Animal Models to Study the Mutational Landscape for Oral Cavity and Oropharyngeal Cancers

Michael T. Spiotto[1], Matthew Pytynia[1], Gene-Fu F. Liu[1], Mark C. Ranck[1], Ryan Widau[1]

[1]Department of Radiation and Cellular Oncology, The University of Chicago, Chicago, Illinois, USA.

Corresponding Author:
Michael T. Spiotto
Department of Radiation and Cellular Oncology
The University of Chicago
KCBD 6142, 900 E. 57th St., Chicago, IL 60637
USA
E-mail: mspiotto@radonc.uchicago.edu

ABSTRACT

Objectives: Cancer is likely caused by alterations in gene structure or expression. Recently, next generation sequencing has documented mutations in 106 head and neck squamous cell cancer genomes, suggesting several new candidate genes. However, it remains difficult to determine which mutations directly contributed to cancer. Here, summarize the animal models which have already validated and may test cancer causing mutations identified by next generation sequencing approaches.
Material and Methods: We reviewed the existing literature on genetically engineered mouse models and next generation sequencing (NGS), as it relates to animal models of squamous cell cancers of the head and neck (HNSCC) in PubMed.
Results: NSG has identified an average of 19 to 130 distinct mutations per HNSCC specimen. While many mutations likely had biological significance, it remains unclear which mutations were essential to, or "drive," carcinogenesis. In contrast, "passenger" mutations also exist that provide no selection advantage. The genes identified by NGS included p53, RAS, Human Papillomavirus oncogenes, as well as novel genes such as NOTCH1, DICER and SYNE1,2. Animal models of HNSCC have already validated some of these common gene mutations identified by NGS.
Conclusions: The advent of next generation sequencing will provide new leads to the genetic changes occurring in squamous cell cancers of the head and neck. Animal models will enable us to validate these new leads in order to better elucidate the biology of squamous cell cancers of the head and neck.

Keywords: head and neck neoplasms; pain, postoperative; analgesics, opioid; pain measurement; systematic review.

Accepted for publication: 23 January 2013
To cite this article:
Spiotto MT, Pytynia M, Liu GF, Ranck MC, Widau R. Animal Models to Study the Mutational Landscape for Oral Cavity and Oropharyngeal Cancers.
URL: http://www.ejomr.org/JOMR/archives/2013/1/e1/v4n1e1ht.pdf
doi: 10.5037/jomr.2013.4101

INTRODUCTION

While our armamentarium used to treat squamous cell cancers of the head and neck (HNSCC) has greatly expanded with the addition of chemoradiation [1], intensity modulated radiation therapy (IMRT) [2], and biological agents [3], the progression free survival rates have increased only slightly while the overall survival (OS) rates have stagnated [4]. Cancers afflicting the oral cavity (OCC) are especially prone to locoregional failure even after aggressive surgery followed by radiation and/or chemotherapy [5,6]. Aside from platinum-based chemotherapies, there has been little development of new systemic agents equipped to eradicate microscopic OCC disease. For example, a major randomized trial investigating the use of an epidermal growth factor receptor (EGFR) antagonist concurrent with radiotherapy in patients with HNSCC did not include those with OCC [3]. This lack of targeted therapies, coupled with the aggressive nature of OCC, highlights the need to identify genes that drive this malignancy in order to identify new targets.

In contrast to OCC, oropharyngeal cancers (OPC) behave more favourably and also frequently demonstrate a distinct gene expression signature. In general, cure rates using standard therapeutic regimens are 10% higher for OPC than for OCC [4]. This difference may partially be explained by the impact of the Human Papillomavirus (HPV), which has been responsible for an OPC epidemic [7]. Those with HPV-positive OPC have a 5-year OS of 70 - 90%, while those with HPV-negative OPC have a 5-year OS of less than 50% [8]. Furthermore, HPV-negative HNSCCs often have mutations in p53, a major tumour suppressor protein controlling genomic integrity, and a correspondingly worse prognosis [9]. Given the more favourable outcomes of patients with HPV-positive OPC, there is a push to de-escalate their treatment [10]. In addition, the unique molecular signature of HPV may enable better ways to target this disease specifically. Therefore, in contrast to OCCs, understanding the distinct mutational landscapes in OPCs may enable us to identify new molecular targets and, therefore, to de-escalate the toxicities associated with the current, non-targeted cytotoxic chemotherapies.

Much of our previous knowledge regarding the molecular characteristics of HNSCC was derived from expression microarrays or other assays quantifying gene expression [11-15]. With half of the studied cancers derived from the oral cavity or oropharynx, these studies demonstrated at least four unique expression patterns in HNSCC, including: an EGFR, a mesenchymal, an epithelial, and an anti-oxidant expression pattern [11]. Most tumours with the EGFR expression pattern recurred within 2 years, while the majority with the epithelial or anti-oxidant pattern never recurred. Furthermore, these expression signatures could differentiate between HPV-positive and HPV-negative cancers, as well as prognosticate responses to therapy [13,15,16]. Nevertheless, these microarray expression patterns could only implicate a large set of genes involved in HNSCC and have difficulty pinpointing the exact genes driving this disease.

In addition, others have used cytogenetic approaches to identify structural changes in chromosomes [16-20]. These studies support the model whereby HNSCC carcinogenesis begins through two distinct pathways: one caused by chemical carcinogens and the other by HPV oncogenes [20]. Later, these pathways share common chromosomal alterations during progression to invasive cancer. However, because these chromosomal changes are only detected on a megabase-pair level, the changes affecting the exact genes that drive the development of HNSCCs remain largely unknown. Therefore, much of our knowledge of genes that drive HNSCC remains limited to p53 mutations, HPV oncogenes, and the EGFR pathway. There may exist additional undiscovered driving mutations that may one day serve as new targets for novel therapies.

The goal of this article is to review recent trends in identifying HNSCC "driver" mutations, especially those occurring in OCCs and OPCs. We define "driver" mutations as mutations in genes that confer a selective advantage to a clone enabling it to better survive or proliferate. This contrasts with "passenger" mutations that have little if any advantageous effect. We will first discuss the use of next generation sequencing (NGS) to catalogue point mutations prevalent in HNSCC. Next, we will review how some of these mutant genes have already been validated in genetically engineered mouse models (GEMM). Finally, we will discuss how GEMMs may complement NGS by testing novel mutations identified by NGS as well as identify pathways observed in NGS analysis. Thus, this review will examine recent trends in the identification and validation of novel targets, which may revolutionize our understanding of HNSCC biology and usher in innovative treatment strategies.

MATERIAL AND METHODS

Literature Search

In the present article, the authors discuss ways to identify genetically engineered mouse models that supported the recent identification of mutant genes which likely acted as "driver" mutations in HNSCCs. We searched

relevant articles on PubMed (www.ncbi.nlm.nih.gov) regarding next generation sequencing and genetically engineered mouse models for head and neck cancer from 1990 to present. First, to identify mutant genes identified by NGS in HNSCC samples we performed two searches. We queried (1) "genome sequencing" head and neck cancer and (2) "exome sequencing" head and neck cancer which returned 10 and 4 results, respectively. We selected two articles that specifically described NGS (specifically whole exome sequencing) in HNSCC [21,22]. From these articles, we compiled a list of commonly mutated genes and searched whether each gene has been described in an autochthonous head and neck cancer model using the query terms "transgenic mice head and neck cancer." The specific genes searched and the resulting citations are: TP53, 55 citations; TP63, 7 citations; SYNE1,2, 0 citations; NOTCH, 4 citations; HPV, 21 citations; PI3KCA, 0 citations, PTEN, 11 citations; RAS, 53 citations; pRB, 13 citations; FBXW7, 0 citations, RIPK4, 0 citations; DICER, 0 citations. In order to identify additional GEMMs for oral cavity/oropharynx cancer, we also searched all citations using the terms "transgenic mice head and neck cancer" that resulted in 392 total articles. We reviewed all article abstracts and selected articles describing GEMMs targeting genes subsequently identified in NGS of HNSCCs. We also selected articles describing GEMMs of genes not identified by NGS to discuss pathways important in cancer but possibly missed by this approach. Given the breadth of genomic analysis in HNSCCs, we regret any omissions of GEMMs for oral cavity/oropharynx cancer.

RESULTS

Next generation sequencing (NGS) for oral cavity and oropharynx cancers (OCCs and OPCs)

Conventional or "low throughput" DNA sequencing provides one sequence read per DNA sample. With this technique, the DNA sample requires a homogeneous DNA template to decipher a maximum sequence of approximately 1000 base-pairs (bp) long. Given that the human genome contains 3.5 billion bp encoding 10 to 30,000 genes, it is not surprising that a single genome once required approximately 13 years and three billion dollars to sequence [23]. NGS can complete the same task in days, at a cost of approaching a few thousand dollars. Its efficiency has revolutionized the sequencing of entire genomes or, more commonly, "exomes" (genomic libraries limited to a cell's expressed sequences) [24]. This has allowed investigators to catalogue mutations in over twenty malignancies, including brain, breast, and prostate cancers (The Cancer Genome Atlas: http://cancergenome.nih.gov/).

NGS is essentially multiple "low throughput" DNA sequencing reactions run in parallel in a single sample. First, DNA or mRNA is isolated from a tumour or tissue. Since the genomic or exomic sequences can be very long, the DNA or cDNA is cut or sheared by mechanical means to generate many smaller fragments. Each individual DNA molecule is then amplified in order to enhance subsequent DNA sequencing detection. These amplified DNA clones are then sequenced in a massively-parralled fashion to generate multiple short DNA sequences or "reads". These shorter reads are then aligned using computer software in order to determine the longer genomic or exomic sequence.

In regards to HNSCC, the Cancer Genome Atlas contains 312 separately sequenced HNSCC genomes at the writing of this article. Furthermore, two recent publications by Agrawal et al. [21] and Stransky et al. [22] have also detailed common mutations in 106 HNSCC samples. Here, the authors performed whole exome sequencing that can identify coding as well as splice-site mutations. However, this approach is limited because cancer causing mutations can occur in non-protein coding regions that affect the regulation of gene expression, and may cause altered expression of a normal gene in a tissue/cell type or at a temporal point in time that is different than in normal cells. Of note, in the study by Stransky et al. [22], 50 of 77 samples were of OCC or OPC. Both publications compared sequences from tumour specimens and those of autologous tissue controls, and samples were micro-dissected to minimize contamination by adjacent stroma. The authors called mutations in tumours if the genetic changes were detected in the tumour but not the autologous control tissues. Subsequently, many of these mutations were further validated in separate cohorts or by additional sequencing and mass spectrometric genotyping analysis. Interestingly, the average number of mutations differed by almost ten-fold (19 [21] vs. 130 [22]) with each group reporting a variety of mutations per tumour. This variation in mutations may reflect technical differences, such as differences in sample size (32 samples vs. 74 samples), as well as the degree of equence coverage for each study (44 to 77-fold vs. 150-fold) which can affect sequence accuracy. In addition, Agrawal et al. [21] analyzed tumours harvested prior to chemotherapy or radiation, which can select for additional mutational events [25,26] and also account for the lower average number of mutations in their study. Finally, these differences may reflect differences in the accumulation of mutations due to cancer progression, additional DNA damage or different stages of cancer. Nevertheless, it remains unclear which mutations were driving carcinogenesis.

NGS has generated unexpected insights. While the finding that tumours arising from smokers had more mutations than those of non-smokers was expected, two tumours from non-smokers had the highest number of mutations in one study, suggesting genomic instability in HNSCC may not be entirely tobacco dependent [22]. Certain germline genetic conditions, such as Fanconi Anemia, can affect DNA repair pathways and predispose individuals to HNSCC. Therefore, these instances of increased mutations and genomic instability may be due to various Mendelian cancer syndromes in addition to carcinogen exposure.

Consistent with epidemiologic studies suggestive of biological differences based on HPV status, HPV positive tumours contained approximately half the number of mutations as HPV negative tumours, independent of smoking status. In addition, TP53 mutations were inversely correlated with HPV positivity and found in up to 78% of HPV-negative tumours [21]. In fact, Westra et al. [27] has shown an inverse relationship between p53 mutations and HPV positivity in HNSCC. Compared to 25% of HPV-positive tumours, 52% of HPV-negative tumours had p53 mutations. Furthermore, only HPV-negative tumours had mutations that disrupted p53 function suggesting that most p53 mutations resulting in a functional significance were exclusive of HPV. Therefore, NGS will continue to identify potential genes that are advantageous for HNSCCs and further elucidate those already known such as HPV oncogenes or mutations in p53.

Overall, genes previously implicated in HNSCC and confirmed by whole-exome sequencing include TP53, CDKN2A, HRAS, PTEN, PI3KCA and RB. In multiple studies, the most commonly dysregulated gene by far was TP53 [21,22]. In addition, NGS has identified new mutations in genes that regulate epithelial differentiation in up to 30% of tumours. This includes newly discovered mutations in NOTCH1, IRF6, TP63 and FBXW7. Inactivating point mutations in NOTCH1 are particularly noteworthy; in one study, point mutations affecting this gene occurred in 11% of the HNSCC tumours and focal deletions were seen in two additional tumours [22]. Importantly, the identification of Notch genes and others may represent the first new targets implicated in the genesis, as well as treatment, of HNSCC.

While advancements in sequencing may further pinpoint the structural changes causing head and neck cancer, these techniques, like previous technologies, fail to separate those changes that drive HNSCC and those passenger mutations that provide no selection advantage. Validation of driver mutations requires additional *in vivo* and *in vitro* models to confirm and to understand their importance in the biology of this disease.

Validation of next generation sequencing (NGS) with existing genetically engineered mouse models (GEMMs)

Using information gleaned from NGS, we may better understand the physiological significance and molecular mechanisms of several candidate genes driving the development of HNSCCs. Previous mouse models of HNSCC relied mainly on chemical carcinogens such as coal tar, cigarette smoke, 9,10-dimethyl-1,2-benzanthracene (DMBA), and 4-nitroquinoline 1-oxide (4NQO) [28,29]. Over the last 20 years, GEMM have been developed to study how changes in the structure or expression of specific genes impact HNSCC development *in vivo*. These mice have been further engineered to express these altered genes in a tissue-specific and temporal manner. Below, we will describe some of these previously known mutations identified in NGS that also cause HNSCCs in GEMMs. These observations indicate that GEMMs can be used to test whether novel mutations identified by NGS "drive" HNSCCs.

Oncogene P53

TP53 is one of the most frequently mutated genes in human cancers, including OCC [9]. Loss of TP53 function may be nearly universal event in the development of HNSCC by mutation, deletion, amplification of MDM2, deletion of CDKN2A or expression of HPV oncogenes [30]. In addition to p53 deletion, the more common way of inactivating p53 in OCC is by mutation, which induces both dominant negative, as well as lesser understood gain-of-function mutations [31]. With gain-of-function mutations, the tumour suppressive activities of p53 are inhibited, while other potentially progrowth functions of p53 are maintained. In fact, these gain-of-function mutations in p53 predict for worse outcome in HNSCCs [9].

The loss of p53 has been shown to be an initiating event in mouse models, where its deletion predisposed *de novo* tumour formation and greatly sensitized mice to chemical carcinogens [32-34]. However, the majority of mice with p53 deletion or mutations in all tissues died rapidly due to lymphoma or other cancers before the impact of their p53 defects caused development of HNSCC or squamous cell carcinomas (SCC) at other cutaneous sites. In murine models where p53 defects successfully led to HNSCC development, mice with mutations or loss of p53 were treated with carcinogens or bred to mice with additional genetic abnormalities. HNSCCs developed in mice which possessed p53 defects in the germline or when p53 was conditionally disrupted in the basal epithelial layer of the oral cavity

and skin. To conditionally delete p53, mice expressing Cre recombinase expressed under the Keratin 5 [35] or Keratin 14 [36] promoter which is active in the basal keratinocyte layer of the epithelium were bred to knock-in mice possessing floxed p53 alleles. Since Cre recombinase was expressing in the basal keratinocyte layer, the mice possessed disrupted p53 pathways in the epithelium of their skin and upper aerodigestive tract. Transgenic mice expressing dominant negative p53 or mice with p53 haploinsufficiency in the germline experienced accelerated HNSCCs after 4-NQO treatment compared to wild type mice [37,38]. In addition, mice that lost p53 expression or had p53 gain-of-function mutations in the basal keratinocytes of the oral cavity developed invasive HNSCC when tumours also expressed a mutant KRAS gene [35,36]. These results confirmed the clinical observations where loss or mutation in p53 was an important event in at least 50% of HNSCCs.

Loss of TP53 and HPV-positivity appear to be exclusive events. In one study using whole-exome sequencing, TP53 mutations were not identified in any of the HPV associated tumours but were found in 78% of the HPV-negative tumours [21,22]. As such, investigation of HPV oncogenes will likely provide future insights into a distinct subset of tumours and will be described below.

Human Papillomavirus (HPV)

HPV-associated cancers likely arise due to the expression of the viral oncoproteins E6 and E7 [39,40]. E7 binds to and inhibits the retinoblastoma protein (Rb) enabling cells to progress through the cell cycle and to divide [41,42]. However, abnormal cell division usually activates p53, which induces cell apoptosis. HPV relies on E6 to bind p53 and to degrade it, enabling HPV infected cells to escape this safeguard [43]. Mice expressing high risk HPV16 oncogenes, E6 and E7, from the promoter of the bovine keratin 6 gene develop focal epithelial hyperplasia on the tongue by 27 weeks of age [44]. However, no tumours develop in these mice suggesting E6 and E7 alone were insufficient to drive tumourigenesis. Furthermore, epithelial cells derived from HPV16 transgenic or from HPV18 immortalized cells cannot form independent colonies in soft agar or tumours in syngeneic or immuno-compromised mice unless they are extensively cultivated *in vitro* [45]. Other available HPV transgenic mice that target expression via the αA crystallin and keratin 14 promoter have a low incidence of epithelial malignancies that develop after 15 months in only 5 - 10% of mice [46]. However, tumour development in the oral cavity has not been noted [47-50]. Taken together, these studies indicate oncogenes E6 and E7 from high-risk HPV can immortalize epithelial cells but additional genetic events are required for transformation.

While E6 and E7 alone are not sufficient to drive tumour formation, mice that co-express mutant RAS or those exposed to chemical carcinogens are highly susceptible to the development of tumours of the oral cavity. Schreiber et al. [51] demonstrated strong synergy between the mutant HRAS and HPV16 E6/E7. In this model, mice expressing HRAS driven by the zeta-globin promoter, were crossed with transgenic mice that express HPV16-E6/E7 in epithelial tissues using a keratin 14 promoter driven. Double transgenic mice developed dysplastic squamous papillomas of the transitional epithelium that involved the mouth, eye and ear beginning around 3 months of age. Furthermore, K14-HPV-E6/E7 mice treated with 4NQO, a chemical carcinogen, developed oral SCC [52]. Importantly, these E6/E7 driven tumours resembled the molecular characteristics of human HPV-positive OCC, including overexpression of p16, a surrogate for HPV infection. In addition, minichromosome maintenance protein 7 (MCM7) was overexpressed in this model of HNSCC, verifying a previous study on human cervical cancer [53]. Although E7 may play a more prominent role than E6 with regard to long term carcinogenesis [54], the development of HNSCCs in mice likely required a synergy between E6 and E7 [55]. It is believed that E7 may be the predominant initiating oncogene whereas E6 is thought to play a more important role in the progression to malignancy. In addition, E7 likely targeted multiple RB family members to cause HNSCC as deletion of both p107 and Rb recapitulates many features of HPV-16 E7 mice after 4NQO treatment [56]. Therefore, the development of HPV-positive HNSCCs require both the inhibition of p53 pathways and RB family members by HPV E6 and E7 respectively as well as additional mutagenic events.

To study the contribution of other genes to the development of HPV-associated cancers, several reports have studied mice that express HPV oncogenes and that harbour additional defects in other cellular genes. Compared to the general population, Fanconi Anemia (FA) patients who reach 50 years of age are more likely to develop a solid tumour [57-60] where the majority of these tumours are squamous cell cancers (SCCs) involving the head and neck [59]. In a study by Kutler et al. [61], 84% of SCCs in FA patients, of which the majority had HNSCCs, tested positive for HPV and none of these SCCs had p53 mutations. By contrast, van Zeeberg et al. [62] did not detect HPV signatures in HNSCCs but did demonstrate that two-thirds of anogenital cancers contained HPV DNA. Although the aetiology of HNSCCs in FA patients remains a hotly debated topic, it is likely that HPV plays an important

role in this process. This relationship was demonstrated by Park et al. [63] who showed that mice expressing HPV16-E7 and deficient in the FA gene FANCD2 developed SCCs of the tongue and oesophagus at a higher frequency than that observed in control mice. Here, the HPV oncogenes were driven by a K14 promoter and expressed in the basal epithelium of the oral cavity and skin. Therefore, HPV oncogenes may cooperate with other cellular genes to cause HNSCC and other cancers.

Mutant RAS oncogene

Although mutations in RAS are present in only 4 - 5% of HNSCC [21,22], alterations of in RAS signalling occurs frequently in cancer. This often includes amplification of chromosome 7p11, the locus for EGFR and a downstream mediator of RAS [64]. In addition, promoter methylation of RASSF1A, a negative regulator or Ras, is frequently observed in OCC [65,66]. Parallel to these observations, mice expressing a G12D mutation in KRAS developed benign squamous papillomas of the oral mucosa, tongue and palate by 16 - 24 weeks [67]. Although highly proliferative, these papillomas never progressed to malignancy suggesting a role for KRAS in the initiation, but not progression to SCC. In another study, mice expressing mutant KRAS in the basal epithelium developed papillomas exclusively located within the oral mucosa [36]. Again, these papillomas failed to progress to carcinoma. However, mice possessing KRAS and mutant p53 did progress to invasive SCCs. This indicates that RAS requires other factors to increase genomic instability and that this ultimately can lead to the development of frank malignancy. Several groups have used these models to study novel therapies for HNSCCs. For example, rapamycin prevented tumour progression of benign or malignant tumours in mice possessing mutant KRAS, with or without loss of p53, respectively [36]. This preclinical model parallels recent finding using this small molecule inhibitor in HNSCC patients [68]. Similarly, Samuel et al. [69] showed that deletion of RAC1 prevented oral papilloma development in mutant KRAS mice, providing another possible therapeutic target for mutant KRAS mice. Studies such as these with mutant KRAS mice demonstrate that genetically engineered mice can be used to identify novel targets and therapeutic regimens for HNSCCs.

Using genetically engineered mouse models (GEMMs) to test for "Driver" mutations identified by next generation sequencing (NGS)

The significant amount of next NGS data provides a starting point to develop novel *in vivo* models for HNSCC in order to better understand the biology and treatment of this disease. Table 1 compares the genes involved in HNSCC identified with NGS and/or GEMMs. As mentioned above, NGS identified inactivating mutations in the NOTCH gene family in 22% of the samples. Originally described in Drosophila, NOTCH family members are transmembrane proteins that regulate cell-cell communication and differentiation. NOTCH mutations consistently mapped to the transactivating C-terminal ankyrin repeat domain. The predicted effect of this mutation is a truncation resulting in a loss-of-function mutant [21,22]. Additional mutations occurred in the extracellular ligand binding domain and splice junctions that were also likely inactivating in nature. These mutations are similar to those recently described for myeloid leukaemia [70] but contrast sharply with NOTCH activating mutations observed in other lymphocytic leukaemia's and lymphomas [71,72]. These results suggest that NOTCH mutations may be context dependent whereby NOTCH inhibition may promote some cancers while inhibiting others.

Along these lines, a clinical trial using a NOTCH inhibitor was stopped due to an unanticipated consequence of increased cutaneous cancers [73]. Similarly, mice with a tissue specific deletion of NOTCH1 in the skin resulted in corneal hyperplasia and skin tumours as early as 8 months post inactivation [74]. Furthermore, DMBA treatment accelerated tumour formation and frequency. This group suggested that loss of NOTCH1 drove skin cancers by elevating β-catenin possibly resulting in de-differentiation of epithelial cells. As no respective GEMMs exist for Notch driven OCC, the precise role of the NOTCH gene family remains unclear in HNSCC and may be context-dependent.

Using NGS approaches, Stransky et al. [22] confirmed previous observations that Cyclin D1 was also amplified in 22% of HNSCC samples. The Cyclin D family promotes cellular proliferation by enabling cells to enter the S phase of the cell cycle in order to synthesize DNA and prepare for cell division. Amplifications or overexpression of Cyclin D1 frequently occurs in SCC leading to dysregulation of the cell cycle [75]. In transgenic mice, expression of Cyclin D1 was directed to the oral-oesophageal squamous epithelium using part of the Epstein-Barr virus ED-L2 promoter (L2-CD1). Expression of Cyclin D1 caused hyperplasia of the basal and suprabasal epithelia of the tongue, oesophagus and forestomach [76,77]. These mice were treated with 20 to 50 ppm of 4NQO for 8 weeks and then observed for an additional 16 weeks. Half of the L2-CD1 mice treated with 50 ppm of 4NQO, exhibited SCC of the tongue and oesophagus by 16 weeks.

Table 1. Non-chemically induced transgenic mice models of oral cavity cancers (OCC)

Pathway/ Gene family	Gene	Function	Freq. in NGS	Epithelial mouse model genotype	Tumour type	Reference
p53 family	TP53	Tumour suppressor involved in apoptosis, activates DNA repair proteins, cell cycle regulation at G_1/S checkpoint	50 - 78%	$p53^{-/-}$ or mutant p53 mice with chemical carcinogens or mutant KRAS	Various HNSCC	Acin, Raimondi [35,36]
	TP63	Involved in development and regulation of apoptosis	8%	Not described	-	n/a
Nesprins	SYNE1,2	Found in the outer nuclear membrane and bind to actin filaments	24%	Not described	-	n/a
Notch	NOTCH1,2,3	Transmembrane proteins that are involved in development by controlling cell fate decisions by regulating interactions between adjacent cells.	22%	$NOTCH1^{-/-}$	Basel Cell Carcinoma	Nicolas [74], Agrawal [21]
HPV Oncogenes	E6	Degrades p53 through ubiquitination	15%	HPV-E6/E7 with chemical carcinogens or mutant Hras	Papillomas of the lip	Schreiber [51]
	E7	Binds pRb to free the transcription factor E2F				
PI3K/AKT/ mTOR	PIK3CA	Oncogene	8%	Not described	-	n/a
	PTEN	Tumor suppressor that regulates AKT	8%	myrAKT $PTEN^{-/-}$	Dysplastic lesions in the palate, cheeks, and lips	Bian, Moral [80,82]
TGF-β pathway	TGF-β	Regulates proliferation and differentiation, angiogenesis, and serves as an immune modulator	Not described	$TGFBR1^{-/-}$	OCC, ears, periorbital, perianal	Bian [81]
	SMAD4	Downstream transcription factor that activates apoptosis	Not described	$SMAD4^{-/-}$	OCC, lymph node mets	Bornstein [83]
	TGFB2	Encodes transmembrane Ser/Thr protein kinase that is activated by TGF-β, amongst other signalling molecules	Not described	Not described	-	Lu [82]
RAS/RAF/ MEK/MAPK	RASSF1A	Tumour suppressor involved in DNA repair and cell cycle arrest; negative regulator of RAS	Not described	G12D mutant KRAS	Benign squamous papillomas of oral mucosa, tongue and palate	Caulin [67]
	EGFR	Epidermal growth factor receptor; downstream mediator of RAS	Not described	Not described	-	n/a
Cyclins	Cyclin D1	Promotes cellular proliferation by enabling cells to enter S phase and synthesize DNA in preparation for cell division	Not described	L2-CyclinD1	Hyperplasia of tongue, oesophagus	Mueller [76], Nakagawa [77]
Pocket protein family	RB1	Tumour suppressor that inhibits cell cycle (G_1/S) and is involved in chromatin remodelling	3%	pRb/p107-deficient pRB/p130-deficient	Head and neck Squamous cell carcinomas	Shin [56]
	RBL1	Gene product p107 is a tumour suppressor involved in cell cycle regulation		pRb/p107-deficient	Head and neck Squamous cell carcinomas	Shin [56]
	NOLC1	Gene product is p130, unclear function		pRb/p130-deficient	Head and neck Squamous cell carcinomas	Shin [56]
Interferon regulator transcription factor family	IRF6	Involved in the formation of connective tissues	Not described	Not described	-	n/a
F-box protein family	FBXW7	Binds to cyclin E and targets it for ubiquitination to prevent progression from G_1 to S phase	Not described	Not described	-	n/a
	RIPK4	Serine/threonine protein kinase that interacts with PKC-δ and can also activate NFkappaB.	3%	Not described	-	n/a
	DICER1	RNA helicase that functions as a ribonuclease in RNA interference and microRNA pathways to repress gene expression	3%	Not described	-	n/a

NGS = next generation sequencing.

By contrast, wild-type mice failed to develop SCC [78]. Furthermore, when mice both overexpressed CCND1 and were haploinsufficient for p53, invasive SCC occurred by 12 months of age [79]. Cancers were evident in the buccal mucosa (12%), tongue (25%) and upper and lower oesophagus (11 - 12%) with 25% containing metastasis to lymph nodes.

Finally, these NGS approaches showed deletion or inactivating mutations in the phosphatase and tensin homolog (PTEN) gene. PTEN functions as a tumour suppressor by regulating AKT which promotes cell survival and metabolism. Mice expressing a myrisolated, and hence, constitutively active, AKT (myrAKT) under control of a bovine Keratin 5 promoter, developed dysplastic lesions in the palate, cheeks and lips [80]. When the epithelial cells expressed myrAKT and also lost p53 expression, mice developed malignant tumours in the oral cavity, palate, tongue and lips with local metastasis to regional lymph nodes. Therefore, as shown with NOTCH1, CCND1, and PTEN, mouse models complimented and were able to confirm candidate genes that drive HNSCC as initially identified by NGS.

However, one notable gene important in HNSCCs but not covered with NGS involves the Transforming Growth Factor (TGF-β) signalling pathway. TGF-β regulates cellular proliferation and differentiation as well as angiogenesis and immune suppression. This pathway is often mutated in cancer cells so that these cells become resistant to the anti-proliferative effects of TGF-β but still benefit from its pro-angiogenic and immunosuppressive functions. Although mutations in TGF-β signalling were not found by whole-genome sequencing [21,22], tumours of the head and neck have frequent loss of chromosome 18q, which contains the SMAD2, SMAD3, SMAD4 and TBR2 genes [17,81]. In addition, TGF-β is well known to cause differentiation of epithelial cells and whole-exome sequencing identified up to 30% mutations in genes that play a role in terminal differentiation [21,22]. Therefore, future studies still require vigilance to examine candidate genes not identified by massive sequencing efforts or other high-throughput technologies.

To this end, several mouse models have shown that loss of the TGF-β signalling pathway in cancer cells resulted in HNSCCs. After chemical carcinogen treatment, mice possessing deletion of TGFBR1 in their epithelium developed SCCs of the oral cavity. These mice also developed regional and distant metastasis within one year after treatment. Furthermore, tumours exhibited enhanced proliferation, reduced apoptosis and the tumour stroma appeared highly inflamed with high levels of TGF-β. Furthermore, the TGF-β signalling pathway may also cooperate with the AKT pathway to cause HNSCCs; mice that had lost both TGFBR1 and the AKT inhibitor PTEN in their epithelia developed oral SCCs within ten weeks [82]. In addition, mice that lost other proteins involved in the TGF-β signalling pathway such as TGFBR2 [83] or the downstream effector molecules SMAD4 [84] also developed HNSCCs mainly affecting the oral cavity and regional lymphatics. Consistent with previous studies [85], tumours and stroma from mutant Smad4 mice had high levels of TGFB1 and inflammation. Thus, these mouse models may provide additional insight into genes mediating HNSCCs that were not observed using NGS and other powerful high throughput techniques.

CONCLUSIONS

As with other high throughput technologies such as expression microarrays and comparative genomic hybridization, recent advances in NGS can identify both new candidates and novel structural information regarding genes that drive HNSCCs. In addition to genes known to be involved in HNSCC such as HPV oncoproteins E6 and E7, p53 and RAS, these studies have also identified novel mutations in genes such at NOTCH1 and PTEN, among others. All of these genes have been show to accelerate the development of SCCs in genetically engineered mice. Furthermore, NGS along with other works have identified mutations in several novel pathways. For example, 22% of tumours contained mutations in spectrin repeat containing, nuclear envelope (SYNE1) which may regulate cytoskeletal regulation. In addition, these studies reported that 3% of cancers had mutations in the endoribonucelase DICER, an important player in miRNA genesis. Still, it remains unclear which candidates actually promotes SCC development as well as the mechanism by which this occurs.

GEMMs provide a novel platform to better understand and validate these novel mutations that have been identified by sequencing HNSCCs genomes. It has been shown that these GEMMs develop SCCs when mice possess mutated genes known to be involved in HNSCCs and continued study will allow the discovery and validation of novel “driver” mutations important in HNSCCs. Understanding how these novel mutations promote malignant transformation may enable us to target HNSCCs more rationally. Furthermore, these models will provide an *in vivo* platform to study the effectiveness of different strategies utilizing cytotoxic chemotherapy as well as other small molecule inhibitors. One caution with this approach centers on the extent to which regional differences in mutations contribute to tumour heterogeneity and possibly response to therapy. For example, Gerlinger et al. [86] showed that more that 60% of all somatic mutations differed among

tumour regions. Despite such heterogeneity, many subclonal populations exhibited convergent tumour evolution with distinct mutations affecting similar pathways. Furthermore, epigenetic alterations also occur in HNSCCs [87,88] and may promote tumour growth. These changes may be missed in NGS and may be difficult to study in GEMMs. Finally, mutations in mitochondrial DNA are associated increased HNSCC aggressiveness [89]. Such mutations can be studied with GEMMs but may be missed with NGS as these events turn off gene expression without causing mutations and lead to further tumour heterogeneity. Therefore, coupling NGS with GEMMs will also be essential to understand which mutations and pathways drive HNSCCs. Thus, coupling NGS approaches with GEMMs will provide important platforms to investigate the best ways to target individual candidate genes and, more generally, those pathways essential to HNSCC.

ACKNOWLEDGMENTS AND DISCLOSURE STATEMENTS

Committee on cancer biology fellowship NIH T32 CA009594 (RCW). MTS is a recipient of the Burroughs Wellcome Fund Career Award for Medical Scientists Award.

REFERENCES

1. Pignon JP, le Maître A, Maillard E, Bourhis J; MACH-NC Collaborative Group. Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): an update on 93 randomised trials and 17,346 patients. Radiother Oncol. 2009 Jul;92(1):4-14. Epub 2009 May 14. [Medline: 19446902] [doi: 10.1016/j.radonc.2009.04.014]
2. Eisbruch A, Ship JA, Dawson LA, Kim HM, Bradford CR, Terrell JE, Chepeha DB, Teknos TN, Hogikyan ND, Anzai Y, Marsh LH, Ten Haken RK, Wolf GT. Salivary gland sparing and improved target irradiation by conformal and intensity modulated irradiation of head and neck cancer. World J Surg. 2003 Jul;27(7):832-7. [Medline: 14509515] [doi: 10.1007/s00268-003-7105-6]
3. Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, Jones CU, Sur R, Raben D, Jassem J, Ove R, Kies MS, Baselga J, Youssoufian H, Amellal N, Rowinsky EK, Ang KK. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med. 2006 Feb 9;354(6):567-78. [Medline: 16467544] [doi: 10.1056/NEJMoa053422]
4. Carvalho AL, Nishimoto IN, Califano JA, Kowalski LP. Trends in incidence and prognosis for head and neck cancer in the United States: a site-specific analysis of the SEER database. Int J Cancer. 2005 May 1;114(5):806-16. [Medline: 15609302] [doi: 10.1002/ijc.20740]
5. Soo KC, Tan EH, Wee J, Lim D, Tai BC, Khoo ML, Goh C, Leong SS, Tan T, Fong KW, Lu P, See A, Machin D. Surgery and adjuvant radiotherapy vs concurrent chemoradiotherapy in stage III/IV nonmetastatic squamous cell head and neck cancer: a randomised comparison. Br J Cancer. 2005 Aug 8;93(3):279-86. [Medline: 16012523] [doi: 10.1038/sj.bjc.6602696] [FREE Full Text]
6. Blanchard P, Baujat B, Holostenco V, Bourredjem A, Baey C, Bourhis J, Pignon JP; MACH-CH Collaborative group. Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): a comprehensive analysis by tumour site. Radiother Oncol. 2011 Jul;100(1):33-40. Epub 2011 Jun 16. [Medline: 21684027] [doi: 10.1016/j.radonc.2011.05.036]
7. Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E, Jiang B, Goodman MT, Sibug-Saber M, Cozen W, Liu L, Lynch CF, Wentzensen N, Jordan RC, Altekruse S, Anderson WF, Rosenberg PS, Gillison ML. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol. 2011 Nov 10;29(32):4294-301. doi: 10.1200/JCO.2011.36.4596. Epub 2011 Oct 3. [Medline: 21969503] [doi: 10.1200/JCO.2011.36.4596] [FREE Full Text]
8. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, Westra WH, Chung CH, Jordan RC, Lu C, Kim H, Axelrod R, Silverman CC, Redmond KP, Gillison ML. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med. 2010 Jul 1;363(1):24-35. Epub 2010 Jun 7. [Medline: 20530316] [doi: 10.1056/NEJMoa0912217] [FREE Full Text]
9. Poeta ML, Manola J, Goldwasser MA, Forastiere A, Benoit N, Califano JA, Ridge JA, Goodwin J, Kenady D, Saunders J, Westra W, Sidransky D, Koch WM. TP53 mutations and survival in squamous-cell carcinoma of the head and neck. N Engl J Med. 2007 Dec 20;357(25):2552-61. [Medline: 18094376] [doi: 10.1056/NEJMoa073770] [FREE Full Text]
10. Psyrri A, Sasaki C, Vassilakopoulou M, Dimitriadis G, Rampias T. Future directions in research, treatment and prevention of HPV-related squamous cell carcinoma of the head and neck. Head Neck Pathol. 2012 Jul;6 Suppl 1:S121-8. Epub 2012 Jul 3. Review. [Medline: 22782231] [doi: 10.1007/s12105-012-0361-8]
11. Chung CH, Parker JS, Karaca G, Wu J, Funkhouser WK, Moore D, Butterfoss D, Xiang D, Zanation A, Yin X, Shockley WW, Weissler MC, Dressler LG, Shores CG, Yarbrough WG, Perou CM. Molecular classification of head and neck squamous cell carcinomas using patterns of gene expression. Cancer Cell. 2004 May;5(5):489-500. [Medline: 15144956] [doi: 10.1016/S1535-6108(04)00112-6]
12. Schlecht NF, Burk RD, Adrien L, Dunne A, Kawachi N, Sarta C, Chen Q, Brandwein-Gensler M, Prystowsky MB, Childs G, Smith RV, Belbin TJ. Gene expression profiles in HPV-infected head and neck cancer. J Pathol. 2007 Nov;213(3):283-93. [Medline: 17893858] [doi: 10.1002/path.2227]

13. Slebos RJ, Yi Y, Ely K, Carter J, Evjen A, Zhang X, Shyr Y, Murphy BM, Cmelak AJ, Burkey BB, Netterville JL, Levy S, Yarbrough WG, Chung CH. Gene expression differences associated with human papillomavirus status in head and neck squamous cell carcinoma. Clin Cancer Res. 2006 Feb 1;12(3 Pt 1):701-9. [Medline: 16467079] [doi: 10.1158/1078-0432.CCR-05-2017] [FREE Full Text]
14. Alevizos I, Mahadevappa M, Zhang X, Ohyama H, Kohno Y, Posner M, Gallagher GT, Varvares M, Cohen D, Kim D, Kent R, Donoff RB, Todd R, Yung CM, Warrington JA, Wong DT. Oral cancer in vivo gene expression profiling assisted by laser capture microdissection and microarray analysis. Oncogene. 2001 Sep 27;20(43):6196-204. [Medline: 11593428] [doi: 10.1038/sj.onc.1204685] [FREE Full Text]
15. Hanna E, Shrieve DC, Ratanatharathorn V, Xia X, Breau R, Suen J, Li S. A novel alternative approach for prediction of radiation response of squamous cell carcinoma of head and neck. Cancer Res. 2001 Mar 15;61(6):2376-80. [Medline: 11289099] [FREE Full Text]
16. van den Broek GB, Wreesmann VB, van den Brekel MW, Rasch CR, Balm AJ, Rao PH. Genetic abnormalities associated with chemoradiation resistance of head and neck squamous cell carcinoma. Clin Cancer Res. 2007 Aug 1;13(15 Pt 1):4386-91. [Medline: 17671120] [doi: 10.1158/1078-0432.CCR-06-2817] [FREE Full Text]
17. Van Dyke DL, Worsham MJ, Benninger MS, Krause CJ, Baker SR, Wolf GT, Drumheller T, Tilley BC, Carey TE. Recurrent cytogenetic abnormalities in squamous cell carcinomas of the head and neck region. Genes Chromosomes Cancer. 1994 Mar;9(3):192-206. [Medline: 7515662] [doi: 10.1002/gcc.2870090308]
18. Singh B, Kim SH, Carew JF, Yu I, Shaha AR, Wolden S, Boyle J, Shah JP, Rao PH. Genome-wide screening for radiation response factors in head and neck cancer. Laryngoscope. 2000 Aug;110(8):1251-6. [Medline: 10942121] [doi: 10.1097/00005537-200008000-00005]
19. Cowan JM, Beckett MA, Ahmed-Swan S, Weichselbaum RR. Cytogenetic evidence of the multistep origin of head and neck squamous cell carcinomas. J Natl Cancer Inst. 1992 May 20;84(10):793-7. [Medline: 1573667] [doi: 10.1093/jnci/84.10.793]
20. Smeets SJ, Braakhuis BJ, Abbas S, Snijders PJ, Ylstra B, van de Wiel MA, Meijer GA, Leemans CR, Brakenhoff RH. Genome-wide DNA copy number alterations in head and neck squamous cell carcinomas with or without oncogene-expressing human papillomavirus. Oncogene. 2006 Apr 20;25(17):2558-64. [Medline: 16314836] [doi: 10.1038/sj.onc.1209275]
21. Agrawal N, Frederick MJ, Pickering CR, Bettegowda C, Chang K, Li RJ, Fakhry C, Xie TX, Zhang J, Wang J, Zhang N, El-Naggar AK, Jasser SA, Weinstein JN, Treviño L, Drummond JA, Muzny DM, Wu Y, Wood LD, Hruban RH, Westra WH, Koch WM, Califano JA, Gibbs RA, Sidransky D, Vogelstein B, Velculescu VE, Papadopoulos N, Wheeler DA, Kinzler KW, Myers JN. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. Science. 2011 Aug 26;333(6046):1154-7. Epub 2011 Jul 28. [Medline: 21798897] [doi: 10.1126/science.1206923] [FREE Full Text]
22. Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, Kryukov GV, Lawrence MS, Sougnez C, McKenna A, Shefler E, Ramos AH, Stojanov P, Carter SL, Voet D, Cortés ML, Auclair D, Berger MF, Saksena G, Guiducci C, Onofrio RC, Parkin M, Romkes M, Weissfeld JL, Seethala RR, Wang L, Rangel-Escareño C, Fernandez-Lopez JC, Hidalgo-Miranda A, Melendez-Zajgla J, Winckler W, Ardlie K, Gabriel SB, Meyerson M, Lander ES, Getz G, Golub TR, Garraway LA, Grandis JR. The mutational landscape of head and neck squamous cell carcinoma. Science. 2011 Aug 26;333(6046):1157-60. Epub 2011 Jul 28. [Medline: 21798893] [doi: 10.1126/science.1208130] [FREE Full Text]
23. Bennett ST, Barnes C, Cox A, Davies L, Brown C. Toward the 1,000 dollars human genome. Pharmacogenomics. 2005 Jun;6(4):373-82. Review. [Medline: 16004555] [doi: 10.1517/14622416.6.4.373]
24. Loyo M, Li RJ, Bettegowda C, Pickering CR, Frederick MJ, Myers JN, Agrawal N. Lessons learned from next-generation sequencing in head and neck cancer. Head Neck. 2013 Mar;35(3):454-63. Epub 2012 Aug 21. [Medline: 22907887] [doi: 10.1002/hed.23100]
25. Liu L, Schwartz S, Davis BM, Gerson SL. Chemotherapy-induced O(6)-benzylguanine-resistant alkyltransferase mutations in mismatch-deficient colon cancer. Cancer Res. 2002 Jun 1;62(11):3070-6. [Medline: 12036916] [FREE Full Text]
26. Danesi CC, Bellagamba BC, Dihl RR, de Andrade HH, Cunha KS, Spanó MA, Reguly ML, Lehmann M. Mutagenic evaluation of combined paclitaxel and cisplatin treatment in somatic cells of Drosophila melanogaster. Mutat Res. 2010 Feb;696(2):139-43. Epub 2010 Jan 18. [Medline: 20083227] [doi: 10.1016/j.mrgentox.2010.01.002]
27. Westra WH, Taube JM, Poeta ML, Begum S, Sidransky D, Koch WM. Inverse relationship between human papillomavirus-16 infection and disruptive p53 gene mutations in squamous cell carcinoma of the head and neck. Clin Cancer Res. 2008 Jan 15;14(2):366-9. [Medline: 18223210] [doi: 10.1158/1078-0432.CCR-07-1402] [FREE Full Text]
28. Vitale-Cross L, Czerninski R, Amornphimoltham P, Patel V, Molinolo AA, Gutkind JS. Chemical carcinogenesis models for evaluating molecular-targeted prevention and treatment of oral cancer. Cancer Prev Res (Phila). 2009 May;2(5):419-22. Epub 2009 Apr 28. Review. [Medline: 19401522] [doi: 10.1158/1940-6207.CAPR-09-0058]

29. Kanojia D, Vaidya MM. 4-nitroquinoline-1-oxide induced experimental oral carcinogenesis. Oral Oncol. 2006 Aug;42(7):655-67. Epub 2006 Jan 30. Review. [Medline: 16448841] [doi: 10.1016/j.oraloncology.2005.10.013]
30. Rothenberg SM, Ellisen LW. The molecular pathogenesis of head and neck squamous cell carcinoma. J Clin Invest. 2012 Jun 1;122(6):1951-7. Review. [Medline: 22833868]
31. Olive KP, Tuveson DA, Ruhe ZC, Yin B, Willis NA, Bronson RT, Crowley D, Jacks T. Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. Cell. 2004 Dec 17;119(6):847-60. [Medline: 15607980] [doi: 10.1016/j.cell.2004.11.004]
32. Jonkers J, Meuwissen R, van der Gulden H, Peterse H, van der Valk M, Berns A. Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. Nat Genet. 2001 Dec;29(4):418-25. [Medline: 11694875] [doi: 10.1038/ng747]
33. Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA Jr, Butel JS, Bradley A. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature. 1992 Mar 19;356(6366):215-21. [Medline: 1552940] [doi: 10.1038/356215a0]
34. Purdie CA, Harrison DJ, Peter A, Dobbie L, White S, Howie SE, Salter DM, Bird CC, Wyllie AH, Hooper ML, et al. Tumour incidence, spectrum and ploidy in mice with a large deletion in the p53 gene. Oncogene. 1994 Feb;9(2):603-9. [Medline: 8290271]
35. Acin S, Li Z, Mejia O, Roop DR, El-Naggar AK, Caulin C. Gain-of-function mutant p53 but not p53 deletion promotes head and neck cancer progression in response to oncogenic KRAS. J Pathol. 2011 Dec;225(4):479-89. Epub 2011 Sep 26. [Medline: 21952947] [doi: 10.1002/path.2971]
36. Raimondi AR, Molinolo A, Gutkind JS. Rapamycin prevents early onset of tumorigenesis in an oral-specific KRAS and p53 two-hit carcinogenesis model. Cancer Res. 2009 May 15;69(10):4159-66. Epub 2009 May 12. [Medline: 19435901] [doi: 10.1158/0008-5472.CAN-08-4645] [FREE Full Text]
37. 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 Nov;163(5):1729-33. [Medline: 14578172] [doi: 10.1016/S0002-9440(10)63531-6] [FREE Full Text]
38. 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 Jun;4(6):401-10. Erratum in: Mol Cancer Res. 2006 Aug;4(8):599. [Medline: 16778087] [doi: 10.1158/1541-7786.MCR-06-0028] [FREE Full Text]
39. Yugawa T, Kiyono T. Molecular mechanisms of cervical carcinogenesis by high-risk human papillomaviruses: novel functions of E6 and E7 oncoproteins. Rev Med Virol. 2009 Mar;19(2):97-113. Review. [Medline: 19156753] [doi: 10.1002/rmv.605]
40. Moody CA, Laimins LA. Human papillomavirus oncoproteins: pathways to transformation. Nat Rev Cancer. 2010 Aug;10(8):550-60. Epub 2010 Jul 1. Review. [Medline: 20592731] [doi: 10.1038/nrc2886]
41. Phelps WC, Yee CL, Münger K, Howley PM. The human papillomavirus type 16 E7 gene encodes transactivation and transformation functions similar to those of adenovirus E1A. Cell. 1988 May 20;53(4):539-47. [Medline: 2836062] [doi: 10.1016/0092-8674(88)90570-3]
42. Werness BA, Levine AJ, Howley PM. Association of human papillomavirus types 16 and 18 E6 proteins with p53. Science. 1990 Apr 6;248(4951):76-9. [Medline: 2157286] [doi: 10.1126/science.2157286]
43. Scheffner M, Münger K, Byrne JC, Howley PM. The state of the p53 and retinoblastoma genes in human cervical carcinoma cell lines. Proc Natl Acad Sci U S A. 1991 Jul 1;88(13):5523-7. [Medline: 1648218] [doi: 10.1073/pnas.88.13.5523] [FREE Full Text]
44. Ocadiz-Delgado R, Marroquin-Chavira A, Hernandez-Mote R, Valencia C, Manjarrez-Zavala ME, Covarrubias L, Gariglio P. Induction of focal epithelial hyperplasia in tongue of young bk6-E6/E7 HPV16 transgenic mice. Transgenic Res. 2009 Aug;18(4):513-27. Epub 2009 Jan 23. [Medline: 19165615] [doi: 10.1007/s11248-009-9243-6]
45. Shen ZY, Xu LY, Chen MH, Shen J, Cai WJ, Zeng Y. Progressive transformation of immortalized esophageal epithelial cells. World J Gastroenterol. 2002 Dec;8(6):976-81. [Medline: 12439909]
46. Arbeit JM, Münger K, Howley PM, Hanahan D. Progressive squamous epithelial neoplasia in K14-human papillomavirus type 16 transgenic mice. J Virol. 1994 Jul;68(7):4358-68. [Medline: 7515971] [FREE Full Text]
47. Griep AE, Herber R, Jeon S, Lohse JK, Dubielzig RR, Lambert PF. Tumorigenicity by human papillomavirus type 16 E6 and E7 in transgenic mice correlates with alterations in epithelial cell growth and differentiation. J Virol. 1993 Mar;67(3):1373-84. [Medline: 8382301] [FREE Full Text]
48. Lambert PF, Pan H, Pitot HC, Liem A, Jackson M, Griep AE. Epidermal cancer associated with expression of human papillomavirus type 16 E6 and E7 oncogenes in the skin of transgenic mice. Proc Natl Acad Sci U S A. 1993 Jun 15;90(12):5583-7. [Medline: 8390671] [doi: 10.1073/pnas.90.12.5583] [FREE Full Text]
49. Herber R, Liem A, Pitot H, Lambert PF. Squamous epithelial hyperplasia and carcinoma in mice transgenic for the human papillomavirus type 16 E7 oncogene. J Virol. 1996 Mar;70(3):1873-81. [Medline: 8627712] [FREE Full Text]
50. Song S, Gulliver GA, Lambert PF. Human papillomavirus type 16 E6 and E7 oncogenes abrogate radiation-induced DNA damage responses in vivo through p53-dependent and p53-independent pathways. Proc Natl Acad Sci U S A. 1998 Mar 3;95(5):2290-5. [Medline: 9482878] [doi: 10.1073/pnas.95.5.2290] [FREE Full Text]

51. Schreiber K, Cannon RE, Karrison T, Beck-Engeser G, Huo D, Tennant RW, Jensen H, Kast WM, Krausz T, Meredith SC, Chen L, Schreiber H. Strong synergy between mutant ras and HPV16 E6/E7 in the development of primary tumors. Oncogene. 2004 May 13;23(22):3972-9. [Medline: 15077191] [doi: 10.1038/sj.onc.1207507]
52. 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 U S A. 2006 Sep 19;103(38):14152-7. Epub 2006 Sep 7. [Medline: 16959885] [doi: 10.1073/pnas.0606698103] [FREE Full Text]
53. Brake T, Connor JP, Petereit DG, Lambert PF. Comparative analysis of cervical cancer in women and in a human papillomavirus-transgenic mouse model: identification of minichromosome maintenance protein 7 as an informative biomarker for human cervical cancer. Cancer Res. 2003 Dec 1;63(23):8173-80. [Medline: 14678972] [FREE Full Text]
54. Strati K, Lambert PF. Role of Rb-dependent and Rb-independent functions of papillomavirus E7 oncogene in head and neck cancer. Cancer Res. 2007 Dec 15;67(24):11585-93. [Medline: 18089787] [doi: 10.1158/0008-5472.CAN-07-3007] [FREE Full Text]
55. Jabbar S, Strati K, Shin MK, Pitot HC, Lambert PF. Human papillomavirus type 16 E6 and E7 oncoproteins act synergistically to cause head and neck cancer in mice. Virology. 2010 Nov 10;407(1):60-7. Epub 2010 Aug 24. [Medline: 20797753] [doi: 10.1016/j.virol.2010.08.003] [FREE Full Text]
56. hin MK, Pitot HC, Lambert PF. Pocket proteins suppress head and neck cancer. Cancer Res. 2012 Mar 1;72(5):1280-9. Epub 2012 Jan 11. [Medline: 22237625] [doi: 10.1158/0008-5472.CAN-11-2833] [FREE Full Text]
57. Kennedy AW, Hart WR. Multiple squamous-cell carcinomas in Fanconi's anemia. Cancer. 1982 Aug 15;50(4):811-4. [Medline: 7093916] [doi: 10.1002/1097-0142(19820815)50:4<811::AID-CNCR2820500432>3.0.CO;2-I]
58. Reed K, Ravikumar TS, Gifford RR, Grage TB. The association of Fanconi's anemia and squamous cell carcinoma. Cancer. 1983 Sep 1;52(5):926-8. Review. [Medline: 6347359] [doi: 10.1002/1097-0142(19830901)52:5<926::AID-CNCR2820520530>3.0.CO;2-T]
59. Rosenberg PS, Greene MH, Alter BP. Cancer incidence in persons with Fanconi anemia. Blood. 2003 Feb 1;101(3):822-6. Epub 2002 Sep 5. Erratum in: Blood. 2003 Mar 15;101(6):2136. [Medline: 12393424] [doi: 10.1182/blood-2002-05-1498] [FREE Full Text]
60. Sarna G, Tomasulo P, Lotz MJ, Bubinak JF, Shulman NR. Multiple neoplasms in two siblings with a variant form of Fanconi's anemia. Cancer. 1975 Sep;36(3):1029-33. [Medline: 171048] [doi: 10.1002/1097-0142(197509)36:3<1029::AID-CNCR2820360328>3.0.CO;2-5]
61. Kutler DI, Auerbach AD, Satagopan J, Giampietro PF, Batish SD, Huvos AG, Goberdhan A, Shah JP, Singh B. High incidence of head and neck squamous cell carcinoma in patients with Fanconi anemia. Arch Otolaryngol Head Neck Surg. 2003 Jan;129(1):106-12. [Medline: 12525204] [doi: 10.1001/archotol.129.1.106]
62. van Zeeburg HJ, Snijders PJ, Wu T, Gluckman E, Soulier J, Surralles J, Castella M, van der Wal JE, Wennerberg J, Califano J, Velleuer E, Dietrich R, Ebell W, Bloemena E, Joenje H, Leemans CR, Brakenhoff RH. Clinical and molecular characteristics of squamous cell carcinomas from Fanconi anemia patients. J Natl Cancer Inst. 2008 Nov 19;100(22):1649-53. Epub 2008 Nov 11. [Medline: 19001603] [doi: 10.1093/jnci/djn366] [FREE Full Text]
63. Park JW, Pitot HC, Strati K, Spardy N, Duensing S, Grompe M, Lambert PF. Deficiencies in the Fanconi anemia DNA damage response pathway increase sensitivity to HPV-associated head and neck cancer. Cancer Res. 2010 Dec 1;70(23):9959-68. Epub 2010 Oct 8. [Medline: 20935219] [doi: 10.1158/0008-5472.CAN-10-1291] [FREE Full Text]
64. Sheu JJ, Hua CH, Wan L, Lin YJ, Lai MT, Tseng HC, Jinawath N, Tsai MH, Chang NW, Lin CF, Lin CC, Hsieh LJ, Wang TL, Shih IeM, Tsai FJ. Functional genomic analysis identified epidermal growth factor receptor activation as the most common genetic event in oral squamous cell carcinoma. Cancer Res. 2009 Mar 15;69(6):2568-76. Epub 2009 Mar 10. [Medline: 19276369] [doi: 10.1158/0008-5472.CAN-08-3199] [FREE Full Text]
65. Taioli E, Ragin C, Wang XH, Chen J, Langevin SM, Brown AR, Gollin SM, Garte S, Sobol RW. Recurrence in oral and pharyngeal cancer is associated with quantitative MGMT promoter methylation. BMC Cancer. 2009 Oct 6;9:354. [Medline: 19807915] [doi: 10.1186/1471-2407-9-354] [FREE Full Text]
66. Lo KW, Kwong J, Hui AB, Chan SY, To KF, Chan AS, Chow LS, Teo PM, Johnson PJ, Huang DP. High frequency of promoter hypermethylation of RASSF1A in nasopharyngeal carcinoma. Cancer Res. 2001 May 15;61(10):3877-81. [Medline: 11358799] [FREE Full Text]
67. Caulin C, Nguyen T, Longley MA, Zhou Z, Wang XJ, Roop DR. Inducible activation of oncogenic KRAS results in tumor formation in the oral cavity. Cancer Res. 2004 Aug 1;64(15):5054-8. [Medline: 15289303] [doi: 10.1158/0008-5472.CAN-04-1488] [FREE Full Text]
68. Ekshyyan O, Mills GM, Lian T, Amirghahari N, Rong X, Lowery-Nordberg M, Abreo F, Veillon DM, Caldito G, Speicher L, Glass J, Nathan CO. Pharmacodynamic evaluation of temsirolimus in patients with newly diagnosed advanced-stage head and neck squamous cell carcinoma. Head Neck. 2010 Dec;32(12):1619-28. [Medline: 20222045] [doi: 10.1002/hed.21374]
69. Samuel MS, Lourenço FC, Olson MF. KRAS mediated murine epidermal tumorigenesis is dependent upon and associated with elevated Rac1 activity. PLoS One. 2011 Feb 15;6(2):e17143. [Medline: 21358804] [doi: 10.1371/journal.pone.0017143] [FREE Full Text]

70. Klinakis A, Lobry C, Abdel-Wahab O, Oh P, Haeno H, Buonamici S, van De Walle I, Cathelin S, Trimarchi T, Araldi E, Liu C, Ibrahim S, Beran M, Zavadil J, Efstratiadis A, Taghon T, Michor F, Levine RL, Aifantis I. A novel tumour-suppressor function for the Notch pathway in myeloid leukaemia. Nature. 2011 May 12;473(7346):230-3. [Medline: 21562564] [doi: 10.1038/nature09999] [FREE Full Text]
71. Puente XS, Pinyol M, Quesada V, Conde L, Ordóñez GR, Villamor N, Escaramis G, Jares P, Beà S, González-Díaz M, Bassaganyas L, Baumann T, Juan M, López-Guerra M, Colomer D, Tubío JM, López C, Navarro A, Tornador C, Aymerich M, Rozman M, Hernández JM, Puente DA, Freije JM, Velasco G, Gutiérrez-Fernández A, Costa D, Carrió A, Guijarro S, Enjuanes A, Hernández L, Yagüe J, Nicolás P, Romeo-Casabona CM, Himmelbauer H, Castillo E, Dohm JC, de Sanjosé S, Piris MA, de Alava E, San Miguel J, Royo R, Gelpí JL, Torrents D, Orozco M, Pisano DG, Valencia A, Guigó R, Bayés M, Heath S, Gut M, Klatt P, Marshall J, Raine K, Stebbings LA, Futreal PA, Stratton MR, Campbell PJ, Gut I, López-Guillermo A, Estivill X, Montserrat E, López-Otín C, Campo E. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. Nature. 2011 Jun 5;475(7354):101-5. [Medline: 21642962] [doi: 10.1038/nature10113] [FREE Full Text]
72. Lee SY, Kumano K, Nakazaki K, Sanada M, Matsumoto A, Yamamoto G, Nannya Y, Suzuki R, Ota S, Ota Y, Izutsu K, Sakata-Yanagimoto M, Hangaishi A, Yagita H, Fukayama M, Seto M, Kurokawa M, Ogawa S, Chiba S. Gain-of-function mutations and copy number increases of Notch2 in diffuse large B-cell lymphoma. Cancer Sci. 2009 May;100(5):920-6. [Medline: 19445024] [doi: 10.1111/j.1349-7006.2009.01130.x]
73. Extance A. Alzheimer's failure raises questions about disease-modifying strategies. Nat Rev Drug Discov. 2010 Oct;9(10):749-51. [Medline: 20885394] [doi: 10.1038/nrd3288]
74. Nicolas M, Wolfer A, Raj K, Kummer JA, Mill P, van Noort M, Hui CC, Clevers H, Dotto GP, Radtke F. Notch1 functions as a tumor suppressor in mouse skin. Nat Genet. 2003 Mar;33(3):416-21. Epub 2003 Feb 18. [Medline: 12590261] [doi: 10.1038/ng1099]
75. Fu M, Wang C, Li Z, Sakamaki T, Pestell RG. Minireview: Cyclin D1: normal and abnormal functions. Endocrinology. 2004 Dec;145(12):5439-47. Epub 2004 Aug 26. Review. [Medline: 15331580] [doi: 10.1210/en.2004-0959] [FREE Full Text]
76. Mueller A, Odze R, Jenkins TD, Shahsesfaei A, Nakagawa H, Inomoto T, Rustgi AK. A transgenic mouse model with cyclin D1 overexpression results in cell cycle, epidermal growth factor receptor, and p53 abnormalities. Cancer Res. 1997 Dec 15;57(24):5542-9. [Medline: 9407965] [FREE Full Text]
77. Nakagawa H, Wang TC, Zukerberg L, Odze R, Togawa K, May GH, Wilson J, Rustgi AK. The targeting of the cyclin D1 oncogene by an Epstein-Barr virus promoter in transgenic mice causes dysplasia in the tongue, esophagus and forestomach. Oncogene. 1997 Mar 13;14(10):1185-90. [Medline: 9121767] [doi: 10.1038/sj.onc.1200937]
78. Wilkey JF, Buchberger G, Saucier K, Patel SM, Eisenberg E, Nakagawa H, Michaylira CZ, Rustgi AK, Mallya SM. Cyclin D1 overexpression increases susceptibility to 4-nitroquinoline-1-oxide-induced dysplasia and neoplasia in murine squamous oral epithelium. Mol Carcinog. 2009 Sep;48(9):853-61. [Medline: 19263437] [doi: 10.1002/mc.20531] [FREE Full Text]
79. Opitz OG, Suliman Y, Hahn WC, Harada H, Blum HE, Rustgi AK. Cyclin D1 overexpression and p53 inactivation immortalize primary oral keratinocytes by a telomerase-independent mechanism. J Clin Invest. 2001 Sep;108(5):725-32. [Medline: 11544278] [FREE Full Text]
80. Moral M, Segrelles C, Lara MF, Martínez-Cruz AB, Lorz C, Santos M, García-Escudero R, Lu J, Kiguchi K, Buitrago A, Costa C, Saiz C, Rodriguez-Peralto JL, Martinez-Tello FJ, Rodriguez-Pinilla M, Sanchez-Cespedes M, Garín M, Grande T, Bravo A, DiGiovanni J, Paramio JM. 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 Feb 1;69(3):1099-108. Epub 2009 Jan 27. [Medline: 19176372] [doi: 10.1158/0008-5472.CAN-08-3240] [FREE Full Text]
81. Jones JW, Raval JR, Beals TF, Worsham MJ, Van Dyke DL, Esclamado RM, Wolf GT, Bradford CR, Miller T, Carey TE. Frequent loss of heterozygosity on chromosome arm 18q in squamous cell carcinomas. Identification of 2 regions of loss--18q11.1-q12.3 and 18q21.1-q23. Arch Otolaryngol Head Neck Surg. 1997 Jun;123(6):610-4. [Medline: 9193222] [doi: 10.1001/archotol.1997.01900060052009]
82. Bian Y, Hall B, Sun ZJ, Molinolo A, Chen W, Gutkind JS, Waes CV, Kulkarni AB. Loss of TGF-β signaling and PTEN promotes head and neck squamous cell carcinoma through cellular senescence evasion and cancer-related inflammation. Oncogene. 2012 Jul 12;31(28):3322-32. Epub 2011 Oct 31. [Medline: 22037217] [doi: 10.1038/onc.2011.494]
83. Lu SL, Herrington H, Reh D, Weber S, Bornstein S, Wang D, Li AG, Tang CF, Siddiqui Y, Nord J, Andersen P, Corless CL, Wang XJ. Loss of transforming growth factor-beta type II receptor promotes metastatic head-and-neck squamous cell carcinoma. Genes Dev. 2006 May 15;20(10):1331-42. [Medline: 16702406] [doi: 10.1101/gad.1413306] [FREE Full Text]
84. Bornstein S, White R, Malkoski S, Oka M, Han G, Cleaver T, Reh D, Andersen P, Gross N, Olson S, Deng C, Lu SL, Wang XJ. Smad4 loss in mice causes spontaneous head and neck cancer with increased genomic instability and inflammation. J Clin Invest. 2009 Nov;119(11):3408-19. Epub 2009 Oct 19. [Medline: 19841536] [doi: 10.1172/JCI38854] [FREE Full Text]

85. Lu SL, Reh D, Li AG, Woods J, Corless CL, Kulesz-Martin M, Wang XJ. Overexpression of transforming growth factor beta1 in head and neck epithelia results in inflammation, angiogenesis, and epithelial hyperproliferation. Cancer Res. 2004 Jul 1;64(13):4405-10. [Medline: 15231647] [doi: 10.1158/0008-5472.CAN-04-1032] [FREE Full Text]
86. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, Martinez P, Matthews N, Stewart A, Tarpey P, Varela I, Phillimore B, Begum S, McDonald NQ, Butler A, Jones D, Raine K, Latimer C, Santos CR, Nohadani M, Eklund AC, Spencer-Dene B, Clark G, Pickering L, Stamp G, Gore M, Szallasi Z, Downward J, Futreal PA, Swanton C. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012 Mar 8;366(10):883-92. Erratum in: N Engl J Med. 2012 Sep 6;367(10):976. [Medline: 22397650] [doi: 10.1056/NEJMoa1113205] [FREE Full Text]
87. Hennessey PT, Ochs MF, Mydlarz WW, Hsueh W, Cope L, Yu W, Califano JA. Promoter methylation in head and neck squamous cell carcinoma cell lines is significantly different than methylation in primary tumors and xenografts. PLoS One. 2011;6(5):e20584. doi: 10.1371/journal.pone.0020584. Epub 2011 May 26. [Medline: 21637785] [doi: 10.1371/journal.pone.0020584] [FREE Full Text]
88. Pattani KM, Soudry E, Glazer CA, Ochs MF, Wang H, Schussel J, Sun W, Hennessey P, Mydlarz W, Loyo M, Demokan S, Smith IM, Califano JA. MAGEB2 is activated by promoter demethylation in head and neck squamous cell carcinoma. PLoS One. 2012;7(9):e45534. Epub 2012 Sep 24. [Medline: 23029077] [doi: 10.1371/journal.pone.0045534] [FREE Full Text]
89. Zhou S, Kachhap S, Sun W, Wu G, Chuang A, Poeta L, Grumbine L, Mithani SK, Chatterjee A, Koch W, Westra WH, Maitra A, Glazer C, Carducci M, Sidransky D, McFate T, Verma A, Califano JA. Frequency and phenotypic implications of mitochondrial DNA mutations in human squamous cell cancers of the head and neck. Proc Natl Acad Sci U S A. 2007 May 1;104(18):7540-5. Epub 2007 Apr 24. [Medline: 17456604] [doi: 10.1073/pnas.0610818104] [FREE Full Text]

To cite this article:
Spiotto MT, Pytynia M, Liu GF, Ranck MC, Widau R. Animal Models to Study the Mutational Landscape for Oral Cavity and Oropharyngeal Cancers.
J Oral Maxillofac Res 2013;4(1):e1
URL: http://www.ejomr.org/JOMR/archives/2013/1/e1/v4n1e1ht.pdf
doi: 10.5037/jomr.2013.4101