Kingsman, Susan

The Macrophage-Exploiting Hypoxia With Cell Based Delivery of GDEPT

S.M. Kingsman, L. Griffiths, K. Binley, S. Iqball, O. Kan, P. Maxwell, C. Lewis, A. Harris & S. Naylor

Oxford BioMedica (UK) Ltd, Medawar Centre, Oxford Science Park, Oxford, OX4 4GA, UK
Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK
Molecular Oncology Laboratory, University of Oxford, Oxford, UK
Department of Pathology, University of Sheffield Medical School, Sheffield, UK

Macrophages can form a significant proportion of the solid tumour mass and consequently play an important role in tumour progression. The use of activated macrophages in the treatment of cancer has been largely ineffectual. By 'arming' these cells with the ability to express a therapeutic gene we demonstrate significant advances in the efficacy of this approach. We have constructed a hypoxia-regulated adenoviral vector to transduce human macrophages with both a reporter (LacZ) and a therapeutic gene encoding human cytochrome P450 2B6. In this way a non-toxic enzyme can be delivered to the tumour and subsequent application of a prodrug provides therapeutic activity at the site of gene expression. The particular enzyme/prodrug combination that we have used, human cytochrome P450/cyclophosphamide, permits significant bystander activity through local diffusion of the activated metabolite. We have investigated the hypoxic response in primary human macrophages via HRE sequences (hypoxia response elements) in both a luciferase reporter assay and using a hypoxia regulated adenoviral vector. These studies have indicated a hypoxic/normoxic induction ratio (0.1%/21% O2) of over 20 fold. Infiltration of hypoxically regulated transduced macrophages into a tumor spheroid results in induction of gene expression. We demonstrate significant tumor cell killing in this model only in the presence of cyclophosphamide via activation by P450 2B6 and show that this can be further targeted to tumors through hypoxia regulated gene expression.

Kingsman, Susan

A Physiologically Regulated Adenoviral Vector for the Treatment of Ischaemic Disease

S.M. Kingsman, K. Binley, L. Griffiths, S. Iqball, H. Spearman, A.J. Kingsman & S. Naylor

Oxford Biomedica (UK) Ltd, Medawar Centre, Oxford Science Park, Oxford, OX4 4GA, UK

Adenoviral vectors can be used for the transient expression of a variety of genes in both dividing and non-dividing cells. Although such a broad tropism can be advantageous there is the possibility that non-target cells might be adversely affected by the expression of a transferred gene. To overcome this limitation we have developed an adenoviral vector that can deliver regulated gene expression in response to physiological stimuli. A large number of pathological conditions are characterised by ischaemia where oxygen and glucose levels are low and the tissues are acidic. These conditions activate a number of genes and in particular low oxygen (hypoxia) is sensed by DNA sequence elements referred to as hypoxia response elements. We have characterised a number of such elements and configured them into adenoviral vectors. In one configuration we have obtained a vector which displays a remarkable degree of regulation in response to hypoxia, basal expression is minimal and maximum expression is equivalent to that obtained with commonly used constitutive promoters. We have characterised the expression of this vector in a range of target tissues including human tumour cells, xenografts and normal tissues including skeletal muscle and hematopoeitic cells. This vector can be used to deliver therapeutic genes such as pro-drug activating enzymes and vascular growth factors for the treatment of diseases such as cancer, peripheral arterial disease and arthritis.