

DMBA-induced hamster buccal pouch carcinoma and VX2-induced rabbit cancer as a model for human oral carcinogenesis

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In this article, we have described and compared the advantages and disadvantages of two potential animal cancer models (the hamster buccal pouch cancer model and the VX2-induced rabbit cancer model) for human squamous cell carcinomas of the oral mucosa. Currently, no animal cancer model is perfectly applicable to human oral squamous cell carcinomas. This is because the hamster buccal pouch cancer model has a different etiology and genetic constitution compared with human oral carcinomas. In addition, the VX2-induced rabbit cancer model is not produced *in situ* and, consequently, its natural behavior is totally reliant on the location of transplantation. Nonetheless, with the use of these two animal cancer models together, researchers could evaluate different aspects of the cellular and molecular biological characteristics or assess potential novel treatment regimens for squamous cell carcinomas of the human oral mucosa.

KEYWORDS: animal model • DMBA • hamster • oral carcinogenesis • rabbit • squamous cell carcinoma • VX2

Squamous cell carcinoma, which arises from the oral mucosa lining, accounts for over 90% of oral malignancies [1]. In addition, squamous cell carcinoma of the oral cavity is the 11th most common global cancer, accounting for 3% of all newly diagnosed cancer cases [2]; it is also the eighth most predominant cancer in males [3]. Owing to the high prevalence of chewing betel quid and/or tobacco in Central and Southeast Asian countries, oral squamous cell carcinoma is the most common carcinoma, comprising approximately one-third of all cancers [4]. In addition, in those countries in which chewing betel quid and/or tobacco is highly popular [5–7], squamous cell carcinoma of the buccal mucosa is the most common oral cancer, which is in contrast to North America and Western Europe (in which it constitutes only 10% of all oral cancers) [8]. Despite the large amount of research into tumor cells and molecular biology, as well as recent advances in oncology and surgery, the rates of both mortality and morbidity in oral squamous cell carcinoma patients have remained unaltered over the past few decades [9]. However, a recent report indicates that the prognosis of oral carcinoma has improved in recent years [10].

Basically, there are three types of animal models for oral cancers available for research purposes. The first is spontaneously occurring tumors, such as auricular squamous cell carcinomas in sheep [11]. The second is tumors induced by topical treatment with chemical carcinogens, such as the induction of mouse [12] or rat [13] squamous cell carcinoma by topical application of 4-nitroquinoline-1-oxide, and hamster buccal pouch carcinoma induced by 9,10-dimethyl-1,2-benzanthracene (DMBA) [14]. The third is subcutaneous and orthotopic xenograft tumors induced by inoculation with cells from a transplantable head and neck cancer cell line in mice [15]. In brief, the ideal animal cancer model would be that of a spontaneously occurring oral cancer; however, spontaneous oral squamous cell carcinomas are very rare in both domestic and laboratory animals. In addition, it is difficult to establish a standardized experimental protocol for a spontaneous cancer model. On the other hand, tumor induction by the topical application of carcinogens is a rather time-consuming method and involves a latency period, while in a subcutaneous xenograft animal model the development of human tumor

cells in immune-suppressed mice is technically easy to perform and simple to manipulate, and quantification of the tumor burden is uncomplicated.

Although no animal model is perfectly applicable to every kind of human cancer, it is generally agreed that the use of a suitable preclinical animal cancer model is helpful in attempting to elucidate the molecular pathogenesis of squamous cell carcinoma of the oral cavity. Among the aforementioned three types of animal cancer models, the hamster buccal pouch model is one of the best characterized and is a useful cancer model for squamous cell carcinoma of the oral cavity [16].

In addition to the commonly used hamster buccal pouch cancer model, a novel application for the transplant of VX2 cancer cells to the buccal mucosa of rabbits as a model for inducing squamous cell carcinomas of the buccal mucosa was recently established in our laboratory [17].

The aim of this article is to summarize and compare the merits and drawbacks of the two aforementioned animal cancer models (i.e., the hamster buccal pouch cancer model and the VX2-induced rabbit cancer model) for squamous cell carcinomas of the human oral mucosa.

Hamster buccal pouch cancer model

Early experimental trials of the chemical induction of carcinomas of the oral mucosa either failed to yield any tumors or the incidence of tumor formation was very low, because the oral mucosa is noticeably more resistant to the actions of chemical carcinogens in comparison to the skin [18]. Success was first achieved reliably by topical application of highly carcinogenic polycyclic aromatic hydrocarbons such as DMBA, 20-methyl cholanthrene and 3,4-benzpyrene to the hamster buccal pouch. In mammalian cells, polycyclic aromatic hydrocarbons are bio-activated through the formation of reactive diol-epoxide metabolites that subsequently bind to adenine and guanine residues in DNA and form adducts [19–21]. Adduct formation has been implicated in the carcinogenic mechanism of polycyclic aromatic hydrocarbons [22–24].

In recent years, the buccal pouch of the Syrian golden hamster (*Mesocricetus auratus*) has become a widely used animal cancer model for the chemical induction of buccal squamous cell carcinoma. Anatomically, the bilateral pouches (one on each side of the mouth) beneath the cheek muscles open into the anterior part of the mouth, extending backwards to the oral cavity, but not as far posteriorly as the pharynx (FIGURE 1A). Histologically, the buccal pouch mucosa is lined with thin keratinizing squamous epithelia (FIGURE 2A). The earliest successful induction of neoplasms in the hamster buccal pouch was reported by Salley in 1954, who, after dissolving it in acetone or ether, painted the potent carcinogenic hydrocarbon DMBA topically onto the pouch three-times a week for 16 weeks [14]. The protocol for chemically induced experimental carcinogenesis with DMBA was subsequently modified and standardized by Morris in 1961 using a 0.5% DMBA solution in acetone and young hamsters (5 weeks of age), which yielded the greatest tumor growth with the least latency without morbidity [15].

Following standard experimental procedures for the application of DMBA to the hamster buccal pouch, upon gross examination there are no apparent changes in untreated (control) buccal pouches. Thickened mucosa with a rough surface of whitish granular appearance is noted in hamster buccal pouches topically treated with DMBA for 3–8 weeks, and a 100% tumor incidence is apparent in hamster buccal pouches subjected to 10–14 weeks of DMBA treatment (FIGURE 1B). Microscopically, no significant histologic changes are observed in untreated (control) pouches. The control hamster buccal pouch is composed of a thin squamous cell layer, connective tissue and a muscle layer, and the mucosal epithelium consists of three to four layers of squamous cells exhibiting slight keratinization (FIGURE 2A). During malignant transformation, varying degrees of hyperkeratosis, basal cell hyperplasia, cellular pleomorphism and nuclear hyperchromatism are observed within 3–9 weeks of DMBA application (FIGURE 2B). In the final stages, exophytic and/or endophytic squamous cell carcinomas are detected in hamster buccal pouch mucosa that have been topically treated with the carcinogen DMBA for 10–14 weeks (FIGURE 2C).

Experimental chemical carcinogenesis

The hamster buccal pouch cancer model has been employed in a wide series of experiments to investigate the various aspects of oral experimental chemical carcinogenesis in our laboratory and others [25–28]. The development of oral squamous cell carcinoma has long been characterized as a multistep process consisting of the phases of 'initiation', 'promotion' and 'progression', which are reflected by accumulated genetic changes, inducing the malignant transformation of control oral mucosa [29]. The concept of field cancerization, postulated as early as 1953, relies on the fact that the epithelial surface of the oral cavity is constantly exposed to the same common carcinogens [30], thus increasing the risk of the development of oral carcinoma. The hamster buccal pouch cancer model has the advantage of being very useful for studying the multistep process of oral epithelial carcinogenesis and, in our laboratory, has been used to establish the promotional effect of betel quid on cancer of the hamster buccal pouch after initiation with DMBA [31,32]. The cancer-promoting effects of 12-*O*-tetradecanoylphorbol-13-acetate and collagenase have also been elucidated in hamster buccal pouch carcinogenesis [33,34], and polycyclic aromatic hydrocarbons and DMBA carcinogenic mechanisms have been thoroughly investigated using this animal cancer model [35].

The hamster buccal pouch model possesses the further advantage of reflecting many essential features of the development of premalignant and malignant human buccal squamous cell carcinomas. Some common features reported by our laboratory include alterations in the activity of enzymes such as creatine kinase isoenzymes [36,57], placental glutathione *S*-transferase isoenzyme [34,35,37,38], γ -glutamyl transpeptidase [39] and inducible nitric oxide synthase [40–43], as well as an increase in low-molecular-weight keratins during hamster buccal pouch carcinogenesis [44]. In addition, aberrant mRNA and protein expression of p53 family proteins (p53, p63 and p73) [43,45–47], and overexpression and

epigenetic regulation of the inhibitors of apoptosis family proteins, have commonly been observed in hamster buccal pouch squamous cell carcinogenesis induced by DMBA [48,49].

A considerable body of evidence suggests that changes in cell products occur during transformation of a control cell into a malignant cell, and that these alterations are determinants of some of the important features of tumor cells, which include loss of contact inhibition, decrease in cell adhesion, enhanced growth, prolonged survival, the production of new antigens and an ability to escape from immune destruction by the host. Abnormalities of cell surface carbohydrates are believed to be implicated in many of these features [50,51]. Studies have been conducted in our laboratory in order to confirm alterations in the levels and distribution of sugar residues reflected by the lectin-binding pattern during the process of experimental carcinogenesis in hamster buccal pouch mucosa upon DMBA tumor induction [52].

In addition, we discovered the chemoprevention of inducible nitric oxide synthase inhibitors, which inhibit the development of premalignant or invasive carcinomas in hamster buccal pouch squamous cell carcinogenesis [53]. Moreover, using this hamster buccal pouch cancer model, the therapeutic effect of fractionated radiation in hamster buccal pouch carcinomas induced by DMBA was recently evaluated by our laboratory [54]. The cancer stem cell model postulates the heterogeneity of tumorigenicity in tumor volume, in which only a certain phenotype is tumor initiating, whereas the majority of the tumor cells are non-tumor initiating [55]. The rate of renewal of control human oral epithelium is estimated to be approximately 14–24 days; therefore, most epithelial cells do not survive long enough to accumulate the genetic alternations required for the formation of oral squamous cell carcinoma because, in accordance with the genetic progression model, the formation of oral squamous cell carcinoma requires a duration of months or years [56]. It is therefore hypothesized that control somatic stem cells, being long-term residents of the oral epithelium, are uniquely susceptible to the accumulation of multiple oncogenic changes giving rise to oral squamous cell carcinomas. Interestingly, stem cells have been successfully isolated and characterized from control hamster buccal pouch mucosa in our laboratory, suggesting that hamster buccal pouch mucosa may also be a potential oral cancer stem cell model [57].

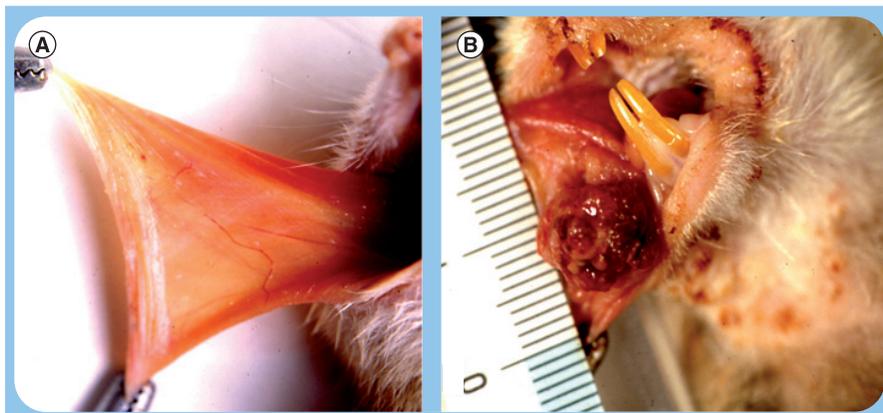


Figure 1. Gross image of the hamster buccal pouch cancer model. (A) A control hamster buccal pouch. **(B)** Representative sample of a macroscopic tumor of a pouch treated for 14 weeks with 9,10-dimethyl-1,2-benzanthracene.

Subsequent to the isolation of control hamster buccal stem cells, the sequential changes of the control stem cells during multistep oral carcinogenesis or their alternations upon irradiation and/or chemotherapy can be followed *in vitro*.

One of the main drawbacks of the hamster buccal pouch cancer model is the labor-intensive procedure and extensive animal and carcinogen handling required, as well as the time-consuming process involved. In accordance with the standard experimental protocol, an animal needs to receive repeated DMBA treatment at least 36–42 times in order for tumor induction to be successful. In addition, chromosomal alterations of hamster buccal pouch carcinomas appear to be different to those of squamous

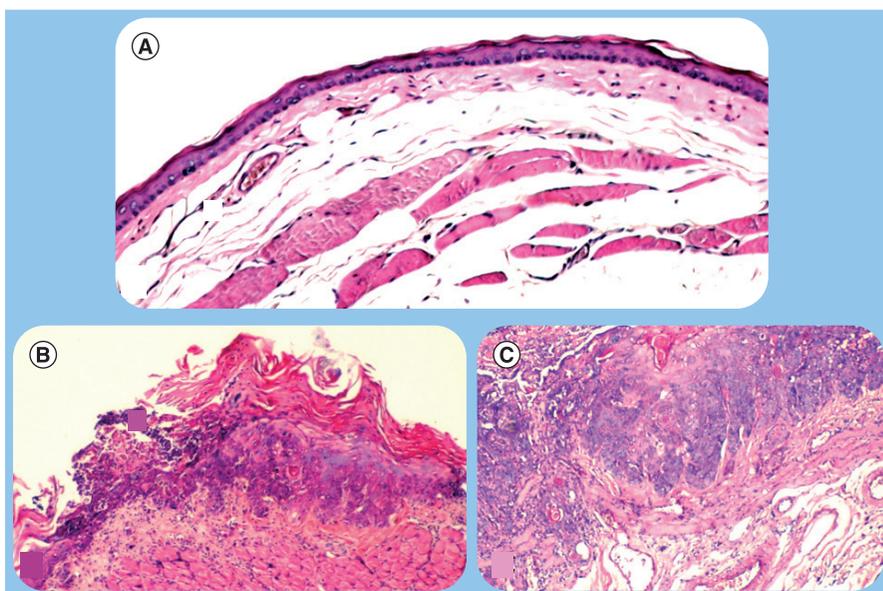


Figure 2. Histological images of the hamster buccal pouch cancer model. (A) The control hamster buccal pouch consists of three to four layers of squamous cells exhibiting slight keratinization (hematoxylin and eosin stain, $\times 40$). **(B)** Dysplastic epithelium is noted approximately 9 weeks after the application of 9,10-dimethyl-1,2-benzanthracene (DMBA; hematoxylin and eosin stain, $\times 200$). **(C)** A well-differentiated squamous cell carcinoma is detected in the 14-week DMBA-treated buccal pouch mucosa (hematoxylin and eosin stain, $\times 200$).

cell carcinomas of the human oral mucosa [58]. The main findings from selected literature for the DMBA-induced hamster buccal pouch cancer model have been summarized in TABLE 1.

Experimental molecular mechanism of metastasis

It is generally accepted that exploration of the molecular mechanism of metastasis of oral cancer would assist the discovery of novel prognostic biomarkers and therapeutic modalities, and result in subsequent improvement of the survival rate of patients with the disease. However, most researchers agree that the carcinomas induced in the hamster buccal pouch cancer model unfortunately often do not produce cervical lymph node metastasis, and distant metastasis is even rarer [59,60]. As a result, a further drawback of this animal cancer model is that it is not applicable for use in either locoregional or distant metastatic studies of oral cancer.

VX2-induced rabbit cancer model

Although the most important and independent environmental risk factors for oral malignancies are betel quid chewing, cigarette smoking and alcohol consumption, there is also evidence to suggest that viruses, most notably the human papilloma virus, are involved in the development of at least a subset of oral squamous cell carcinomas [61].

From a review of the literature, it can be seen that the idea of an association between viruses and malignancies can be traced as far back as 1933, when Shope and Hurst found that papillomas of the skin of domestic rabbits can be induced naturally by a virus: these papillomas change into squamous cell carcinomas upon malignant transformation [62]. Subsequent to that report, in 1936 Kidd *et al.* were able to produce cutaneous carcinomas after inoculation of extracts of the Shope-papilloma virus for a duration of 10 months [63]. These carcinomas were designated 'V1'; however, tumor growth failed in the second trial of transfer. Subsequent experiments resulted in the development of the 'V2' carcinoma cell line: the transplantation rate was low in the beginning (successful in only 5% of the inoculated Dutch belted rabbits), but in the third tumor generation the success rate increased to 21% of the inoculated animals, and successful

inoculation has been reported to continue until the 14th generation [63]. It was subsequently reported that these V2-induced squamous cell carcinomas quickly grew in most animals into which they were transplanted [64]. By then, after serial inoculations the V2 tumor had become progressively more anaplastic histologically. Current stocks of cell lines across the world consist of different transplant generations and, hence, the carcinoma has been designated the 'VX2' tumor. A thorough depiction of the pathology of the V2 tumor in the New Zealand white rabbit was documented by Stewart and colleagues of the Armed Forces Institute of Pathology (AFIP) in 1959 [65]. Nowadays, the New Zealand white rabbit is employed for most experimental studies using this animal cancer model.

In 1976, Galasko and Haynes reported that a culture medium containing 10% dimethyl sulfoxide (DMSO; an antifreeze compound) is necessary in order to sustain sufficient cell viability after thawing for successful stocking of VX2 cells under liquid nitrogen (at -196°C) [66]. In 1978, Shah and Dickson further reported on the *in vitro* preservation of the VX2 tumor and noted that the capacity of the VX2 cell line to regrow *in vivo* was eventually halted, with VX2 tumor cells grown in culture for more than 3 weeks unable to induce tumors when transplanted into rabbits [67]. However, by 1982 a VX2 cell line had been developed that could be successfully reimplanted following several months of continuous culture [68]. The ability to preserve VX2 tumors in culture has the advantage of allowing a variety of *in vitro* experiments without the need for serial transplantation in rabbits, thereby decreasing the quantity of animals required for experiment. However, in practice, most investigators, including our laboratory, favor propagation of the VX2 line *in vivo* via intramuscular passage in the hind paw of rabbits [69-74], the developed hind paw tumors then acting as the donor site for subsequent studies (FIGURE 3A).

Subsequently, inoculation of VX2 tumor cells to the donor sites can be achieved by injecting either fresh or cryopreserved tumor cell suspensions [73,74], or by implanting fresh solid tumor pieces [75,76]. The use of cell suspension is, of course, technically simple and has the benefit of simple determination of the

Table 1. Summary of the main findings from selected literature on the DMBA-induced hamster buccal pouch cancer model.

Item	Summary of main findings	Ref.
Cancer chemoprevention	Among one of the most recent studies demonstrating the application of the DMBA-induced hamster pouch model for cancer chemoprevention evaluation by topical application of resveratrol complexed with 2-hydroxypropyl- β -cyclodextrin	[28]
Tumor markers	Comparative assessment of carcinogen activation, DNA damage, cell proliferation, apoptosis, invasion and angiogenesis in oral cancer patients and hamster buccal pouch carcinomas has been performed	[25]
Model for radiotherapy	New applications of BNCT and radiobiology of BNCT to improve its therapeutic efficacy has been studied using the DMBA-induced hamster cheek pouch oral cancer model	[26]
	First establishment of the therapeutic effect of fractionated radiation on DMBA-induced hamster buccal pouch squamous cell carcinomas	[54]
Sequential carcinogenesis	The DMBA-induced hamster buccal pouch model has been confirmed to be suitable for sequential oral squamous cell carcinogenesis	[27]

BNCT: Boron neutron capture therapy; DMBA: 9,10-dimethyl-1,2-benzanthracene.

percentage of vital cells injected by trypan blue exclusion, rendering it feasible to inject approximately equal amounts of vital cells in each subsequent inoculation, resulting in tumors of more-or-less equal size. However, the injection pressure can lead to the unintentional infusion of tumor cells into vessels, producing early pulmonary metastases. This phenomenon is referred to as 'seeding at implantation' and also causes early lymph node metastases [77], which decreases the survival rate of the rabbits and may be a disadvantage in studies aiming to evaluate treatment modalities for locoregional intra-arterial tumor therapy. Furthermore, during injection of a tumor cell suspension into a site in the oral cavity, the exact depth of injection is difficult to gauge, with tumor cells spreading out uncontrollably within the area of injection. In addition, limitation of mouth opening due to anesthesia and the limited working field lead to additional difficulty in the intraoral injection of a tumor cell suspension, and the dead cells injected may have an immunization effect, stimulating an effective immune response that could prevent tumor growth.

VX2 model for head & neck squamous cell carcinoma

VX2 cells have been used to successfully produce squamous cell carcinomas in rabbits in the head and neck region [78–80], including the oral cavity [17,79,81,82]. Furthermore, a cervical lymph node metastasis model of pyriform sinus VX2 carcinoma of rabbits was recently reported by Shen and coworkers, who observed deep cervical lymph node metastasis in all rabbits after 14, 21 and 28 days of VX2 tumor transplantation [83]. In addition, Wu and coworkers performed an interesting experimental study to investigate the feasibility of using indirect computed tomography lymphography as a guide for sentinel lymph node biopsy in VX2 lingual carcinoma with cervical lymph node metastasis [84]. Lingual VX2 carcinoma with metastasis to the cervical lymph node was induced in rabbits by injecting a suspension of VX2 carcinoma into the lingual submucosa. Sentinel lymph node biopsy was then performed under the guidance of indirect computed tomography lymphography, and lymph node identification was attained by indirect computed tomography lymphography combined with the injection of blue dye in the rabbits. The tongue sentinel lymph nodes were identified preoperatively by indirect computed tomography lymphography, and the blue-stained sentinel lymph nodes were visualized intraoperatively. The authors reported that indirect computed tomography lymphography may be valuable for guiding sentinel lymph node biopsies in cases of lingual carcinoma. Employing a combination of indirect computed tomography lymphography and blue dye injection may improve the preoperative and intraoperative identification of the sentinel lymph node [84]. In addition, Taniguchi and colleagues studied the relationship between malignant tumor tissue and nerve fibers using transplanted VX2 carcinoma in the tongue of the rabbit in 1995, and found that in most of the neural tissue, as well as other host tissues, serious degenerative changes were observed as the transplanted tumor grew [82]. These degenerative changes were mainly limited to the site anterior to the tumor, suggesting that these changes were possibly irritated by the compressive effects of the quickly growing transplanted tumor. These results could, perhaps, be applied

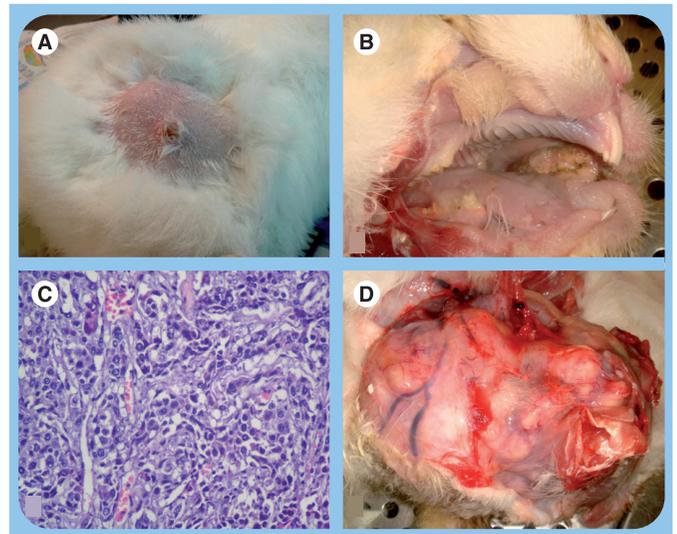


Figure 3. VX2 model for buccal carcinoma. (A) A hind paw tumor is formed through propagation of the VX2 line *in vivo* via intramuscular passage in the hind paw of the rabbit. **(B)** An induced ulcerated buccal rabbit carcinoma. **(C)** The induced buccal tumors are moderately-to-poorly differentiated squamous cell carcinomas (hematoxylin and eosin stain, $\times 200$). **(D)** Cervical lymph node metastases of a rabbit with induced buccal squamous cell carcinoma.

to attempt to elucidate clinical symptoms such as paralysis or the disturbance of tongue movement occasionally noted in some patients with lingual carcinomas [82].

In 1989, Jefferis and Berenbaum demonstrated that the rabbit VX2 tumor could be used as a model for carcinomas of the tongue and larynx, with a tumor uptake rate of 92.6% (25 out of 27 individuals), but the condition of cervical lymphadenopathy was not discussed [79]. In 2002, Chikui and colleagues were also successful in inducing squamous cell carcinomas of the floor of the mouth using the VX2 rabbit cancer model, and 20 metastatic cervical lymph nodes to be detected by ultrasonography were identified; however, the tumor uptake rate was not stated in that study [81]. Most recently, a 100% buccal tumor uptake rate (moderately-to-poorly differentiated squamous cell carcinoma) and a 50% cervical lymph node metastasis rate with a relatively short duration of tumor induction (6 weeks) were observed in our laboratory (FIGURE 3B–D). Remarkably, in one rabbit, mandibular alveolar bone invasion and dental pulp involvement of the cancer cells was also observed. In addition, as all previous studies of the use of the rabbit VX2 cancer model to induce oral squamous cell carcinomas have been performed via intraoral injection of a VX2 tumor cell suspension [78,79,81], in our laboratory a comprehensive extraoral approach was used to induce squamous cell carcinomas of the buccal mucosa via the implantation of a fresh solid VX2 tumor mass beneath the epithelium [17].

The existence of tumor-specific lymphocytes has been reported in both experimental tumor animal models [85] and in cancer patients [86]. Accordingly, we assume that after VX2 tumor transplantation in the oral cavity of the rabbits an early immune

response develops. On the other hand, squamous cell carcinoma of the head and neck has been reported to be associated with impaired cell-mediated immune reactivity [86]. Hence, in line of this viewpoint, with the increasing tumor burden of the rabbit transplanted with VX2 tumor, the cell-mediated immune reaction would be hampered, along with the progression of tumor development. Therefore, stimulation of immune reaction with immune-modulating agents such as IL-2 may possess therapeutic potential. Animal models would aid in evaluating suitable doses, time intervals and site of injections for clinical trials using IL-2. Using the VX2-induced rabbit auricular cancer model, Van Es *et al.* have confirmed a peritumor infiltrate containing macrophages, lymphocytes and granulocytes in all biopsy tissues, suggesting that the effects of tumor cell degeneration and disruption of intratumor angiogenesis are produced [87]. Hence, these findings demonstrated a therapeutic effect of the peritumoral IL-2 treatment regimen for the VX2-induced rabbit auricular cancer model. The main findings from selected literature for the VX2-induced rabbit cancer model for head and neck squamous cell carcinomas have been summarized in TABLE 2.

Molecular imaging of angiogenesis

Angiogenesis is defined as the formation of new blood vessels from pre-existing vasculature [88]. There is an increasing body of experimental evidence indicating that tumor growth and metastasis are reliant on tumor angiogenesis [88–90]. Hence, tumor angiogenesis acts as a promising target for new therapeutic modalities. Nevertheless, only limited investigations have studied the association between the effects of chemotherapy and tumor angiogenesis for oral carcinomas using the VX2-induced rabbit cancer model [91]. Takagi and colleagues recently evaluated the association between blood vessel density and the concentration of free platinum during intra-arterial chemotherapy in experimentally VX2-induced tongue tumors in rabbits. They also elucidated the relationship between blood vessel density and the clinical response of patients who had received intra-arterial chemoradiotherapy of carboplatin. Consequently, this study confirmed a close correlation between blood vessel density and anticancer drugs in tissue. In addition, blood vessel density of the tumor has been a convincing indicator for the effects of chemotherapy for oral carcinoma [91].

Our knowledge of the molecular mechanisms fundamental to angiogenesis has advanced notably over the past decade, leading to the development of antiangiogenic therapy [92–94]. The accomplishment of antiangiogenic therapy has triggered the development of accurate, noninvasive and reproducible molecular imaging to monitor tumor angiogenesis. Most recently, by using the VX2-induced rabbit cancer model (implantation of VX2 tumors into the right thigh muscle of the rabbits), Wang and coworkers have compared 3D computed tomography angiography (3D-CTA) and 4D contrast-enhanced magnetic resonance angiography (4D-CE-MRA) for the *in vivo* monitoring of tumor angiogenesis. They found that tumor angiogenesis could be dynamically monitored *in vivo* by 3D-CTA and 4D-CE-MRA. For these two methods, 3D-CTA had better spatial resolution; however, 4D-CE-MRA provided temporal resolution of tumor angiogenesis [95].

Clinical implications

Most recently, there have been a number of very interesting and significant experimental reports regarding cancer studies related to stem cells, novel treatment regimens, and various kinds of imaging using the VX2-induced rabbit cancer model [96–103].

In 2010, Zhang and colleagues presented interesting data showing that grafted bone marrow mesenchymal stem cells can differentiate into vascular endothelial cells, a process attributed to angiogenesis in the tumor microenvironment, which may be the chief pathway of the promotion of tumor growth in bladder carcinomas in a VX2-induced rabbit cancer model [96]. On the other hand, the anti-tumor effect of docetaxel-loaded lipid microbubbles combined with ultrasound-targeted microbubble activation on VX2 rabbit hepatic tumors was reported by Kang and coworkers in 2010 [97]. The authors claimed that this combined treatment with docetaxel-loaded lipid microbubbles could inhibit the growth of VX2 rabbit liver tumors by deferring proliferation and promoting apoptosis, which may provide a novel targeted strategy for chemotherapy to treat hepatic carcinoma [97]. In addition, Shafirstein and colleagues investigated the potential anti-tumor effect of conductive interstitial thermal therapy to inhibit recurrence and metastasis in New Zealand white rabbits implanted with VX2 tumor intramuscularly in the rear thigh [98]. The authors inferred that the device effectively ablated partially resected VX2 rabbit thigh carcinomas and inhibited recurrence and metastasis in this

Table 2. Summary of the main findings from selected literature on the VX2-induced rabbit cancer model for head and neck squamous cell carcinoma.

Item	Summary of main findings	Ref.
Cancer model	Establishment of the VX2-induced rabbit cancer model for human buccal mucosa squamous cell carcinomas	[17]
	Establishment of the VX2-induced rabbit auricle cancer as a model for head and neck cancer in humans	[78]
	Establishment of the rabbit VX2 tumor as a model for carcinomas of the tongue	[79]
Cancer imaging	Application of the VX2-induced rabbit cancer model for head and neck ultrasonography study	[81]
Immunotherapy	Application of the VX2-induced rabbit cancer model for local and systemic therapeutic effects of the peritumoral IL-2 regimen	[87]
Intra-arterial chemotherapy	Application of VX2-induced rabbit cancer model for the study of blood vessel density correlated with the effects of targeted intra-arterial carboplatin infusion with concurrent radiotherapy	[91]

Table 3. Comparison of some of the features of the hamster buccal pouch cancer model with those of the VX2-induced rabbit cancer model.

Item	Hamster buccal pouch cancer model	VX2-induced rabbit cancer model
Method of tumor induction	Topical application of DMBA onto the mucosa	Implantation of fresh VX2 tumor mass beneath the mucosa or injection of a VX2 suspension
Induction time	Long (12–14 weeks)	Shorter (6 weeks)
Frequency of treatment	Three times a week	Once only
Tumor differentiation	Well-differentiated exophytic tumors; moderately differentiated endophytic tumor	Moderately-to-poorly differentiated
Multiple-site tumor induction	Not applicable	Applicable
Cervical lymph node metastatic study	Not applicable	Applicable
Jawbone invasion study	Not applicable	Applicable
Distant metastatic study	Not applicable	Not applicable
Multistage carcinogenesis study	Applicable	Not applicable

DMBA: 9,10-dimethyl-1,2-benzanthracene.

VX2-induced rabbit cancer model. Conductive interstitial thermal therapy evokes an inflammatory response that may be linked to the mechanism involved in reducing metastatic spread [98]. In 2009, Xiao and colleagues evaluated 5-amino-4-oxopentanoic acid photodynamic diagnosis-guided microsurgery and photodynamic therapy in New Zealand white rabbits implanted with VX2 brain tumors, and reported that photodynamic-guided surgery and photodynamic therapy significantly reduced or delayed local recurrence, enhanced the effectiveness of radical resection and prolonged the survival time of tumor-bearing rabbits [99]. This combination has the potential to be applied as a rapid and highly effective treatment for metastatic brain tumors [99].

Furthermore, a study by Luo and colleagues examined the role of sonography in the implantation process of a VX2 rabbit liver tumor model and evaluated the results, finding that sonographically guided implantation acquired a good success rate with a good inoculation performance. Conventional grayscale, color Doppler, contrast-enhanced pulse inversion harmonic and contrast-enhanced color Doppler sonography have been very helpful in the sequential assessment of tumor growth and characteristic vascularity [100]. In 2010, Choi and coworkers prospectively compared the diagnostic performance of 1.5- and 3.0-T ultrasmall superparamagnetic iron oxide-enhanced MRI techniques for the detection of metastases of the lymph node in VX2 carcinomas transplanted into the thigh, and concluded that 3.0-T imaging demonstrated a higher specificity compared with 1.5-T imaging, without a significant difference in sensitivity in the rabbit VX2 cancer model [101]. In 2009, Liang and coworkers established a reliable rabbit rectal VX2 carcinoma model for the study of rectal carcinoma via injection of a suspension of VX2 cells into the rectal wall under the guidance of x-ray fluoroscopy. In addition, CT scan and MRI were used to observe tumor growth and metastasis in different phases [102]. Histological alterations and survival time of the rabbits have also been recorded [102]. Significantly, in 2009 Liu and colleagues explored the differences between tumor and inflammatory cells using fluoro-18-fluorodeoxyglucose-PET/computed

tomography uptake kinetics by comparing VX2 tumor lesions obtained by transplantation of VX2 tumor cells into one forelimb muscle and inflammatory lesions in rabbits induced by turpentine oil in the contralateral forelimb, and observed differences in the kinetic parameters of inflammatory and tumor lesions [103].

Expert commentary

Potential clinical implications

In view of the potential clinical implications of the VX2-induced rabbit cancer model and hamster buccal pouch cancer model, we conclude that the VX2-induced rabbit cancer model appears to be superior to the hamster buccal pouch cancer model. Due to the small caliber of the branches of the external carotid artery in the hamster, microsurgical techniques are inevitably required for drug delivery if the hamster buccal pouch cancer model is used for studies of intra-arterial chemotherapy. By contrast, in the VX2-induced rabbit cancer model, the caliber of the external carotid artery branches in the rabbit is suitable for the delivery of drugs. In addition, in line with the aforementioned valuable experimental findings [96–103], we proposed that the VX2-induced rabbit cancer model would be applicable for future experimental studies for squamous cell carcinoma of the human oral mucosa, especially for various novel treatment regimens concerning the assessment of antiangiogenic therapy, and different imaging modalities such as molecular imaging of angiogenesis.

Comparison of the two animal cancer models

In this article, we have described two animal cancer models (the hamster buccal pouch cancer model and the VX2-induced rabbit buccal cancer model) that can potentially be employed to study squamous cell carcinoma of the human oral mucosa. The chief characteristics of the hamster buccal pouch cancer model and the VX2-induced rabbit buccal cancer model are summarized and compared (TABLE 3), and we propose that the hamster buccal pouch cancer model and the VX2-induced rabbit buccal cancer model are complementary to one another. The process of induction of buccal

carcinoma using the hamster buccal pouch cancer model is rather time consuming, with carcinomas produced after 12–14 weeks of thrice-weekly DMBA application. Moreover, in contrast with human oral squamous cell carcinomas, the induced squamous cell carcinomas of the hamster buccal pouch mucosa do not always yield infiltrating carcinomas, and only in rare cases is lymph node metastasis to the cervical region observed [59,60]. However, the VX2-induced rabbit cancer model has been reported to induce squamous cell carcinoma successfully in the oral mucosa after only 6 weeks of tumor induction with only one instance of VX2 tumor transplantation. Furthermore, mandibular alveolar bone invasion and tooth pulp involvement of the cancer cells was also noted [17]. All these findings imply that the locoregional, metastatic and bony invasion behavior of VX2-induced rabbit buccal squamous cell carcinomas is stronger than that of the hamster buccal pouch model and more closely reflects human squamous cell carcinoma of the buccal mucosa. Then again, chemically induced carcinomas have merit over transplanted tumors in that the tumors originate in the epithelium of the test animal, despite the fact that the tumors require a significantly longer development time. One inadequacy of the rabbit buccal cancer model is that rabbits do not grow tumors spontaneously in the oral mucosa, and all of the tumors had to be implanted beneath the overlying oral epithelium; however, all of the induced tumors in our laboratory had ulcerated surfaces and were compatible with those of epithelial origin in humans. In addition, the rabbit cancer model is of further merit in that tumors could be implanted at several sites at the same time in the same animal, which is not the case in the hamster buccal pouch model.

Different stages of oral experimental carcinogenesis, from control mucosa to hyperkeratosis, hyperplasia, dysplasia, carcinoma *in situ*, well-differentiated oral squamous cell carcinoma and, finally, moderately differentiated oral squamous cell carcinoma, can be elucidated using the hamster buccal pouch cancer model, while this sequential aspect of tumor production cannot be studied using the VX2-induced rabbit cancer model, because in the latter model a fully developed tumor is usually obtained after a relatively short period of time. In the hamster buccal pouch cancer model, there is additional mortality of the animals due to

the cumulative toxicity of the potent carcinogen (i.e., DMBA), while for the VX2-induced rabbit cancer model, the rabbit may die owing to cancer cachexia accompanied by severe weight loss (which occurs in >10–15% of animals), together with other important signs of tachypnea and cyanosis. Finally, neither animal cancer model is applicable for studies of distant metastasis of squamous cell carcinoma of the human oral mucosa.

Five-year view

The human oral cavity obviously lacks a similar pouch to the hamster, and the hamster buccal pouch mucosa tissue is histologically dissimilar to human buccal mucosa and is significantly thinner, with a single submucosal connective tissue [104]. The hamster buccal pouch cancer model differs in etiology and genetic composition compared with human oral carcinomas. However, despite these anatomic, histologic and genetic variations between hamster pouch mucosa and human buccal tissue, experimental carcinogenesis protocols for the former induce premalignant changes and carcinomas that resemble those that occur during analogous progression in human oral mucosa [60]. The VX2-induced rabbit cancer model, on the other hand, has not been produced *in situ* and hence, its biological behavior is totally reliant on the location of transplantation. As a result, we should be aware that, currently, no animal model is perfectly applicable to human oral squamous cell carcinoma. Nevertheless, with the application of these two animal cancer models simultaneously, oral oncologists or pathologists in future would be able to investigate multiple aspects of the cellular and molecular biological characteristics or evaluate potential new treatment regimens for squamous cell carcinomas of the human oral mucosa.

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Key issues

- Despite some recent reports indicating that the prognosis of oral squamous cell carcinoma has been improved in recent years, the rates of mortality and morbidity in oral carcinoma patients remain to be enhanced further.
- Identification of an adequate animal cancer model is vital in order to study the biologic characteristics of, or evaluate potential new treatment modalities for, squamous cell carcinoma originating in the human oral mucosa.
- The hamster buccal pouch cancer model and the VX2-induced rabbit cancer model are two potential animal models for use in the research of human oral squamous cell carcinomas.
- The most significant advantage of the hamster buccal pouch cancer model is that it can simulate the multistage oral carcinogenesis of squamous cell carcinomas of the human oral mucosa, while its main drawback is that it is not applicable to locoregional metastatic study.
- The most significant advantage of the VX2-induced rabbit cancer model is that it can be used for locoregional metastatic and alveolar bone invasion studies for human buccal squamous cell carcinoma, but it cannot be used to study the multiple stages of oral carcinogenesis.
- The VX2-induced rabbit cancer model has the potential to be used for the future assessment of various novel treatment regimens, as well as different imaging modalities for squamous cell carcinoma of the human oral mucosa.
- The hamster buccal pouch cancer and the VX2-induced rabbit cancer models appear to be complementary to each other, and therefore application of these two animal cancer models simultaneously enables the examination of various aspects of the cellular and molecular biological characteristics of, or the evaluation of potential new treatment regimens for, squamous cell carcinomas of the human oral mucosa.

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