# Contents

**Preface**  xi
**List of Contributors**  xiii

1  **Peptides as Drugs: Discovery and Development**  1  
*Bernd Groner*

1.1  Discovery of New Potential Drug Targets and the Limitations of Druggability  1

1.2  Protein Interaction Domains Are at the Core of Signaling Pathways  4

1.3  Peptides as Inhibitors of Protein Interactions  5

References  7

2  **Mimics of Growth Factors and Cytokines**  9  
*Jürgen Scheller, Joachim Grötzinger, and Stefan Rose-John*

2.1  Introduction  9

2.2  The Cytokines  9

2.2.1  The Receptors  11

2.2.2  “Simple” Receptors  12

2.2.3  “Complex” Receptors  13

2.3  Defining Receptor Recognition Sites in Cytokines Using Chimeric Proteins  15

2.4  Receptor Recognition Sites are Organized as Exchangeable Modules  17

2.5  The Concept of Fusing the Cytokine to the Soluble Receptor: Hyper-IL-6  19

2.6  Antagonists Specifically Inhibiting IL-6 Trans-Signaling  20

2.7  *In Vitro* Evolution of Peptides and Proteins  22

2.7.1  Platforms for the Selection of High-Affinity Binders  24

2.7.2  Agonists and Antagonists of Cytokines and Growth Hormones  27

2.8  Concluding Remarks  28

References  29
3 Peptides Derived from Exon v6 of the CD44 Extracellular Domain Prevent Activation of Receptor Tyrosine Kinase and Subsequently Angiogenesis and Metastatic Spread of Tumor Cells 35
Helmut Ponta and Véronique Orian-Rousseau

3.1 Introduction 35
3.2 CD44 Proteins and Their Involvement in RTK Activation 36
3.3 CD44v6 Acts as a Coreceptor for c-Met and Ron 37
3.4 Three Amino Acids in CD44 Exon v6 Are Crucial for the CD44v6 Coreceptor Function, and Small Peptides Can Interfere with This Function 38
3.5 The Ectodomain of CD44v6 Binds to HGF 42
3.6 Peptides Corresponding to Exon v6 of CD44 Inhibit Metastatic Spread of Tumor Cells 43
3.7 The Significance of the Collaboration between CD44v6 and c-Met In Vivo 45
3.8 The CD44v6 Peptides Interfere with Angiogenesis 46
3.9 Outlook 48
References 49

4 Peptide Aptamers Targeting the Viral E6 Oncoprotein Induce Apoptosis in HPV-positive Cancer Cells 57
Felix Hoppe-Seyler, Susanne Dymalla, Markus A. Moosmeier, and Karin Hoppe-Seyler

4.1 Human Papillomaviruses and Oncogenesis 57
4.1.1 Cervical Cancer 58
4.1.2 The E6 and E7 Genes 59
4.2 Peptide Aptamers Targeting the HPV E6 Oncoprotein 61
4.3 E6-Targeting Peptide Aptamers: Therapeutic Perspectives 64
4.3.1 Therapeutic Target Protein Evaluation by Peptide Aptamers 64
4.3.2 The Intrinsic Therapeutic Potential of Peptide Aptamers 65
4.3.3 Identification of Functional Peptide Mimics by Displacement Screening 67
4.4 Perspectives 68
References 69

5 The Prevention of HIV Infection with Viral Entry Inhibitors 73
Lisa Egerer, Anne Hubert, Dorothee von Laer, and Ursula Dietrich

5.1 Introduction: The Potential of Peptides as Drugs in the Treatment of HIV Infection 73
5.2 The HIV Entry Process 75
5.3 Peptides that Inhibit Receptor or Coreceptor Binding 77
5.3.1 Physiological Antimicrobial Peptides 77
5.3.1.1 Defensins 77
5.3.2 Chemokines 78
5.3.3 Synthetic Peptides and Peptidomimetics 79
## 5.4 Inhibitors of the Viral and Cellular Membrane Fusion Process 81

## 5.5 Entry Inhibitory Peptides Selected by the Phage Display Technology 83

## 5.6 Limitations of Peptides in the Treatment of HIV Infection 84

## 5.7 Strategies to Prolong the *In Vivo* Half-Life of Antiviral Peptides 85

## 5.8 Antiviral Peptides in Gene Therapy of HIV Infection 88

## References 93

## 6 Intracellular Expression of Peptides 103

*Christian Wichmann, Yvonne Becker, and Manuel Grez*

### 6.1 Introduction 103

### 6.2 Peptide Design and Expression Cassettes 103

### 6.3 Stable Delivery and Expression of Peptides: Gamma-Retro- and Lentiviral Vectors 106

### 6.4 Gamma-Retroviral Vectors 109

### 6.5 Lentiviral Peptide Delivery 111

### 6.6 Vectors for Transient Expression of Peptides: Adenoviruses and Adeno-Associated Viruses 114

### 6.7 Perspective 119

Acknowledgments 120

## References 120

## 7 The Internalization Mechanisms and Bioactivity of the Cell-Penetrating Peptides 125

*Mats Hansen, Elo Eiste, and Ülo Langel*

### 7.1 Introduction 125

### 7.2 Discovery and Classification of CPPs 125

### 7.3 Internalization Mechanisms of Cell-Penetrating Peptides 126

### 7.4 Models of CPP Uptake 128

### 7.5 The Current View of CPP Uptake 129

### 7.6 CPPs as Cargo Delivery Vehicles 130

### 7.7 Delivery of Proteins 131

### 7.8 CPPs in Gene Delivery 131

### 7.9 Delivery of Oligonucleotides 131

### 7.10 Cytotoxicity of Cell-Penetrating Peptides 133

### 7.11 *In Vivo* Drug Delivery with CPPs 134

### 7.12 CPPs for Targeted Delivery 136

### 7.13 Conclusions 136

Acknowledgments 137

## References 137

## Abbreviations 137
8 Production and Purification of Monomeric Recombinant Peptide Aptamers: Requirements for Efficient Intracellular Uptake and Target Inhibition

Corina Borghouts and Astrid Weiss

8.1 Introduction 145
8.2 Protein Production 146
8.2.1 Bacterial Systems 148
8.2.2 Yeast Systems 150
8.2.3 Baculovirus Systems 152
8.2.4 Chemical Synthesis 153
8.3 Protein Purification 154
8.3.1 Ammonium Sulfate Fractionation 154
8.3.2 Affinity Chromatography 155
8.3.3 Buffer Exchange and Desalting 156
8.3.4 Ion-Exchange Chromatography 156
8.3.5 Hydrophobic Interaction Chromatography 156
8.3.6 Size-Exclusion Chromatography 157
8.4 Isolation of Monomeric, Natively Folded Proteins 157
8.4.1 Correct Refolding versus Aggregation 157
8.4.2 Techniques for Protein Folding 158
8.4.3 Factors Influencing Refolding 159
8.5 Increasing Peptide Production, Purification and Efficacy by Using Scaffolds 161
8.5.1 Properties and Requirements of Scaffolds 161
8.6 The Use of Cell-Penetrating Peptides for Cellular Uptake of Purified Proteins 162
8.6.1 Uptake of Proteins by Lipid Raft-Dependent Macropinocytosis 164
8.6.2 Points of Consideration for the Use of CPPs 165
8.7 Classification of Therapeutic Peptides 167
8.7.1 Bioactive Peptides 167
8.7.2 Peptide Aptamers 168
8.7.3 Designed Peptides 171
8.7.4 Antibodies 172
8.8 Production and Administration of Therapeutic Peptides In Vivo 172
8.8.1 Extracellular Protein Therapeutics and Peptides with Extracellular Targets 172
8.8.2 Peptides Targeting Intracellular Targets In Vivo 173
8.9 Concluding Remarks 175
References 176

9 Peptide Arrays on Solid Supports: A Tool for the Identification of Peptide Ligands

Mike Schutkowski, Alexandra Thiele, and Joachim Koch

9.1 Introduction 187
9.2 Synthesis of Peptide Arrays 188
9.2.1 Fmoc-Based Synthesis of Peptides on Cellulose Membranes 188
9.2.2 Fabrication of CelluSpots 190
9.2.3 Generation of Peptide Arrays with a Laser Printer 191
9.2.4 Generation of Peptide Arrays on a Compact Disc Device 191
9.2.5 Generation of Peptide Arrays by Chemoselective Immobilization 191
9.3 Applications of Peptide Arrays 193
9.3.1 Generation of Small Amounts of Soluble Peptide Libraries 193
9.3.2 Epitope Mapping of Monoclonal Antibodies 194
9.3.3 Investigation of Antibody Epitopes in Polyclonal Sera 195
9.3.4 Investigation of Protein–Protein Interactions 196
9.3.4.1 General Considerations 196
9.3.4.2 Identification of Enzyme Substrates 197
9.3.5 Mapping of Protein–Nucleic Acid Interactions 202
9.3.6 Screening for Antimicrobial Peptides 202
9.3.7 Identification, Characterization, and Optimization of Peptidic Ligands 204
9.3.8 Identification of Metal Ion-Selective Peptides 204
9.4 Challenges in High-Throughput Screening (HTS) 205
9.5 Future Perspectives 206
Acknowledgments 207
Abbreviations 207
References 207

Index 219