Impact of Age on Disease Progression and Microenvironment in Oral Cancer

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Abstract

Despite the recognized link between aging and cancer, most preclinical studies in experimental tumor models are conducted with 6- to 8-wk-old rodents. The goal of the present study was to examine the impact of age on tumor incidence, growth, and microenvironmental characteristics in mouse models of head and neck squamous cell carcinoma (HNSCC). Experimental studies were conducted with the 4-nitroquinoline-oxide (4NQO) oral carcinogenesis model and orthotopic FaDu HNSCC xenografts, established in young (7 to 12 wk of age) and old (65 to 70 wk of age) female C57BL/6 mice ($n = 44$; 4NQO model) and severe combined immunodeficient mice ($n = 13$; HNSCC xenografts). Noninvasive whole body magnetic resonance imaging revealed increased subcutaneous and visceral fat in aging animals of both strains. On histologic examination, a higher incidence ($P < 0.001$) of severe dysplasia/invasive squamous cell carcinoma was observed in old mice (92%) as compared with young mice (69%). Old C57BL/6 mice exposed to 4NQO exhibited increased incidence of oral and extraoral (peritoneal masses) neoplasms (42%) versus their young counterparts ($P < 0.05$). The incidence of extraoral neoplasms was significantly lower (16%) in the younger cohort. Interestingly, no difference in growth rate and oxygen saturation was observed between orthotopic FaDu xenografts established in old and young severe combined immunodeficient mice. Our observations suggest that host age may have an impact on the growth kinetics and progression of HNSCC in the immunocompetent 4NQO model. Further investigation into the impact of aging on tumor response to preventive and therapeutic intervention is warranted.

Keywords: aging, oral carcinogenesis, 4NQO, MRI, US, PAI

Introduction

It is widely accepted that cancer is a disease of aging (Dix et al. 1980; DePinho 2000). In humans, with a few exceptions (e.g., pediatric malignancies), most cancers are diagnosed in the sixth or seventh decades of life (Siegel et al. 2017). In the case of head and neck squamous cell carcinomas (HNSCCs), the age range at diagnosis is 60 to 78 y for smoking-related HNSCC and 40 to 55 y for human papilloma virus–positive head and neck cancers (Marur et al. 2010; Vigneswaran and Williams 2014). Despite this recognition, host age as a variable is often overlooked or unaccounted for in experimental cancer research. Most preclinical studies are conducted with 8- to 12-wk-old rodents that are analogous to adolescents or young adults (Flurkey et al. 2007; Dutta and Sengupta 2016; An et al. 2018). Observations in experimental tumor models suggest that the influence of age on tumor biology is tumor cell and microenvironment dependent. For example, B16-F10 melanomas (Pili et al. 1994) and subcutaneous Lewis lung carcinomas (Klement et al. 2007) were reported to grow slowly in old mice as compared with young animals. In contrast, robust growth of TRAMP C2 prostate tumors was reported in 20-mo-old mice versus 4-mo-old mice (Reed et al. 2007). Much of the published evidence on aging in cancer models pertains to the use of transplanted cell lines or xenografts. The impact of age on growth and progression of HNSCC has not been extensively examined. In the present study, we examined the impact of age on carcinogenesis, tumor growth, and microenvironmental parameters (perfusion and oxygenation) with 2 widely utilized,

clinically relevant animal models of HNSCC: 1) an oral squamous cell carcinoma (OSCC) model that involves exposure of immunocompetent C57BL/6 mice to the carcinogen 4-nitroquinoline oxide (4NQO; Kanojia and Vaidya 2006) and 2) orthotopic FaDu xenografts (Nelson et al. 2005; Seshadri and Toth 2009) established in severe combined immunodeficient (SCID) mice (Fig. 1A). To enable noninvasive longitudinal assessment of disease progression in vivo, we utilized a multi-modal imaging approach based on magnetic resonance imaging (MRI), photoacoustic imaging (PAI), and ultrasound (US). MRI offers high spatial resolution of the whole animal and the capacity to simultaneously obtain information related to anatomy and physiology (Nieman et al. 2005), whereas PAI/US enables functional assessment of tumor growth and oxygen saturation (sO_2) in a cost-effective and high-throughput

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A supplemental appendix to this article is available online.

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manner (Kruger et al. 1995; Wang 2009). Outcome measures in the 4NQO model included assessment of tumor burden (oral/extraoral neoplasms) on MRI with histologic evaluation of the incidence of precancer (severe dysplasia) and invasive OSCC and survival analysis. Assessment in orthotopic FaDu xenografts included tumor volume (US), sO₂ and hemoglobin concentration (HbT; both PAI), and correlative histology.

Materials and Methods

Animals

Experimental studies were carried out with young (7 to 12 wk) and old (65 to 70 wk) female C57BL/6NCR mice (Charles River) and CB.17 (C.B-Igh-1^b/IcrTac-Prkdc^{scid}) SCID mice (Laboratory Animal Resource, Roswell Park Comprehensive Cancer Center). Animals were housed in microisolator cages in a laminar flow unit in air-conditioned and light-controlled rooms (12-h cycles) and fed a house chow diet. All experimental procedures were performed in accordance with approved protocols at the Roswell Park Comprehensive Cancer Center.

4NQO Oral Carcinogenesis Model

The carcinogen 4NQO was dissolved in propylene glycol at 4 mg/mL and diluted in autoclaved water to a final concentration of 100 µg/mL (Bothwell et al. 2015). Young and old C57BL/6 mice were exposed to 4NQO in drinking water for 16 wk (Fig. 1B). The water was changed every 7 to 10 d. Regular autoclaved water was provided following 16 wk of carcinogen exposure. Control animals received autoclaved water at all times. Animals were monitored for tumor growth by visual examination of the oral cavity under white light once every 2 d throughout the duration of the study. Mice were humanely euthanized when sustained weight loss (>20% of their highest body weight) was observed or at end point (wk 32).

Orthotopic FaDu Xenograft Model of HNSCC

Orthotopic HNSCC xenografts were established in young and old female SCID mice by transcervical injection of FaDu cells (1×10^6) as described previously (Seshadri and Toth 2009). Animals were monitored for changes in body weight during the study period.

Figure 1. Experimental models and study design. **(A)** The impact of age on carcinogenesis and tumor growth was studied in 2 clinically relevant animal models of head and neck squamous cell carcinoma (HNSCC): an oral carcinogenesis model that involves exposure of immunocompetent C57BL/6 mice to the carcinogen 4-nitroquinoline oxide (4NQO) and a model based on orthotopic FaDu xenografts established in severe combined immunodeficient (SCID) mice. The experimental design, study timeline, and end points for studies in the **(B)** 4NQO and **(C)** orthotopic FaDu xenograft models are shown. Outcome measures in the 4NQO model included assessment of tumor burden (oral/extraoral neoplasms) on magnetic resonance imaging (MRI) with histologic evaluation of the incidence of precancer (severe dysplasia) and invasive oral squamous cell carcinoma (OSCC) and survival analysis. Assessment in orthotopic FaDu xenografts included tumor volume (ultrasound [US]), oxygen saturation and hemoglobin concentration (photoacoustic imaging [PAI]), and correlative histology.

Magnetic Resonance Imaging

MRI examinations were performed with a 4.7-T/33-cm horizontal bore magnet (GE NMR Instruments) as described previously (Bothwell et al. 2015). Baseline imaging was performed at week 15 following start of 4NQO exposure with subsequent examinations performed once every 3 to 4 wk. When feasible, additional imaging examinations were performed prior to euthanasia depending on the health status of individual animals.

PAI with Coregistered US

PAI with coregistered US was performed with the Vevo LAZR system (Visual Sonics Inc.): transducer, LZ-250; frequency, 21 MHz; depth, 20.00 mm; width, 23.04; wavelength, 750/850 nm; acquisition mode, sO_2 /Hbt. Three-dimensional PA images were acquired for the whole tumor to obtain whole tumor estimates of tumor percentage sO_2 (% sO_2) and HbT. Postprocessing of data was performed with the FujiFilm VisualSonics workstation suite (Vevo Lab 2.2; Visual Sonics Inc.) by tracing a 3-dimensional region of interest over the entire tumor. High-resolution US was performed to obtain tumor volumes on days 4, 6, 11, and 15. PAI of tumor hemodynamics was performed on days 4, 8, and 16 (Fig. 1C). Quantitative estimates and parametric maps of tumor % sO_2 and HbT were obtained with the 2-wavelength approach, as previously reported (Rich and Seshadri 2016).

Histopathologic Assessment

Whole tongue specimens were fixed in 10% neutral-buffered formalin (Sigma) for histology. In the 4NQO model, hematoxylin and eosin-stained sections of the tongue were evaluated for hyperkeratosis and hyperplasia, dysplasia (mild, moderate, and severe), carcinoma in situ, and invasive squamous cell carcinoma as described previously (Warnakulasuriya 2001; Warnakulasuriya et al. 2008). Hyperplasia and hyperkeratosis were defined as thickened epithelium with prominent surface keratinization with or without elongated rete ridges. Oral epithelial dysplasia was diagnosed when the epithelium exhibited cellular changes (increased nuclear:cytoplasmic ratio, hyperchromatic nuclei, increased or abnormal mitotic figures, nuclear pleomorphism) and architectural changes (loss of polarity in the epithelial cells, dyskeratosis, drop-shaped rete ridges) in the lower third parabasal layers (mild dysplasia), potentially extended to the middle third (moderate dysplasia) and upper third (severe dysplasia / carcinoma in situ) layers (Warnakulasuriya 2001; Warnakulasuriya et al. 2008). Squamous cell carcinoma was defined by the presence of atypical epithelial cells invading into the stroma/connective tissues.

Sample Sizes and Statistical Considerations

For the 4NQO model, young (7 wk of age, $n = 19$) and old (70 wk of age, $n = 25$) C57BL/6 mice were exposed to 4NQO in drinking water for 16 wk. MRI and histology were not

obtained for 1 animal in the old cohort. MRI was also performed on a control group ($n = 5$) of naïve C57BL/6 mice (beginning at 70 wk of age) without 4NQO exposure. For studies in the xenograft model, orthotopic FaDu tumors were established in young (12 wk of age, $n = 6$) and old (65 wk of age, $n = 7$) SCID mice. The ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines were utilized to design and report the results of animal experiments. Differences in tumor volumes and PAI values calculated with US/PAI were analyzed with 2-way analysis of variance. Differences in the incidence of lesions on MRI and histology were analyzed with the chi-square test. P values <0.05 were considered statistically significant. All statistical analyses were performed with Graph Pad Prism 6.00 for Windows (Graph Pad Software; <http://www.graphpad.com>).

Results

Longitudinal MRI and Histology of Oral Lesions in Young and Old Mice Following 4NQO Exposure

We utilized noninvasive T2-weighted MRI (T2W-MRI) to longitudinally monitor the effects of 4NQO exposure in old and young C57BL/6 mice. In a previous study, T2W-MRI of 6- to 8-wk-old mice that were not exposed to the carcinogen 4NQO did not show any evidence of oral or extraoral neoplasms (Bothwell et al. 2015). Similarly, MRI examination performed on naïve mice of different ages without intervention (i.e., 4NQO exposure) did not show any evidence of oral neoplasms (Appendix Figs. 1–3). The panel in Figure 2 represents axial T2W-MRI of three 70-wk-old (Fig. 2A) and three 7-wk-old (Fig. 2B) C57BL/6 mice exposed to 4NQO. A single slice from multislice imaging data sets at different times after carcinogen exposure is shown for all 6 animals. Exposure to 4NQO resulted in thickening on the lateral borders of the tongue (yellow arrows) in old (Fig. 2A, a) and young (Fig. 2B, a) mice. Corresponding histology of tongue sections from old mice revealed papillary structures covered by severe dysplastic epithelium (Fig. 2A, d) and hyperkeratotic stratified squamous epithelium exhibiting mild dysplasia (Fig. 2A, e). T2W-MRI also revealed hyperintense masses (white arrows) on the dorsum of the tongue in old mice that exhibited consistent (Fig. 2A, b) and aggressive (Fig. 2A, c) growth over time. Tongue sections revealed parakeratinized stratified squamous epithelium exhibiting hyperplasia (Fig. 2A, f) and papillary structures covered by severe dysplastic epithelium (Fig. 2A, g). The tongue lesion of mouse m3 exhibited hyperplastic surface-stratified squamous epithelium exhibiting moderate epithelial dysplasia (Fig. 2A, h) and invasive carcinoma characterized by infiltration of the underlying connective tissue with nests, islands, and sheets of neoplastic epithelial cells. The neoplastic cells exhibited nuclear and cellular pleomorphism and marked nuclear hyperchromatism (Fig. 2A, i). Tongue lesions in young mice also exhibited dysplasia and invasive squamous cell carcinoma (Fig. 2B, d–i). The tongue lesion of mouse m1 exhibited papillary structures covered by severe dysplastic epithelium

Figure 2. Magnetic resonance imaging (MRI) and histology of 4-nitroquinoline oxide (4NQO)-induced disease progression in (A) old and (B) young C57BL/6 mice. (a–c) Axial T2-weighted MRI (T2W-MRI) of 3 old and young mice at different times from start of 4NQO exposure. MRI revealed thickening of the lateral borders of the tongue (yellow arrows; m1) and exophytic lesions on the dorsum (m2, m3; white arrows). The animal m3 in both cohorts was euthanized prior to the last time point due to aggressive tumor growth (indicated by X). (d–i) Histologic assessment of tongue tissue demonstrated severe dysplasia and oral squamous cell carcinoma. Hematoxylin and eosin (H&E) images at (d, f, h) 10× magnification (scale bars: 200 μm) and (e, g, i) 20× magnification (scale bars: 100 μm). No histologic differences were observed between the preneoplastic and neoplastic lesions observed in old and young mice.

(Fig. 2B, d, e) whereas the tongue lesions from m2 and m3 exhibited infiltrating nests of squamous cell carcinoma in the stroma/connective tissue (Fig. 2B, f–i).

Quantitative evaluation revealed differences in the incidence of oral carcinogenesis between old and young mice. Figure 3 shows the number of mice in the old and young

Figure 3. Incidence of severe dysplasia and oral squamous cell carcinoma (OSCC) in old and young C57BL/6 mice from start of 4-nitroquinoline oxide exposure. The total number of animals that exhibited severe dysplasia and OSCC across the duration of the study was higher in old mice (22 of 24, ~92%) than in young mice (13 of 19, ~69%).

cohorts that showed severe dysplasia + OSCC over the entire study duration. The combined incidence of severe dysplasia and OSCC across the entire study duration was higher in old mice (22 of 24, ~92%) as compared with their young counterparts (13 of 19, ~69%; $P = 0.05$). Temporal analysis revealed earlier onset of severe dysplasia and OSCC in older mice (~92%) versus their young counterparts (43%) by weeks 22 to 27, suggestive of a longer tumor latency period in young mice. No difference in disease incidence was observed between old and young mice past week 28.

Whole Body MRI of Old and Young Mice Following 4NQO Exposure

Noninvasive MRI was also used to examine physiologic and pathologic changes in old and young mice at the whole body level. Figure 4 shows coronal T2W-MRI of 2 animals from the old cohort and 2 animals from the young cohort at different times after carcinogen exposure. Longitudinal MRI of aging animals with (Fig. 4A) or without (Appendix Fig. 3) 4NQO exposure revealed increased adipose tissue primarily in the form of subcutaneous fat deposits (yellow arrows) with splenic enlargement (outlined in green). Histologic assessment of splenic sections from old mice revealed the presence of granulomas and disorganization of the red and white pulp (mostly white pulp expansion) and proliferative histiocytosis. However, no evidence of extraoral masses was detected in naïve mice of different ages without 4NQO exposure (Appendix Figs. 1–3). In comparison, extraoral neoplasms (abdominal and peritoneal masses) were detected in 46% (11 of 24) of older animals (Fig. 4A, outlined in yellow) versus 16% (3 of 19) in young mice (Fig. 4C, $P < 0.05$). Histologic examination ($n = 6$) of peritoneal masses from older mice exposed to 4NQO showed presence of high-grade large cell lymphomas (Appendix Fig. 4).

Effect of Host Age on Tumor Growth and Microenvironmental Characteristics in HNSCC Xenografts

Finally, we examined the effect of host age on the growth and vascular characteristics of orthotopic HNSCC xenografts with US and PAI. Orthotopic FaDu xenografts were established in old and young SCID mice and monitored with US. Figure 5A shows B-mode US images of orthotopic FaDu tumor growth in old and young SCID mice over a 15-d period postimplantation. US-based tumor volume measurements did not reveal any significant difference in tumor volume or growth kinetics between the cohorts (Fig. 5B). Consistent with the observations in the 4NQO model, hematoxylin and eosin-stained tumor sections did not reveal any differences in histology between FaDu xenografts established in old and young SCID mice (Fig. 5C). Tumors from both cohorts exhibited squamous histology with the presence of intercellular bridges, hyperchromatic and pleomorphic nuclei, numerous abnormal mitotic figures, and increased nuclear:cytoplasmic ratio. To determine if host age influenced tumor angiogenesis in HNSCC, PAI was performed at different times postimplantation, and changes in tumor %sO₂ and HbT were estimated. The panel in Figure 5D represents pseudo-colored %sO₂ maps of FaDu tumors (outlined in black) in old and young mice at different times postimplantation. Tumors in young and old cohorts appeared well oxygenated at all time points of assessment during the 2-wk period. Quantitative estimates of tumor %sO₂ levels did not reveal any differences between tumors established in young and old mice (Fig. 5E). Tumors in young and old animals showed evidence of decline in HbT levels at later time points (Fig. 5F). However, differences in tumor HbT levels between cohorts and time points were not statistically significant.

Discussion

The overall goal of the present study was to examine the impact of age on carcinogenesis, growth, and functional microenvironmental characteristics in HNSCC. To this end, studies were conducted with an immunocompetent 4NQO model of HNSCC and human HNSCC xenografts established in immunodeficient mice. Our observations highlight the impact of aging on the progression of HNSCC in experimental model systems.

The 4NQO model is one of the widely utilized preclinical models for studying oral carcinogenesis (Kanojia and Vaidya 2006), the chemopreventive efficacy of nutritional supplements (e.g., metformin; Vitale-Cross et al. 2012), and the response to targeted therapy (Leeman-Neill et al. 2011). Consistent with these published studies, 4NQO exposure in drinking water resulted in the development of oral premalignant and malignant lesions in young and old mice. We previously utilized MRI to characterize the growth kinetics of 4NQO-induced OSCC in young C57BL/6 mice (Bothwell et al. 2015). Similarly, T2W-MRI enabled noninvasive longitudinal visualization of OSCC progression in young and old mice. Ex vivo histologic

Figure 4. Whole body magnetic resonance imaging (MRI) of old and young mice following 4-nitroquinoline oxide (4NQO) exposure. Coronal T2-weighted MRI of (A) old and (B) young C57BL/6 mice at different times from start of 4NQO exposure (weeks 15, 27, and 31/32). Images for 2 animals from each cohort are shown. MRI revealed increased subcutaneous fat deposits (yellow arrows) with splenic enlargement (outlined in green). Whole body MRI examination revealed presence of extraoral (abdominal and peritoneal masses) neoplasms in 46% (11 of 24) of older animals (outlined in yellow), as opposed to 16% (3 of 19) of young mice ($P < 0.05$). (C) Incidence of extraoral neoplasms in old and young mice over time. At 21 wk, 21% of the old mice showed radiologic evidence of extraoral tumor masses (vs. 0% of young mice). The incidence rose to 42% by week 27 (vs. 16%) and 46% by week 33 (vs. 16%).

assessment of the tongue confirmed the presence of invasive squamous cell carcinoma in the 4NQO model. While no differences in histology were observed between tumors that developed in old and young mice, a higher incidence of severe dysplasia and OSCC was observed in old mice as compared with their young counterparts. One plausible explanation for

Figure 5. Impact of age on growth and oxygenation of orthotopic FaDu xenografts. **(A)** B-mode ultrasound images of orthotopic FaDu tumor growth (outlined in white) in old and young severe combined immunodeficient mice. **(B)** Ultrasound-based tumor volume measurements revealed comparable tumor growth kinetics in young and old mice. Values are presented as mean $\pm$ SE. **(C)** Photomicrographs of hematoxylin and eosin–stained FaDu tumors established in old and young mice. Images are shown at 20 $\times$ magnification (scale bar: 100 μm). Tumors from both cohorts exhibited squamous histology with the presence of intercellular bridges, hyperchromatic and pleomorphic nuclei, numerous abnormal mitotic figures, and an increased nucleus:cytoplasm ratio. **(D)** Pseudocolored photoacoustic imaging oxygen saturation (sO_2) maps of FaDu tumors in old (left) and young (right) mice at 16 d postimplantation. Quantitative estimates of tumor **(E)** sO_2 levels and **(F)** hemoglobin concentration (HbT) did not reveal any differences between young and old mice. Values are presented as mean $\pm$ SD.

our observation is increased susceptibility of aging animals to tumor-promoting effects of carcinogens such as 4NQO due to immunosenescence. Multiple hallmarks of aging, including reduced functionality, decline in immune function (“immunosenescence”), and impaired tissue regeneration or repair capacity, can directly affect oral cancer progression (López-Otín et al. 2013; An et al. 2018). Furthermore, in our study, MRI revealed increased subcutaneous and visceral fat in aging animals of both strains and in aging animals not exposed to 4NQO. Visceral fat accretion is a hallmark of aging that increases risk of disease (Hunter et al. 2010). It is therefore possible that aging-related change in visceral fat may have also contributed to the increased incidence of extraoral neoplasms in the 4NQO model. Consistent with our finding, shorter tumor latency periods were previously reported in aged C57BL/6 mice following oral dosing of the carcinogen dimethylbenzanthracene (Bojovic and Crowe 2010). While advanced age was also shown to have a negative effect on the survival of C57BL/6 mice bearing intracranial GL261 gliomas (Ladomersky et al. 2016), Kaplan-Meier analysis did not reveal any significant difference in median survival between old and young C57BL/6 mice exposed to 4NQO (Appendix Fig. 5). However, radiographic evidence of carcinogen-induced changes in the oral cavity was apparent by week 15 in old mice, and similar observations on MRI of young mice were apparent only at a later time point (week 27). Consistent with this finding, 40% of the animals in the aged cohort were alive at week 26 (10 wk postcompletion of 4NQO exposure), as opposed to 80% animals in the young cohort, suggestive of earlier onset of disease in old mice (Appendix Fig. 5).

Noninvasive MRI enabled whole body imaging in these animals and revealed the presence of extraoral (peritoneal) masses in older mice. Histologic evaluation was performed on 6 of 11 extra oral masses found in older animals. Examination of peritoneal masses revealed the presence of nonepithelial malignancy consistent with high-grade large cell lymphomas. This suggests that the extraoral masses are likely independent primary neoplasms rather than metastases from the oral lesions. Szymanska et al. (2014) documented that C57BL/6 mice are prone to hematopoietic cancers and malignant lymphomas with age. However, we did not observe such tumor masses in age-matched, non-carcinogen-exposed controls. While aging C57BL/6 mice are susceptible to hematopoietic neoplasms, our results suggest that carcinogen exposure in older animals renders them even more susceptible to neoplastic transformation.

Building on our findings in the 4NQO model, we investigated the influence of aging on tumor growth and vascularity with orthotopic FaDu tumors, a well-characterized xenograft model of HNSCC. For these experiments, noninvasive PAI with coregistered US was utilized to longitudinally study tumor growth and perfusion. However, we did not observe any difference in tumor volume or hemodynamic parameters (HbT and sO₂) over time between FaDu xenografts established in old and young SCID mice. The contrasting observations between the 4NQO and FaDu models further point to the potential influence of aging-associated immunosenescence on OSCC progression.

Future studies should address the limitations of the present study. First, aging is a complex variable that can influence carcinogenesis through multiple mechanisms (López-Otín et al. 2013). It would therefore be important to understand the mechanisms that regulate oral carcinogenesis in aging hosts. The potential of chemopreventive intervention against oral premalignant lesions and invasive cancer should also be studied in the context of aging animals. Second, while our observations illustrate the influence of host age on disease progression, we did not study the impact of host age on therapeutic response. As such, the impact of aging-associated immunosenescence in dental, oral, and craniofacial tissues is being increasingly recognized (An et al. 2018). It would therefore be interesting to evaluate the response of tumors in old and young immunocompetent hosts to immune checkpoint inhibitors. Finally, the issue of sex as an experimental variable warrants further attention. Unlike sex-specific cancers (e.g., ovarian, prostate), oral cancers occur in both sexes albeit with a higher incidence in males. Only female animals were used in the present study. It would therefore be critical to examine how physiologic differences between male and female sexes influence disease biology in experimental systems.

In summary, the current study adds to the limited knowledge on the influence of aging in preclinical models of head and neck cancer. Host age is often an overlooked variable in preclinical studies due to economic and feasibility constraints. Our results suggest that host age could have an impact on disease progression following carcinogen exposure in immunocompetent mouse models of HNSCC.

Author Contributions

V.K. Vincent-Chong, L.J. Rich, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; H. DeJong, A. Patti, contributed to design, data acquisition, and analysis, drafted and critically revised the manuscript; M. Merzianu, P.A. Hersherberger, contributed to conception, data analysis, and interpretation, drafted and critically revised the manuscript; M. Seshadri, contributed to conception, design, data analysis, and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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