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REVIEW ARTICLE

Applications and Limitations of Mouse Models in Oral Oncology: A Critical Appraisal

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ABSTRACT

Background: The first step in a biomedical investigation of a disease entity involves framing a research hypothesis. The hypothesis is framed based on the pre-existing information. Evidence for the hypothesis is usually gathered starting with epidemiological studies to estimate the prevalence, etiology, associated risk factors of the disease. Once an epidemiological association is established then the investigation involves the use of experimental (*in vivo, in vitro*) studies to decode the molecular biology of the disease based on which we can formulate appropriate interventions. The major limiting factors in this approach is translating the data obtained from experimental studies on to clinical trials. These limitations are because of the inability of the *in vivo* and *in vitro* studies to replicate the microenvironment of the disease in humans. Experimental studies capable of closely simulating disease environment in humans would aid in eliciting the true nature of these diseases. At present, mouse models are largely being used to study human diseases including cancer.

Aim and clinical significance: Although mouse models are considered better than other experimental models, it is vital that researchers select appropriate mouse models which would suit the purpose of the study. Thus, the present manuscript aims to critically review the applications and limitations of all mouse models employed in oral oncology which may aid the researchers in selecting the most optimal mouse models for their respective research.

Keywords: Head and neck cancer, Metastasis, Mouse models, Oral squamous cell carcinoma, Orthotopic model, Tumor microenvironment, Xenografts.

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INTRODUCTION

The past two decades have seen substantial stride being made in cancer diagnostics and therapeutics. At present, the focus of cancer therapeutics is on the development of targeted therapy. *In vivo* and *in vitro* based experimental studies using targeted therapy have shown increased treatment response while eliminating the complications associated with convention treatment modalities. Although experimental studies have shown success in laboratory settings, they have been relatively ineffective in a clinical setting. The major cause for the lack of translational value of experimental studies is due to the inability of the experimental models to replicate the human tumor microenvironment and heterogenicity. Thus, in the present review, we review the applications and limitations of the mouse models used in oral oncology.

Xenograft or Xenotransplant refers to the transplantation of living cells, tissues or organs from one species to another. Xenotransplantation of human tumor cells into immunocompromised mice is a research technique frequently used in pre-clinical oncology research. Grafts may include the cells from primary patient samples (from a primary tumor or from the metastatic site) or patient samples previously passed through the immunodeficient mice.[1] Patient-derived xenograft models exhibit some disadvantages like reduced engraftment rate and slow rate of tumor development and progression, expensive, less reproducible and sometimes requiring multiple passages in mouse models to obtain enriched cancer cells.[2]

The hierarchical stem cell model proposes that a cancerous growth is dominated and controlled by a distinct stem cell which helps in propagating cancer. In contrary to rare stem cell hierarchy, in a stochastic model of engraftment, most of the cells can propagate a tumor. Sampling from different sites containing different sub-clones with different properties might possibly represent the whole disease.[1] It is believed that samples from patients and those from cell lines may show a different tumor-propagating capacity which modulates the tumor growth, invasion and metastasis, and thus different engraftment kinetics. Although cell lines do retain the original driver mutations, serial passaging of cell lines over time can cause genotypic and phenotypic variation and may not represent the molecular complexity of the disease at presentation. Therefore, researchers

should be careful about only relying on data generated by cell lines.[1] Comparing the molecular and phenotypic characteristics of orthotopic xenografts with the tumors with orthotopic patient-derived xenograft tumors might possibly provide the information into the mechanisms of oral squamous cell carcinoma (OSCC) and various candidate drugs.[1]

Most preferred animal models for cancer research are the mice with the advantage of small size, breeding in captivity, 2 to 3 years lifespan, fully sequenced genome and many similar physiological and molecular similarities to the humans.[3]

National Cancer Institute in 1955, first began to use the mouse models by injecting leukemic cells into the mouse peritoneally. The first xenografts in head and neck cancer research were published in the 1980s.[4] Earlier xenografts comprised of human cancer cells from the cell lines engrafted into nude mice subcutaneously (ectopic) by injection. However, it was necessary to ensure that the mice were immunodeficient (athymic or severely immunodeficient mice). Athymic mouse was used because there was a loss of T-cell function allowing the xenografted cells to survive in a different species without being rejected. This prevented the interaction between the tumor cells and the host stromal microenvironment and its effect on tumor development could not be ascertained. These conventional models could not recapitulate regional or distant metastasis probably due to the lack of a site or organ-specific environment.[5]

Numerous mouse models to study oral cancer pathophysiology have been suggested in the published literature which include chemically induced mouse models, genetically engineered mouse models, humanized mice, autochthonous models, and orthotopic models.

Chemically induced mouse models are used to study the genetic and epigenetic changes following exposure to 4-Nitroquinoone oxide (4NQO) which serves as an alternative to the tobacco carcinogens. Development of cancer occurs in a stepwise manner from dysplasia to carcinoma in situ and frank carcinoma following exposure to 4NOQ. It takes around 2 to 3 months to develop a primary tumor.[6] In addition to 4NOQ, Dimethyl-1,2, benzanthracene (DMBA) dissolved in benzene or acetone is also used to induce cancer. The major disadvantage of chemical-induced models is that it does not allow the study of genes involved in carcinogenesis.[3]

Humanized mice refer to immunodeficient mice or athymic mice which receive human stem cells or lymphocytes to create a stromal environment like the humans. Thus, provide realistic heterogeneity of tumor cells. These models are used to study the interaction between a tumor and the stromal environment. They can simulate the drug response of a tumor in a cancer patient. However, they are expensive and technically complicated.[7-9]

Like humanized mice, genetically engineered mouse models (GEM) where a human gene is inserted into the mouse genome are used to study specific species associated with the phenotype. Studies on *p 53*, *k-ras* oncogene utilize these type of animal models since the mutations in mice are similar to those seen in human tumors. Tumor development can be monitored from early points in these models. They also provide a realistic microenvironment with the presence of immunocompetent cells. However, these models have not proved their role yet in drug discovery due to variation in the tumor histotype. Genetically altered mouse models have helped us to understand the role of genes in tumor progression and suppression better. They have some demerits such as extended time, expensive, technic sensitive, differences which are species-specific, and intellectual property restrictions.[10,11] Mimicking the tumor heterogeneity as in humans is challenging and might not be reliable. Furthermore, the drug response of a mouse tumor may not be a true representation as that seen in humans. List of all oral lesions studies in genetically engineered mouse models is enlisted in Table 1. Thus, drugs may not be introduced in clinical practice directly. Reproducing the genetic characteristics of human tumors in GEM models is complex and challenging.[12] A combination of chemically induced and GEM models have also been proposed which combine the genetic modifications with chemical carcinogen treatment. Such models exhibit enhanced tumor development and metastasis into regional lymph nodes and lungs.[13] Some of the commonly used combined models in OSCC include the XPA_/_; p53+/_ mouse + 4NQO, L2D1++ 4NQO, PIK3CA-GEMM mouse + 4NQO, K14-GFP-miR-211 transgenic mouse + 4NQO, Dusp1_/_ mouse + 4NQO.[3]

Table 1: Oral lesions studied in genetically engineered mouse models

Oral lesions to be studied	*Genetically engineered mouse models*
Papillomas of the oral cavity	LSL-KrasG12D; K5-rTA, LSL-Kras G12D; K5 or K14 CrePR1
Oral hyperplasia, and OSCC	LSL-Kras G12D; K5 or K14 CrePR1
Invasive oral-esophageal SCC	L2D1+ p53+/_ and L2D1+ p53_/_
Well-differentiated OSCC	Tgfbr1/Pten 2cKO
OSCC with metastasis	p53 R172H; K5 CrePR1 and p53 flox/flox K5 CrePR1

Autochthonous models reveal the spontaneous development of cancer and chemical, viral, or carcinogen-induced tumors. However, the spontaneous occurrence is quite rare in the laboratory animals.[14] They resemble the human tumors more than other transplanted tumors. They show orthotopic growth, display no changes in histology due to transplantation procedures, and exhibit tumor invasion and metastasis. There are some disadvantages to these models. The time required varies from months to years, there is variation in the induction of a tumor, and a number of animals are required. Hence, the use of these models is restricted for confirmation studies only.[15]

Ectopic mouse models implanted with human cancer cells did not demonstrate metastasis thereby doubting the validity of these models. This was attributed to the possible role of the host stroma at the implanted site. The influence of host stromal environment on the growth and sustenance of the tumor cells has received a lot of attention in recent time. Site-specific tumor environment is said to modulate the proliferation and invasion of the tumor cells. It is believed that the tumor cells establish themselves better in the tissue of their origin than in an ectopic (subcutaneous) site. Thus, the orthotopic mouse models gained importance.[3]

These models produced a high rate of spontaneous metastasis than the ectopic subcutaneous xenografts. The intrinsic property of tumor cells and host stromal environment dictates the pattern of metastasis.[11] It was shown that the orthotropic implantation of human cancer cells in a mouse tongue and buccal mucosa resulted in rapid tumor development and regional metastasis to the cervical lymph nodes similar to that seen in humans. Orthotopic implantation was preferred than the ectopic implantation of human cancer cells keeping in mind the chances of host rejection as well as site-specific stromal response. Tumorigenicity and tumor architecture in the host environment depends on the intrinsic properties of the cancer cell lines, their origin and that metastasis might occur through a heterogeneous tumor-host interaction. These tumor models are mainly used to study the tumor growth at the implanted site, local invasion, and metastasis both regional as well as distant. Orthotopic xenograft models can simulate the drug response similar to that of a tumor in a cancer patient, provide realistic heterogeneity of tumor cells, and allow rapid evaluation of tumor response to a therapeutic regime as in humans. The limitation of these models is that it demands higher technical skill, time-consuming, expensive and causes animal morbidity and mortality.[11] In general, although mouse models are indispensable in cancer research, they have major disadvantages such as their inability to fully reproduce the complexity of genetic profile in human tumors. Thus, at present based solely on mouse cancer models, it is also not possible to predict therapeutic response in human cancer.[11] A summary of all the advantages and disadvantages in the mouse models used in oral oncology is enlisted in Table 2.

In recent times, noninvasive methods to measure the tumor volume have been developed with cell labeling and imaging techniques like small animal MRI, PET scan, reporter genes with specific fluorescence protein and Luciferase gene which allow the researchers to monitor the metastatic process and to quantitate the tumor growth. Reports of some inherent difficulties with the orthotopic model, like clot formation on the control tumor interfering and blocking the photon coming out, and subsequent decreased total photon number of a tumor affecting the final outcomes are also found in the literature.[5]

Chemically induced mouse models and transgenic models are beneficial to observe and study the early stage of carcinogenesis such as precancers. While GEM models with metastasis would be needed to study the chemoprevention and drug development studies. Orthotopic mouse models provide molecular and cellular mechanisms responsible for the metastasis process. The results should be substantiated with further studies with immunocompetent models and analysis of oral cancer specimens obtained from cancer patients. Orthotopic human tumor xenograft is best suited for monitoring drug response in human tumors, and the GEM model is mostly used to explore the role of a gene in tumor growth and progression.[3]

Few studies using the xenograft models in HNSCC and OSCC are published in the literature. Shirako et al.[16] studied the growth and metastatic potential and phenotypic property of five different human OSCC cell lines from a tongue tumor and metastatic lymph nodes in a xenograft mouse model. They commented that the cell lines from the metastatic site revealed invasive and metastatic properties. The interaction of cancer cells with the host environment varies depending on their origin and intrinsic properties which further modulates their survival and ability to form tumor mass. They concluded that the tumor establishment and metastasis depend on the host-tumor interactions apart from the epithelial-mesenchymal transition.

Szaniszlo et al.[17] have concluded few points from their study such as xenografts of different HNSCC cell lines show distinct survival pattern and primary tumor growth, metastasis to regional nodes in the neck depends on the injected cell line, different HNSCC cell line xenografts result in tumors of different histological subtypes,

Table 2: Summary of advantages and disadvantages of mouse models used in oral cancer research

Category	*Advantage and Disadvantage*
Chemically induced mouse models	*Advantage:* Carcinoma is induced in a stepwise manner from dysplasia to carcinoma in situ and frank carcinoma simulating human OSCC *Disadvantage:* Genes responsible for the carcinogenic transformation cannot be studied. Such models develop a restricted subset of tumor types and grades with incomplete penetrance and variable latency
Genetically engineered mouse models	*Advantage:* The presence of immunocompetent cells provides a realistic tumor microenvironment *Disadvantage:* Extended time, expensive, technic sensitive, differences which are species-specific, and intellectual property restrictions
Combined (chemically induced plus genetically engineered) models	*Advantages:* Provides enhanced tumor development and metastasis into regional lymph nodes and lungs *Disadvantage:* Expensive, technic sensitive differences which are species-specific, and intellectual property restrictions
Humanized mice	*Advantage:* Provide realistic heterogeneity of tumor cells; Simulate the drug response of a tumor in a cancer patient *Disadvantage:* Expensive, technique sensitive
Autochthonous models	*Advantage:* Resemble the human tumors more than other transplanted tumors; Show orthotopic growth, display no changes in histology due to transplantation procedures, and exhibit tumor invasion and metastasis *Disadvantage:* Time required varies from months to years; There is variation in the induction of a tumor. The required number of animals are relatively more
Ectopic models	*Advantage:* There is an ease of approach during engraftment procedure as the injections are either subcutaneous or intra-cutaneous; Ectopic models allow the study of the stages of tumor progression more easily than the orthotropic model which requires either imaging and/or sacrificing models frequently. *Disadvantage:* There is a lack of real heterogeneity in tumor cells, thus lacking appropriate therapeutic responses; The lack of site/organ-specific environment does not allow for recapitulating regional or distant metastasis; Graft rejection and site-specific stromal response are frequent in cases of immunocompetent mouse

and injected cells of any type can reach the lymph nodes in the neck within a day after injection.

Bais et al.[2] also suggested that the orthotopic implantation of human oral cancer cell lines rather than xenografts in the oral tissues is more likely to represent the mechanism of invasion and metastasis as in humans.

To study invasion of oral cancer into muscle and bone, Dinesman et al.[18] performed transcutaneous injection into the tissue deep to the mylohyoid muscle beneath the floor of mouth through a submandibular route. They noticed regional metastasis of 5% and pulmonary metastasis of 40%. The injection differs from the submucosal route in the sense that the tumor spillage and seeding enhanced hematogenous spread and pulmonary metastasis contradicting the orthotopic approach.

The success of these animal experiments depends on a number of factors such as the intrinsic properties of the tumour cells such as type (solid, leukemia, or metastatic), selection of appropriate cell lines for greater metastatic potential, number of cells engrafted, host environment, anatomic site of implantation and its accessibility, type of engrafting (ectopic or orthotopic), technical challenges, labour intensity, health and maintenance of the mouse, type of mouse model and so on. Also, calculating the time required to get a predetermined tumor volume, determining the maximum tolerated dose, route, schedule, duration of therapy, evaluation of anti-tumor response and determining endpoint timing is challenging in drug discovery and response studies.[11]

CONCLUSION

The advent of a new treatment modality for oral cancer in humans requires an evidence-based proof of safety as well as efficacy which is possible only through prior simulations. At present among in-vivo studies on oral cancer, mouse-based models remain to be the most optimal means for replicating human tumor microenvironment. It is vital that future studies aim to improve

the translational value of *in vivo* mouse model studies to human clinical trials.

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