Generation of Patient Specific Reagents for the Study of Multiple Myeloma Tumour Progenitor Cells

By

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Chapter 2 : Materials, Reagents and Solutions

List of Abbreviations

3'	three prime
5'	five prime
APS	ammonium persulphate
Az	sodium azide
bp	base pair
BSA	bovine serum albumin
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
ECL	enhanced chemi-luminescence
EDTA	ethylenediamine tetra-acetic acid
ELISA	enzyme linked immunosorbent assay
Fab	fragment antigen binding
FBS	fetal bovine serum
g	centrifugal force
g3p	gene III coat protein of filamentous bacteriophage
HRP	horse-raddish peroxidase
i.p.	intraperitoneal
i.v.	intravenous
IgG	immunoglobulin, γ isotype
IPTG	isopropylthio-β-D-galactopyranoside
kDa	kilodalton
mAb	monoclonal antibody
MW	molecular weight
nm	nanometre
NTA	nitrilotriacetic acid
OD	optical density
OPD	o-phenylene diamine
PAGE	polyacrylamide gel electrophoresis
PBS(-T)	phosphate buffered saline (-Tween)
PEG	polyethylene glycol
pfu	plaque forming units
rpm	revolusions per minute
RU	resonance units
SA	streptavidin
scFv	single chain variable fragment
SDS	sodium dodecyl sulphate
TBS(-T)	tris buffered saline (-Tween)
tu	transducing units
UV	ultraviolet
V	volts
V_H	heavy chain variable region
V_L	light chain variable region
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-galoctoside

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Abstract

Hybridoma production was investigated as a means to produce patient specific reagent for the characterisation of putative tumour precursors in multiple myeloma. Purified Fab fragments were used as immunogen so as to avoid an immune response towards antigenic regions within the Fc. However, all of the paraprotein-reactive murine antibodies isolated also demonstrated reactivity against pooled normal human immunoglobulin. Induction of tolerance towards human immunoglobulin was investigated as a means focus the immune response towards idiotypic determinants. Although successful in ablating the response towards unrelated immunoglobulins, serum antibody response against immunising paraprotein was also absent.

Three linear libraries were screened against a total of six IgG patient paraproteins, either as immobilised targets or in solution. Three different peptides were recovered (P3-20, P6-15 and P4-7), each of which recognised a different patient paraprotein. All three peptides exhibited binding in ELISA to the original target paraprotein and an absence of binding to normal human immunoglobulin or unrelated paraprotein when expressed by phage. P3-20 retained its binding characteristics in synthetic form, as determined by surface plasmon resonance. However, P6-15 and P4-7 failed to bind to their respective targets as synthetic peptides. The loss of function for P6-15 (VLLFHEPAGLPVYFW) may be attributed to the highly hydrophobic nature of this motif.

It was hypothesised that the presence of structural elements within the minor coat protein were required for P6-15 to adopt the appropriate conformation for binding to target paraprotein. Recombinant proteins were engineered containing the peptide sequence fused to the first N-terminal domain of g3p. Expressed protein did not exhibit the desired binding to paraprotein and neither did a second recombinant protein containing the first and second

domains together. P3-20 expressed as recombinant g3p fusion proteins also failed to bind to its target.

In conclusion, this work has demonstrated the value of phage-displayed peptide libraries for the generation of reagents specific towards myeloma tumour paraproteins. It is reasonable to predict that the extension of this work with additional patient subjects would enable exciting research into the true nature of the malignant precursor involved in multiple myeloma.

Declaration

This work contains no material which has been accepted for the award of any degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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