Construction of a Recombinant Immunotoxin

by

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Chapter 1

IMMUNOTOXINS IN CANCER THERAPY

Declaration

The experiments presented in this thesis were carried out by myself, except where indicated in the text. None of the material has been presented previously for the purpose of obtaining any other degree.

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ABSTRACT

In recent years a number of therapeutically useful immunotoxins have been produced using recombinant gene technology. In general, this involves fusion of a toxin gene with sequence encoding a variety of clinically relevant proteins or peptides. Using these techniques a recombinant immunotoxin has been engineered by fusing the genes encoding an antibody fragment with the sequence of a small cytolytic peptide, melittin. The antibody fragment consists of the antigen binding site derived from a murine monoclonal antibody K-1-21, which binds to human free *kappa* light chains and recognises a specific epitope (KMA) expressed on the surface of human myeloma and lymphoma cells. The toxic portion of the molecule is melittin, a 26 amino acid, membrane lytic peptide which is a major component of bee venom. Using PCR a single chain Fv (scFv) was constructed by linking V_H and V_L genes with an oligonucleotide encoding a flexible, hydrophilic peptide. The melittin gene was synthesised as an oligonucleotide and extended by PCR. Nucleotide sequence encoding a linker peptide was added to the 5' end and a primer encoding a FLAG peptide was used to extend the 3' end. This gene construct was then ligated into the recombinant expression vector, pPOW scFv, to create the fusion gene encoding the recombinant immunotoxin. The gene construct was expressed in the periplasm of *E.coli* (TOPP2) using the secretion signal pelB. Expression of the foreign protein was monitored by western blot using a monoclonal antibody which recognises the FLAG peptide encoded at the carboxy terminal region of the gene construct. Expression of the recombinant immunotoxin was optimised and the resulting protein was purified using anti-FLAG M2 affinity chromatography. Antigen binding activity was assessed by ELISA and flow cytometry using a human myeloma cell line, HMy2, which expresses the KMA antigen.Binding of the immunotoxin to a control human cell line, K562, which does not express KMA on the cell surface was also assessed. The results indicated that the recombinant immunotoxin retained antigen binding specificity and it was cytotoxic towards the target cell line (HMy2).

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LIST OF ABBREVIATIONS

AEBSF	4-(2-Aminoethyl)-benzenesulphonyl fluoride hydrochloride
dATP	deoxy-adenosine 5'-triphosphate
ABMT	autologous bone marrow transplantation
ADCC	antibody dependent cell mediated cytotoxicity
az	sodium azide
BCIP	bromochloroindolylphosphate
BSA	bovine serum albumin
CD	cluster of differentiation
CDR	complementarity determining region
DMSO	dimethyl sulphoxide
DT	diphtheria toxin
EDTA	ethylenediamine tetra acetic acid
Eth.Br	ethidium bromide
flag	an octapeptide which is recognised by the monoclonal antibody anti-FLAG M2
FITC	fluorescein isothiocyanate
HAMA	human anti-mouse antibody
HMy2	*kappa*-myeloma cell line, LICR LON/HMy2
Ig	immunoglobulin
IL-2	interleukin-2
IL-2R	interleukin-2 receptor
IL-4	interleukin-4
IL-6	interleukin-6
KMA	*kappa* myeloma antigen
mAb	monoclonal antibody
MWt	molecular weight
N	Avogadros number
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PE	*Pseudomonas* exotoxin
PEG	polyethylene glycol
RTA	ricin toxin A chain
SDS	sodium dodecyl sulphate

SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
T-ALL	T-cell acute lymphoblastic leukemia
X-GAL	5-bromo-4-chloro-3-indolyl-ß-D-galactopyranoside