Oncolytic activity of Coxsackievirus A21 (CAVATAK™) in human pancreatic cancer

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Abstract

Coxsackievirus A21 (CAVATAK™) is known to induce lytic oncolytic activity both in vitro and in vivo in a number of human cancers including melanoma, multiple myeloma, breast cancer, prostate cancer, and glioma. CAVATAK™ is currently under clinical evaluation in patients with late stage melanoma, breast cancer, prostate cancer and head/neck cancer. Coxsackievirus A21 requires interaction with a surface expressed human ICAM-1 molecule for cellular attachment and lytic cancer cell infection. Historical immunohistochemical studies have reported high levels of ICAM-1 expressed in biopsies obtained from both localised and metastatic pancreatic cancer tissues.1,2 The present study evaluated the oncolytic potential of the genetically modified wild-type human CAVATAK™, in both in vitro cultures and in vivo mouse models of human pancreatic cancer. In vitro studies established that CAVATAK™ was able to express a lytic phenotype at relatively high levels on the surface of pancreatic cancer cells, thereby facilitating rapid CA21-induced lytic infection in such cancer cell cultures. In vivo CAV21 challenge studies employing human pancreatic xenograft mouse models are discussed. Based on findings to date, administration of wild-type CA21 may provide novel therapeutic avenues for the treatment of metastatic human pancreatic cancer.

Methods

Cells:

As-PC-1, CAPAN-2 and PANC-1 cells were obtained from the American Type Culture Collection (ATCC). As-PC-1 cells were maintained in RPMI, CAPAN-2 in McCoy’s 5A Modified Media Formulation and PANC-1 cells in DMEM (all with 5-10% FCS) incubated at 37°C with 5% CO2. PANC-1 cells were generated using a lentiviral transduction vector as described previously1 to allow the assessment of tumor burden using bioluminescent imaging.

Virus:

Coxsackievirus A21 Fuyuendan strain (CAVATAK™) [Viralytics Ltd].

Tissue arrays and immunohistochemical staining levels:

A panel of formalin-fixed normal human pancreatic tissues and cancer specimens were examined by immunohistochemistry using PANC-1 cells. The tissue arrays were obtained from the Vectorlinkshion.com. Primary monoclonal antibody ICAM-1 (Santa Cruz) was used at 1:250.

Flow cytometric evaluation of ICAM-1 and DAF receptors:

Quantifiable flow cytometry (for ICAM-1 and DAF) and FACS (fluorescence-activated cell sorting) receptor levels were performed using FcBlock™ PE beads (BD #560405) as a calibrator to determine the number of antibodies bound per cell. PANC-1 cells were harvested before staining with PE-conjugated antibodies against ICAM-1 (al280012) and DAF (al280005) (Alexis, Sante Sifone).

Evaluation of virus induced cytokopathic effect (CPE):

The panel of pancreatic cancer cell lines were incubated with serially diluted CAVATAK™ moctas for 7 days and cell viability was examined by a neutral red (0.5% (phenylamino)carbonyl-3,4-tetrazolium)bis-[4-(methylyldene)-2-(4-rroxy)tetrazo] (XTT) cell proliferation assay.

One step viral growth curve of CAVATAK™ in PANC-1 and PANC-1-luc cells:

To investigate the replication of CAVATAK™ in pancreatic cancer cells, viral growth curves were established in PANC-1 and PANC-1-luc cell lines. Confluent cell monolayers in 24-well plates were infected with a multiplicity of infection (MOI) of 10 TCID50 of CAVATAK™. The infection was allowed to proceed for 3 h at 37°C before being washed 3 times with PBS. The monolayers were replenished with 1 ml of 2% FCS and then the cell supernatants were harvested every 3 h until viral cytopathic effect was observed. Viruses from confluent cultures were then collected and used to infect three consecutive passage-free cultures, before TCD50 assays were performed using SK-Mel-28 melanoma cells to determine the virus yield at each time point.

PANC-1 xenograft mouse model of oncologic disease:

Female SCID (severe combined immunodeficiency) mice (n=20), were subcutaneously inoculated with PANC-luc cells (2x106 cells in a volume of 100 μl). After six days of tumor growth, mice were treated with a single intratumoral (i.t.) injection of either saline (100 μl per group) or of the oncolytic virus agent, CAVATAK™ (1x106 TCID50 in 100 μl (i.t. group)). Eight days post tumor inoculation, mice were given rectal treatment of saline or CAVATAK™ (1x106 TCID50 in volumes of 100 μl). Tumor development was monitored on a weekly basis using electronic bioluminescence imaging. Tumors were examined using Xenogen IVIS100 bioluminescent live imaging system.

Results

1. Immunohistochemical staining of relative ICAM-1 receptor expression in biopsy samples from pancreatic cancer patients compared to normal pancreatic tissues

2. ICAM-1 surface expression on a panel of pancreatic cancer cells

3. Oncolytic activity of CAVATAK™ on a panel of human pancreatic cancer cells

4. Validation of luciferase tagged PANC-1 cell line for use in vivo

5. CAVATAK™ oncolytic virotherapy of PANC-1-luc tumours in SCID Balb/C mice

Conclusions

Overall these pre-clinical findings support the continued development of CAVATAK™ for the treatment of human pancreatic cancers.

References


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