Bioluminescent Imaging of HPV-Positive Oral Tumor Growth and Its Response to Image-Guided Radiotherapy

Rong Zhong, Matt Pytynia, Charles Pelizzari, and Michael Spiotto

Abstract

The treatment paradigms for head and neck squamous cell cancer (HNSCC) are changing due to the emergence of human papillomavirus (HPV)-associated tumors possessing distinct molecular profiles and responses to therapy. Although patients with HNSCCs are often treated with radiotherapy, preclinical models are limited by the ability to deliver precise radiation to orthotopic tumors and to monitor treatment responses accordingly. To better model this clinical scenario, we developed a novel autochthonous HPV-positive oral tumor model to track responses to small molecules and image-guided radiation. We used a tamoxifen-regulated Cre recombinase system to conditionally express the HPV oncogenes E6 and E7 as well as a luciferase reporter (iHPV-Luc) in the epithelial cells of transgenic mice. In the presence of activated Cre recombinase, luciferase activity, and by proxy, HPV oncogenes were induced to 11-fold higher levels. In triple transgenic mice containing the iHPV-Luc, K14-CreERT2, and LSL-Kras transgenes, tamoxifen treatment resulted in oral tumor development with increased bioluminescent activity within 6 days that reached a maximum of 74.8-fold higher bioluminescence compared with uninduced mice. Oral tumors expressed p16 and MCM7, two biomarkers associated with HPV-positive tumors. After treatment with rapamycin or image-guided radiotherapy, tumors regressed and possessed decreased bioluminescence. Thus, this novel system enables us to rapidly visualize HPV-positive tumor growth to model existing and new interventions using clinically relevant drugs and radiotherapy techniques. Cancer Res; 74(7); 2073–81. ©2014 AACR.

Introduction

Given their distinct oncogenic and mutational pathways (1, 2), head and neck squamous cell cancers (HNSCC) may differentially respond to radiation and/or chemotherapy. Existing reports indicate that treatment outcomes depend on the p53 mutational status and human papillomavirus (HPV) oncogene expression (3–5). To better understand HNSCC biology, several models exist to study the development of autochthonous head and neck tumors. Many groups have relied on chemical carcinogens such as 4-Nitroquinolone 1-oxide (4-NQO) to induce cancers with undefined DNA lesions that mimic those caused by cigarette smoking (6, 7). To generate tumors with more homogeneous genetic profiles, other groups have genetically engineered mice to overexpress mutant cellular oncogenes such as Kras (8) or to delete tumor suppressors such as Tp53 (9–12). Furthermore, groups have engineered mice to express some of these oncogenes in a spatiotemporal manner using systems such as ligand-regulated Cre recombinases (8, 9, 13).

However, understanding how other oncogenes such as the HPV oncogenes E6 and E7 (E6E7; ref. 14) impact oral tumor responses to therapy are limited by the availability preclinical models, the accurate delivery of radiotherapy, and the assessment of treatment responses. Although several xenotransplant models exist for HPV-associated HNSCCs, these tumors were transplanted into immunodeficient mice and may be biologically distinct from the parental cancer (15–18). Furthermore, oral tumors developed in HPV-transgenic mice treated with 4-NQO (19), but these mice constitutively expressed HPV oncogenes, which may impact immune tolerance and tumor development. In addition, irradiation of oral tumors has been limited to 2 to 6 Gy due to the proximity of tumors to the central nervous system and other vital structures (17). Finally, monitoring treatment responses to autochthonous oral tumors has been mostly constrained to crude measurements such as survival and weight loss. Thus, understanding how the tumor genotype dictates response to therapy would benefit from novel preclinical models that monitor the response of primary HPV-positive tumors to radiation and other targeted therapies.

Here, we developed a novel head and neck tumor model to monitor the growth of HPV-positive tumors and their response to therapy using bioluminescence. We used a ligand-regulated Cre recombinase to induce the HPV oncogenes E6E7 and a luciferase reporter in vitro and in vivo. Oral tumors arose in mice when E6E7 and mutant KrasG12D oncogenes were induced in the basal epithelial layer. These oral tumors expressed HPV-associated biomarkers and grew faster than tumors arising in control mice harboring only a mutant Kras oncogene. HPV tumors gained bioluminescence over time, which was modulated by...
tumorcidal agents, including small-molecule inhibitors and image-guided radiotherapy (IGRT).

Materials and Methods

**Generation of iHPV-Luc transgenic vector and mice**

The p-B-actin E6E7 plasmid containing the HPV-16 E6E7 was a generous gift from Karl Munger (Harvard University, Boston, MA; ref. 20) and was obtained from Addgene (plasmid 13712). The E6E7 gene was amplified by the 5’ primer 5’-TTGAGGCACCGGCACACACGAAGAGAATTCG-3’ and 3’ primer 5’-TTCTGGAATTGTTCTGAGACAGATGG-3’. The E6E7 PCR product was digested with Eco RI-Xho I and ligated to MSCV IRES Luciferase plasmid, a generous gift of Scott Lowe (Memorial Sloan Kettering Cancer Center, New York, NY; Addgene plasmid 18760). An Eco RI-Sal I fragment of HPV-Luc to generate iHPV-Luc. The resulting plasmid was linearized with SalI-BamHI and transgenic mice were made by microinjection into the nuclei of FVB/N (The Jackson Laboratory) zygotes. Mice were maintained on an FVB/N background.

**Mice**

All mice were maintained under specific pathogen-free conditions and used according to protocols approved by The University of Chicago (Chicago, IL) Institutional Animal Care and Use Committee and Institutional Biosafety Committee. B6.129S4-Kras^{tm1(Ify)}/J (LSL-Kras) mice were purchased from The Jackson Laboratory. Transgenic mice STOCK-Tg(KRT14-cre/ERT2)20/Eh/J (K14-CreER{	extsuperscript{tam}}) have been described (22). K14-CreER{	extsuperscript{tam}} mice were backcrossed to the FVB background using MAX BAX speed congenics Taconic for seven generations and shared 99.74% similarities to FVB mice with 2 non-Max polymorphisms adjacent to the K14-CreER{	extsuperscript{tam}} transgene. K14-CreER{	extsuperscript{tam}} mice were bred with iHPV mice to generate K14-CreER{	extsuperscript{tam}} × iHPV-Luc mice (KH mice) on an FVB background. KH mice were then bred with LSL-Kras mice to generate KHR mice or KR mice on a C57BL/6 × FVB F1 background. Mice used for experiments were between 30 and 40 days old.

**Reagents**

Rapamycin was obtained from Sigma Chemical Co. and reconstituted (0.2% carboxymethylcellulose and 0.25% Tween-80 and injected into mice intraperitoneally; i.p.) at 4 mg/kg/d for 3 days. D-Luciferin Potassium Salt was obtained from Gold Biotechnology. Tamoxifen was purchased from Sigma. Tamoxifen was dissolved in sunflower seed oil vehicles at 20 mg/mL and 0.1 mL was injected intraperitoneally daily for 5 days. All oligonucleotides were synthesized by IDT.

**Fluorescence cytometry**

A total of 2 × 10^5 293 HEK cells (American Type Culture Collection; ATCC) were transfected with iE6E7 vector with or without PGK-Cre-bpA vector, a gift of Klaus Rajewski (Harvard University, Boston, MA; Addgene plasmid 11543) using Fugene HD (Promega). Cells were trypsinized after 48 hours and EGFP fluorescence was assessed. Peripheral blood lymphocytes were isolated by retroorbital bleeding and red blood cells were lysed with red blood cell lysis buffer (eBioscience). Cells were analyzed by FACscan and data were analyzed by FlowJo software.

**Western blot analysis**

A total of 10^6 293 HEK cells were transfected with iHPV-Luc vector with or without iCre vector using Fugene HD (Promega). Forty-eight hours later, cells were trypsinized and lysed with a modified radioimmunoprecipitation assay buffer [50 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, 1 mmol/L EDTA, 1% NP40, 1 μg/mL aprotinin, 1 μg/mL leupeptin, and 10 μmol/L phenylmethylsulfonylfluoride] for 30 minutes on ice. Samples were subjected to SDS-PAGE using a 4% stacking gel and a 10% resolving gel. The protein gel was then transferred to nitrocellulose, blocked, and probed using a 1:200 dilution of primary antibody and subsequently a 1:1000 dilution of a secondary horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G antibody (Pierce). The membrane was developed using enhanced chemiluminescence (Amersham).

**Luciferase assays**

A total of 2 × 10^5 293 HEK cells (ATCC) were transfected with iE6E7 vector with or without PGK-Cre-bpA vector, a gift of Klaus Rajewski using Fugene HD (Promega). Forty-eight hours later, cells were lysed in Bright Glo lysis buffer (Promega) for 20 minutes and luciferase activity was assessed using a Turner TD 20/20 Luminometer (Turner Designs) and luciferase activity was normalized to protein concentration of the sample.

**In vivo imaging**

Luciferase activity was measured noninvasively using the IVIS-200 imaging system (Caliper LifeSciences). Mice were injected intraperitoneally with luciferin (300 mg/kg dissolved in PBS; Caliper) and anesthetized via inhaled 3% isoflurane (Abbott Laboratories Ltd.). Exposure time for all images ranged from 1 to 5 seconds. All images were analyzed using Living Image software (Caliper) with a binning of 8. In vivo bioluminescent signal was quantified by taking the total photon counts for each region of interest.

**Histology**

Tumors were isolated and placed into 4% paraformaldehyde solution for 24 hours and then dehydrated with 70% ethanol. Tissues were embedded in paraffin, sectioned and stained with hematoxylin and eosin or the indicated antibodies at the University of Chicago Immunohistochemistry core facility.
Excision Kras-specific PCR

For detection of iE6E7 recombination, tumors were digested in lysis buffer supplemented with protease K and genomic DNA was isolated from tumors using ethanol precipitation. The iE6E7 gene was amplified by PCR using the primers: forward primer: 5'-CAACCTGTTATTGTGCGT-3' corresponding to the 3' end of the CAG promoter and the reverse primer: 5'-GGTAACTTTCTGGGTCGCTCCT-3' corresponding the 5' region of the E6E7 gene with a 58°C annealing temperature and 72°C extension temperature repeated over 33 cycles. The 1,629 bp amplicon represents the unexcised HPV vector and the 388 bp amplicon represents the excised iE6E7 gene.

For detection of the LSL-Kras recombination, gDNA was amplified by PCR using the forward primer: GTCTTTCCCCAGCACAAGTCG and the reverse primer: GGATGGCATCTTGAGCCTTTA that flank the floxed stop gene cassette. The resulting amplicon was digested with HindIII that cuts the unique restriction site engineered into the second exon of the mutant allele. The undigested 947 bp amplicon represents the wild-type allele and the digested 323 bp and 624 bp amplicons represent the mutant allele.

Image-guided radiotherapy

Mice bearing 14 to 21-day-old tumors were irradiated using an XRAD 225Cx (Precision Xray) small animal image-guided irradiator. Anesthetized mice were immobilized in the supine position and a cone beam computed tomography (CT) was obtained using 40 kVp and 250 mA. For each mouse, the isocenter was placed in the midpoint of the tumor and anterior to the mouth to cover the oral tumor using half of a 1.5-cm collimator. A radiation dosimetry plan was generated using opposed lateral fields. Mice were treated with 225 kVp X-rays at 13 mA to a dose of 20 Gy.

In vivo tumor growth

Tumors were measured every 3 days. Given the ellipsoid growth of oral tumors, we reasoned that the oral tumor volume would approximate a solid ellipsoid minus a cylindrical volume to account for space of the oral aperture. To measure the tumor volume, we measured the intercommisural distance (a) as well as two additional orthogonal measurements (b and c). In addition, the lip thickness was measured on each side (d and e) to calculate the diameter of the oral aperture. The oral tumor volume (mm³) was then calculated as: (1/6πa'b'c') – π((a-d-e)²/2)c. We illustrate this method in Supplementary Fig. S1.

Statistical analysis

Two-tailed independent Student t tests were done to analyze the results of in vitro luciferase assays, in vivo bioluminescence, or tumor growth time points.

Results

Generation of a Cre-loxP regulated inducible HPV transgenic mouse

Current HPV transgenic mouse models constitutively expressed E6 and/or E7 and, therefore, do not recapitulate the HPV infections that occur in young adults who are first exposed to exogenous HPV oncogenes. To generate an inducible HPV mouse model that better reflects the clinical scenario, we generated a transgenic vector where E6E7 expression was dependent on Cre recombinase (iHPV-Luc; Fig. 1A). To monitor oncogene activation and tumor development, the E6E7 cassette was followed by an internal ribosomal entry site (IRES)-luciferase gene that enabled bicistronic expression of the E6E7 oncogenes and the luciferase reporter. iHPV-Luc contained a floxed EGFP gene that inhibited expression of the E6E7-IRES-luciferase gene cassette expression. In the absence of Cre recombinase, cells transfected with iHPV-Luc had higher levels of EGFP and lower levels of E6 expression and luciferase activity (Fig. 1B–D). In the presence of Cre recombinase, cells expressed lower levels of EGFP and 10.1-fold higher levels of luciferase activity and E6 expression consistent with the Cre-mediated recombination of the floxed EGFP cassette and induction of the E6E7 genes and the luciferase reporter.

We next generated transgenic mice containing this iHPV construct. Of the 49 founder mice, 24 mice contained the iHPV-Luc transgene. In transgene-positive mice, we then assessed EGFP expression in the peripheral blood leukocytes that acted as a surrogate for the potential level of oncogene induction. We selected one mouse that expressed EGFP 290-fold above background (Fig. 2A). To assess the induction of the transgene, we bred iHPV-Luc mice to Rosa-CreERTm mice to generate HRosa mice. Treatment with tamoxifen activated the CreERTm fusion protein that was ubiquitously expressed by all tissues. After a 5-day course of tamoxifen treatment, HRosa mice had increased bioluminescence in the non-fur bearing skin that was 11-fold above background (tamoxifen treated: 10.3 ± 1.2-fold vs. vehicle treated: 0.9 ± 0.2-fold; Fig. 2B and C).

Generation of an autochthonous oral HPV tumor model

We next generated mice that developed autochthonous HPV-positive oral tumors. On the basis of previous reports, mice induced to express the KrasG12D mutant in the basal epithelial layer developed oral papillomas over the course of 2 months (8, 9). Similarly, we first bred K14-CreERTm mice to iHPV-Luc mice to generate KH mice (Fig. 3A). The K14-CreERTm transgene expresses the ligand-regulated Cre recombinase driven by the basal keratinocyte promoter, K14. KH mice were then bred to LSL-Kras mice to generate KR mice containing the K14-CreERTm and LSL-Kras transgene or KHR mice containing all three transgenes (K14-CreERTm × LSL-Kras × iHPV-Luc; Fig 3A). Tamoxifen treatment of KH, KR, or KHR mice resulted in excision of the respective LSL-Kras and/or iHPV-Luc transgenes (Fig. 3B).

We next monitored tumor growth in KR and KHR mice. Although oral tumors formed in KR and KHR mice, KHR tumors grew faster than in KR tumors (Fig. 3C). Histologic analysis of KHR tumors revealed papillomas that expressed the HPV-biomarkers p16 and MCM7 (Fig. 3D). Compared with oral tumors in KR mice, oral tumors developing in KHR mice had increased MCM7 expression and similar p16 expression.
KHR tumor growth was associated with increased bioluminescence

We then assessed the kinetics of bioluminescence after tamoxifen treatment (Fig. 4A). Within 3 days of initiating tamoxifen treatment, mice had increased bioluminescence compared with untreated mice. By 24 days after tamoxifen treatment, KHR mice had 65.7-fold higher bioluminescent signal compared with untreated controls (Fig. 4B). KHR mice that developed oral tumors had progressively increasing oral bioluminescence that correlated with tumor growth ($P < 0.0001$, $r = 0.88$; Fig. 4C). In contrast, bioluminescence around the oral cavity plateaued in KH mice within 6 days at 7.4-fold above background. KR mice treated with tamoxifen developed oral tumors that did not possess bioluminescence signal above background.

In vivo bioluminescent monitoring of response to tumoricidal agents

Because bioluminescent signal correlated with tumor volume, we tested the extent to which bioluminescent signal in autochthonous tumors would enable monitoring response to
agents that impacted tumor growth (9). Rapamycin has been shown to impact the growth in autochthonous oral tumor models driven by a mutant Kras, in transplanted head and neck squamous cell carcinoma models and in clinical trials. Fourteen days after tamoxifen treatment, we treated KHR mice with rapamycin for 3 days and monitored tumor growth. Compared with the vehicle-treated controls, mice treated with rapamycin had a transient decrease in tumor growth with regrowth after rapamycin was discontinued (Fig. 5A). Compared with untreated tumors, tumors treated with rapamycin displayed 3.3-fold decreased bioluminescent signal at the completion of treatment (Fig. 5B and C).

Because oral tumors in KHR mice possessed decreased bioluminescence that correlated with rapamycin treatment, we next tested the extent to which IGRT impacted KHR tumor growth and bioluminescence (Fig. 6). IGRT is rapidly being implemented in the clinic to treat patients with HNSCC given the increased ability to target tumors (23). As in patients, cone beam CT images were obtained for each tumor and the isocenter was placed according to the tumor extent (Fig. 6A). We developed individual radiotherapy plans using opposed lateral radiation beams to treat mice to 20 Gy. Fourteen days after tamoxifen treatment, mice were irradiated with 20 Gy and tumors regressed (Fig. 6B), whereas unirradiated tumors grew progressively. The final oral tumor volume was significantly smaller for irradiated KHR mice (31.63 $\pm$ 22.03 mm$^3$; n = 9) compared with unirradiated mice (414.01 $\pm$ 80.96 mm$^3$; n = 10; P = 4.96 $\times$ 10$^{-7}$). Six days after radiotherapy, irradiated tumors had 3-fold less bioluminescence compared with nonirradiated controls consistent with tumor regression. Therefore, bioluminescent signal was a surrogate for response to different targeted therapies in autochthonous oral tumors.

**Discussion**

Here, we developed a preclinical model to mimic the development and treatment of autochthonous HPV-positive oral tumors. Similar to patients who are first infected with HPV as young adults, we generated an inducible HPV oral tumor model to control HPV oncogene expression in a spatiotemporal manner. Furthermore, compared with previous models, this bioluminescent signal, linked to HPV oncogene expression, provided a noninvasive method to monitor the growth of intraoral tumors that were otherwise difficult to assess. Finally, we used this model to track responses to IGRT and small molecules, two interventions currently being used in the clinic. Therefore, we intend our novel model to study how HPV oncogenes alone or in cooperation with other genotypes impact tumor growth and response to therapy.

We recognize the limitations for our model to accurately reflect the clinical scenario of patients with HNSCC. First, mice developing HPV-positive tumors also required mutant Kras for tumor development. Although mutations in the ras family account for approximately 5% of HNSCCs (1, 2), several groups have shown that the ras–MAPK pathway was aberrantly activated in HNSCCs as well as in other HPV-positive tumors (24–27).
Second, HPV oncogene expression was driven by a cytomegalovirus promoter, which may express oncogenes at artificial levels that do not reflect human HPV-positive tumors. Nevertheless, most, if not all, HPV-positive cancers have viral sequences that nonspecifically integrated throughout the host genome, likely resulting in a range of expression levels (28). In addition, HPV oncogene expression accelerated oral papilloma growth but we did not find any obvious invasive cancer. Still, given the accelerated tumor growth, our model may not have had sufficient time to develop invasive disease. Finally, HPV-positive mice developed oral tumors and not oropharyngeal cancers that are seen in patients with HNSCC. Still, these oral tumors occurred at murine anatomic sites with epithelial transitional zones that are classically affected by HPV oncogenes (29).

![Figure 4](image-url)

**Figure 4.** The increase in bioluminescence correlated with tumor growth in KHR mice. A, time course for bioluminescence of KHR mice treated with vehicle or tamoxifen. Mice were imaged every 3 days after the initiation of treatment. B, kinetics of bioluminescence in KHR (n = 7), KR (n = 2), and KH (n = 4) mice treated with tamoxifen (Tam) or KHR mice (n = 5) treated with vehicle. *, P < 0.05, significant differences between vehicle-treated or tamoxifen-treated KHR mice. Error bars, ±1 SD. C, correlation of tumor volume with bioluminescence.

![Figure 5](image-url)

**Figure 5.** Bioluminescence tracked oral tumor response to small-molecule therapy. A, oral tumors in tamoxifen (Tam)-treated KHR mice regressed after rapamycin treatment. Mice bearing 14-day-old oral tumors were treated with 4 mg/kg of rapamycin for 3 days (n = 3) or vehicle (n = 5) and tumor growth was monitored every 3 days. Similar results were observed in two independent experiments. *, P < 0.05, significant differences between vehicle-treated or rapamycin-treated KHR mice. Error bars, ±1 SD. Arrow, rapamycin (Rap) treatment. B, five days after rapamycin treatment, oral tumors displayed lower bioluminescence levels. C, quantitation of bioluminescence in KHR mice bearing oral tumors treated with rapamycin (n = 3) or vehicle (n = 3).
genes expression was induced in the basal epithelial layer and oncogenes altered the biology of oral tumors. Studied, our model will enable us to further examine how HPV cooperation of HPV and Ras oncogenes have been widely tion of the cell cycle and/or cell invasiveness (29, 33). As the tumor aggressiveness through mechanisms such as dysregulation of the cell cycle and/or cell invasiveness (29, 33). As the cooperation of HPV and Ras oncogenes have been widely studied, our model will enable us to further examine how HPV oncogenes altered the biology of oral tumors.

Our system mimics the HPV’s life cycle where HPV oncogenes expressed in the basal epithelial layer and continued as cells differentiated into the suprabasal layers. In contrast, the establishment of HPV oncogenes using tissue-specific promoters including keratin 10 and keratin 14 that restricted oncogene expression based on the differentiation state of the cell (34–37). In addition, the induction of these oncogenes may minimize immune tolerance that occurs with constitutively expressed HPV antigens to better mimic immune responses to exogenous viral proteins (38). Thus, our inducible model may more physiologically reflect viral oncogene expression to enhance our understanding of HPV oncogenesis and response to therapy.

As in previous models (8, 9), we used a tamoxifen-regulated Cre recombinase expressed in the basal epithelial layer to induce HPV-positive oral tumors. Previous models of autochthonous oral tumors have relied on chemical carcinogens (6) or genetically engineered transgenes (8, 9, 13). These genetically defined models expressed mutant Kras and/or mutant Trp53 as well as deleted Notch1 (12) and/or Tgfbr1 (11). In addition, mouse models with inducible promoters have been used to drive oncogenes in the basal epithelial layer, resulting in squamous tumors (30, 31). Finally, other groups have directly fused oncogenes to hormonal receptors that spatially sequesters oncogene activity within the cell (32). Upon treatment with estrogen or other ligands, these mice developed squamous papillomas and invasive cancers. However, these inducible promoters and oncogene fusion proteins act only transiently while the ligand is present, which may impact tumorigenesis. Therefore, in our model, Cre-mediated recombination enabled sustained tissue-specific expression of HPV-oncogenes.

In our study, HPV oncogene expression has had functional consequences as HPV-positive oral tumors grew faster than HPV-negative tumors and gained expression of MCM7, a known HPV biomarker. These results are consistent with previous studies demonstrating that the ras–MAPK pathway cooperated with HPV oncogenes in vitro and in vivo to increase tumor aggressiveness through mechanisms such as dysregulation of the cell cycle and/or cell invasiveness (29, 33). As the cooperation of HPV and Ras oncogenes have been widely studied, our model will enable us to further examine how HPV oncogenes altered the biology of oral tumors.

In Vivo Imaging of Inducible HPV Oral Tumors

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Here, we applied a bioluminescent signal to monitor the development of autochthonous HPV-positive tumors. Bioluminescent systems have overcome the difficulties in monitoring the growth of tumors transplanted in orthotopic sites such as the oral cavity, which is difficult to assess (39, 40). However, orthotopic transplants were often studied in immunodeficient hosts that may not recapitulate the heterogeneous tumor biology seen in primary tumors (18). Other groups have adapted in vivo imaging technologies to monitor the development of autochthonous tumors including prostate (41, 42), pancreas (42), lymphoma (43), and others. However, in the majority of these models, the reporter transgenes were not linked to initiating oncogenic events and, therefore, it remained unclear whether regional differences existed between oncogene expression and reporter activity. In addition, others have linked reporter genes to tissue-specific promoters such as prostate-specific antigen and CD19 (43, 44) that are expressed in both malignant and nonmalignant parenchyma. Yet, these tissue-specific promoters may not be active in poorly differentiated tumors and, therefore, may not adequately track tumor growth.
Thus, genetically linking oncogene expression to reporter activity more faithfully reflects tumor growth.

Finally, we have used our novel autochthonous HPV tumor model to monitor responses to IGRT and small molecules that are currently used in the clinic. Although previous studies demonstrated that rapamycin prevented the outgrowth of autochthonous HPV-negative oral tumors (9), we observed that rapamycin also caused regression of HPV-positive tumors. Because rapamycin inhibits mTOR, our results suggest that the mTOR–PI3K pathway was active in our tumor model and are consistent with HNSCC sequencing data describing mutations in PIK3CA and PTEN, leading to the activation of the mTOR pathway (1, 2, 45). As the phosphoinositol 3-kinase (PI3K) pathway is often mutated in HNSCCs, we are applying forward and reverse genetic approaches to study the mTOR–PI3K pathway in our HPV mouse model (M. Spiotto; unpublished observations). Paralleling the clinical setting, IGRT involves tumor localization with a cone beam CT, real-time dosimetry planning, and subsequent dose delivery (46, 47).

Recently, several groups have treated autochthonous sarcomas and non–small cell lung cancers as well as normal tissues (8, 48–50). However, irradiation of these tumors at most resulted in growth arrest, whereas, in our model, we observed tumor regression. Although previous studies treated the head and neck region with low doses of radiation, we were able to deliver therapeutic doses of radiation using IGRT. Furthermore, IGRT will enable us to test how the tumor genotype impacts responses to biologically relevant doses delivered using different fractionation schemes (15). Thus, IGRT will more accurately target autochthonous HPV-positive tumors and enable a better understanding for how the tumor genotype impacts the radiation response.

In conclusion, we have developed a novel model to monitor the growth of HPV-positive oral tumors and their response to clinically relevant treatments. This model induced HPV oncogenes in the basal epithelial layer, mimicking the natural history of this disease. Furthermore, the bioluminescent signal from these oral tumors acted as a surrogate for tumor growth and response to therapy. Finally, we have validated this model to monitor responses to small molecules and to IGRT, two modalities that are currently being used in patients. We anticipate this system to serve as a platform to better test preclinical concepts in vivo as well as to address underlying mechanisms for how the genotype of autochthonous tumors impacts responses to existing and novel therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Concept and design: M. Spiotto

Development of methodology: R. Zhong, C. Pelizzari, M. Spiotto

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R. Zhong, M. Pytynia, C. Pelizzari, M. Spiotto

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Spiotto

Writing, review, and/or revision of the manuscript: R. Zhong, C. Pelizzari, M. Spiotto

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Pytynia, M. Spiotto

Study supervision: M. Spiotto

Acknowledgments

The authors thank Dr. Linda Degenstein and the University of Chicago Translational Core Facility for expert help in producing the dHPV transgenic mouse, the University of Chicago Cytometry Core Facility, Dr. Lara Leoni and the University of Chicago Optical Imaging Facility for expert advice and assistance, and Terry Li and Dr. Mark Lingen of the University of Chicago Human Tissue Resource Center for expert assistance with immunohistochemistry.

Grant Support

This work was supported by the Burroughs Wellcome Career Award for Medical Scientists and the Fanconi Anemia Research Fund (M. Spiotto). Acquisition of the image-guided animal irradiation was supported by NIH Shared Instrumentation Grant 1S10RR026747-01.

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Received October 18, 2013; revised January 14, 2014; accepted January 15, 2014; published OnlineFirst February 13, 2014.

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*Cancer Res* 2014;74:2073-2081. Published OnlineFirst February 13, 2014.

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