Low Pathogenic Enteroviruses as Novel Oncolytic Agents Against Human Prostate Cancer

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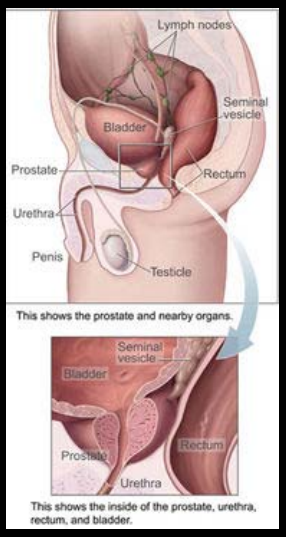
ABSTRACT:

The use of replication-competent viruses to preferentially target and lyse cancer cells compared to normal cells has emerged as a novel form of cancer therapy known as oncolytic viral therapy. Following the successful results obtained from both *in vitro* and *in vivo* testing of Coxsackievirus A21 as an oncolytic agent against malignant melanoma, several low pathogenic enteroviruses, Coxsackievirus A21 (CVA21) and a bio-selected Coxsackvirus-A21 DAF variant (CVA21-DAFv) were investigated as novel oncolytic agents against prostate cancer cell lines *in vitro* and *in vivo*.

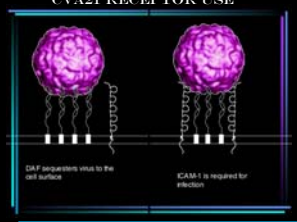
INTRODUCTION:

Prostate cancer is the most commonly diagnosed cancer in males. The incidence continues to escalate in Australia with 10,512 new cases estimated in 2000. Current therapies include surgery, radiation, hormone ablation therapy and chemotherapy to remove malignant tissue but these remain inadequate and often have serious side effects that reduce quality of life. Once prostate cancer has metastasised to distant regions it is considered incurable.

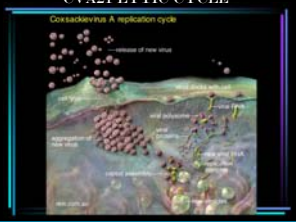
Intercellular adhesion molecule-1 (ICAM-1) and/or the complement regulatory protein Decay-accelerating factor (DAF) are often over-expressed on cancer cells compared to normal cells in the surrounding tissue. These molecules conveniently form the target receptors for attachment and entry of a subset of enteroviruses. Coxsackievirus A21 requires ICAM-1 and/or DAF, bio-selected CVA21-DAFv requires DAF only for attachment and infection.



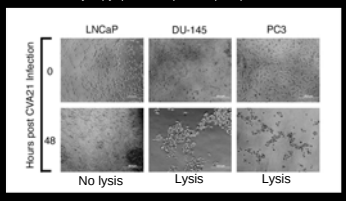
CVA21 RECEPTOR USE



CVA21 LYTIC CYCLE



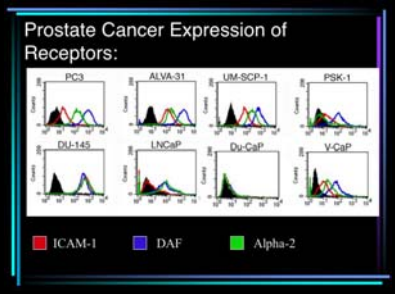
Virus induced lysis (cytopathic effect) of susceptible prostate cancer cells



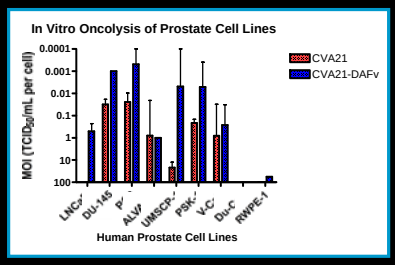
METHODS/RESULTS:

A panel of prostate cancer cell lines were screened for their expression of ICAM-1 and/or DAF by flow cytometry and subsequent *in vitro* susceptibility to CVA21 and CVA21-DAFv. Of the eight prostate cancer cell lines examined six lines expressed the receptors of interest (shift in expression relative to conjugate alone negative control flow staining (black histogram), and were subsequently susceptible to lytic infection (lower multiplicity of infection (MOI) indicates a greater sensitivity to virus and thus less virus is required to induce lysis of cancer cells). CVA21-DAFv was able to increase the therapeutic range of this therapy by lysing the CVA21-resistant LNCaP cell line, which has little to no expression of ICAM-1 but adequate DAF. An *in vivo* xenograft model of prostate cancer utilising the PC3 cell line was established and used to assess the oncolytic capacity of CVA21 and CVA21-DAFv for comparison. After palpable tumours had developed, viruses administered IV as a single dose caused significant ($p<0.001$) tumour regression from day 4 and over the full course of the experiment relative to PBS treated controls which had to be sacrificed at day 29 in accordance with the ethical limits for maximal tumour growth.

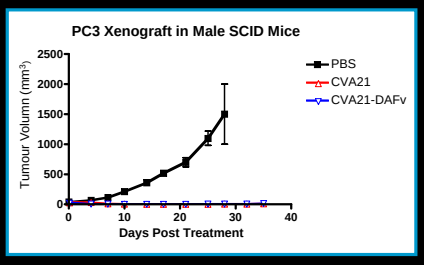
VIRUS RECEPTOR EXPRESSION



SUSCEPTIBILITY TO ONCOLYSIS *IN VITRO*



SUSCEPTIBILITY TO ONCOLYSIS *IN VIVO*



CONCLUSIONS:

Human prostate cancer cell lines with increased expression of ICAM-1 and DAF on their surface were susceptible to rapid oncolysis by CVA21 and CVA21-DAFv both *in vitro* and *in vivo*.These findings highlight the potential of naturally occurring, low pathogenic, replication competent human enterovirus CVA21 and of CVA21-DAFv as novel oncolytic agents against human prostate cancer.

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