating these mutations preceded metastatic spread (73).

Organoids also serve as an invaluable tool for studying the tumor microenvironment, exploring the supportive niche created by malignant cells and their surrounding environment, which opens avenues for therapeutic targeting. Traditional in vivo models fall short in capturing the intricate paracrine interactions within cancer organoid cultures, necessitating the development of models that simulate these interactions between cancer cells and the tumor microenvironment (75).

Engrafting organoids into murine models establishes organoid xenografts that enable in vivo studies of human cancer biology, offering insights into malignancies like breast and bladder cancer. For instance, orthotopically transplanted pancreatic cancer organoids can recreate a microenvironment closely resembling human pancreatic cancer, overcoming limitations seen in GEMMs of colon cancer, which typically develop tumors in the small intestine. Orthotopic transplantation into the murine cecum offers a more accurate model for colon cancer (76).

Despite their transformative impact on cancer research, organoids have limitations, including the lack of a complete microenvironment, restricting them to epithelial layer studies. The development of non-epithelial organoid cultures and overcoming challenges in drug response, gene expression, and signaling pathways impacted by growth factors are areas needing further exploration (77,78).

COMPUTATIONAL MODELING IN CANCER TREATMENT AND RESEARCH

In the face of challenges such as tumor heterogeneity, disease complexity, and inadequate clinical diagnostics, leveraging the unique genetic constitution, pharmacokinetic properties, and individual characteristics of patients promises to refine therapy personalization for tumor management. Personalized medicine, emphasizing treatments tailored to individual genetic profiles, is poised to redefine future healthcare paradigms. The leap in systems biology, alongside the surge in high-throughput methodologies and the detailed analysis of various -omics, has shifted the research landscape from traditional hypothesis-led investigations to data-centric studies, fostering the advent of precision medicine for complex conditions like cancer (79).

Computational cancer modeling encompasses digital simulations related to cancer therapies and tumor biology (80). These models, applied extensively in cancer diagnosis, monitoring, and growth prediction, utilize 3D imaging for visual representations of tumors or tissues. Despite their reliance on algorithms and diverse software tools, these models face challenges in consistency and replicability, unlike in vitro cancer models (81).

Currently, expansive computational models are under development to decode signal transduction within human cells, employing platforms like PyBioS3 for designing, modeling, and simulating cellular systems. These models, integrating around 50 cancer-associated signaling pathways, draw on data reflecting the impacts of genetic variations and drug mechanisms (82,83).

Personalized prognoses are rendered by customizing models with next-generation sequencing (NGS) derived-omics data, where biological data science furnishes essential resources and effective tools for simulating biological processes. This enables the construction of robust cancer models based on experimental data, disease progression, and therapeutic strategies. Computational and mathematical models elucidate cancer evolution, offering insights into potential biomarkers within signaling pathways and promising therapeutic targets. Cancer signaling network models, grounded in time-lapse experimental data on protein expression and activity, support the validation of drug target effectiveness and simulation forecasts (84).

Enhancing the translation of findings from cancer models to patient care necessitates aligning experimental outcomes with computational model predictions. For instance, assessing drug effects in digital cell or animal models, followed by model adjustments, allows for personalized patient adaptation (83). Computational systems further aid in cancer research and treatment by facilitating image analysis and interpretation, with computerized tomography recently proposed for assessing personalized cancer responses (83).

Advanced computational models promise to refine experimental designs, reducing reliance on animal models, cutting costs, and enhancing the translational relevance of research outcomes. These models offer insights into molecular changes in disease pathways and serve as effective screens for selecting promising candidates, enriching our understanding of disease mechanisms and drug responses (83).

However, current computational models do not capture the full complexity of the biological systems they simulate. A significant barrier to their application in research and clinical settings is the accuracy of their predictions. One approach to overcoming this challenge involves simplifying models through reduction techniques to manage the complexity and improve predictive validity (83,85).

CONCLUSION

In summarizing the extensive landscape of experimental cancer models in laboratory animals, it becomes evident that the field has made significant strides toward understanding and combatting this complex disease. From the utilization of xenograft models, which bridge the gap between in vitro studies and human clinical scenarios, to the sophisticated GEMMs that offer a closer approximation of human cancer genetics and behavior, researchers have a broad arsenal at their disposal for the exploration of cancer biology and the development of therapies.

The advent of organoid cancer modeling and computational cancer models further underscores the rapid evolution of cancer research methodologies, offering more personalized and precise insights into tumor dynamics and treatment responses. Organoids provide a three-dimensional, microenvironment-aware platform that more accurately reflects the cellular complexity and heterogeneity of tumors, enabling targeted therapy testing and the study of cancer-stem cell interactions within a controlled setting. Meanwhile, computational models stand at the forefront of precision medicine, offering predictive insights into cancer progression and treatment outcomes based on individual genetic profiles, thereby paving the way for customized patient care.

As we advance, the integration of these models -each with its unique strengths and limitations- into a cohesive research framework will be paramount. The synergy between traditional in vivo models, innovative organoid cultures, and computational simulations promises to enhance our understanding of cancer's molecular underpinnings, improve the efficacy of therapeutic interventions, and ultimately, lead to more effective, personalized cancer treatments.

The future of cancer research and treatment lies in the continued refinement of these models, increased collaboration between disciplines, and the integration of emerging technologies. By leveraging the distinct advantages of each model and addressing their respective challenges, the scientific community can hope to unravel the complexities of cancer, offering new hope for patients around the world. The journey from bench to bedside, while fraught with challenges, is illuminated by the potential of these experimental models to transform cancer diagnosis, treatment, and prevention, moving us closer to the ultimate goal of curing this multifaceted disease.

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