

Contents

Preface xiii

1

Introduction 1

1.1 The Basics of Molecular Medicine 2

1.1.1 Topics of Molecular Medicine 2

1.1.2 Stages of Drug Development 3

1.2 The Human Cell 4

1.2.1 Organelles 4

1.2.1.1 The Nucleus 4

1.2.1.2 Mitochondria 6

1.2.1.3 Endoplasmic Reticulum and Golgi Apparatus 7

1.2.1.4 Peroxisome and Lysosome 8

1.2.2 Cell Cycle 8

1.2.3 Apoptosis 9

1.3 DNA Replication and Gene Expression 10

1.3.1 DNA Replication 11

1.3.2 Mutations 13

1.3.3 Transcription 14

1.3.4 Epigenetic Regulation of Gene Expression 19

1.3.5 Translation 21

1.3.6 Protein Degradation 24

1.4 Biological Communication 25

1.4.1 Neurotransmitters 26

1.4.2 Hormones 27

1.4.3 Signal Transduction 28

1.5 The Immune System 30

1.5.1 The Innate Immune System 30

1.5.1.1 The Complement System 31

1.5.2 The Adaptive Immune System 33

1.5.2.1 Cellular Immunity 33

1.5.2.2 Humoral Immunity 34

References 36

2

Methods in Molecular Medicine 37

2.1 DNA Microarrays 38

2.2 Quantitative Polymerase Chain Reaction 40

2.3 Next-Generation Sequencing 45

2.4 Animal Models in Biomedical Research 51

2.5 Additional Methods 56

2.5.1 Fluorescence Microscopy 56

2.5.2 Flow Cytometry and Fluorescence-Activated Cell Sorting 58

2.5.3 Surface Plasmon Resonance 59

References 59

3

Genetic Disorders 61

3.1 Single-Gene Disorders 62

3.1.1 Autosomal Dominant Disorders 64

3.1.1.1 Familial Hypercholesterolemia 65

3.1.1.2 Polycystic Kidney Disease 67

3.1.1.3 Marfan's Syndrome 67

3.1.1.4 Huntington's Disease 68

3.1.2 Autosomal Recessive Disorders 69

3.1.2.1 Cystic Fibrosis 70

3.1.2.2 Tay-Sachs Disease 71

3.1.2.3 Phenylketonuria 72

3.1.2.4 Xeroderma Pigmentosum 73

3.1.3 X-Linked Recessive Disorders 74

3.1.3.1 Red-Green Color Blindness 75

3.1.3.2 Duchenne and Becker Muscular Dystrophy 75

3.1.4 Mitochondriopathies 77

3.2 Polygenic Disorders 80

3.2.1 Asthma 80

3.2.2 Diabetes Mellitus 81

References 83

4

Molecular Oncology 85**4.1 Molecular Biology of Breast Cancer and Its Clinical Implications 88**

- 4.1.1 Intrinsic Subtypes of Breast Cancer 88
 - 4.1.1.1 Luminal 88
 - 4.1.1.2 Subclassification of TNBC 89
- 4.1.2 Molecular Profiling of Breast Cancer 89
- 4.1.3 Signaling Pathways 89
 - 4.1.3.1 The Role of the Estrogen Pathway in Breast Cancer 90
 - 4.1.3.2 Endocrine Therapy Resistance 90
 - 4.1.3.3 The mTOR/PI3K Pathway and Endocrine Resistance 90
 - 4.1.3.4 The CDK 4/6 Pathway 90
 - 4.1.3.5 HER2 Pathway and HER2 Targeted Therapy 91
- 4.1.4 Angiogenesis Pathway 92
 - 4.1.4.1 PARP Inhibitors 92
- 4.1.5 Other Biological Therapies/Approaches 93

4.2 Lung Cancer 93

- 4.2.1 Genetic Alterations in Non-Small Cell Lung Cancer 93
 - 4.2.1.1 Epidermal Growth Factor Receptor 93
 - 4.2.1.2 Anaplastic Lymphoma Kinase 94
 - 4.2.1.3 Kirsten Rat Sarcoma (KRAS) 94
 - 4.2.1.4 The Proto-Oncogene ROS1 95
 - 4.2.1.5 The Proto-Oncogene BRAF 95
 - 4.2.1.6 The Human Epidermal Growth Factor Receptor 2 (HER2) 95
 - 4.2.1.7 The RET Proto-Oncogene 95
 - 4.2.1.8 The MET Proto-Oncogene 95
 - 4.2.1.9 Phosphatidylinositol-3-Kinase (PI3K) 95
 - 4.2.1.10 Immune Checkpoint Inhibition 96

4.3 Hepatocellular Carcinoma 96

- 4.3.1 Risk Factors for Hepatocellular Carcinoma 96
- 4.3.2 Molecular Biology of Hepatocellular Carcinoma 97
- 4.3.3 Development of Sorafenib for the Treatment of Hepatocellular Carcinoma 97
- 4.3.4 Complexity of Cancer 98

4.4 Molecular Biology of Colorectal Cancer and Its Clinical Implications 99

- 4.4.1 Colorectal Cancer Carcinogenesis 99
 - 4.4.1.1 Chromosomal Instability Pathway 100
 - 4.4.1.2 Microsatellite Instability Pathway 100
 - 4.4.1.3 CpG Island Methylator Phenotype (CIMP) Pathway 101
- 4.4.2 Hereditary Colorectal Cancers 101

- 4.4.2.1 Familial Adenomatous Polyposis 101

- 4.4.2.2 Management of FAP Patients 101

- 4.4.2.3 Hereditary Non-Polyposis Colorectal Cancer 102

- 4.4.2.4 Management of HNPCC-Associated Germline Mutation Carriers 103

- 4.4.2.5 MUTYH-Associated Colorectal Cancer 103

- 4.4.2.6 Management of MAP Patients 103

- 4.4.3 Clinical Impact of Molecular Markers on the Management of Colorectal Cancer 103

- 4.4.3.1 MSI-H Status and Colorectal Cancer 103

- 4.4.3.2 Epidermal Growth Factor Receptor Pathway Targeting and Colorectal Cancer 103

- 4.4.3.3 RAS Mutations and Response to Anti-EGFR Therapy 104

- 4.4.3.4 BRAF Mutations and Colorectal Cancer 104

4.5 Molecular Biology of Renal Cell Carcinoma 105

- 4.5.1 Biology of Clear Cell Renal Cell Carcinoma 105
- 4.5.2 Approved Drugs for the Treatment of Clear Cell Renal Cell Carcinoma 106
- 4.5.3 Investigational Approaches for the Treatment of Clear Cell Renal Cell Carcinoma 107
- 4.5.4 Biology and Treatment of Papillary Renal Cell Carcinoma 108
- 4.5.5 Biology and Treatment of Chromophobe Renal Cell Carcinoma 108
- 4.5.6 Further Subtypes of Renal Cell Carcinoma 108

4.6 Molecular Biology of Prostate Cancer 109

- 4.6.1 Genes Associated with Hereditary Prostate Cancer 109
- 4.6.2 Tumor Suppressor Genes in Sporadic Prostate Cancer 110
- 4.6.3 Oncogenes 111

4.7 Molecular Biology of Hematological Malignancies 114

- 4.7.1 The Importance of Cytogenetics in Diagnosis and Treatment Decision-Making 115
- 4.7.2 Recognition of a Genetic Basis for the Hematological Malignancies 117
- 4.7.3 Targeted Therapeutics for Hematological Malignancies 119
- 4.7.4 Risk-Adapted Therapies 120
- 4.7.5 Epigenetics and Hematological Malignancies 120
- 4.7.6 The Unknown Unknowns – The Future of Molecular Oncology 120

References 121

5**Molecular Virology 123****5.1 The Basics of Virology 124**

- 5.1.1 Human Immunodeficiency Virus 127
- 5.1.2 Hepatitis B Virus 130
- 5.1.3 Influenza Virus 130

5.2 Vaccination 132

- 5.2.1 Live Vaccines 133
- 5.2.2 Recombinant Virus Vaccines 136
- 5.2.3 Inactivated Virus Vaccines 136
- 5.2.4 Subunit Vaccines 137
- 5.2.5 DNA Vaccines 139
- 5.2.6 HIV Vaccines 139

5.3 Detection of Viruses 139

- 5.3.1 Cytopathic Effects 139
- 5.3.2 Electron Microscopy 140
- 5.3.3 Hemagglutination Assay 140
- 5.3.4 Enzyme-Linked Immunosorbent Assay (ELISA) 140
- 5.3.5 Indirect ELISA 140
- 5.3.6 Polymerase Chain Reaction (PCR) 140
- 5.3.7 Antiviral Susceptibility Testing 142

5.4 Antiviral Therapy 142

- 5.4.1 Human Immunodeficiency Virus (HIV) 145
- 5.4.2 Hepatitis C Virus 149
- 5.4.3 Influenza Virus 149
- 5.4.4 Other Viruses 149

5.5 Prions 151**References 151****6****Bacteria and Eukaryotic Pathogens 153****6.1 Bacteria 154**

- 6.1.1 Pathogenic Bacteria 155
- 6.1.2 Bacterial Vaccines, Diagnostic, and Antibiotics 160
 - 6.1.2.1 Vaccines 160
 - 6.1.2.2 Diagnostic 160
 - 6.1.2.3 Antibiotics 160

6.2 Eukaryotic Pathogens 166**References 168****7****Genomics and Proteomics 169****7.1 Whole Genome Sequencing 170**

- 7.1.1 Cloning of a Genome 170
- 7.1.2 Mapping and Assembly of the Genome 172
- 7.1.3 Sequencing of a Large Genome 173

7.2 The Human Genome 174

- 7.2.1 Sequencing of the Human Genome 174
- 7.2.2 The International HapMap Project 183
- 7.2.3 The 1000 Genomes Project and the Personal Genome Project 184
- 7.2.4 Encyclopedia of DNA Elements (ENCODE) 186

7.3 Proteomics 188

- 7.3.1 Two-Dimensional Gel Electrophoresis and Mass Spectrometry 189
- 7.3.2 Quantitative and Shotgun Proteomics 192
- 7.3.3 Structural Proteomics 194

References 194**8****Genetic Testing 197****8.1 Types of Genetic Tests 198**

- 8.1.1 Postnatal Genetic Tests 198
- 8.1.2 Prenatal Genetic Tests 200

8.2 Chromosome Abnormalities 202

- 8.2.1 Conventional Karyotyping 203
- 8.2.2 Fluorescence In Situ Hybridization 203
- 8.2.3 Comparative Genomic Hybridization 205

8.3 Molecular Diagnosis 207

- 8.3.1 PCR-Based Methods 207
- 8.3.2 DNA Sequencing 209
- 8.3.3 DNA Microarray-Based Methods 212

References 213**9****Pharmacogenetics/Pharmacogenomics 215****9.1 Uptake and Transport of Drugs 217****9.2 Drug Metabolism 218**

- 9.2.1 Cytochrome P450 Enzymes 218
- 9.2.2 Other Drug Metabolizing Enzymes 220

9.3 Drug Targeting 222**9.4 Drug Toxicity and Hypersensitivity 226****9.5 Drug Development and Individual Pharmacotherapy 226****References 227****10****Recombinant Protein Drugs 229****10.1 Production of Recombinant Proteins 232**

- 10.1.1 Bacteria 233
- 10.1.2 Yeast and Other Fungi 234

10.1.3 Insect Cells 235
10.1.4 Mammalian Cells 235
10.1.5 Transgenic Animals and Plants 236
10.2 Classes of Recombinant Drugs 238
10.2.1 Monoclonal Antibodies 239
10.2.2 Hormones 245
10.2.3 Growth Factors 247
10.2.4 Fusion Proteins 249
10.2.5 Cytokines 250
10.2.6 Blood Coagulation Factors: Anticoagulants and Thrombolytics 251
10.2.7 Therapeutic Enzymes 254
10.2.8 Recombinant Vaccines 254
References 255

11

Gene Therapy 257

11.1 Types of Gene Therapy 258
11.2 Methods of Gene Transfer 259
11.2.1 Retroviral Vectors 260
11.2.2 Adenoviral Vectors 262
11.2.3 Adeno-Associated Virus Vectors 264
11.2.4 Nonviral Gene Transfer 266
11.3 Tissue Specificity of Gene Transfer and Gene Expression 267
11.4 Applications of Gene Therapy 270
11.4.1 Gene Therapy of Monogenic Diseases 271
11.4.2 Gene Therapy of Cancer 272
11.4.3 Other Diseases 273
11.5 Future Prospects 275
References 276

12

Stem Cells 277

12.1 Embryonic Stem Cells 279
12.1.1 Generation and Properties of Embryonic Stem Cells 279
12.1.2 Therapeutic Cloning 281
12.2 Adult Stem Cells 282
12.3 Induced Pluripotent Stem Cells 286
12.3.1 Generation of Induced Pluripotent Stem Cells 286
12.3.2 Properties of Induced Pluripotent Stem Cells 288
12.4 Transdifferentiation and Direct Reprogramming 289
12.5 Differentiation of Stem Cells 291

12.6 Medical Applications of Stem Cells 293
12.6.1 Adult Stem Cell Therapies 293
12.6.2 Pluripotent Stem Cells for Biomedical Research 296
12.6.3 Therapeutic Applications of Pluripotent Stem Cells 299
12.6.3.1 Diabetes 299
12.6.3.2 Heart Disease 300
12.6.3.3 Neurodegenerative Diseases 300
12.6.3.4 Combinations of Stem Cell and Gene Therapy 301
12.6.3.5 Clinical Trials 301
References 302

13

Antisense, Ribozyme, and RNA Interference Strategies 303

13.1 Antisense Oligonucleotides 305
13.1.1 Mechanism of Action of Antisense Oligonucleotides 305
13.1.2 Development and Stabilization of Antisense Oligonucleotides 306
13.1.3 Clinical Applications 308
13.2 Ribozymes 311
13.2.1 Classification of Ribozymes 311
13.2.2 Development of Ribozymes for Medical Applications 312
13.2.3 Clinical Applications of Ribozymes 314
13.3 RNA Interference 315
13.3.1 Mechanism of RNA Interference 316
13.3.2 Nonspecific Side Effects 319
13.3.3 Delivery 320
13.3.4 Preclinical Applications of RNA Interference 321
13.3.5 Clinical Trials 322
13.4 MicroRNAs 325
13.4.1 The Biology of MicroRNAs 325
13.4.2 MicroRNAs and Disease 327
References 330

14

Aptamers 333

14.1 Selection of Aptamers 335
14.2 Modifications of Aptamers 337
14.3 Clinical Development of Aptamers 339
14.4 Decoy and Immunostimulatory Oligonucleotides 342
References 344

15
Ethics in Molecular Medicine 345

15.1 The Basis of Bioethics 346

15.2 Fields of Application 348

15.2.1 Genetic Testing and the Right
“Not to Know” 348

15.2.2 Stem Cell Research 350

15.2.3 Preimplantation Genetic Diagnosis 352

Abbreviations 355

Glossary 363

Index 367

