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Preclinical development of peptide vaccination combined with oncolytic MG1-E6E7 for HPV-associated cancer

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ABSTRACT

Human papilloma virus (HPV)-associated cancer is a significant global health burden and despite the presence of viral transforming antigens within neoplastic cells, therapeutic vaccinations are ineffective for advanced disease. HPV positive TC1 cells are susceptible to viral oncolysis by MG1-E6E7, a custom designed oncolytic Maraba virus. Epitope mapping of mice vaccinated with MG1-E6E7 enabled the rational design of synthetic long peptide (SLP) vaccines against HPV16 and HPV18 antigens. SLPs were able to induce specific CD8+ immune responses and the magnitude of these responses significantly increased when boosted by MG1-E6E7. Logically designed vaccination induced multi-functional CD8+ T cells and provided complete sterilising immunity of mice challenged with TC1 cells. In mice bearing large HPV-positive tumours, SLP vaccination combined with MG1-E6E7 was able to clear tumours in 60% of mice and these mice were completely protected against a long term aggressive re-challenge with the TC1 tumour model. Combining conventional SLPs with the multi-functional oncolytic MG1-E6E7 represents a promising approach against advanced HPV positive neoplasia.

1. Introduction

Priming and expanding specific anti-tumour T cells within the patient are the primary tenets of therapeutic cancer vaccination and numerous approaches have been designed to enable this [1,2]. Synthetic long peptides (SLPs) are versatile vaccine platforms capable of inducing specific CD8+ and CD4+ T cell responses and have been evaluated in many pre-clinical and clinical settings [1]. Peptide vaccines offer a variety of potential advantages over other platforms including being easily and rapidly tailor made, cost effective to manufacture, straightforward to clinically administer and unlikely to induce anaphylaxis [3]. The advent of other successful immunotherapeutics has helped re-ignite interest in various vaccine platforms including SLPs with optimism surrounding the clinical combination of vaccines with other novel immunotherapeutics [2].

HPV-associated cancers are a significant contributor to recent global estimates of cancer burden [4]. Prophylactic vaccination and screening measures aim to reduce the incidence of HPV-associated cancer, however, an epidemiologic study from the UK predicted that current preventative measures would result in only relatively minor reductions in new cases of HPV-associated cancer by 2040 [5]. Expression of the transforming viral oncogenes E6 and E7 seemingly make HPV-associated cancers an attractive target for therapeutic vaccination [6]. Pre-clinically administration of peptide-based vaccines induces specific CD8+ immunity against the viral antigens and has curative potential in syngeneic murine tumour models, this has been documented using a variety of approaches [7–9]. Against the early stage HPV-associated cancer, vulvar intraepithelial neoplasia, SLPs from E6 and E7 of HPV16 were able to induce specific anti-tumour immunity and resulted in clinical improvement in 12 of 20 women treated, complete responses were observed in nearly 50% of patients [10]. Whilst specific immune responses were again observed in patients with advanced HPV-associated cancer, SLP vaccination did not delay disease progression in this setting however, the treatments were well

Abbreviations: Ad, adenovirus; HPV, human papilloma virus; IFN-γ, interferon γ; IL1β, Interleukin 1β; MG1, MG1 strain of Maraba virus; MOI, multiplicity of infection; OV, oncolytic virus; PBMC, peripheral blood mononuclear cell; SLP, synthetic long peptide; TAA, tumour-associated antigen; TNF-α, tumour necrosis factor α; TME, tumour microenvironment.

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tolerated [11]. Vaccination with SLPs generates specific anti-tumour immunity in HPV-associated cancer but is only efficacious for early stage disease.

Maraba virus is a potent oncolytic virus and can be engineered to boost T cell responses against specific tumour-associated antigens (TAAs) [12–14]. A customised oncolytic Maraba virus (MG1-E6E7) has been manufactured for the treatment of HPV-associated cancer [15]. This virus contains a tetravalent transgene based on attenuated sequences of E6 and E7 from HPV16 and 18 [15]. When mice are primed with a replication deficient adenovirus expressing the same attenuated transgene and boosted with MG1-E6E7, large and specific CD8+ T-cell responses against an HPV16 E6 and an HPV16 E7 epitope have been detected, furthermore this regimen is able to ablate advanced HPV16 positive TC1 tumours in mice [15]. We have also demonstrated that HPV-associated head and neck tumour biopsies significantly enhance the replication of MG1 Maraba virus within the tumour compared to HPV negative samples as the presence of E6 and E7 decrease innate protection of tumour cells against rhabdoviral infection [15]. Finally we have shown that MG1-E6E7 is able to rapidly alter the immune transcriptome within the microenvironment leading to decreased IL1β transcription [15]. Recently increased expression of IL1β in head and neck cancer has been documented [16]. The cytokine IL1β has pro-metastatic and pro-angiogenic properties [17], suppression of anti-tumour immune effector cells by IL1β have also been documented in a number of malignancies [18] and as such it is a putative therapeutic target. The excellent oncolytic potential of MG1 Maraba and the impressive ability of MG1-E6E7 to boost immune responses formed the rationale for combining this custom made biologic with SLP vaccination.

Combining SLP vaccination with an oncolytic virus for cancer treatment is reported here for the first time in the preclinical setting of HPV-associated cancer. By using a previously designed oncolytic vaccination strategy we are able to show the induction of specific immunity against multiple HPV-specific CD8+ T cell epitopes and this data facilitated the informed design of SLP vaccines. SLP vaccination is able to induce specific CD8+ immunity against HPV epitopes and the magnitude of immune responses is increased when SLPs are combined with MG1-E6E7. Finally the efficacy of SLPs and MG1-E6E7 against large HPV16 positive murine neoplasms is demonstrated and as such forms the basis of a potential alternative therapeutic approach for HPV-associated cancer.

2. Materials and methods

2.1. Cell culture

TC1 cells were grown in RPMI containing 10% foetal bovine serum (Invitrogen, Waltham, MA), 10 mmol/l HEPES (Invitrogen, Waltham, MA), 2 mmol/l L-glutamine (Invitrogen, Waltham, MA) and 400 μg/ml G418 (Gold Biotechnology, St Louis, MO). Vero 76 cells were cultured in αMEM containing 8% foetal bovine serum and 2 mmol/l L-glutamine.

2.2. Recombinant viruses

The codon optimised attenuated fusion E6E7 transgene was manufactured (GenScript, Piscataway, NJ). Ad-E6E7 (encoding the E6E7 transgene) is a human serotype 5, E1/E3 deleted adenovirus manufactured using the AdEasy™ system (Agilent Technologies, Santa Clara, CA). MG1-E6E7 (expressing the attenuated E6E7 transgene between the G and L viral genes) was manufactured in the Stojdl Lab (University of Ottawa, Canada) and was based upon the previously identified oncolytic double mutant MG1-Maraba virus [14].

2.3. In vitro infections

Six well plates containing confluent TC1 (approximately 1.5×10^6 cells per well) were infected at decreasing multiplicity of infection (MOI) with MG1-E6E7 (from 10 to 0.001 alongside an uninfected control well) in 200 μl of culture medium for 45 min, following infection fresh medium was added and at 48 h post-infection, cells were fixed and stained with 0.1% crystal violet (Sigma-Aldridge St Lois, MO) in 20% ethanol for viability.

2.4. Mice

Six to eight week old C57BL/6 female mice were purchased from Charles River (Wilmington, MA) and housed in specific pathogen-free conditions. Animal studies were approved by McMaster University's Animal Research Ethics Board and complied with Canadian Council on Animal Care guidelines.

2.5. Tumour challenge

Mice were engrafted with 1×10^6 TC1 cells subcutaneously under gaseous general anaesthesia. The longest axis of the tumour (length) and the axis perpendicular (width) to this were measured every 2–3 days and tumour volume was calculated using the following formula:

$$\text{Volume} = 4/3\pi((0.5\text{length})((0.5\text{width})^2))$$

Mice reached end point when tumours grew to a volume of 1500 mm^3. For the TC1 model end points also included the mouse losing 20% of its body weight relative to weight recorded prior to tumour engraftment or if the mouse was deemed moribund due to weight loss.

2.6. Viral titration from tumour samples

TC1 tumours were removed from 5 mice at 12 h and 4 mice at 24 h after treatment with 1×10^9 PFU of MG1-E6E7 IV and were snap frozen in 1 ml of PBS on dry ice prior to storage at −80 °C. The tumours were subsequently thawed and weighed prior to homogenisation. The tissue was then centrifuged for 12 min at 225 g at 4 °C, the supernatant was then transferred to a fresh microfuge tube, vortexed for 30 s and centrifuged at 225 g for 5 min at 4 °C. Standard plaque assay was used to determine titres, briefly confluent Vero 76 cells in 60 mm tissue culture dishes were infected by a dilution series of supernatants from homogenised tumour tissue for 45 min at 37 °C, 5% CO_2 and 95% relative humidity. Vero 76 cells were then overlaid with 0.5% agarose in 2xMEM F11 with 8% FBS and incubated overnight prior to plaque counting. Tumour weights and homogenate volumes were recorded and used to calculate PFU per gram of tumour tissue.

2.7. Immunohistochemistry

Tumours were harvested from 4 mice 24 h after treatment with 1×10^9 PFU of MG1-E6E7 IV and from 4 untreated mice concurrently. Tissues for examination were formalin-fixed and paraffin-embedded. An IL1β antibody (rabbit polyclonal, Abcam, Cambridge, U.K.) was used with the Bond RX IHC auto-stainer (Leica, Wetzlar, Germany) and the Bond Polymer Refine Detection system (utilising DAB substrate chromogen and haematoxylin) (Leica, Wetzlar, Germany) as per the manufacturers instructions. Images were obtained using an Olympus VS120 Slide Scanner (Olympus, Tokyo, Japan).