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Review Article

MOUSE MODELS OF ORAL CANCER: CHALLENGES AND OPPORTUNITIES

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ABSTRACT
Oral Cancer is one of the most fatal and widely prevalent diseases especially among male population and occurs most commonly due to smoking and alcohol consumption. It carries immense clinical significance owing to its delayed diagnosis, poor prognosis and expensive therapy. Hence Oral cancer has been one of the priorities of animal experimentation. Although there have been significant advances in the development of mouse models over the years, recapitulating the clinical form of the disease still appears to be farfetched owing to its complicated molecular process. The present review throws light on the significance, advantages, and limitations of various mouse models of oral cancer and alternative techniques that can be adopted to overcome the shortcomings.

KEY WORDS: Oral Cancer, mouse, animal models.

INTRODUCTION

Oral cancer stands sixth among the common cancers in the world (Jemal *et al*., 2011; Garewal and Meyskens, 2014). It has high prevalence among male population especially those associated with smoking and alcohol consumption (Rao *et al.*, 2013). It has reduced survival rate due to diagnosis at an advanced stage resulting in poor prognosis, lack of specific biomarkers and expensive therapy (Rivera, 2015). Also the metastasis of this disease that target the distant organs like mediastinal lymph node, lungs, liver and bones remain occult during the diagnosis of the primary tumour (Noguti *et al*., 2012). These factors turn oral cancer into one of the most important priorities in cancer research .The most common form of oral cancer is oral squamous cell carcinoma (OSCC) accounting to more than 96 percent of the total cancers of the oral cavity (Siegel *et al*., 2012). Oral cancer mainly includes malignant transformations of the oral cavity which consists of the mouth, lip, tongue, gums, cheeks, salivary and parotid glands (Jung *et al*., 2015). At a molecular level, oral cancer develops as a result of a complex process including activation and inactivation of oncogenes and tumour suppressor genes respectively leading to preneoplastic lesions and eventually malignant neoplasm (Fukuda *et al*., 2012). The major preneoplastic lesions developed are leukoplakia, erythroplakia. However neither of these lesions guarantees the onset of oral cancer (Watanabe *et al*., 2015; Yardimci *et al*., 2014). Also various underlying molecular mechanisms of initiation, promotion and metastasis of oral cancer still need to be elucidated to construct effective therapeutic strategies.

Development of animal models is one of the best modus to understand the molecular mechanisms underlying cancer and metastasis which in turn will help to develop novel therapeutic interventions as well predict the efficacy of biomarkers (Milas *et al*., 2009; Denayer *et al*., 2014). Mouse and Hamster are most commonly preferred laboratory animal species for development of preclinical model for oral cancer. However, although the hamster buccal pouch model is one of the most commonly used models for experiments, the significant difference between the the hamster cheek pouch mucosa and the human oral mucosa makes the extrapolation of the results ambiguous (Smith and Thomas, 2006). On the other hand, mice models are easy to handle, economical, have short gestation period, comparatively easy to perform genetic manipulations and closely mimic the human pathophysiology of oral cancer (Cheon and Ursulic, 2011; Lu *et al*., 2006). Hence, mouse models have gained momentum in the development of animal models for oral cancer. The present review enumerates the pros and cons of different mouse models of oral cancer with possible alternatives that can be incorporated to overcome the limitations.

1. Chemical Carcinogenesis models

Chemically induced mouse models, although one of the earliest models developed, is still the best to study the progressive stages in oral cancer and closely mimic the clinical form of the disease (Holzapfel *et al*., 2015; Ide *et al*., 2003). The principle of this model involves administration or application of a chemical carcinogen (similar to that observed in clinical settings) to the animal for a specified period followed by evaluation of the preneoplastic lesions, neoplastic lesions and metastasis. The commonly used chemicals include 4-nitroquinoline 1-oxide (4NQO) and 7, 12-Dimethylbenz (a) anthracene (DMBA) (Wong, 2009; Tanaka and Ishigamori, 2011; Yanaida *et al*., 2002; El-Bayoumy *et al*., 2016). 4NQO is preferred over DMBA as the latter is a highly irritating and produces extensive inflammatory response and

necrosis which makes the evaluation of lesions undesirable (Nauta *et al*., 1996; Vitale-Cross *et al*., 2009; Kanojia and Vaidya, 2006). Activity of of ethanol acting as a promoter to 4-nitroquinoline 1-oxide induced carcinogen has also been reported (Guo *et al*., 2012). However chemical induced carcinogenesis model carries with it some genuine disadvantages. The major limitation is delayed induction leading to to delay in initiation of treatment and difficulty to identify the exact time points to commence the treatment due to chronic nature of the disease development (Schoop *et al*., 2009). Since chemical carcinogenesis in induced in immunocompetent animals there is high risk of the tumour cells to be recognized by the immune system leading to tumour rejection (Dudley and Roopenian, 1996). The resistance extended by the oral mucosa due the sebaceous glands and saliva also poses a hurdle to the development of oral carcinoma (Nishioka *et al*., 1981). Evaluation of the animal model with respect to specific gene becomes difficult in a chemically induced model and manual manual handling of the carcinogen involves high risk (Kim, 2009; Mognetti *et al*., 2006). Chemical carcinogenesis are poor inducers of metastasis and hence not suitable to study molecular mechanisms of metastasis in oral carcinogenesis (Myers, 2009). These challenges can be overcome by adopting certain modifications in the development of models. Preneoplastic lesions can be determined by with the help of autofluroscence, in vivo bioluminescence and magnetic resonance imaging (MRI) technologies. MRI models that use organ specific contrast agents are preferred over non specific contrast agents as the former have better diagnostic capabilities to differentiate between malignant lesions and surrounding tissues (Liu *et al*., 2015; Close *et al*., 2012; Ni *et al*., 2009). Metastasis can be identified by high frequency ultrasound imaging and recently developed DsRed protein in vivo imaging technology (Bais *et al*., 2015). The success of In vivo staining techniques using toluedene blue and methylene blue are only limited to clinical cases, however rose Bengal staining in combination with fluorescence spectroscopy has been reported to be a good diagnostic marker for preneoplastic lesions of oral squamous cell carcinoma (Riaz *et al*., 2013; Anand *et al*., 2013; Liu *et al*.,2016). Also, recently a Rose Bengal conjugated gold nanorod (RB-GNR) technique has been developed for optical detection of premalignant lesions of oral cancer; however this technique needs clinical validation (Wang *et al*., 2013). To overcome the interference of immunity in development of cancer and to study specific gene effects on oral cancer, co carcinogenesis models can be developed which is explained in the later section in this review. Manual handling of the carcinogen can be minimized by adopting alternative administration techniques such as impregnation method wherein the carcinogen is impregnated in cotton sutures that enable slow release of chemical (Heller *et al*., 1996).

2. Transplantation mouse models

Transplantation mouse models are developed by transplantation of tumour cell lines of experimental or clinical origin into the primary site (orthotopic) or a secondary site (eg, subcutaneous) of genetically related animals (allograft models) or unrelated animals (xenograft models) to study different stages of tumour initiation, promotion and progression. Orthotopic xenograft models are ideal to study metastasis as the process mimics the clinical tumour (Sano and Myers, 2009; Masood *et al*., 2013). Although this model assures quick tumour development, lack of functional immunity limits its use to study tumour host interactions as the model is developed in immunodefecient mice (Lie *at al*., 2016). Also Generating large cohort of induced animals is a tedious task owing to the spontaneous nature of development of these tumours (Lum *et al*., 2012). Subcutaneous xenograft limit the study of interactions and molecular process occurring between the tumour and the native environment due to the nonspecific site of induction and posses has poor metastatic potential (Kubota, 1995; Arjona *et al*., 2006; Killion *et al*., 1996). To overcome the challenges involving use of immunocompromised animal models, immunocompetant animal model systems such as syngeneic and humanized mouse models are developed. These animal models are realistic and are also efficient in producing metastasis (Kambe *et al*., 2001; Wong and Feinberg, 1990). Commonly preferred Syngeneic mouse models include murine cutaneous squamous cell carcinoma cell line (SCCVII) and AT-84 cell line induced oral squamous cell carcinoma in C3H/HeJ mice with the former one showing cervical lymph node and pulmonary metastasis.(Mognetti *et al*., 2006; O'Malley *et al*.,1997; Hier *et al*., 1995). Both the cell lines are effective in orthotpic model in immunocompetent system, however are not successful in subcutaneous model (Mognetti *et al*., 2006).Humanized mouse models are generated by grafting immune system components such as human haematopoetic stem cells (HSC'S), peripheral blood mononuclear cells or CD34+ and maintained by exogenous sources of hormones, growth factors and cytokines thus creating an artificial immune system in the body of the animal. These mouse models are excellent to study genetic and epigenetic factors involved in carcinogenesis, however time requirement and reproducibility are serious limitations to this model (Hiramatsu *et al*., 2003; Macchiarini *et al*., 2005; Holzapfel *et al*., 2015; Rongvaux *et al*., 2014). Yet there is immense scope for development.

3. Transgenic mouse models

Transgenic mouse models include animal models with manipulated genetic modifications and are one of the efficient models to study oral cancer. These are one of the fastest models to develop and help in studying all the stages of cancer development (Nair and Reddy, 2016). Some of the successful transgenic models include are Epstein barr virus targeted cyclin D1 transgenic model, P53 suppressed model and c-myc overexpressed model (Nakagawa *et al*., 1997).However transgenic models consist of a heterologous promoter leading to non physiologic levels of transgene product and multiple oncogene activation creates a different tumour microenvironment than that in spontaneous tumour models as only a few cells undergo mutations in latter (Kim, 2009; Mognetti *et al*., 2006). Also in transgenic models, the stromal cells too carry the transgene and these models possess low penetrance of tumour and metastatic

capability (Pearson and Pouliot, 2000). The drawbacks in transgenic models can be overcome either by developing targeted or inducible transgenic models (Lu *et al*., 2006) or by generating co-carcinogenesis models. A K-ras and p53 two-hit genetic manipulation has been reported to develop an oral specific carcinogenesis model (Raimondi *et al*., 2009). Also, a novel targeted and inducible transgenic mouse model targeting removal of transforming growth factor β receptor type II (TGFBR2) and E-cadherin (CDH1) genes has proven to be a good model to study oral squamous cell carcinoma (Andl *et al*., 2014).

4. Co-Carcinogenesis models

Co-carcinogenesis models are developed by a combined 2-way induction process. This process is most commonly achieved by chemical induction in transgenic mouse models. It is the best model in terms of recapitulating the human form of disease. 4-NQO chemical induction in c-Ha-ras proto-oncogene activated and p53 supressed as well as combination of xeroderma pigmentosum A (XPA) gene and p53 inactivated model (Zhang *et al*., 2006; Ide *et al*., 2003), are few of the successful co carcinogenesis oral squamous cell carcinoma model systems. Similarly c-Ha-*ras* proto-oncogene transgenic rats chemically induced with 4-NQO-are useful to study early stages of oral carcinoma and chemoprevention mechanisms (Tsuda *et al*., 2001; Miyamoto *et al*., 2008). However there is still a dearth of co-carcinogenesis animal models and sufficient research has to be directed towards developing such models in the field of oral cancer.

CONCLUSION

Animal models play a vital role in determining the underlying molecular mechanisms of different stages of oral carcinogenesis such as initiation, promotion, proliferation, apoptosis, angiogenesis and inflammation to name a few. Animal models not only benefit in validation of various biomarkers for oral cancer but also support in development of effective and safe therapeutic interventions. Each type of animal model carries with it certain advantages and limitations and it is practically impossible to develop an “ideal” or “fool proof” animal model for oral carcinogenesis. However certain modifications in development of the model can fetch better results. A researcher should select an animal model based on the purpose and requirement of the study. For example, in order to study tumour host relationship and immune response, immunocompetent models should be preferred than immunodefecient models. Nevertheless, it is essential that the animal model should be able to closely mimic the clinical pathophysiology to enable a holistic study of the underlying molecular mechanisms.

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