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Review

Current mouse models of oral squamous cell carcinoma: Genetic and chemically induced models

Kazuhisa Ishida [a,b], Hiroyuki Tomita [a,*], Takayuki Nakashima [a,b], Akihiro Hirata [c], Takauji Tanaka [d], Toshiyuki Shibata [b], Akira Hara [a]

[a] Department of Tumor Pathology, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan
[b] Department of Oral Maxillofacial Surgery, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan
[c] Division of Animal Experiment, Life Science Research Center, Gifu University, 1-1 Yanagido, Gifu 501-1194, Japan
[d] Department of Diagnostic Pathology (DDP) and Research Center of Diagnostic Pathology (RC-DiP), Gifu Municipal Hospital, 7-1 Kashima-cho, Gifu City, Gifu 500-8513, Japan

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ABSTRACT

Oral squamous cell carcinoma (OSCC) patients have a low 5-year survival rate and poor prognosis. To improve survival and prognosis, the causes and processes involved in lesion development should be evaluated. For this purpose, the use of OSCC mouse models, such as chemically induced mouse models, genetically modified mouse models, and transplanted (xenograft) models, is crucial. These OSCC models exhibit both advantages and disadvantages when studying OSCC development and progression. Until a model resembling human OSCC is developed, both the advantages and disadvantages of each model should be carefully considered. In this review, we discuss OSCC mouse models and their use in cancer research worldwide.

Introduction

Ninety percent of head and neck cancers are squamous cell carcinomas involving mucosal surfaces of the oral cavity, pharynx, and larynx. This cancer can affect any site in the oral mucosa, typically the tongue and floor of the mouth [1]. Oral squamous cell carcinoma (OSCC), a subset of head and neck squamous cell carcinoma, is one of the most common human malignancies worldwide [2,3]. Further, OSCC is the most common malignant tumor of the head and neck, and its incidence has increased in recent years [4].

OSCC is a challenging disease to manage in the field of head and neck cancer. The standard treatment for OSCC is a combination of surgery, radiation, and chemotherapy. The 5-year survival rate of OSCC is only 50%, which has remained unchanged for a decade [5]. This poor prognosis is considered to stem from aggressive local invasion and metastasis, leading to recurrence. Further, a lack of applicable markers for early detection and the failure of advanced lesions to respond to chemotherapy contribute to poor OSCC outcomes.

OSCC lesions accumulate genetic alterations including chromosomal aberrations, DNA mutations, amplifications, or deletions, and/or epigenetic alterations, such as changes in DNA methylation, which affect genetic regulation [6]. These events are affected by exposure to environmental agents, including tobacco smoke, alcohol, and viruses (e.g. human papillomavirus), which are the major risk factors [7,8].

Insight into the mechanisms of OSCC initiation and progression is needed, and identification of novel molecular targets to assist the development of new therapeutic strategies is crucial [9]. New therapies can be investigated both *in vitro* and *in vivo*. Animal models, particularly mouse models, enable the study of complex biological processes [10]. An optimal animal model would exhibit spontaneously occurring oral cancer; however, spontaneous OSCC is extremely rare in laboratory animals [11].

In mouse models of OSCC, xenograft models and chemical carcinogen-induced models are widely used. Xenograft models with implanted human OSCC cells are frequently used to study human tumor growth and spread as well as to develop and test new antitumor drugs [10].

As a chemical carcinogen-induced model, the 4-nitroquinoline 1-oxide (4NQO) mouse model has been used worldwide. The dynamics of gene expression alterations in the 4NQO mouse model of human oral carcinogenesis that lead to the development of preneoplastic histological changes such as hyperplasia and dysplasia followed by SCC development can be characterized in this model [12–16].

* Corresponding author.
E-mail address: h_tomita@gifu-u.ac.jp (H. Tomita).

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Mice are one of the best model organisms for cancer research because of the animals' small size, propensity to breed in captivity, life span of 2–3 years, many physiological and molecular similarities to humans, and fully sequenced genome [17].

Here, we review mouse models used to investigate OSCC pathogenesis and discuss both the advantages and disadvantages of their application in cancer research.

Chemical carcinogen-induced mouse models

4-Nitroquinolone oxide (4NQO) model

The 4NQO molecule forms DNA adducts, causes substitutions of adenosine for guanosine, and evokes intracellular oxidative stress, resulting in genetic mutations and DNA strand breaks. All these effects are similar to the genetic and epigenetic alterations resulting from exposure to tobacco carcinogens, and therefore, 4NQO treatment serves as an alternative for tobacco exposure in animal experiments of oral cancers [13]. 4NQO induces carcinoma in the tongue and esophagus, and the carcinogenesis model of 4NQO exposure imitates many situations of human oral squamous carcinogenesis.

Historically, 4NQO, the most commonly used carcinogenic drug, is a DNA adduct-forming agent that causes DNA damage similar to the damage induced by cigarette smoke. In rodent models, the rat oral cavity model was introduced in the early 1970s [18]. In this landmark study, 4NQO dissolved in water produced palatal cancers in all experimental rats by seven months. In addition, 75% of these animals developed carcinomas on the dorsum of the tongue and 20% developed lesions on the gingival tissue [18]. 4NQO has also been broadly used in mice and is useful for investigating the effects of anti-tumor drugs.

Regarding mouse models, the 4NQO OSCC model involves a protracted multi-step process in which invasive SCC is eventually reached after several months, and 100% of 4NQO-treated mice exhibit precancerous lesions and tumors on the tongues and oral mucosa [19]. Pathological analyses indicate that these lesions and tumors are flat squamous dysplasias (from mild to severe atypia), papillary squamous tumors (papillomas), carcinoma *in situ* (non-invasive SCC), and invasive SCC, even after 4NQO is withdrawn (Fig. 1) [6,20]. Multiple lesions are considered to constitute the multi-step carcinogenesis of OSCC; this multi-step process is one advantage of the 4NQO model, and the development of fully malignant SCC is preceded by increasing grades of dysplastic changes that mimic oral cancer development in humans. SCCs are typically detected between 12 and 33 weeks after applying 4NQO (via drinking water) for 16 weeks [20,21].

Regarding the molecular pathology of the 4-NQO model, the SCC tumors produced display some of the molecular changes observed in human SCC; however, the detailed pathological and molecular mechanisms remain unclear.

Dimethyl-1,2,benzanthracene (DMBA) model

This model involves the administration of polycyclic hydrocarbon 9,10 dimethyl-1,2 benzanthracene (DMBA) dissolved in benzene or acetone to the cheek pouch of hamsters [22]. DMBA induces DNA adduct formation and is mutagenic in mice [23,24]. However, the DMBA mouse model is not commonly used, and only one study using this model has been reported [25]. In this study, several mice treated with DMBA for 2–6 weeks developed papillomas and papillomas with dysplasia.

Overall, carcinogen-induced models do not allow for the study of specific genes in the process of oral carcinogenesis. For this purpose, utilizing xenograft models or transgenic mouse models is necessary.

Genetically modified mouse models

LSL-Kras^{G12D} mouse

Another approach involves a novel inducible mouse model that allows oncogene activation and/or tumor suppressor inactivation solely in the stratified epithelia of the oral cavity (Table 1). Two transgenic mouse models of oral cancer have been described related to *K-ras* mutations. These models utilize the keratin 5 (K5) or keratin 14 (K14) promoter to overexpress the oncogene *K-ras*^{G12D} in the oral epithelium of mice [26,27]. These promoters are optimal for targeting transgene expression to the oral cavity.

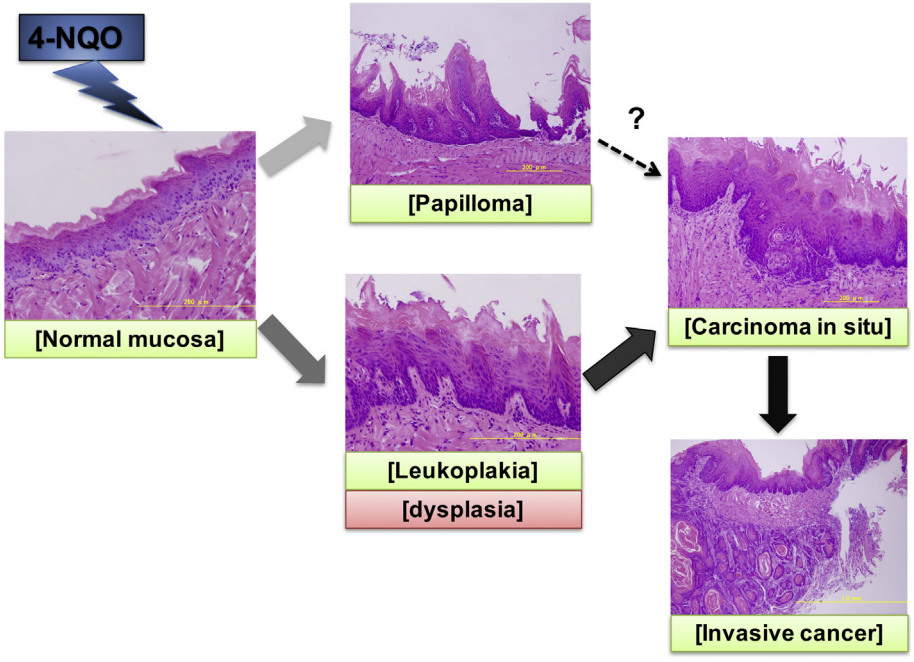

Fig. 1. 4NQO-induced tongue lesions in mice. 4-NQO treatment induces tumors mimicking human oral squamous cell carcinogenesis.