Abstract
Coxsackievirus A21 (CAVATAK™) exhibits oncolytic activity both in vitro and in vivo in a number of human cancers including melanomas, multiple myelomas, breast cancer, prostate cancer and malignant gliomas. CAVATAK™ is currently under clinical evaluation in patients with late stage melanomas.1Breast cancer is the most common cancer in women and breast cancer is also a frequent cause of death in women.2Coxsackievirus A21 (CAVATAK™) has been found to selectively infect and replicate in many types of human cancer cells, with low levels of replication in normal cells.3,4 The present study evaluated the oncolytic potential of the genetically modified enterovirus CAVATAK™ in both in vitro cultures and in vivo mouse models of human pancreatic cancer. In vitro studies established that CAVATAK™ was expressed at relatively high levels on the surface of human pancreatic cancer cells, thereby facilitating rapid CAVATAK™ induced lysis in such cancer cell cultures. In vivo CAVATAK™ challenges studies employing human pancreatic cancer xenograft mouse models are discussed. Based on findings to date, administration of wild-type CAVATAK™ may provide new therapeutic avenues for the treatment of metastatic human pancreatic cancer.

Methods

Cells
AsPC-1, CAPAN-2 and PC3 cells were obtained from the American Type Culture Collection (ATCC). PANC-1 cells were maintained in RPMI (Panel cells maintained in McCoy’s 5A Modified Media Formulation and PANC-1 cells were maintained in a xenograft mouse model of human pancreatic cancer). The cells were cultured in 75 cm² flasks (Corning, NY), with RPMI medium supplemented with 10% FBS and sodium bicarbonate. The pH of the medium was adjusted to 7.3. The cells were maintained at 37°C in a humidified 5% CO₂ incubator. The cells were passaged 2-3 times a week using 0.05% trypsin-EDTA and sub-cultured in 25 cm² flasks in T-25 flasks (Corning, NY).

Virus
Coxsackievirus A21 Kuykendall strain (CAVATAK™) [Viralytics Ltd] was used in this study. virus stock was titrated in 96 well plates using CRL-1560 cells and expressed as p.f.u. per mL. CAVATAK™ was serially diluted in RPMI (100 μL) and 10 μL of virus was added per well. The cells were incubated at 37°C for 48 hours. 

Results

1. Immunohistochemical staining of relative ICAM-1 receptor levels 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. Combination of CAVATAK™ and gemcitabine in AspC-1 cells in vitro

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

Conclusions

Immunohistochemical staining failed to detect ICAM-1 expression in biopsies of normal pancreatic tissue, while significant levels of ICAM-1 were detected in numerous pancreatic cancer biopsies from varying stages of disease.

Moderate to high levels of cell surface ICAM-1 expression were observed following flow cytometric analysis of Panc-1 cell and CAPAN-2 cultures in vitro. Low levels of surface ICAM-1 were detected on AspC-1 cells. DAF expression levels were similar across the three cell lines tested.

PANC-1 were highly sensitive to rapid multi-cycle replication and cell cytolysis following in vivo challenge with CAVATAK™. CAPAN-2 were moderately sensitive, however AspC-1 were less sensitive to CAVATAK™ challenge.

The combination of CAVATAK™ and gemcitabine generated highly synergistic oncolytic activity within in vitro cultures of AspC-1 cells.

Intestinal or intravenous administration of two doses of CAVATAK™ to tumor bearing SCID Balb/C mice resulted in rapid cancer cell destruction and subsequent reductions in tumor burden.

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

References

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