Chapter 12
Preclinical Models of Head and Neck Squamous Cell Carcinoma

Michiel W.M. van den Brekel, C.L. Zuur, Stephen P. Malkoski, and Xiao-Jing Wang

Abstract Model systems are irreplaceable to study cancer. Although the best model system is human cancer in man, research in patients is restricted by ethical and financial restraints. Furthermore, experiments cannot be repeated and patient numbers are limited. Tumor cell cultures are the most versatile system to study cancer cells. They allow for repeated experiments in controlled conditions, are relatively inexpensive and are ideal to study genes, pathways, and tumor response. Mouse models enable to study cancer behavior and carcinogenesis in vivo and many different model systems are available. Apart from xenografts in immune compromised mice, transplantation of oral mice tumors in syngeneic mice, animals developing oral cancer using carcinogen exposure and genetically modified mice can be used. All these models, however, have advantages and limitations that will be discussed in this chapter.

Keywords Squamous cell cancer • Mouse • Model system • Xenograft • Cell culture • Head and neck • Transgenic

Head and neck squamous cell carcinomas (HNSCCs) represent 3–5% of all newly diagnosed cancers each year in the western world with 5-year survival rates in the order of 25–95% depending on disease site and stage. The limited survival rates in most patients indicate the need for novel treatment strategies with new potent drugs. In addition, these survival rates yield a widely divergent individual response of similar histopathological cancers to the applied treatment regimen. Currently, the decision on therapy relies mainly on the outcome of both retrospective data as well as various well-performed prospective trials and meta-analyses. However, so far no prospective trial has been conducted using biomarkers for treatment selection and thus we have not been able to stratify patients based on individual tumor properties since knowledge regarding the biological basis of variations in tumor response to chemotherapeutics was and is still limited.

HNSCCs are characterized by a rather large genetic diversity, possibly caused by the long duration of carcinogenic exposure and the genetic instability of most head and neck carcinomas [1]. However, several pathways are almost always invariably involved in carcinogenesis, such as the P53 and INK4a pathways [2, 3]. The fact that these tumors are genetically highly heterogeneous and instable has hampered the development of drugs specifically targeting pathways relevant in head and neck cancer. So far, only the inhibition of the EGFR receptor has proven to have clinical benefit for a subpopulation of head and neck cancer patients, especially when combined to radiotherapy [4]. However, in preclinical models, several other targeted therapies have shown promising results, such as drugs targeting phosphoinositol (PI)-3-kinase–AKT, insulin-like growth receptor, BCL2, MET, and several others [5–8].

Although the role of human papilloma virus (HPV) was postulated a long time ago, only recently it was recognized that HNSCCs can be divided really into those that are and those that are not associated with HPV [9]. The HPV16 papillomavirus oncogenes E6 and E7 have been detected in HPV genomes in HNSCC and its oncoproteins are known for their ability to bind and inactivate tumor suppressor proteins p53 and retinoblastoma (pRb) [10]. Tumors with HPV infection occur at a younger age, are less related to smoking and alcohol and do not have P53 mutations or loss of P16 (INK4a) function by mutations, deletions, or methylation. Instead, these pathways are deregulated by the E4 and E6 proteins expressed by the virus [11]. HPV status does not only predict treatment outcome, but likely, should also be used to guide treatment [12]. However, as stated above, at the moment we lack the knowledge and reliable trials to personalize treatment regimens in HNSCC.

Studying cancer in humans poses enormous ethical, financial, and practical hurdles, due to the limited number of patients and tumor material, the enormous costs, and the ethical dilemmas in clinical research. Therefore, preclinical

M.W.M. van den Brekel (✉)
Otorhinolaryngology – Head and Neck Surgery Department, Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital and Academic Medical Center, University of Amsterdam, Plesmanlaan 121, Amsterdam 1066 CX, The Netherlands
e-mail: vd.brekel@nki.nl

J. Bernier (ed.), *Head and Neck Cancer: Multimodality Management*,
DOI 10.1007/978-1-4419-9464-6_12,

models are an important tool for exploring tumor initiation and progression, cancer genetics, and novel therapeutic approaches. A variety of HNSCC model systems have been developed, including cancer cell lines derived from human HNSCC, exposure of animals to oral carcinogens, and genetically engineered mouse models (GEMMs). Each system has important strengths and weaknesses that must be appreciated to interpret data derived from these models. To maximize clinical relevance, model systems should resemble human HNSCC as closely as possible. For example, cell lines should harbor the genetic and epigenetic alterations common to HNSCC and carcinogen exposures should mimic the routes and chemicals associated with human HNSCC. Similarly, GEMMs should examine the genetic alterations frequently observed in human HNSCC. To overcome the intrinsic limitations of a given model, results should be validated by multiple approaches in different systems; however, ultimately, all results obtained in model systems must be validated in human samples or subjects.

HNSCC Cell Lines

Cancer cell lines are the most versatile model system wherein cancer cells can be characterized and even manipulated genetically. Genetic manipulation techniques have enabled us to study the influence of specific genetic abnormalities or correction of these abnormalities on tumorigenesis, tumor behavior, or treatment response [5]. Using these techniques, one can study human genes in mouse cell lines as, e.g., the influence of known mutagens on human P53 [13]. In addition, it has been shown that differences in response can be attributed to differences in the genetic make-up of HNSCC cell lines, them being either HPV positive or negative [8] and Li et al. showed that by blocking SRC kinase, cetuximab resistant tumors can become sensitive again [14]. Such experiments, that can only be done using well-characterized cancers, can give us insights that in the near future may lead to more individualized and more effective treatment protocols. Although in HNSCC the routine use of molecular markers for treatment selection is not established yet, in several other tumor types such as breast, colon, and lung, this molecular knowledge has already been translated into important predictive assays used in treatment selection [15, 16]. Currently, there is a strong urge to find, validate, and implement markers for a more individualized treatment selection in HNSCC.

A major advantage of cell lines is that experiments can be done within several days and are relatively inexpensive. Using cell lines, the same tumors can be tested over and over again with multiple new drugs, combinations of drugs, or genetic interventions. This enables testing numerous radiotherapy doses or drugs on the same tumor as well as the mechanisms or conditions by which tumors become resistant to treatment. These mechanisms can then be targeted to avoid resistance. For instance, in human HNSCCs, ligand activation of the epidermal growth factor receptor (EGFR) leads to downstream signaling of several prosurvival cascades (pathways) eventually promoting cell proliferation, angiogenesis, invasion, and metastasis. Therefore, EGFR is one of the most promising molecular targets in cancer therapy. Recently, Li et al. described several mechanisms of acquired resistance of SCC to cetuximab (an EGFR-blocking antibody) and performed research to investigate the role of nEGFR (EGFR translocated from plasma membrane to nucleus) in this phenomenon using nonsmall cell lung carcinoma cell lines. In these cells, an increased Src family kinase (SFK) activity was found, linked to the translocation of the EGFR to the nucleus, suggesting that a combined modality treatment regimen of blocking SFKs together with cetuximab may be a future clinical trial treatment design for patients with EGFR resistant tumors [14]. In the field of studying radiotherapy and radiosensitization in head and neck cancer, much work has been done using cell lines [17]. It has been shown that hypoxia, DNA repair, and repopulation, as well as intrinsic tumor cell characteristics play an important role in radioresistance [18, 19].

The role of HPV has also been studied in head and neck cell lines. Mouse tonsil epithelial cell lines (MTECs) become immortalized by HPV 16 E6–E7 transfection and allow for extensive research to determine what viral genes are required for this immortalization and anchorage-independent growth and, eventually, malignant growth in vivo. Hoover et al. in 2007 reported that HPV viral oncogenes alone were indeed sufficient to induce anchorage-independent growth of MTECs in vitro, but additional H-ras oncogene expression was needed to form invasive cancers in vivo [20].

However, cancer cell lines also have critical limitations. Most importantly, they represent a homogeneous clonal population capable of growing in vitro; in fact, the majority of individual tumors and cancer cells within an individual tumor are incapable of growing in tissue culture. Hence, cultured cells typically fail to reflect the genetic heterogeneity of the native tumor from which they were derived. Interestingly, patients whose tumors can establish cell lines have worse clinical prognosis [21], suggesting that characteristics supporting in vitro growth are indicative of aggressive tumor behavior in vivo. Furthermore, as cells are passaged, there is increased selective pressure for in vitro growth and after many passages, cultured cancer cells may differ from the original tumor from which they were derived. For example, tumor lines and native tumors may exhibit different chemosensitivity patterns and this can be influenced by the number of in vitro passages [22–24]. Culture conditions can also influence the responses to cytotoxic therapies; e.g., cells

grown as anchorage-independent spheroids can have different responses to cytotoxic agents than the same cells grown as anchorage-dependent monolayers [25]. Accordingly, cell lines cannot be used to predict treatment response in individual patients [26]. Many of these issues have potentially been accentuated in HNSCC as there is a relative paucity of well characterized lines [21, 27], and a lack of standardized tissue culture techniques that can limit reproducibility [28–34]. A final important limitation of cultured cells is an inability to study the interactions between tumor epithelial cells and key components of the tumor stroma, including fibroblasts, immune cells, and the vasculature.

Despite these limitations, much of our basic mechanistic understanding of the roles of specific molecules has been derived from cell culture data. Perhaps the best successful example of basic biologic understanding directly improving cancer outcome occurred in chronic myelogenous leukemia (CML) where the observation that inhibition of the bcr–abl fusion protein reduced growth of leukemic cell lines led to the successful clinical deployment of imatinib [35, 36]. Unfortunately, because HNSCC appears to be more genetically heterogeneous than CML, HNSCC may not be susceptible to inhibition of a single oncogenic pathway [1]. It is probable that using a combination of drugs, inhibiting several pathways, such as EGFR inhibitors in combination with, e.g., blockade of the PI-3-kinase–AKT pathway, insulin-like growth factor receptor (IGFR), BCL2, or cMET holds promise for the future and has been studied in preclinical models [5–8].

Although cell lines are the optimal system to study pathways and the role of specific genes for carcinogenesis and treatment response, it has proven very difficult to find reliable markers from cell line experiments. We recently studied radioresistance in cell lines obtained from Grenman in Turku (Finland) and made a gene expression profile correlating with radiosensitivity [19, 37, 38]. Unfortunately, this expression profile was not predictive of local control after radiotherapy of laryngeal cancer in patients. This again shows the difficulty of extrapolating in vitro findings to clinical practice.

Short Term Cultures

As cell lines are difficult to establish, as they represent only a fraction of a tumor and as in every passage additional genetic changes occur, cell lines cannot be used to guide treatment of an individual patient. To augment individual treatment planning, short-term culturing techniques are used. In this technique, a small tissue biopsy sample that includes both tumor epithelial cells and tumor stromal cells (e.g., fibroblasts) is cultured and then tested for sensitivity to chemotherapeutic agents in vitro. In this setting, it appears that a single biopsy (100 mg) sample is representative of the entire tumor with respect to chemoresponsiveness [39] and that coculture of tumor and stromal cells increases the predictive value of this assay [40] (Fig. 12.1). One technical difficulty of this approach is the overgrowth of the fibroblast subpopulation; however, this can be overcome by avoiding enzymatic digestion and allowing both tumor epithelial and stromal cells to grow out of multicellular tumor particles. Short-term cultures can also be grown on a collagen sponge-gel-supported matrix to maintain tissue architecture and facilitate cell–cell interactions that may be important in chemotherapy response. Using these systems, a culture sufficient for in vitro drug testing can be established 80% of the time [41, 42] and in vitro testing can occur within a few days. In addition, short-term cultures can be subsequently used to establish xenograft models if desired (see below) [43].

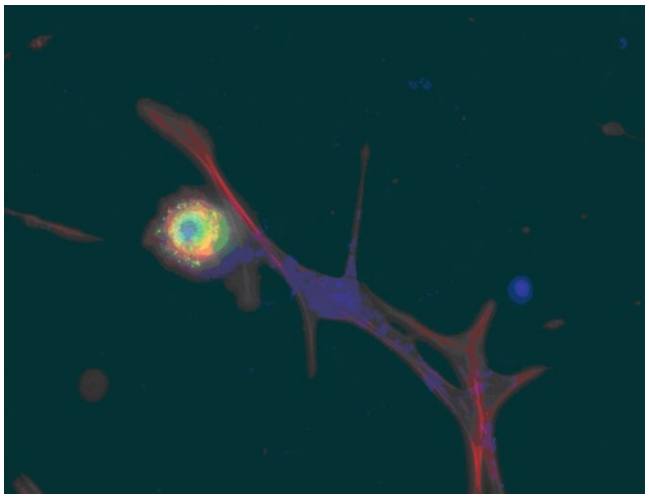

Fig. 12.1 Digital immunofluoroscopy of short-term cultured cells from a oropharyngeal carcinoma A tumor cell and fibroblast are shown (64×). Staining: cell nucleus: Hoechst staining double-stranded DNA (*blue*); cell actin: Alexa Fluor® 568 phalloidin (*red*); SCC cytokeratin: Mouse anti-human pan Cytokeratin and Alexa Fluor® anti-mouse IgG 568 (*green*)

In these experiments, the typical read out is cell count or proliferation after treatment with a cytotoxic agent [40, 44, 45], however, qualitative data, such as cell cycle arrest, differentiation, and morphology can also be collected to assess the specific response of the tumor and stroma cells to the (cancer) drug applied. For mass screening the HTS immunofluorescent automated microscopy with high-content imaging is possible using the CellProfiler program [46].

The tumor clonogenic assay is one of the most intensely studied in vitro methods for chemosensitivity testing and evaluates colony formation of cancer (stem) cells with the potential for anchorage-independent growth in semisolid media in which individual cells develop into colonies. Comparing response to drugs in this clonogenic assay and in patients, Fiebig et al. found that 62% of the comparisons for drug sensitivity and 92% of the comparisons for drug resistance were

correct [41]. In further experiments in head and neck cancer using the histoculture drug-response assay, the correlation between clinical response to induction chemotherapy and the prediction in the assay was almost 78% [45].

An alternative method for short-term cultures is a collagen sponge-gel-supported histoculture in which tissue architecture is maintained. The architecture allows more cell–cell interaction which might be important in chemotherapy response. A histoculture drug response assay for individualizing chemotherapy has been developed and proved to be very predictive. As an end-point, the MTT assay can be used [40, 44, 45]. Comparison between all these culture methods has not been performed.

Although small patient numbers were used, primary tumor cell culture models have been shown to predict the individual tumor sensitivity for different cancer drugs [45, 47–49]. However, there are so far no phase III studies demonstrating a significant increase in survival rates compared to empirically determined standard chemotherapy regimens. Therefore, the tissue culture has not yet found a routine role in the individualization of patient therapy.

Xenograft Mouse Models

Another approach for amplifying, studying, and testing tumors in vivo is to subcutaneously implant human cancers into immunologically compromised nude or SCID mice. Depending on the original tumor subsite (oral cavity, oropharynx, larynx, and hypopharynx), 70–80% of the patient HNSCCs were successfully xenografted and short-term cultured in the mouse [43]. Once established, the system allows for in vivo testing of novel cancer drugs, as studying the response of human tumors in subcutaneous, e.g., ectopic tissue sites of the mouse to the various cancer drugs applied may produce relevant and predictive information to the clinic, provided that pharmacokinetic parameters (especially dosing) are employed. Alternatively, orthotopic transplantation is suggested to facilitate metastatic spread thereby increasing the models' clinical predictive value as various drugs can then be tested on either (or both) the primary tumor growing in a physiologically relevant site and distant metastatic disease, especially in case therapy is initiated at the point when metastases are macroscopic in nature [50].

To study the changes in gene expression with transformation and metastatic tumor progression of squamous cell carcinomas, oral tumors and cell lines derived from mice were transplanted into inbred syngeneic recipients [51]. Other examples include the oral SCC VII/SF cell line (from C3H/HeJ mice) and the transformed PAM 212 cell line (from BALB/c keratinocytes) [52] and similar models have been described using a hamster buccal pouch carcinoma of rat oral carcinoma [53, 54]. In addition, tumor cell lines can be manipulated ex vivo then transplanted to study the roles of specific molecules or pathways during tumor progression and metastases. For example, while induction of HPV genes E6 and E7 can immortalize mouse tonsil epithelial cells in vitro, additional H-ras transduction is necessary to form invasive cancers [20].

Compared to cell lines, direct xenograft mouse models preserve key features that cells in culture derived from the same tumor samples irreversibly lose [55], perhaps by preserving the human stroma and immune cells important for tumor growth and metastases [56].

Xenografts derived directly from patient biopsies, with minimal in vitro manipulation, appear to retain better the morphological and molecular marker of the source tumors, despite serial passing across several generations of mice [57]. In addition, human tumors can be serially transplanted into other immunocompromised mice providing additional tumor material for downstream molecular or cellular analysis or additional tumor-bearing mice for in vivo testing of therapeutic compounds. These systems may be better suited for studying invasiveness and metastases than cell culture systems [58–60], particularly if coupled with evolving imaging techniques such as micro PET-CT.

However, because the xenograft model involves implanting human tumors cells, it cannot be used to study early stage carcinogenesis, tumor initiation, or chemoprevention. Also, when tumors are transplanted they will still require angiogenesis and supporting tumor stroma from the murine host, and as recipient mice are immunocompromised, this model is not suitable for evaluating tumor immunology. Moreover, agent metabolism and pharmacodynamics are different in mice, and, as with immortalized cell lines, serial passaging of tumor xenografts can change the tumor characteristics by selecting for tumor cell populations suited to growing in an immunocompromised host [61, 62]. Finally, compared to experiments with cell lines, xenografting experiments are more time-consuming and expensive.

Cancer Induction by Chemical Carcinogens

Mice, rats, and hamsters can be exposed to carcinogens to induce cancer. Although exposures can be laborious and time-consuming, these models are especially useful to study carcinogenesis and chemopreventive strategies as there is usually a long latency between exposure and tumor development and animals frequently develop premalignant lesions [63]. Depending on the mutagen, exposure route, and dose, oral tumors with different genetic alterations and behaviors can be produced. Like human HNSCC, chemically induced HNSCC harbor a variety of genetic lesions, however, chemically

induced tumors are typically more homogenous than their human counterparts as animals are only exposed to one carcinogen that produces characteristic genetic alterations as opposed to being exposed to a complex mixture of compounds each with different genotoxic effects (i.e., cigarette smoke) [64, 65]. Finally by applying carcinogenesis protocols to genetically engineered mice, the specific roles of molecules and pathways in promoting or inhibiting tumor initiation, growth, or metastases can be assessed.

One well-characterized HNSCC model is application of the H-ras mutagen 7,12-dimethylbenz(a)anthracene (DMBA) to the hamster buccal pouch [66, 67]. In this model, oral DMBA is applied three times weekly for 10–24 weeks. Squamous cell carcinomas will occur in the majority of the hamsters, and lymph node metastases are sometimes found [68]. Because these tumors are almost all H-ras initiated, they do not typically have the genetic instability seen in human HNSCC where chromosome breaks and aneuploidy are frequent. Nonetheless, this is a clinically relevant genetic alteration as HNSCC arising in Asian patients is frequently initiated by activated Ras signaling [69]. One downside of this model is that the tools and reagents for hamsters are more limited than those for mice, however, DMBA can also be used to induce skin SCC in mice (when combined with tumor promotion by a phorbol ester) or to induce oral SCC in genetically susceptible animals [70]. Although DMBA is not a tobacco carcinogen, it is a convenient way of introducing H-ras mutations to the oral epithelium to evaluate the interactions of other experimental systems on Ras-initiated tumors.

In a study of Chang et al. in 2000, *N*-methyl-*N*-benzylnitrosamine (MBN) was applied to hamster buccal pouches to characterize the MBN-induced tumors with regard to the frequency of p53 and H-ras mutations, as these are among the specific molecular alterations observed in human HNSCCs [71]. In this analysis, the alterations in p53, H-ras, and telomerase activity observed in the model are similar in many respects to the analogous human lesions of the head and neck, suggesting that this model system may be particularly useful for the development of cancer chemoprevention regimens and cancer therapies.

Rats and mice also develop oral squamous cell carcinomas after application of the chemical carcinogen 4-nitroquinoline *N*-oxide (4-NQO) for 2–6 months in their drinking water or application in a concentrated solution to the oral cavity for 12–16 weeks [72]. 4-NQO, although not a natural tobacco derivative, causes a spectrum of DNA damage similar to that caused by tobacco-associated carcinogens. In addition, in p53 transgenic mice, the incidence of oral cancer was increased from 0 to 67% when treated with 4-NQO thrice weekly for 16 weeks and a maximum follow-up of 32 weeks [73]. Also in HPV16-transgenic mice treated with 4-NQO, the incidence of SCC was increased significantly compared to their nontransgenic counterparts and histopathological analyses demonstrated progressive neoplastic disease in the oral cavity with remarkable similarities to human HPV-positive HNSCC. Using this model, the investigators reported to have identified a biomarker that distinguishes between HPV-positive and HPV-negative HNSCC [74].

The 4-NQO-induced cancer model has also been used to induce salivary gland cancer [75]. Furthermore, other carcinogens can be used, such as benzo[a]pyrene (B[a]P), *N*-nitroso-*N*-methylurea (NMU), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), or nitrosonornicotine (NNN). The oral cavity of hamsters, rats, and mice or the skin of these animals can be used to induce cancer [65, 76, 77].

As these animals first develop premalignant lesions, these models are especially useful to study inhibitory (chemopreventive) or promoting stimuli. It has been shown that DMBA-induced carcinogenesis in the hamster cheek pouch can be counteracted by long-term (18 weeks) topical application of GW2974, a dual inhibitor of EGFR and ErbB2 tyrosine kinase, decreasing the incidence, number, and size of both visible tumors and microscopic lesions such as hyperplasia, dysplasia, and SCC significantly [78]. In another study, celecoxib (a highly selective inhibitor of cyclooxygenase (COX)-2, known to be overexpressed in human (pre)malignant oral lesions) was applied for 7 weeks in the oral cavity of hamsters after they were painted for 5 weeks with DMBA. Celecoxib was effective in delaying the onset of early lesions and able to slow down the growth of the oral tumor [79]. The antilipidperoxidative and antioxidant potential of curcumin and piperine were reported to be crucial in the biochemical mechanistic pathway of their chemoprevention in DMBA-induced oral carcinogenesis [80, 81].

A major improvement of using carcinogen induced cancer has been made by using these carcinogens in genetically predisposed mice of cell lines, such as the P53 knock-out mice, or the HPV 16 E–E7 transfected immortalized oral cell lines [73, 74, 82]. Using carcinogens in these models causes cancer or transformation in a much faster and controlled way [83]. Using this model system, one can study the role of different genes on carcinogenesis. Apart from P53, also Xeroderma pigmentosa A (XPA) knock-out or cycline D1 overexpression have been shown to increase and accelerate oral cancer formation in 4-NQO-treated mice [84, 85].

Transgenic Mouse Models

GEMMs have been an enormous step forward for cancer modeling and allow evaluation of discrete genetic alterations in specific organs in vivo in an immunocompetent animal. Additional benefits of GEMMs include the ability to evaluate how multiple genetic defects interact to promote or inhibit

cancer and the opportunity to evaluate whether specific targeted therapies are active against tumors with a defined genetic composition. Drawbacks are that human cancers are more genetically complex and heterogeneous than tumors produced in mouse models and differences in the human and mouse immune systems may complicate studies of tumor immunology.

Advances in murine embryology and genetics initially facilitated targeted mutagenesis of the mouse germ line by homologous recombination in ES cells leading to the creation of classic "knock out" mice. If a genetic modification is not lethal during embryonic development, heterozygotes can be crossed to create mice homozygous for a particular gene deletion. While knockout mice can occasionally be used to study deletion of tumor suppressors, there are critical limitations to this approach. First, global gene deletion of putative tumor suppressors is frequently embryonic lethal and this prevents the assessment of many genes using this strategy. For similar reasons, it is difficult, if not impossible, to study combinations of genetic modifications using this technique [86]. In addition, because the genetic modification is present in all tissues, tumors can develop in multiple anatomic locations, potentially hindering study of the tumor of interest. Finally, the fact that tumor stromal cells (fibroblasts, immune cells, and vasculature) also harbor the same genetic modification can impact overall tumor behavior in unanticipated ways. So far, germ-line deletions have not provided HNSCC specific insight. A step toward conditional mutagenesis is to place oncogenes under control of a tissue-specific promoter. Examples are K14-HPV16 mice that express E6/E7 in K14 expressing cells. These mice develop hyperplasia and some strains also oral SCCs [87]. Recently, also AKT activation or Ras activation in combination with loss of P53 has been shown to induce oral cancer [88, 89].

With the development of conditional genetic manipulation systems, many of these problems have been overcome [90]. In these systems, a target gene is flanked by loxP restriction sites that are the target of the *Escherichia coli* bacteriophage P1 Cre recombinase; Cre recombinase then excises sequences between loxP sites, allowing conditional gene deletion. As animals harboring conditional alleles are phenotypically normal in the absence of genetic recombination mediated by Cre recombinase, this system avoids many of the problems of embryonic lethality or infertility associated with germ-line deletions. By placing a loxP-flanked stop codon upstream of an oncogene (e.g., $Kras^{G12D}$), this approach can also be used to "knock-in" tumor initiators [91, 92] or specific p53 mutations.

Tissue restricted genetic manipulation is achieved by delivery of Cre recombinase to the cells of interest. While this can be done with adenoviral vectors [91], this approach has largely given way to transgenic approaches that use a tissue-specific promoter to target Cre recombinase expression to the cells of interest [93]. In this setting, genetic manipulation then occurs only in cells that express the targeted Cre recombinase transgene. The Epstein–Barr virus ED-L2 promoter as well as keratin 5 (K5) and keratin 14 (K14) promoters have been used to target gene manipulations to the oral epithelium [94–98], however, because keratins are robustly expressed in a variety of epithelial tissues, especially the skin and mammary tissue, an additional layer of control is required to restrict Cre recombinase expression to the head and neck epithelium. This is achieved by using a ligand-inducible Cre recombinase fusion protein whose expression is restricted by a K5 or K14 promoter. Currently available constructs include both tamoxifen-inducible truncated estrogen receptor fusions, such as $K14CreER^{T}$ and $K5CreER^{T2}$ [93, 95] (Figs. 12.2 and 12.3); and RU486-inducible truncated progesterone receptor fusions, such as K14CrePR or K5CrePR [99], although only the CrePR constructs have so far been used to generate mouse models of HNSCC [96]. Another system in which genes can be turned on and off is the tetracycline-inducible system (tet-on and tet-off receptor) targeted to epithelial cells combined with oncogene under the control of tet-regulated responsive elements. On doxycycline administration the oncogene can be expressed [100, 101]. The main advantage of ligand-dependent systems is that they allow tissue-specific, spatial, and temporal control of recombination. Because these systems can be used to introduce

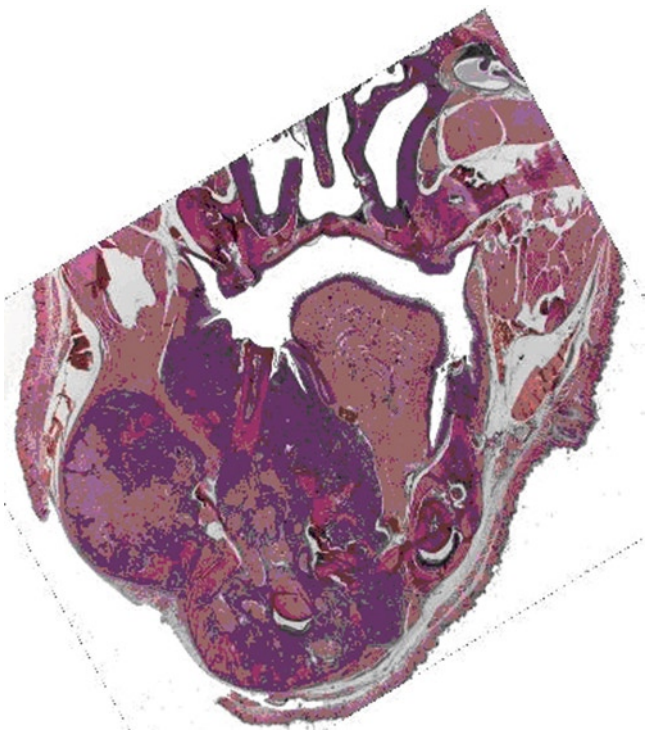

Fig. 12.2 Coronal histopathological section through the mouth and nose of a K14P53FF transgenic mouse (nonfunctioning P53 in all K14 expressing cells). Some of these animals develop oral squamous cancer as visible on the right side around the mandible

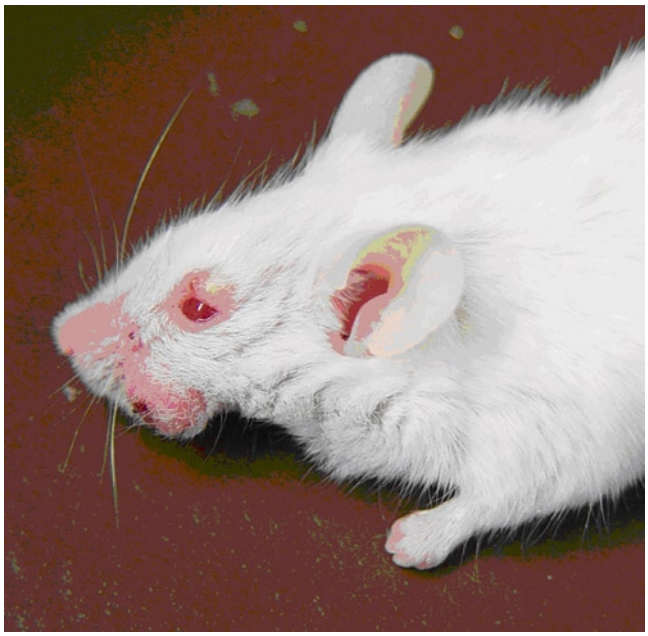

Fig. 12.3 Transgenic mouse with K14P53FF and P16 knockout with a cheek cancer

multiple somatic genetic alterations simultaneously into a target tissue interactions between different oncogenes and tumor suppressors can be evaluated in vivo. Disadvantages of this system are that most inducible Cre recombinase systems have some level of background activity and toxicity, and that there may be variability in recombination efficiency for different genes, partially related to the distance between LoxP sites [102, 103]. Apart from using tissue-specific promoters and ligands, an alternative is to use an adenoviral vector: adenoCre. This system is used in pulmonary cancer [91].

GEMMs can be used to test whether alterations in specific pathways or combinations of pathways are sufficient for HNSCC development and the mechanisms by which specific molecular alterations contribute to HNSCC development [96, 98, 104]. For example, although knock-in of oncogenic $Kras^{G12D}$ in the oral cavity causes benign papilloma formation [97], simultaneous deletion of transforming growth factor beta type II receptor (TGFbetaRII) with $Kras^{G12D}$ activation causes full penetrance HNSCC [70]. Thus it appears that Kras activation functions as a tumor initiator while defective TGFbeta signaling causes tumor progression, especially as TGFbetaRII deletion in the oral epithelium does not cause HSNCC. Interestingly, in contrast to TGFbetaRII, Smad deletion in the oral epithelium causes spontaneous HNSCC, suggesting that although both these molecules are components of the TGFbeta signaling pathway that they have distinct nonoverlapping functions in HNSCC [96, 104]. GEMMs can also be used to suggest novel therapeutic avenues. For example, HNSCC induced by Smad4 deletion have increased genetic instability and may hence be more susceptible to either ionizing radiation or poly(ADP-ribose) polymerase (PARP) inhibitor-induced cell death. Given that Smad4 expression is frequently reduced in HNSCC this may have substantial clinical implications [96].

There is a great need to develop more reliable and different GEMMs for HNSCCs. When these are established, they should be validated for predicting treatment responses in human HNSCCs. Also the influence of different genetic make-up on tumor behavior and treatment response is an important aspect to be studied. So far HNSCC model systems are not as well developed as breast cancer models and pulmonary cancer models. It is a challenge for the next years to catch up with these research fields.

References

1. Reshmi SC, Saunders WS, Kudla DM, Ragin CR, Gollin SM. Chromosomal instability and marker chromosome evolution in oral squamous cell carcinoma. Genes Chromosom Cancer. 2004;41:38–46.
2. Leng K, Schlien S, Bosch FX. Refined characterization of head and neck squamous cell carcinomas expressing a seemingly wild-type p53 protein. J Oral Pathol Med. 2006;35:19–24.
3. Perez-Ordonez B, Beauchemin M, Jordan RC. Molecular biology of squamous cell carcinoma of the head and neck. J Clin Pathol. 2006;59:445–53.
4. Bonner JA, Harari PM, Giralt J, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med. 2006;354:567–78.
5. Crowe DL, Sinha UK. p53 apoptotic response to DNA damage dependent on bcl2 but not bax in head and neck squamous cell carcinoma lines. Head Neck. 2006;28:15–23.
6. Wilsbacher JL, Zhang Q, Tucker LA, et al. Insulin-like growth factor-1 receptor and ErbB kinase inhibitor combinations block proliferation and induce apoptosis through cyclin D1 reduction and Bax activation. J Biol Chem. 2008;283:23721–30.
7. Seiwert TY, Jagadeeswaran R, Faoro L, et al. The MET receptor tyrosine kinase is a potential novel therapeutic target for head and neck squamous cell carcinoma. Cancer Res. 2009;69:3021–31.
8. Gupta AK, Lee JH, Wilke WW, et al. Radiation response in two HPV-infected head-and-neck cancer cell lines in comparison to a non-HPV-infected cell line and relationship to signaling through AKT. Int J Radiat Oncol Biol Phys. 2009;74:928–33.
9. Park NH, Li SL, Xie JF, Cherrick HM. In vitro and animal studies of the role of viruses in oral carcinogenesis. Eur J Cancer B Oral Oncol. 1992;28B:145–52.
10. Chung CH, Gillison ML. Human papillomavirus in head and neck cancer: its role in pathogenesis and clinical implications. Clin Cancer Res. 2009;15:6758–62.
11. Hafkamp HC, Mooren JJ, Claessen SM, et al. P21 Cip1/WAF1 expression is strongly associated with HPV-positive tonsillar carcinoma and a favorable prognosis. Mod Pathol. 2009;22:686–98.
12. Fallai C, Perrone F, Licitra L, et al. Oropharyngeal squamous cell carcinoma treated with radiotherapy or radiochemotherapy: prognostic role of TP53 and HPV status. Int J Radiat Oncol Biol Phys. 2009;75:1053–9.
13. Liu Z, Muehlbauer KR, Schmeiser HH, Hergenhahn M, Belharazem D, Hollstein MC. p53 mutations in benzo(a)pyrene-exposed human p53 knock-in murine fibroblasts correlate with

p53 mutations in human lung tumors. Cancer Res. 2005;65: 2583–7.
14. Li C, Iida M, Dunn EF, Ghia AJ, Wheeler DL. Nuclear EGFR contributes to acquired resistance to cetuximab. Oncogene. 2009;29:3801–13.
15. Zwart W, Griekspoor A, Rondaij M, Verwoerd D, Neefjes J, Michalides R. Classification of anti-estrogens according to intramolecular FRET effects on phospho-mutants of estrogen receptor alpha. Mol Cancer Ther. 2007;6:1526–33.
16. Gerber DE. EGFR inhibition in the treatment of non-small cell lung cancer. Drug Dev Res. 2008;69:359–72.
17. Begg AC, van der Kolk PJ, Dewit L, Bartelink H. Radiosensitization by cisplatin of RIF1 tumour cells in vitro. Int J Radiat Biol Relat Stud Phys Chem Med. 1986;50:871–84.
18. Begg AC, Vens C. Genetic manipulation of radiosensitivity. Int J Radiat Oncol Biol Phys. 2001;49:367–71.
19. Eschrich SA, Pramana J, Zhang H, et al. A gene expression model of intrinsic tumor radiosensitivity: prediction of response and prognosis after chemoradiation. Int J Radiat Oncol Biol Phys. 2009;75:489–96.
20. Hoover AC, Spanos WC, Harris GF, Anderson ME, Klingelhutz AJ, Lee JH. The role of human papillomavirus 16 E6 in anchorage-independent and invasive growth of mouse tonsil epithelium. Arch Otolaryngol Head Neck Surg. 2007;133:495–502.
21. Pekkola K, Raikka A, Joensuu H, Minn H, Aitasalo K, Grenman R. Permanent in vitro growth is associated with poor prognosis in head and neck cancer. Acta Otolaryngol. 2004;124:192–6.
22. Engelholm SA, Vindelov LL, Spang-Thomsen M, et al. Genetic instability of cell lines derived from a single human small cell carcinoma of the lung. Eur J Cancer Clin Oncol. 1985;21:815–24.
23. Ferguson PJ, Cheng YC. Phenotypic instability of drug sensitivity in a human colon carcinoma cell line. Cancer Res. 1989;49: 1148–53.
24. Kruczynski A, Kiss R. Evidence of a direct relationship between the increase in the in vitro passage number of human non-small-cell-lung cancer primocultures and their chemosensitivity. Anticancer Res. 1993;13:507–13.
25. Griffon-Etienne G, Merlin JL, Marchal C. Evaluation of Taxol in head and neck squamous carcinoma multicellular tumor spheroids. Anticancer Drugs. 1997;8:48–55.
26. Johnson JI, Decker S, Zaharevitz D, et al. Relationships between drug activity in NCI preclinical in vitro and in vivo models and early clinical trials. Br J Cancer. 2001;84:1424–31.
27. Spiegel J, Carey TE, Shimoura S, Krause CJ. In vitro sensitivity and resistance of cultured human squamous carcinoma cells to cisplatinum and methotrexate. Otolaryngol Head Neck Surg. 1984;92: 524–31.
28. Masuda N, Fukuoka M, Takada M, Kudoh S, Kusunoki Y. Establishment and characterization of 20 human non-small cell lung cancer cell lines in a serum-free defined medium (ACL-4). Chest. 1991;100:429–38.
29. Verschraegen CF, Hu W, Du Y, et al. Establishment and characterization of cancer cell cultures and xenografts derived from primary or metastatic Mullerian cancers. Clin Cancer Res. 2003;9: 845–52.
30. Liu B, Wang T, Qian X, Liu G, Yu L, Ding Y. Anticancer effect of tetrandrine on primary cancer cells isolated from ascites and pleural fluids. Cancer Lett. 2008;268:166–75.
31. Mouriquand J, Mouriquand C, Petitpas E, Mermet MA. Long-term tissue cultures of human pleural effusions: a cytological follow-up. In Vitro. 1978;14:591–600.
32. Lam DC, Girard L, Suen WS, et al. Establishment and expression profiling of new lung cancer cell lines from Chinese smokers and lifetime never-smokers. J Thorac Oncol. 2006;1:932–42.
33. Ince TA, Richardson AL, Bell GW, et al. Transformation of different human breast epithelial cell types leads to distinct tumor phenotypes. Cancer Cell. 2007;12:160–70.
34. Inagaki T, Matsuwari S, Takahashi R, Shimada KK, Fujie K, Maeda S. Establishment of human oral-cancer cell-lines (Kosc-2 and Kosc-3) carrying P53 and C-Myc abnormalities by geneticin treatment. Int J Cancer. 1994;56:301–8.
35. O'Brien SG, Kirkland MA, Melo JV, et al. Antisense BCR-ABL oligomers cause non-specific inhibition of chronic myeloid leukemia cell lines. Leukemia. 1994;8:2156–62.
36. Cohen MH, Williams G, Johnson JR, et al. Approval summary for imatinib mesylate capsules in the treatment of chronic myelogenous leukemia. Clin Cancer Res. 2002;8:935–42.
37. Grenman R, Carey TE, McClatchey KD, et al. In vitro radiation resistance among cell lines established from patients with squamous cell carcinoma of the head and neck. Cancer. 1991;67: 2741–7.
38. de Jong MC, Pramana J, van der Wal JE, Lacko M, et al. CD44 expression predicts local recurrence after radiotherapy in larynx cancer. Clin Cancer Res. 2010;16:5329–38.
39. Horn IS, Wichmann G, Mozet C, et al. Heterogeneity of epithelial and stromal cells of head and neck squamous cell carcinomas in ex vivo chemoresponse. Cancer Chemother Pharmacol. 2010;65: 1153–63.
40. Singh B, Li R, Xu L, et al. Prediction of survival in patients with head and neck cancer using the histoculture drug response assay. Head Neck. 2002;24:437–42.
41. Fiebig HH, Maier A, Burger AM. Clonogenic assay with established human tumour xenografts: correlation of in vitro to in vivo activity as a basis for anticancer drug discovery. Eur J Cancer. 2004;40:802–20.
42. Wichmann G, Horn IS, Boehm A, et al. Single tissue samples from head and neck squamous cell carcinomas are representative regarding the entire tumor's chemosensitivity to cisplatin and docetaxel. Onkologie. 2009;32:264–72.
43. Law JH, Whigham AS, Wirth PS, et al. Human-in-mouse modeling of primary head and neck squamous cell carcinoma. Laryngoscope. 2009;119:2315–23.
44. Furukawa T, Kubota T, Hoffman RM. Clinical applications of the histoculture drug response assay. Clin Cancer Res. 1995;1: 305–11.
45. Hasegawa Y, Goto M, Hanai N, et al. Evaluation of optimal drug concentration in histoculture drug response assay in association with clinical efficacy for head and neck cancer. Oral Oncol. 2007;43:749–56.
46. Kuijl C, Savage ND, Marsman M, et al. Intracellular bacterial growth is controlled by a kinase network around PKB/AKT1. Nature. 2007;450:725–30.
47. Dollner R, Granzow C, Werner JA, Dietz A. Is there a role for chemosensitivity tests in head and neck cancer? Onkologie. 2004;27:310–5.
48. Dollner R, Granzow C, Helmke BM, Ruess A, Schad A, Dietz A. The impact of stromal cell contamination on chemosensitivity testing of head and neck carcinoma. Anticancer Res. 2004;24: 325–31.
49. Yoshimasu T, Ohta F, Oura S, et al. Histoculture drug response assay for gefitinib in non-small-cell lung cancer. Gen Thorac Cardiovasc Surg. 2009;57:138–43.
50. Kerbel RS. Human tumor xenografts as predictive preclinical models for anticancer drug activity in humans: better than commonly perceived – but they can be improved. Cancer Biol Ther. 2003;2:S134–9.
51. Dong G, Loukinova E, Chen Z, et al. Molecular profiling of transformed and metastatic murine squamous carcinoma cells by differential display and cDNA microarray reveals altered expression of multiple genes related to growth, apoptosis, angiogenesis, and the NF-kappaB signal pathway. Cancer Res. 2001;61:4797–808.
52. OMalley BW, Cope KA, Johnson CS, Schwartz MR. A new immunocompetent murine model for oral cancer. Arch Otolaryngol Head Neck Surg. 1997;123:20–4.

53. Tezabwala BU, Nouri AME, Cannell H, Symes M. Local anergy rather than systemic anti-tumour immunity to explain tumour growth in an animal model of oral squamous cell carcinoma. Oncol Rep. 1997;4:883–8.
54. Thomas Dw, Matthews Jb, Patel V, Game Sm, Prime Ss. Inflammatory cell infiltrate associated with primary and transplanted tumors in an inbred model of oral carcinogenesis. J Oral Pathol Med. 1995;24:23–31.
55. Daniel VC, Marchionni L, Hierman JS, et al. A primary xenograft model of small-cell lung cancer reveals irreversible changes in gene expression imposed by culture in vitro. Cancer Res. 2009;69:3364–73.
56. De Wever O, Mareel M. Role of tissue stroma in cancer cell invasion. J Pathol. 2003;200:429–47.
57. Rubio-Viqueira B, Jimeno A, Cusatis G, et al. An in vivo platform for translational drug development in pancreatic cancer. Clin Cancer Res. 2006;12:4652–61.
58. Langdon SP, Hendriks HR, Braakhuis BJ, et al. Preclinical phase II studies in human tumor xenografts: a European multicenter follow-up study. Ann Oncol. 1994;5:415–22.
59. Shigeta T, Umeda M, Komatsubara H, Komori T. Lymph node and pulmonary metastases after transplantation of oral squamous cell carcinoma cell line (HSC-3) into the subcutaneous tissue of nude mouse: detection of metastases by genetic methods using beta-globin and mutant p53 genes. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;105:486–90.
60. Henriksson E, Baldetorp B, Borg A, et al. p53 mutation and cyclin D1 amplification correlate with cisplatin sensitivity in xenografted human squamous cell carcinomas from head and neck. Acta Oncol. 2006;45:300–5.
61. Hashitani S, Noguchi K, Manno Y, et al. Changes of histological and biological features by serial passages in a human adenoid cystic carcinoma line transplantable in nude mice. Oncol Rep. 2005;13:607–12.
62. Kelland LR. Of mice and men: values and liabilities of the athymic nude mouse model in anticancer drug development. Eur J Cancer. 2004;40:827–36.
63. Frame S, Crombie R, Liddell J, et al. Epithelial carcinogenesis in the mouse: correlating the genetics and the biology. Philos Trans R Soc Lond B Biol Sci. 1998;353:839–45.
64. Mitsunaga SI, Zhang SY, Ruggeri BA, et al. Positive immunohistochemical staining of p53 and cyclin D in advanced mouse skin tumors, but not in precancerous lesions produced by benzo[a] pyrene. Carcinogenesis. 1995;16:1629–35.
65. Ruggeri BA, Bauer B, Zhang SY, Klein-Szanto AJ. Murine squamous cell carcinoma cell lines produced by a complete carcinogenesis protocol with benzo[a]pyrene exhibit characteristic p53 mutations and the absence of H-ras and cyl 1/cyclin D1 abnormalities. Carcinogenesis. 1994;15:1613–9.
66. Shklar G, Schwartz J, Grau D, Trickler DP, Wallace KD. Inhibition of hamster buccal pouch carcinogenesis by 13-cis-retinoic acid. Oral Surg Oral Med Oral Pathol. 1980;50:45–52.
67. Gimenezconti IB, Bianchi AB, Stockman SL, Conti CJ, Slaga TJ. Activating mutation of the Ha-ras gene in chemically induced tumors of the hamster-cheek pouch. Mol Carcinog. 1992;5:259–63.
68. Take Y, Umeda M, Teranobu O, Shimada K. Lymph node metastases in hamster tongue cancer induced with 9, 10-dimethyl-1, 2-benzanthracene: association between histological findings and the incidence of neck metastases, and the clinical implications for patients with tongue cancer. Br J Oral Maxillofac Surg. 1999;37: 29–36.
69. Saranath D, Chang SE, Bhoite LT, et al. High frequency mutation in codons 12 and 61 of H-ras oncogene in chewing tobacco-related human oral carcinoma in India. Br J Cancer. 1991;63:573–8.
70. Lu SL, Herrington H, Reh D, et al. Loss of transforming growth factor-beta type II receptor promotes metastatic head-and-neck squamous cell carcinoma. Genes Dev. 2006;20:1331–42.
71. Chang KW, Sarraj S, Lin SC, Tsai PI, Solt D. P53 expression, p53 and Ha-ras mutation and telomerase activation during nitrosamine-mediated hamster pouch carcinogenesis. Carcinogenesis. 2000;21:1441–51.
72. Schoop RA, Noteborn MH, de Jong Baatenburg RJ. A mouse model for oral squamous cell carcinoma. J Mol Histol. 2009;40: 177–81.
73. Zhang Z, Wang Y, Yao R, Li J, Lubet RA, You M. p53 transgenic mice are highly susceptible to 4-nitroquinoline-1-oxide-induced oral cancer. Mol Cancer Res. 2006;4:401–10.
74. Strati K, Pitot HC, Lambert PF. Identification of biomarkers that distinguish human papillomavirus (HPV)-positive versus HPV-negative head and neck cancers in a mouse model. Proc Natl Acad Sci USA. 2006;103:14152–7.
75. Ide F, Kitada M, Sakashita H, Kusama K. Reduction of p53 dosage renders mice hypersensitive to 7, 12-dimethylbenz(alpha) anthracene-induced salivary gland tumorigenesis. Anticancer Res. 2003;23:201–4.
76. Yuan B, Heniford BW, Ackermann DM, Hawkins BL, Hendler FJ. Harvey ras (H-ras) point mutations are induced by 4-nitroquinoline-1-oxide in murine oral squamous epithelia, while squamous cell carcinomas and loss of heterozygosity occur without additional exposure. Cancer Res. 1994;54:5310–7.
77. Tang XH, Knudsen B, Bemis D, Tickoo S, Gudas LJ. Oral cavity and esophageal carcinogenesis modeled in carcinogen-treated mice. Clin Cancer Res. 2004;10:301–13.
78. Sun Z, Sood S, Li N, et al. Chemoprevention of 7, 12-dimethylbenz[a]anthracene (DMBA)-induced oral carcinogenesis in hamster cheek pouch by topical application of a dual inhibitor of epidermal growth factor receptor (EGFR) and ErbB2 tyrosine kinases. Oral Oncol. 2008;44:652–7.
79. Feng L, Wang Z. Chemopreventive effect of celecoxib in oral precancers and cancers. Laryngoscope. 2006;116:1842–5.
80. Manoharan S, Balakrishnan S, Menon VP, Alias LM, Reena AR. Chemopreventive efficacy of curcumin and piperine during 7, 12-dimethylbenz[a]anthracene-induced hamster buccal pouch carcinogenesis. Singapore Med J. 2009;50:139–46.
81. Letchoumy PV, Mohan KV, Stegeman JJ, Gelboin HV, Hara Y, Nagini S. In vitro antioxidative potential of lactoferrin and black tea polyphenols and protective effects in vivo on carcinogen activation, DNA damage, proliferation, invasion, and angiogenesis during experimental oral carcinogenesis. Oncol Res. 2008;17: 193–203.
82. Zhong LP, Pan HY, Zhou XJ, et al. Characteristics of a cancerous cell line, HIOEC-B(a)P-96, induced by benzo(a)pyrene from human immortalized oral epithelial cell line. Arch Oral Biol. 2008;53:443–52.
83. Attardi LD, Donehower LA. Probing p53 biological functions through the use of genetically engineered mouse models. Mutat Res. 2005;576:4–21.
84. Ide F, Kitada M, Sakashita H, Kusama K, Tanaka K, Ishikawa T. p53 haploinsufficiency profoundly accelerates the onset of tongue tumors in mice lacking the xeroderma pigmentosum group A gene. Am J Pathol. 2003;163:1729–33.
85. Wilkey JF, Buchberger G, Saucier K, et al. Cyclin D1 overexpression increases susceptibility to 4-nitroquinoline-1-oxide-induced dysplasia and neoplasia in murine squamous oral epithelium. Mol Carcinog. 2009;48:853–61.
86. Berns A. Cancer. Improved mouse models. Nature. 2001;410: 1043–4.
87. Coussens LM, Hanahan D, Arbeit JM. Genetic predisposition and parameters of malignant progression in K14-HPV16 transgenic mice. Am J Pathol. 1996;149:1899–917.
88. Moral M, Segrelles C, Lara MF, et al. Akt activation synergizes with Trp53 loss in oral epithelium to produce a novel mouse model for head and neck squamous cell carcinoma. Cancer Res. 2009;69:1099–108.

89. Raimondi AR, Molinolo A, Gutkind JS. Rapamycin prevents early onset of tumorigenesis in an oral-specific K-ras and p53 two-hit carcinogenesis model. Cancer Res. 2009;69:4159–66.
90. Akagi K, Sandig V, Vooijs M, et al. Cre-mediated somatic site-specific recombination in mice. Nucleic Acids Res. 1997;25: 1766–73.
91. Meuwissen R, Linn SC, van der Valk M, Mooi WJ, Berns A. Mouse model for lung tumorigenesis through Cre/lox controlled sporadic activation of the K-Ras oncogene. Oncogene. 2001;20: 6551–8.
92. Chan IT, Kutok JL, Williams IR, et al. Conditional expression of oncogenic K-ras from its endogenous promoter induces a myeloproliferative disease. J Clin Invest. 2004;113:528–38.
93. Bindels EM, van den Brekel MW. Development of a conditional mouse model for head and neck squamous cell carcinoma. Adv Otorhinolaryngol. 2005;62:1–11.
94. Opitz OG, Harada H, Suliman Y, et al. A mouse model of human oral-esophageal cancer. J Clin Invest. 2002;110:761–9.
95. Vasioukhin V, Degenstein L, Wise B, Fuchs E. The magical touch: genome targeting in epidermal stem cells induced by tamoxifen application to mouse skin. Proc Natl Acad Sci USA. 1999;96:8551–6.
96. Bornstein S, White R, Malkoski S, et al. Smad4 loss in mice causes spontaneous head and neck cancer with increased genomic instability and inflammation. J Clin Invest. 2009;119:3408–19.
97. Caulin C, Nguyen T, Longley MA, Zhou Z, Wang XJ, Roop DR. Inducible activation of oncogenic K-ras results in tumor formation in the oral cavity. Cancer Res. 2004;64:5054–8.
98. Lu SL, Herrington H, Wang XJ. Mouse models for human head and neck squamous cell carcinomas. Head Neck. 2006;28:945–54.
99. Matsumoto T, Kiguchi K, Jiang J, et al. Development of transgenic mice that inducibly express an active form of c-Src in the epidermis. Mol Carcinog. 2004;40:189–200.
100. Vitale-Cross L, Amornphimoltham P, Fisher G, Molinolo AA, Gutkind JS. Conditional expression of K-ras in an epithelial compartment that includes the stem cells is sufficient to promote squamous cell carcinogenesis. Cancer Res. 2004;64:8804–7.
101. Paillard F. "Tet-on": a gene switch for the exogenous regulation of transgene expression. Hum Gene Ther. 1998;9:983–5.
102. Higashi AY, Ikawa T, Muramatsu M, et al. Direct hematological toxicity and illegitimate chromosomal recombination caused by the systemic activation of CreERT2. J Immunol. 2009;182: 5633–40.
103. Leonhard WN, Roelfsema JH, Lantinga-van Leeuwen IS, Breuning MH, Peters DJ. Quantification of Cre-mediated recombination by a novel strategy reveals a stable extra-chromosomal deletion-circle in mice. BMC Biotechnol. 2008;8:18.
104. Opitz OG, Quante M, von Werder A, Heeg S, Blum HE. A mouse model of oral-esophageal carcinogenesis. Onkologie. 2005;28: 44–8.