Table of Contents

Preface — V

List of contributing authors — VII

Table of Contents — XI

Introduction to Volume 1: Kallikrein-related Peptidases. Characterization, Regulation, and Interactions Within the Protease Web — 1

Bibliography — 3

1 Genomic Structure of the KLK Locus — 5

1.1 Introduction — 5

1.2 Kallikreins in rodents — 6

1.2.1 The mouse kallikrein gene family — 7

1.2.2 The rat kallikrein gene family — 8

1.3 Characterization and sequence analysis of the human KLK gene locus — 9

1.3.1 Locus overview — 9

1.3.2 Repeat elements and pleomorphism — 11

1.4 Structural features of the human KLK genes and proteins — 12

1.4.1 Common structural features — 12

1.5 Sequence variations of human KLK genes — 13

1.6 Regulation of KLK activity — 14

1.6.1 At the mRNA level — 14

1.6.2 Locus control of KLK expression — 15

1.6.3 Epigenetic regulation of KLK gene expression — 17

1.7 Isoforms and splice variants of human KLKs — 18

1.8 Evolution of KLKs — 21

Bibliography — 22

2 Single Nucleotide Polymorphisms in the Human KLK Locus and Their Implication in Various Diseases — 31

2.1 Introduction — 31

2.2 KLK SNPs — data-mining from SNPdb and 1000 Genomes — 32

2.3 Functional annotations using web-based prediction tools — 34

2.4 Experimentally validated functional KLK SNPs — 35

2.5 KLK SNP haplotypes and tagging — 36

2.6 Malignant and non-malignant diseases and association with KLK SNPs — 38

2.6.1 Association studies on high-risk variants in KLK genes — 39
2.6.2 Association studies on low-risk variants in KLK genes —— 39
2.7 Conclusions —— 71
Bibliography —— 71

3 Evolution of Kallikrein-related Peptidases —— 79
3.1 Introduction —— 79
3.2 Basic elements of phylogenetic analysis —— 80
3.3 Evolutionary trends at the KLK locus —— 80
3.4 Evolution of the KLK1–KLK4 sublocus —— 82
3.4.1 KLK2 and KLK3 originate from a duplicated segment containing both KLK1 and KLK15 —— 82
3.4.2 A large number of KLK1 tandem repeats in the house mouse —— 85
3.4.3 The rat KLK1 sublocus consists of 10 large repeats —— 87
3.4.4 Four duplications of KLK1 and KLK15 in the dog —— 88
3.4.5 A large repeat containing KLK4 in the horse —— 90
3.5 KLK genes in non-mammalian species —— 92
3.6 General conclusions and remarks on the evolution of KLK genes —— 93
Bibliography —— 94

4 Structural Aspects of Kallikrein-related Peptidases —— 97
4.1 Introduction —— 97
4.2 Individual KLK structures —— 98
4.2.1 Tissue kallikrein (KLK1) —— 98
4.2.2 Prostate specific antigen (PSA/KLK3) —— 100
4.2.3 Prostase (KLK4) —— 101
4.2.4 Stratum corneum tryptic enzyme (SCTE/KLK5) —— 104
4.2.5 Myelencephalon-specific protease or neurosin (MSP/KLK6) —— 106
4.2.6 Stratum corneum chymotryptic enzyme (SCCE/KLK7) —— 108
4.2.7 Neuropsin (KLK8) —— 110
4.2.8 Other mammalian KLK structures —— 111
Bibliography —— 112

5 Molecular Recognition Properties of Kallikrein-related Peptidases on Synthetic and Endogenous Substrates —— 117
5.1 Introduction —— 117
5.2 Substrate specificities of individual kallikrein-related peptidases —— 121
5.2.1 The classical kallikreins (KLK1, KLK2, KLK3) —— 121
5.2.2 KLK4/KLK5/KLK7 —— 125
5.2.3 KLK6/KLK13/KLK14 —— 127
5.2.4 KLK8/KLK10/KLK12 —— 129
5.2.5 KLK9/KLK11/KLK15 — 130
Bibliography — 132

6 Natural, Engineered and Synthetic Inhibitors of Kallikrein-related Peptidases — 141

6.1 Introduction — 141
6.2 KLK diversity — 141
6.3 The KLK superfamily: Structure and catalytic mechanism — 141
6.4 KLK inhibition: Rationale and mechanisms — 143
6.5 Proteinaceous inhibitors — 144
6.5.1 Kunitz domain inhibitors — 144
6.5.2 Kazal domain inhibitors — 146
6.5.3 Other canonical inhibitors — 147
6.5.4 Serpins — 147
6.6 Naturally occurring small molecule kallikrein inhibitors — 148
6.7 Engineered KLK Inhibitors — 149
6.7.1 Approaches to inhibitor design — 150
6.7.2 Pharmacological challenges for therapeutic inhibitors — 150
6.7.3 Serpins — 150
6.7.4 Ecotin — 151
6.7.5 Sunflower Trypsin Inhibitor (SFTI) — 152
6.7.6 Warhead inhibitors — 153
6.8 Conclusions and outlook — 154
Acknowledgements — 154
Bibliography — 154

7 Kallikrein-related Peptidases as Pharmaceutical Targets — 161

7.1 Introduction — 161
7.2 KLK disease markers as potential therapeutic targets — 162
7.3 KLKs in oncology — 165
7.3.1 Prostate cancer — 165
7.3.2 Ovarian and pancreatic cancer — 167
7.4 KLKs in inflammatory skin diseases — 169
7.4.1 Kallikrein expressions and activities in skin — 169
7.4.2 Netherton Syndrome as most relevant clinical model — 170
7.4.3 Atopic dermatitis, the potential major indication for kallikrein targeting — 171
7.4.4 Psoriasis and relevance of kallikreins — 172
7.4.5 Other potential skin disorders with kallikrein involvement — 172
7.5 KLKs in neurological disorders — 173
7.5.1 Alzheimer’s disease and dementia — 173
7.5.2 Multiple sclerosis (MS) — 173
7.6 Kallikrein inhibitors to treat human diseases — 174
7.6.1 Design of KLK inhibitors and clinical development — 174
7.6.2 KLK inhibitors in oncology — 176
7.6.3 KLK inhibitors in dermatology — 179
7.7 Conclusions and Outlook — 180
Bibliography — 181

8 Expression of Kallikrein-related Peptidases under (Patho-)Physiological Conditions — 187
8.1 Introduction — 187
8.2 KLK expression in tissues and biological fluids under physiological conditions — 188
8.2.1 KLKs in the central and peripheral nervous system — 188
8.2.2 KLKs in the female reproductive system — 192
8.2.3 KLKs in the male reproductive system — 196
8.2.4 Cellular distribution of KLKs in the gastrointestinal system — 198
8.2.5 KLKs in the skin and skin appendages — 203
8.2.6 KLKs in the respiratory system — 207
8.2.7 KLKs in the urinary system — 207
8.2.8 KLKs in lymphatic and endocrine organs (adrenal glands, thyroid gland, parathyroid glands, pituitary gland) — 207
8.2.9 KLKs in the cardiovascular system — 211
8.2.10 KLKs in the skeletomuscular system — 211
8.3 Expression of KLKs in non-malignant diseases — 212
8.3.1 Non-malignant diseases of the CNS — 212
8.3.2 Inflammatory-related conditions — 215
8.4 Expression of KLKs in cancer tissues — 217
8.4.1 Cancers of the brain — 222
8.4.2 Cancers of the female reproductive system — 222
8.4.3 Cancers of the male reproductive system — 223
8.4.4 Cancers of the gastrointestinal system — 224
8.4.5 Cancers of the skin — 225
8.4.6 Lung cancer — 226
8.4.7 Cancers of the urinary system — 226
8.5 Conclusion — 227
Abbreviations — 227
Bibliography — 228

9 Kallikrein-related Peptidases within the Proteolytic Web — 251
9.1 Introduction — 251
9.2 KLKs as actors and targets during the initiation and amplification of extracellular proteolytic activity — 252
### Table of Contents

9.2.1 The KLK-dependent KLK activome —— 252  
9.2.2 Cross- and reciprocal activation of KLK and non-KLK proteases —— 256  
9.2.3 Inactivation of protease inhibitors —— 260  
9.3 KLKs in the termination of proteolytic activity —— 260  
9.3.1 Proteolytic inactivation of (non-)KLK proteases —— 260  
9.3.2 Processing of the uPA receptor —— 261  
9.3.3 Disarming of the proteinase-activated receptors —— 262  
9.4 Conclusion —— 263  
Bibliography —— 264

10 Kallikrein-Kinin Cascade: Bioregulation by Human Tissue Kallikrein 1 (hK1, KLK1) —— 271  
10.1 Discovery of classical (true) tissue kallikrein and kinins —— 271  
10.2 Cellular localization —— 272  
10.3 Genomics and molecular structure —— 273  
10.4 Inhibitors of hK1 —— 276  
10.5 Modulation of membrane receptors —— 277  
10.6 Epigenetic regulation —— 277  
10.7 Kinin receptors and signaling —— 278  
10.7.1 Receptor subtypes —— 278  
10.7.2 Kinin receptor signaling —— 279  
10.7.3 Regulation of kinin receptor signaling —— 280  
10.8 Human disease —— 281  
10.8.1 Hypertension and renal damage —— 281  
10.8.2 Cardiac protection —— 283  
10.8.3 Inflammation and neutrophil function —— 283  
10.8.4 Cancer —— 286  
10.8.5 Angiogenesis —— 286  
10.9 Conclusion —— 287  
Abbreviations —— 288  
Bibliography —— 289

11 Role of KLK4 in Dental Enamel Formation —— 295  
11.1 Introduction —— 295  
11.2 Early studies implicated proteases in dental enamel formation —— 295  
11.3 Investigations of enamel proteases discovered KLK4 —— 296  
11.4 KLK4 and amelogenesis imperfecta —— 297  
11.5 Klk4^{lacZ/lacZ} mice —— 297  
11.6 Other enamel specific genes —— 302  
11.7 Role of KLK4 in enamel formation —— 304  
11.8 Conclusion —— 307  
Bibliography —— 307