Oral Oncology 56 (2016) 25–31

Contents lists available at ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

Oncolytic adenoviruses targeted to Human Papilloma Virus-positive head and neck squamous cell carcinomas

Christopher J. LaRocca [a], Joohee Han [a], Amanda O. Salzwedel [a], Julia Davydova [a,b], Mark C. Herzberg [c], Rajaram Gopalakrishnan [c], Masato Yamamoto [a,b,d,*]

[a] Department of Surgery, University of Minnesota, United States
[b] Masonic Cancer Center, University of Minnesota, United States
[c] Department of Diagnostic & Biological Sciences, School of Dentistry, University of Minnesota, United States
[d] Institute of Molecular Virology, University of Minnesota, United States

ARTICLE INFO

Article history:
Received 3 September 2015
Received in revised form 18 January 2016
Accepted 29 February 2016
Available online 18 March 2016

Keywords:
Human papillomavirus
Head and neck cancer
Squamous cell carcinoma
Oncolytic adenovirus

SUMMARY

Objectives: In recent years, the incidence of Human Papilloma Virus (HPV)-positive head and neck squamous cell carcinomas (HNSCC) has markedly increased. Our aim was to design a novel therapeutic agent through the use of conditionally replicative adenoviruses (CRAds) that are targeted to the HPV E6 and E7 oncoproteins.
Methods: Each adenovirus included small deletion(s) in the E1a region of the genome (Δ24 or CB016) intended to allow for selective replication in HPV-positive cells. In vitro assays were performed to analyze the transduction efficiency of the vectors and the cell viability following viral infection. Then, the UPCI SCC090 cell line (HPV-positive) was used to establish subcutaneous tumors in the flanks of nude mice. The tumors were then treated with either one dose of the virus or four doses (injected every fourth day).
Results: The transduction analysis with luciferase-expressing viruses demonstrated that the 5/3 fiber modification maximized virus infectivity. In vitro, both viruses (5/3Δ24 and 5/3CB016) demonstrated profound oncolytic effects. The 5/3CB016 virus was more selective for HPV-positive HNSCC cells, whereas the 5/3Δ24 virus killed HNSCC cells regardless of HPV status. In vivo, single injections of both viruses demonstrated anti-tumor effects for only a few days following viral inoculation. However, after four viral injections, there was statistically significant reductions in tumor growth when compared to the control group ($p < 0.05$).
Conclusion: CRAds targeted to HPV-positive HNSCCs demonstrated excellent in vitro and in vivo therapeutic effects, and they have the potential to be clinically translated as a novel treatment modality for this emerging disease.

Introduction

In the United States, approximately 55,000 new cases of head and neck cancers were diagnosed in 2014 [1]. These cancers encompass a heterogeneous group of malignances including oral, oropharyngeal, pharyngeal, and laryngeal tumors. Importantly, the vast majority of these malignancies are squamous cell carcinomas [2]. In recent years, the incidence of Human Papilloma Virus (HPV)-positive head and neck squamous cell carcinomas (HNSCC) has markedly increased [3]. Specifically, the population level incidence of HPV-positive oropharyngeal squamous cell carcinomas increased by 225% from 1998 to 2004, while there was a concomitant 50% decline in the incidence of their HPV-negative counterparts during the same time period [4]. Additionally, the annual number of HPV-positive oropharyngeal cancers is projected to surpass that of cervical cancers by 2020 [4].

HPV-positive HNSCCs are considered distinct biologic entities when compared to those without the viral association. When compared to HPV-negative HNSCCs, HPV-positive tumors tend to occur in younger individuals without exposures to the traditional risk

Abbreviations: HPV, Human Papilloma Virus; HNSCC, head and neck squamous cell carcinoma; CRAd, conditionally replicating adenovirus; ADP, adenovirus death protein; Luc, luciferase.

* Corresponding author at: Division of Basic and Translational Research, University of Minnesota, Department of Surgery, 420 Delaware Street SE, MMC 195, Minneapolis, MN 55455, United States. Tel.: +1 612 624 9131 (office), cell: +1 763 354 9483; fax: +1 612 624 9080.
E-mail address: yamam016@umn.edu (M. Yamamoto).

http://dx.doi.org/10.1016/j.oraloncology.2016.02.014
1368-8375/

factors such as smoking and alcohol use [5,6]. While HPV-positive HNSCCs do have an improved survival when compared to HPV-negative tumors [7], distant recurrences often have atypical presentations. Distant metastases associated with HPV-positive tumors have been shown to occur in multiple organs, unusual sites, and after longer intervals when compared to those in HPV-negative tumors [8]. These distant lesions are known to be a major cause of death in patients with oropharyngeal squamous cell carcinomas [9].

Much of the aforementioned differences in behavior can be attributed to the distinct mechanisms of oncogenesis in HPV-positive tumors. Among the various HPV subtypes, HPV 16 and 18 (high risk) are most often associated with malignant transformation, and 95% of HPV-positive tumors within the oral cavity and oropharynx contained HPV 16 DNA [10,11]. Unlike their low-risk counterparts, DNA from HPV 16 and 18 are known to contribute to the immortalization of human keratinocytes [12]. More specifically, it is the E6 and E7 oncoproteins encoded by only the high risk subtypes that have been shown to transform and immortalize primary human keratinocytes [13,14]. The E6 protein suppresses the function of p53 by promoting its degradation through the ubiquitin-dependent protease system [15,16] and downregulates p53 by binding to the co-activator p300/CBP [17]. E7 binds to the retinoblastoma (Rb) protein to disrupt interactions with the E2F transcription factor, which results in the release of free E2F in its active form to potentiate tumorigenesis [18,19].

The increasing incidence of HPV-positive HNSCCs and their unique biology have necessitated the development of novel treatment methods. Traditionally, chemotherapy and radiotherapy have been part of the standard therapy for this disease process, but these treatments can be quite morbid. Adenovirus-based vectors have emerged as a powerful tool for the treatment of many types of cancers, and could have a great deal of applicability for the treatment of HPV-positive HNSCCs. When used in conjunction with standard therapies, adenovirus-based vectors may be able to lessen the required dosages of chemoradiotherapy, which could improve a patient's quality of life. Specifically, conditionally replicative adenoviruses (CRAd) are attractive as therapeutic agents as they have the potential to selectively replicate within the target cells of interest. Importantly, adenoviruses and the HPV E6/E7 oncoproteins interact with similar regulatory proteins to modulate the cell cycle. The adenovirus E1A protein is a key component for successful adenoviral replication. The CR1 region of the adenovirus E1A protein has been shown to bind to p300, which then suppresses the function of p53 [20]. Additionally, the CR2 region of the E1A protein is able to disrupt the interaction between the pRb and E2F complex, rendering the retinoblastoma protein unable to perform its normal cell cycle checkpoint functions [19,21].

Adenoviral vectors can be designed with genomic deletions to confer replication selectivity. Due to the similarities in the way the HPV and adenovirus interact with cell cycle regulators, deletion in the adenovirus E1 region can be used to target HPV positive tumors. For example, a 24 base pair deletion (Δ24) in the E1A CR2 region (the area normally responsible for binding to the retinoblastoma protein) has been used to selectively target HPV-positive tumors [22]. Furthermore, another CRAd (CB016) that contains an additional deletion in the adenovirus E1A CR1 region (along with the Δ24 deletion) has been developed [23]. The deletions in the adenoviral genome within the Δ24 and CB016 viruses do not allow for the encoding of key viral protein products that would normally allow for viral replication. However, within HPV positive cells that express the E6 and E7 oncoproteins, adenoviral replication proceeds due to functional transcomplementation from the HPV proteins [24,25].

In this study, we employed the Δ24 and CB016 conditionally replicating adenoviruses in the treatment of HPV-positive HNSCCs. The vectors' ability to selectively replicate within and kill HPV-positive HNSCCs was demonstrated in vitro. Thereafter, the oncolytic potential of the adenoviruses was studied in a xenograft model using nude mice. The results of these experiments provide insight into the clinical utility of adenovirus-based therapy for the treatment of HPV-positive HNSCCs.

Materials and methods

Cell lines and culture conditions

Two human HPV-negative (SCC-4, SCC-15 [provided by Dr. David Wong, University of California Los Angeles]) and three HPV-positive HNSCC cell lines (93VU147T [provided by Dr. Hans Joenje, VU University Medical Center, The Netherlands], UPCI SCC 090 [gift from Dr. Suzanne Gollin, University of Pittsburgh], and UM047 [obtained from Dr. Thomas Carey, University of Michigan]) were cultured in Dulbecco's modified Eagle medium (DMEM) (Mediatech, Herndon, VA). All cell lines were supplemented with 10% (V/V) fetal bovine serum and a 1% penicillin–streptomycin mixture. They were maintained as adherent monolayers at 37 °C in a humidified incubator with 5% CO_2 in air.

Adenoviral vectors

The 5/3 Δ24 ΔE3-ADP-Luc and 5/3 CB016 ΔE3-ADP-Luc vectors (hereafter referred to as 5/3 Δ24 and 5/3 CB016 respectively) were based on adenovirus type 5 (Ad5) (Fig. 1). The adenoviral vector plasmids encoding Δ24 and CB016 mutations (pAdΔ24 and pCB016 respectively) [22,23] (provided by Drs. Ramon Alemany and Cristina Balgué) were recombined with the pAd-ΔE3-ADP-Luc adenoviral backbone as previously described [26]. Briefly, in the pAd-ΔE3-ADP-Luc structure, most of the non-essential adenovirus E3 genes were deleted (with the exception of the adenovirus death protein (ADP) which is designed to facilitate viral spread and oncolysis) and replaced with the luciferase reporter gene [26,27].

For infectivity enhancement analysis, CMV promoter-driven luciferase expressing vectors with a RGD-modified Ad5 fiber (RGD-CMV-Luc), Ad5/Ad3-chimeric fiber (5/3-CMV-Luc), or wild type Ad5 fiber (Ad5-CMV-Luc) were used. All of these replication-incompetent Ad vectors are identical, except for their fibers. The Ad 5/3 chimeric fiber replaces the knob region of Ad5 with that of Ad3, while the RGD fiber adds an arginine–glycine–aspartate-containing peptide into the HI loop of the fiber knob domain [28]. The wild type Ad5 (Ad5Wt) and the 5/3 ΔE3-ADP-Luc viruses were utilized as non-selective replicative control vectors [26].

All viruses were propagated in the 293 cell line, purified by double cesium chloride density gradient ultracentrifugation, and dialyzed against phosphate-buffered saline (PBS) with 10% glycerol. The vectors were titrated using a plaque-forming assay, and the viral particle (vp) number was measured spectrophotometrically.

Analysis of fiber structure for optimized infectivity

The human HNSCC cell lines (SCC-4, SCC-15, 93VU147T, UPCI SCC 090, and UM047) were cultured as above in 24-well plates (5×10^4 cells/well). On the following day, they were infected with replication-deficient (CMV promoter-driven), luciferase expressing vectors at a titer of 100 (vp)/cell. Two days after infection, the cells were lysed with 100 μl of cell culture lysis buffer, and the luciferase activity was determined with the Luciferase Assay System (Promega, Madison, WI). Results were standardized with cellular protein concentration as quantitated using the DC protein assay (Bio-Rad, Hercules, CA).