Barts and The London School of Medicine and Dentistry

Institute of Cancer

Targeting Inflammatory Cytokines Using Adenoviruses: gene delivery of biological therapies in ovarian cancer

Michael A. Salako[1,2], Hagen Kulbe[1], Iain A. McNeish[2] and Frances R. Balkwill[1]

[1]Centre for Cancer and Inflammation, [2]Centre for Molecular Oncology and Imaging, Institute of Cancer and the CR-UK Clinical Centre, UK

INTRODUCTION

TNF-α

The cytokine TNF-α is central to initiating the inflammatory reactions of the innate immune system. Constitutive TNF-α expression is characteristic of the malignant ovarian surface epithelium and is a major player in a tumour-promoting cytokine network.

Oncolytic adenoviral vectors

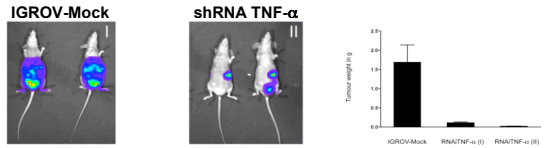
Replication-selective oncolytic viruses are a rapidly expanding therapeutic platform for cancer . E1A CR2 deleted adenoviral mutants hold great promise as gene therapy vectors. However, like all adenoviruses, their efficacy is hindered by an inflammatory cascade orchestrated by TNF-α.

Adenovirus mediated delivery of shRNA

We hypothesised that delivering TNF-α shRNA to ovarian cancer cells using an oncolytic adenovirus could reduce the inflammatory reaction that is generated by adenoviruses and also have direct anti-tumour activity on the cancer cells.

RESULTS 1

In vivo effect of TNF-α knockdown on ovarian cancer growth

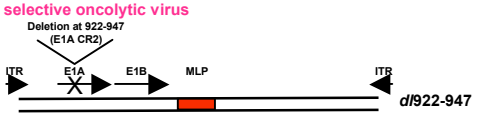


➢Panels I & II are representative biolumenescence images *in vivo* of IGROV-Mock and stable shRNAi TNF-α IGROV ovarian cancer cells 42d after i.p. injection

➢The graph illustrates the significant reduction in tumour burden observed in the TNF-α shRNAi harbouring mice compared to controls

VIRUSES 1

Replication selective oncolytic virus



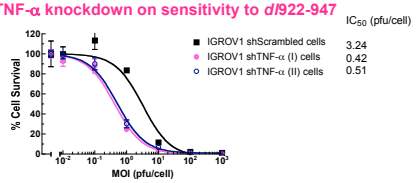
➢*dl*922-947 contains a 24 b.p. deletion in E1A CR2

➢E1A CR2 normally binds to host cell Rb protein thereby driving cells into S-phase

➢Due to this deletion, the virus can only replicate in cells with an abnormal Rb pathway, which is seen in 90% of cancers including ovarian

RESULTS 2

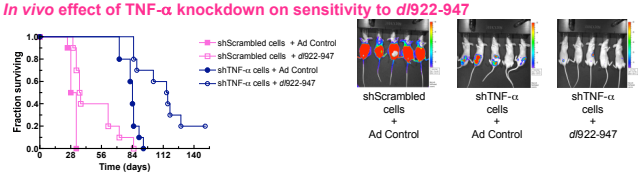
In vitro effect of TNF-α knockdown on sensitivity to *dl*922-947



➢Knockdown of TNF-α sensitizes ovarian cancer cells to oncolytic adenoviruses

➢*dl*922-947 had a 1 log increase in efficacy on the knockdown cells compared to the shScrambled control cells

In vivo effect of TNF-α knockdown on sensitivity to *dl*922-947

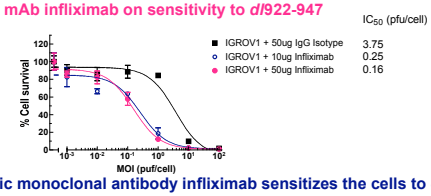


➢Treatment of female nude mice bearing shScrambled IGROV1 cells with non-replicating virus (Ad-control) or *dl*922 had little anticancer effect

➢Mice bearing shTNF-α cells treated with *dl*922 survived significantly longer

RESULTS 3

Effect of the TNF-α mAb infliximab on sensitivity to *dl*922-947



➢The TNF-α specific monoclonal antibody infliximab sensitizes the cells to *dl*922

CONCLUSION

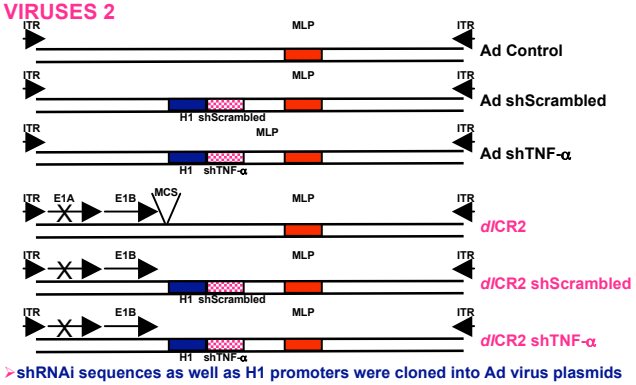
➢The anti-tumour effect of oncolytic Ad viruses is increased by inhibiting TNF-α

➢Viruses containing shTNF-α RNAi have a similar effect

➢Future work will investigate the mechanism of this increased anti-tumour effect

Once pre-clinical studies are complete our aim will be to use the virus in clinical trials to treat women with advanced ovarian cancer

VIRUSES 2

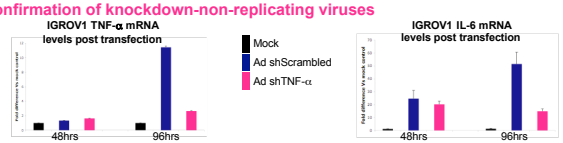


➢shRNAi sequences as well as H1 promoters were cloned into Ad virus plasmids to generate either non-replicating (black label) or replicating (pink label) viruses

➢*dl*CR2 contains the same E1A CR2 deletion as *dl*922-947 as well as a MCS

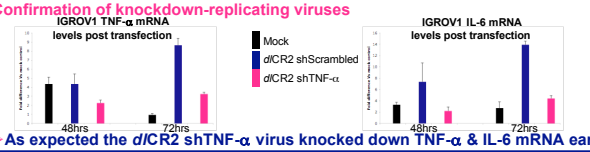
RESULTS 4

Confirmation of knockdown-non-replicating viruses



➢Ad shTNF-α as well as knocking down TNF-α knocks down the cytokine IL-6

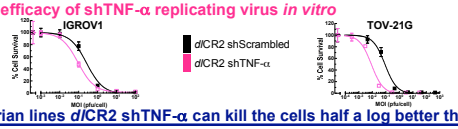
Confirmation of knockdown-replicating viruses



➢As expected the *dl*CR2 shTNF-α virus knocked down TNF-α & IL-6 mRNA earlier

RESULTS 5

Oncolytic efficacy of shTNF-α replicating virus *in vitro*



➢In 2 ovarian lines *dl*CR2 shTNF-α can kill the cells half a log better than the control

Ovarian cancer action

MRC Medical Research Council

CANCER RESEARCH UK

www.cancer.qmul.ac.uk/research/cancer_inflammation