

Summary
Zusammenfassung

1. HISTORY OF SINGLE CHAIN FRAGMENT VARIABLE (SCFVS) ANTIBODIES DEVELOPMENT 21

1.1. Abstract 22

1.2. Introduction 22

1.3. Recombinant antibody technology 23

1.3.1. ScFv antibodies 24

1.3.2. Expression of scFv antibodies 25

1.3.3. Phage display using recombinant libraries 25

1.3.4. Ribosomal display technology 27

1.3.5. Affinity maturation of scFvs selected from phage display libraries 27

1.3.6. Antigen exposure for the scFvs selection process 27

1.3.7. Advantages of scFv antibodies over full-size monoclonal antibodies 28

1.4. Application of scFvs 28

1.4.1. Medical application 28

1.4.1.1. ScFvs in tumor therapy 28

ScFvs as neutralizing antibodies 29

ScFvs as recombinant immunotoxins 29

ScFvs as cancer vaccine 29

ScFvs as anticancer intrabodies 29

1.4.1.2. Application of scFvs in neurodegenerative diseases 31

Alzheimer's disease (AD) 31

1.4.1.3. ScFvs against HIV infection 31

1.4.2. *In vivo* imaging 32

1.4.3. Diagnostic applications 32

1.5. Discussion 33

Aim of the thesis 34

2. DEVELOPMENT AND <i>IN VITRO</i> APPLICATION OF NOVEL VEGFR-2 INHIBITORY SINGLE CHAIN FRAGMENT VARIABLE ANTIBODIES	35
2.1. Abstract	36
2.2. Introduction	36
2.3. Materials and methods	39
2.3.1. Cell culture	39
2.3.2. Transient transfection	39
2.3.3. VEGFR-2 kinase activity assay	39
2.3.4. Immunofluorescence microscopy	40
2.3.5. ETH-2 Gold library	40
2.3.6. ScFvs selection	40
2.3.7. ScFv A7	41
2.3.8. Enzyme-linked immunosorbent assay (ELISA)	41
2.3.9. Expression and purification of scFvs	42
2.3.10. Size-exclusion chromatography (SEC)	42
2.3.11. Fluorescence size-exclusion chromatography (FSEC)	43
2.3.12. Binding affinity determination by ITC	43
2.3.13. HUVEC tube formation assay	43
2.3.14. HUVEC migration assay	44
2.3.15. Squash analysis of VEGFR-2 internalization	44
2.3.16. Trypsin digestion of cell surface exposed receptor	44
2.3.17. Statistical analysis	44
2.4. Results	45
2.4.1. Selection, production, and purification of scFvs antibodies	45
2.4.2. Binding of scFvs to recombinant and endogenous VEGFR-2	50
2.4.3. Functional inhibition of VEGFR-2 phosphorylation with scFvs	51
2.4.4. Effect of antibodies on HUVEC tube formation and migration	52
2.4.5. VEGF and scFvs induce internalization of VEGFR-2	55
2.5. Discussion	57

3. REFORMATTING SCFV ANTIBODIES TO FRAGMENT ANTIGEN BINDING (FAB) ANTIBODY FRAGMENTS 59

3.1. Introduction 60

3.2. Materials and methods 60

3.2.1. Cloning strategy for reformatting scFvs to Fabs 60

3.2.2. Expression and purification of soluble Fabs 61

3.2.3. Receptor kinase activity assay and HUVEC tube formation assay 61

3.3. Results 62

3.3.1. Construction of Fab format 62

3.3.2. Expression and purification of Fabs 62

3.3.3. Affinity determination with ITC 62

3.3.4. Receptor kinase activity assay and HUVEC tube formation assay 65

3.4. Discussion 67

4. OBTAINING FULL-LENGTH IGGs FROM PREVIOUSLY CHARACTERIZED VEGFR-2 INHIBITORY SCFVS 69

4.1. Introduction 70

4.2. Material and methods 70

4.2.1. Cloning, production, and purification 70

4.2.1.1. Cloning pcDNA3 vectors 70

4.2.1.2. Reformatting with MultiPrime expression system 71

4.2.1.3. IgGs purification 71

4.2.2. Fluorescence size-exclusion chromatography (FSEC), receptor kinase activity assay, tube formation assay 71

4.3. Results 72

4.3.1. IgG cloning and purification 72

4.3.2. Cloning into pcDNA3 vectors 72

4.3.3. Cloning with MultiPrime 73

4.3.4. Determination of IgGs binding to VEGFR-2 by FSEC 76

4.3.5. Functional inhibition of VEGFR-2 phosphorylation with IgGs 77

4.3.6. HUVEC tube formation assay 78

4.4. Discussion 79

5. SELECTION AND CHARACTERIZATION OF SCFVS SPECIFIC FOR THE MOUSE VEGFR-2 ECD	81
5.1. Introduction	82
5.2. Materials and methods	83
5.2.1. Production of recombinant mouse VEGFR-2 ECD	83
5.2.2. Selection of mouse specific scFvs against VEGFR-2	83
5.2.2.1. Selection of mouse specific scFvs from ETH-2 Gold library	83
5.2.2.2. Selection of species cross-reactive scFv antibodies against mouse and human VEGFR-2 ECD	83
5.2.2.3. Selection of mouse specific scFvs from R3 EPFL library	84
5.2.3. Sequencing of selected mouse scFvs	84
5.2.4. Testing protein expression to select optimal bacterial strain	85
5.2.5. Expression and purification of scFvs against mouse VEGFR-2	85
5.2.5.1. Expression and purification of soluble scFvs from ETH-2 Gold library ...	85
5.2.5.2. Expression and purification of scFvs from R3 EPFL library	85
5.2.6. ELISA for binder specificity	86
5.2.7. Cell culture	86
5.2.8. Receptor kinase activity assay	86
5.2.9. HUVEC tube formation assay	86
5.3. Results	87
5.3.1. Selection, production, and purification of scFvs antibodies	87
5.3.1.1. ETH-2 Gold library	87
5.3.1.2. R3 EPFL library	87
5.3.2. Functional inhibition of VEGFR-2 phosphorylation with mouse specific scFvs ..	90
5.3.3. Effect of antibodies on endothelial cell tube formation	92
5.4. Discussion	93

6. TUMOR TARGETING WITH RADIOLABELED DARPINS 95

6.1. Introduction 96

6.2. Materials and methods 96

6.2.1. Cell lines used in the study 96

6.2.2. DARPins 96

6.2.3. Fluorescence activated cell sorting (FACS) 97

6.2.4. Immunohistochemistry 97

6.2.5. Labeling of DARPins with 99mTc(CO)3 97

6.2.6. *In vivo* imaging 97

6.2.6.1. Animals 97

6.2.6.2. Cancer cell injections 97

6.2.6.3. Tumor targeting with radiolabeled DARPins 97

6.2.6.4. SPECT/CT imaging 97

6.2.7. Biodistribution 99

6.3. Results 99

6.4. Discussion104

7. CONCLUSIONS AND OUTLOOK105

8. ACKNOWLEDGEMENTS107

9. REFERENCES109