IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

A Review On The Various Animal Model Use For Gastric Cancer

Pratik Sunil Kapse[1*], Monika Shyamsundar Jangid[2], Prasad Shubhash Wagh[3], Nilesh Dhanaji Zanzane[4], Shital Shankarrao Shendge[5]

Department of Pharmaceutical chemistry

PDEA's Seth Govind Raghunath Sable College of Pharmacy College in Saswad, Pune, Maharashtra, India 412301

ABSTRACT

Animal models have greatly enriched our understanding of the molecular mechanisms of numerous types of cancers. Gastric cancer is one of the most common cancers worldwide, with a poor prognosis and high incidence of drug-resistance. However, most inbred strains of mice have proven resistant to gastric carcinogenesis. To establish useful models which mimic human gastric cancer phenotypes, investigators have utilized animals infected with *Helicobacter* species and treated with carcinogens. In addition, by exploiting genetic engineering, a variety of transgenic and knockout mouse models of gastric cancer have emerged, such as INS-GAS mice and TFF1 knockout mice. Investigators have used the combination of carcinogens and gene alteration to accelerate gastric cancer development, but rarely do mouse models show an aggressive and metastatic gastric cancer phenotype that could be relevant to preclinical studies, which may require more specific targeting of gastric progenitor cells. Here, we review current gastric carcinogenesis mouse models and provide our future perspectives on this field.

Keywords: gastric cancer, mouse model, metaplasia, *Helicobacter Felis*, *Helicobacter pylori*, INS-GAS mice.

Introduction

With the effective control of severe infectious diseases and the extension of human life expectancy, cancer has become one of the major diseases that seriously endanger human health. According to 2015 estimates by the World Health Organization (WHO), cancer is the first or second leading cause of death among people under the age of 70 in 91 of these countries.[1] Under the combined influence of population aging and population growth, the number of new cancer cases each year is expected to rise from 18.1 million in 2018 to 29.4 million in 2040.[2] Due to the late diagnosis of most cancers and inadequate prevention measures, cancer is becoming a heavy burden on residents in low-and middle-income countries. The development and research of new diagnostic methods and innovative treatment tools are essential to reduce the global incidence of cancer. The animal experiment is an important bridge between cell experiment and clinical experiment. Under certain conditions, the occurrence and development of animal diseases are similar to that of human beings, and animals have similar anatomy, physiology and heredity to human beings. Therefore, animal models are often used to study human diseases. In cancer research, the use of animal models can help us understand the genetic basis of cancer and the role of specific genes and gene mutations in the occurrence and development of cancer, which also facilitates the development and testing of antineoplastic drugs.[3]With the continuous development of precision medicine and personalized medicine, researchers are looking for standardized and personalized tumour models that are more similar to human tumours.[4] There are many animal types and construction methods used to construct cancer animal models, and the progress of each animal model in tumour research has its own characteristics, which will be described below (Figure 1).

Mouse Model

The mouse genome is highly homologous to the human genome, which can simulate a series of biological characteristics such as the occurrence, development and metastasis of human cancer cells in vivo,[5] and has the advantages of convenient feeding, low price and easy gene modification. It provides a good tool for cancer research and a valuable platform for drug discovery and verification. At present, there are four commonly used methods to construct mouse cancer model: chemically induced model, cell line-derived xenograft (CDX) model, patient derived xenograft (PDX) model and genetically engineered mouse model (GEMM).[6]The chemical induction model refers to the model of experimental tumour induced by chemical carcinogens, which has the advantage of imitating the occurrence of human cancer from the beginning of the carcinogenic process.[7] But the main disadvantage of this method is that it takes 30–50 weeks to form a tumour after using carcinogens.[8] The cell line-derived xenograft (CDX) model refers to the xenotransplantation model produced by subcutaneous injection of cancer cell lines into immunodeficient mice.[9] The establishment of this model is simple and takes a short time to form a tumour, but after long-term culture in vitro, the biological behaviour and tumour heterogeneity of human tumour cell lines are quite different from those of the original tumour tissue.[10] The patient-derived xenograft (PDX) model is an animal model established by directly implanting tumour tissue samples from tumour patients into mice, which well maintains the characteristics of tumour histopathology and genetics.[11] The genetically

engineered mouse model (GEMM) is to induce tumorigenesis by promoting the expression of oncogenes (such as BRAF V600E in melanoma)[12] or the deletion of tumour suppressor genes (such as PTEN in prostate cancer)[13] by genetic engineering. Compared with the above two transplantation models, GEMM formed an orthotopic tumour in an innate immune maturation micro- environment (natural immune-proficient micro-environment), simulating the process of tumorigenesis.[14] However, due to the species differences of the immune system among mammals, these existing models cannot accurately predict the interaction between the human immune system and tumour. Many antineoplastic drugs with a good therapeutic effect in preclinical animal models cannot play a corresponding role in tumour patients. Therefore, it is necessary to establish an animal model

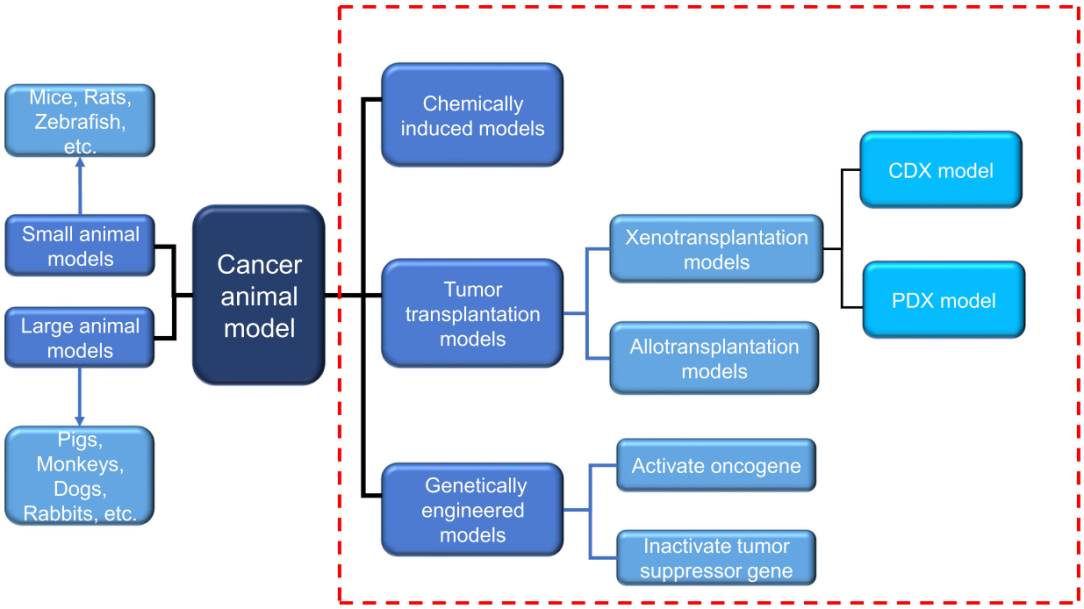

Figure 1 Two commonly used classification methods of cancer animal models. Dashed red box represents the classification according to different modeling methods. Another classification is carried out according to different species. Blue arrows indicate the species of animals included in this classification.

Abbreviations: PDX, patient-derived tumour xenograft; CDX, cell line-derived xenograft. that cannot only replicate the tumour microenvironment, but also have a “humanized” immune system at the same time. The humanized mouse model of the human immune system is a mouse model that reconstructs the human immune system by implanting human hematopoietic cells, lymphocytes or tissues into immunodeficient mice.[15] On this basis, the implantation of human tumour cells or tumour tissue can be used to study tumour growth in the environment of the human immune system and evaluate anti-tumour therapy, especially the effect of immunotherapy and related mechanism. At present, a variety of human tumour cell lines have been successfully established in humanized mice, such as lymphoma, glioma, breast cancer, colorectal cancer, kidney cancer and prostate cancer cell line.[16–19] According to the method of human immune system reconstruction, the humanized mouse models of the immune system are divided into three categories: Hu-BLT (human bone marrow, liver and thymus) model, Hu-HSCs (human hematopoietic stem cell) model and Hu-PBL (human peripheral blood lymphocyte) model (Figure 2).

Hu-BLT Model

The model is established by co-transplanting human embryonic liver and thymus into the renal capsule of immunodeficient mice and injecting liver hematopoietic stem cells from the same embryo into mice.[20] It can reconstruct the human

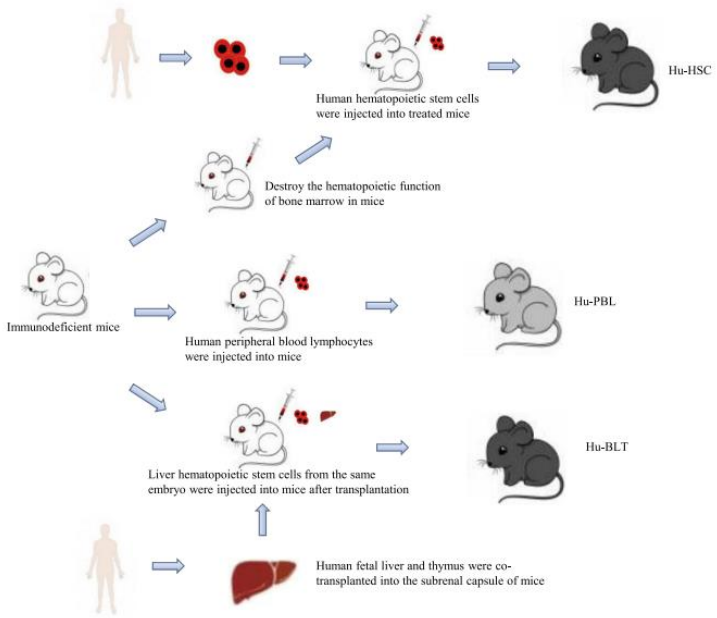

Figure 2 Construction method of humanized mice of human immune system. The construction of humanized mice needs to use immunodeficient mice as a tool. By transplanting different human immune organs or cells into immunodeficient mice, three different humanized mice can be constructed. Among them, the Hu-HSC model also needs to destroy the hematopoietic function of bone marrow in mice.

Abbreviations: Hu-HSC, human hematopoietic stem cell; Hu-PBL, human peripheral blood lymphocyte; Hu-BLT, human bone marrow, liver and thymus.

Zebrafish Leukaemia Model

The similarity between zebrafish and human hematopoietic systems has led to the increasing use of zebrafish to simulate leukaemia.[21] The establishment of the zebrafish leukaemia model plays an important role in understanding the occurrence, development and drug research of human leukaemia. Lange nauetal.[22] injected rag2, encoding a lymphocyte specific promoter into zebrafish to drive the expression of the mouse-derived c-Myc gene. It was found that the fluorescence-labelled leukaemia cells in zebrafish were implanted into the immunodeficient thymus, suggesting that the protooncogene c-Myc is involved in the formation of zebrafish tumour. Gutierrezetal.[13] found that 4-hydroxytamoxifen (4HT) can activate Myc, to induce acute T-lymphoblastic leukaemia in zebrafish by constructing MYC-ER transgenic zebrafish. Corkeryetal.[24] successfully established a leukaemia zebrafish Casper model by implanting K562 and NB-4 human leukaemia cell lines into zebrafish Casper embryos, and conducted targeted inhibitor intervention experiments on this model, which laid a good foundation for tumour research in this whole animal. Since the

construction of the first leukemic zebrafish model in 2003, zebrafish has made a great contribution to the study of leukaemia. Through these studies, we not only have a deeper understanding of the pathogenesis of leukaemia, but also proved a variety of anti-leukaemia drugs, including Nimesulide, Lenaldekar and Perphenazine. [25–27]

K-ras Transgenic Mice

K-ras is one of the most commonly mutated proto-oncogenes in a variety of human cancers [28]. While normally its activity is tightly regulated, somatic mutations occur that render its activity constitutive and thereby oncogenic [29]. Oncogenic activations of K-ras have been found in human gastric cancers, although they are not as common (0–18%) in both intestinal type and diffuse type gastric cancers as reported in other solid tumour, such as pancreatic or colorectal cancer [30]. While oncogenic K-ras leads to increased signalling through a number of proliferative (e.g., MAPK) pathways, it has also been strongly linked to the development of chronic inflammation and cancer [31]. In genetically engineered mouse models of pancreatic cancer based on PDX1-directed K-ras mutations, significant inflammatory and stromal responses correlate with cancer progression [32]. To analyze the function of oncogenic K-ras on the stomach cancer development in mice, the K19-promoter, which targets expression to the progenitor zone of the gastric neck/isthmus [33], was used to direct expression of K-ras-V12 mutant gene. K19-K-ras-V12 transgenic mice (F2 mixed C57BL/6 × DBA background) showed an early upregulation of chemokines such as CXCL1 and recruitment of bone marrow-derived inflammatory cells and fibroblasts, following by the gradual development of parietal cell loss, metaplasia and dysplasia, in a manner that closely resembled H. felis-induced gastric preneoplasia and carcinogenesis [33,34]. Thus, these data suggest that K-ras-dependent chronic inflammation, leading to the recruitment of bone marrow-derived cells that contribute to the stromal microenvironment, can initiate gastric carcinogenesis. In a separate study, investigators introduced a conditional K-ras G12D mutation in the K19-positive lineage in adult mice by crossing K19-CreERT knock-in mice with LoxP-STOP-LoxP-KrasG12D mice. The phenotype of these mice included numerous hyperplasias, metaplasias and adenomas in the stomach as well as in the oral cavity, colon and lungs [35]. Another group bred UBC9-CreERT transgenic mice with LoxP-STOP-LoxP-KrasG12D mice in order to determine the effect in mice of widespread, systemic activation of K-ras [36]. Ubiquitous K-ras activation in mice had rapid and dramatic effects on both the forestomach and glandular stomach, and resulted in severe inflammation, hyperplasia, metaplasia, and activated progenitor cells, although neoplastic changes in other organs were not detected. These latter results suggest that, amongst all the tissues in which K-ras is activated, the stomach appears to be unusually susceptible to the effects of K-ras mutation at early time points, pointing to a crucial role of K-ras activation in initiation of gastric precancerous changes.

Conclusion

Numerous mouse models with various gastric phenotypes are now available for studies of gastric carcinogenesis. These include transgenic mice, knockout mice, Helicobacter infection, and carcinogen (MNU) models. These models have demonstrated that gender, diet, bacterial flora, inflammatory cytokines, T helper immune response, acid secretion, virulence, colonization properties of H. pylori strains, and host genetic background may all have roles in mediating the development of gastric cancer. Reasonable mouse models of gastric cancer are available for studies of early-stage pathogenesis and cancer therapy, which have distinct mechanisms and different tumour phenotypes, with variations in the time course, location, and pathology of the disease. Thus, researchers are able to utilize appropriate mouse models for their studies. Newly suggested research methods, including lineage tracing or genome-wide analysis, should prove valuable for understanding the causes of gastric cancer, and thereby facilitating the discovery of a cure for this disease.

Compliance with ethical standards

Acknowledgments

The authors are thankful to Dr. Rajashri S. Chavhan, PDEA's Seth Govind Raghunath Sable College of Pharmacy College in Saswad, Maharashtra, India 412301 for providing the research facilities and encouragement.

Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424. doi:10.3322/caac.21492
2. Wild CP. The global cancer burden: necessity is the mother of prevention. Nat Rev Cancer. 2019;19(3):123–124. doi:10.1038/ s41568-019-0110-3
3. Schachtschneider KM, Schwind RM, Newson J, et al. The oncopig cancer model: an innovative large animal translational oncology platform. Front Oncol. 2017;7:190. doi:10.3389/ fonc.2017.00190
4. Xu C, Wu S, Schook LB, Schachtschneider KM. Translating human cancer sequences into personalized porcine cancer models. Front Oncol. 2019;9:105. doi:10.3389/fonc.2019.00105
5. Mural RJ, Adams MD, Myers EW, et al. A comparison of whole-genome shotgun-derived mouse chromosome 16 and the human genome. Science. 2002;296(5573):1661–1671. doi:10.1126/science.1069193

6. Mendes N, Dias Carvalho P, Martins F, et al. Animal models to study cancer and its microenvironment. Adv Exp Med Biol. 2020;1219:389–401.
7. Liu Y, Yin T, Feng Y, et al. Mammalian models of chemically induced primary malignancies exploitable for imaging-based preclinical theragnostic research. Quant Imaging Med Surg. 2015;5 (5):708–729. doi:10.3978/j.issn.2223-4292.2015.06.01
8. De Minicis S, Kisseleva T, Francis H, et al. Liver carcinogenesis: rodent models of hepatocarcinoma and cholangiocarcinoma. Dig Liver Dis. 2013;45(6):450–459. doi:10.1016/j.dld.2012.10.008
9. Brennecke P, Arlt MJ, Campanile C, et al. CXCR4 antibody treatment suppresses metastatic spread to the lung of intratibial human osteosarcoma xenografts in mice. Clin Exp Metastasis. 2014;31(3):339–349. doi:10.1007/s10585-013-9632-3
10. Ye F, Chen C, Qin J, Liu J, Zheng C. Genetic profiling reveals an alarming rate of cross-contamination among human cell lines used in China. FASEB J. 2015;29(10):4268–4272. doi:10.1096/fj.14-266718
11. Hidalgo M, Amant F, Biankin AV, et al. Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer Discov. 2014;4(9):998–1013. doi:10.1158/2159-8290.CD-14-0001
12. Hooijkaas AI, Gadiot J, van der Valk M, Mooi WJ, Blank CU. Targeting BRAFV600E in an inducible murine model of melanoma. Am J Pathol. 2012;181(3):785–794. doi:10.1016/j. ajpath.2012.06.002
13. Chen Z, Trotman LC, Shaffer D, et al. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. Nature. 2005;436(7051):725–730. doi:10.1038/nature03918
14. Kersten K, de Visser KE, van Miltenburg MH, Jonkers J. Genetically engineered mouse models in oncology research and cancer medicine. EMBO Mol Med. 2017;9(2):137–153. doi:10.15252/emmm.201606857
15. Shultz LD, Brehm MA, Garcia-Martinez JV, Greiner DL. Humanized mice for immune system investigation: progress, promise and challenges. Nat Rev Immunol. 2012;12(11):786–798. doi:10.1038/nri3311
16. Sanmamed MF, Rodriguez I, Schalper KA, et al. Nivolumab and urelumab enhance antitumor activity of human T lymphocytes engrafted in Rag2-/-IL2Rgammanull immunodeficient mice. Cancer Res. 2015;75(17):3466–3478. doi:10.1158/0008-5472. CAN-14-3510
17. Ashizawa T, Iizuka A, Nonomura C, et al. Antitumor effect of programmed death-1 (PD-1) blockade in humanized the NOG-MHC double knockout mouse. Clin Cancer Res. 2017;23 (1):149–158. doi:10.1158/1078-0432.CCR-16-0122 18.
18. Roth MD, Harui A. Human tumor infiltrating lymphocytes cooperatively regulate prostate tumor growth in a humanized mouse model. J Immunother Cancer. 2015;3:12. doi:10.1186/s40425- 015-0056-2
19. Wang L, Wen W, Yuan J, et al. Immunotherapy for human renal cell carcinoma by adoptive transfer of autologous transforming growth factor beta-insensitive CD8+ T cells. Clin Cancer Res. 2010;16(1):164–173. doi:10.1158/1078-0432.CCR-09-1758
20. Melkus MW, Estes JD, Padgett-Thomas A, et al. Humanized mice mount specific adaptive and innate immune responses to EBV and TSST-1. Nat Med. 2006;12(11):1316–1322. doi:10.1038/nm1431
21. Jing L, Zon LI. Zebrafish as a model for normal and malignant hematopoiesis. Dis Model Mech. 2011;4(4):433–438. doi:10.1242/dmm.006791

22. Langenau DM, Traver D, Ferrando AA, et al. Myc-induced T cell leukemia in transgenic zebrafish. Science. 2003;299 (5608):887–890. doi:10.1126/science.1080280
23. Gutierrez A, Grebliunaite R, Feng H, et al. Pten mediates Myc oncogene dependence in a conditional zebrafish model of T cell acute lymphoblastic leukemia. J Exp Med. 2011;208 (8):1595–1603. doi:10.1084/jem.20101691
24. Corkery DP, Dellaire G, Berman JN. Leukaemia xenotransplantation in zebrafish–chemotherapy response assay in vivo. Br J Haematol. 2011;153(6):786–789. doi:10.1111/j.1365- 2141.2011.08661.x
25. Yeh JR, Munson KM, Elagib KE, Goldfarb AN, Sweetser DA, Peterson RT. Discovering chemical modifiers of oncogene-regulated hematopoietic differentiation. Nat Chem Biol. 2009;5(4):236–243. doi:10.1038/nchembio.147
26. Gutierrez A, Pan L, Groen RW, et al. Phenothiazines induce PP2A-mediated apoptosis in T cell acute lymphoblastic leukemia. J Clin Invest. 2014;124(2):644–655. doi:10.1172/ JCI65093
27. Ridges S, Heaton WL, Joshi D, et al. Zebrafish screen identifies novel compound with selective toxicity against leukemia. Blood. 2012;119(24):5621–5631. doi:10.1182/blood-2011-12-398818
28. Bos, J.L. Ras oncogenes in human cancer: A review. Cancer Res. 1989, 49, 4682–4689.
29. Ellis, C.A.; Clark, G. The importance of being K-Ras. Cell. Signal. 2000, 12, 425–434.
30. Ushijima, T.; Sasako, M. Focus on gastric cancer. Cancer Cell 2004, 5, 121–125.
31. Frame, S.; Balmain, A. Integration of positive and negative growth signals during ras pathway activation in vivo. Curr. Opin. Genet. Dev. 2000, 10, 106–113.
32. Hingorani, S.R.; Petricoin, E.F.; Maitra, A.; Rajapakse, V.; King, C.; Jacobetz, M.A.; Ross, S.; Conrads, T.P.; Veenstra, T.D.; Hitt, B.A.; et al. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell 2003, 4, 437–450.
33. Brembeck, F.H.; Schreiber, F.S.; Deramaudt, T.B.; Craig, L.; Rhoades, B.; Swain, G.; Grippo, P.; Stoffers, D.A.; Silberg, D.G.; Rustgi, A.K. The mutant K-ras oncogene causes pancreatic periductal lymphocytic infiltration and gastric mucous neck cell hyperplasia in transgenic mice. Cancer Res. 2003, 63, 2005–2009.
34. Okumura, T.; Ericksen, R.E.; Takaishi, S.; Wang, S.S.; Dubeykovskiy, Z.; Shibata, W.; Betz, K.S.; Muthupalani, S.; Rogers, A.B.; Fox, J.G.; et al. K-ras mutation targeted to gastric tissue progenitor cells results in chronic inflammation, an altered microenvironment, and progression to intraepithelial neoplasia. Cancer Res. 2010, 70, 8435–8445.
35. Ray, K.C.; Bell, K.M.; Yan, J.; Gu, G.; Chung, C.H.; Washington, M.K.; Means, A.L. Epithelial tissues have varying degrees of susceptibility to Kras(G12D)-initiated tumorigenesis in a mouse model. PLoS One 2011, 6, e16786.
36. Matkar, S.S.; Durham, A.; Brice, A.; Wang, T.C.; Rustgi, A.K.; Hua, X. Systemic activation of K-ras rapidly induces gastric hyperplasia and metaplasia in mice. Am. J. Cancer Res. 2011, 1, 432–445.