Properties of Nanobodies and their discovery via phage display

Rob Roovers
26-05-2010
Agenda

• Introduction to Nanobodies and their properties

• Phage display
  • Construction of antibody libraries
  • Selection cycle
  • Selection for function

• Conclusions
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Structure of heavy chain antibodies

Combining benefits of antibodies and small chemicals

Conventional Antibody
- Heavy and light chains
- Both chains required for antigen binding and stability

Heavy-Chain Antibody
- Only heavy chains
- Full antigen binding capacity and highly stable

Ablynx’s Nanobody®
The smallest functional fragment of a naturally occurring single-chain antibody
Properties of VHH

Small-molecule properties of Nanobodies
- High Stability
- Alternative routes of administration
- High tissue penetration

Antibody properties of Nanobodies
- High affinity/potency
- High target selectivity
- Low inherent toxicity

Unique properties of Nanobodies
- Ease of manufacture in bacteria and yeasts
- Low potential immunogenicity
- Format flexibility
- Very low cost of goods
- Half-life tailoring
- Wider range of epitopes, including cavities
- Low potential immunogenicity
- Ease of manufacture in bacteria and yeasts
- Format flexibility
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- Half-life tailoring
- Wider range of epitopes, including cavities
Hallmark residues within VHH

Hypervariable Regions

H1
H2
H3

Hallmark Residues

FR2
FR1/3
FR4
37, 44
6, 11
103
45, 47
74
104
83, 84
108
Address targets inaccessible to conventional Ab’s: cavity binding and enzyme inhibition

- Extended CDR3 loop inhibits enzymes and binds in receptor clefts and canyon sites of viral envelopes
- Access to small-molecule targets but with antibody specificity and affinity
- Most known therapeutic targets are either enzymes (28%) or receptors (45%)

Nature Structural Biology 1996, 3 (9)
Formatting of VHH

To increase half-life from few hrs to 3 weeks

To increase potency, gain function due to avid binding/cross-linking

To acquire an effector mechanism

To expand beyond one specificity
Multi-valent VHH

- Up to 4 of the same VHH, fused N- to C- terminus made as a genetic fusion and expressed in *E. coli*
- Increase in potency observed with the addition of each unit
- Consistently demonstrated against a number of multimeric targets and cell surface antigens

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Discovery of VHH via phage display

- B-cells
- PCR
- Cloning
- Rescue
- Antibodies

Expression

\[ \text{VH} \]
\[ \text{VL} \]

Fusion to minor coat protein gene III

Phagemid DNA

Antibody repertoire on phage (library) $\geq 10^9$ clones
Analysis of RNA isolated from PBLs
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Phage display based selection on target binding

DNA manipulation to create library of variants (e.g. peptides, proteins)

Display variants on surface of phage

Bind

Analyse

Amplify

The Phage cycle

Elute

Wash

Waste
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Selection for function: isolation of antagonistic antibodies

1. Antibody library
2. Incubate with immobilized cytokine
3. Washing
4. Elution by competition with excess of receptor
5. Analyze phage antibodies
6. Removal unbound phage
The Her family of RTK as cancer targets

- Over-expression of these growth factor receptors is often observed in epithelial tumours

- EGFR is a well studied RTK that contributes to different stages of carcinogenesis:
  - Cell proliferation
  - Resistance to apoptosis
  - Cell motility
  - Angiogenesis

- Several anti-EGFR whole antibodies have been successfully introduced into the clinic: e.g. c225 (CetuxiMab) and 425 (MatuzuMab). Their mode of action is being increasingly well understood.

- Resistance to mono-therapy (e.g. EGFR) is associated with increased signalling via other RTK (IGF-1R; Her3)
2 different conformations of the EGFR

Inactive, auto-inhibited ‘tethered’ conformation

Active, EGF-bound ‘extended’ dimer

Ferguson, KM
Bioch. Soc. Trans. 2004
Model of activation of the EGFR

A - Tethered monomer
B - Untethered monomer
C - Ligand-stabilized extended conformation
D - Ligand-induced activated dimers
Structure Fab Erbitux-sEGFR

Ferguson et al., 2005
Mechanism of action Erbitux

1. Blocking EGF binding sites on domain III
2. Steric hindrance of conformational change
Receptor cross-talk
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Conclusions

- Nanobodies (VHH) originate from Camelid heavy chain antibodies
- Nanobodies combine advantages of antibodies and small molecules
- Nanobodies can be easily formatted into multivalent construct
- Nanobodies can be identified from immune libraries via phage display using PBLs from immunized animals
- Phage display permits selection based on function
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