

CONTENTS

<i>Contributors</i>	<i>xiii</i>
<i>Preface</i>	<i>xxi</i>
<i>Volumes in Series</i>	<i>xxiii</i>
1. Initiation of Autophagy by Photodynamic Therapy	1
David Kessel and Nancy L. Oleinick	
1. Introduction	2
2. Photosensitizing Agents	3
3. Additional Factors Unique to PDT	5
4. A Typical PDT Protocol	5
5. Identification and Characterization of Autophagy after PDT	6
6. Effects of Autophagy on PDT Responses	9
Acknowledgments	15
References	15
2. Autophagic Cell Death	17
Michael J. Lenardo, Christina K. McPhee, and Li Yu	
1. Introduction	18
2. Methods to Quantify Cell Death	19
3. Methods to Measure Autophagy	26
4. Methods to Establish Autophagy as the Cause of Cell Death	27
5. Autophagy Genes for RNAi Silencing	29
6. Transfect RNAi by Electroporation (Amaxa Nucleofection)	29
7. Conclusion	29
References	30
3. Autophagic Neuron Death	33
Yasuo Uchiyama, Masato Koike, Masahiro Shibata, and Mitsuho Sasaki	
1. Introduction	34
2. Experimental Models of Neurodegeneration	35
3. Measurements of Neuron Death	41
References	48

4. Assessing Metabolic Stress and Autophagy Status in Epithelial Tumors	53
Robin Mathew, Vassiliki Karantza-Wadsworth, and Eileen White	
1. Introduction	55
2. Mouse Epithelial Cell Models for Studying the Role of Autophagy in Cancer	57
3. Protocols for Monitoring Autophagy in iBMK Cells and iMMECs <i>In Vitro</i>	61
4. Protocols for Monitoring Autophagy in Tumors <i>In Vivo</i>	64
5. Monitoring Chromosomal Instability Due to Autophagy Defects	67
6. Concluding Remarks and Future Perspectives	78
References	79
5. Autophagic Clearance of Aggregate-Prone Proteins Associated with Neurodegeneration	83
Sovan Sarkar, Brinda Ravikumar, and David C. Rubinsztein	
1. Introduction	84
2. Aggregate-Prone Intracytoplasmic Proteins Associated with Neurodegenerative Disorders are Autophagy Substrates	86
3. Assays for the Clearance of Aggregate-Prone Proteins	87
4. Measurement of Autophagic Flux Using Bafilomycin A ₁	102
5. Concluding Remarks	107
Acknowledgments	107
References	107
6. Monitoring Autophagy in Alzheimer's Disease and Related Neurodegenerative Diseases	111
Dun-Sheng Yang, Ju-Hyun Lee, and Ralph A. Nixon	
1. Introduction	112
2. General Approaches to Investigations of Human Neurodegeneration	113
3. Characterization of Autophagic Vacuoles, Evaluation of Autophagosome and Autolysosome Formation, and Autolysosomal Clearance	119
4. Metabolic Analyses of Autophagy in Neuronal and Nonneuronal Cell Models	132
5. Isolation and Characterization of Autophagic Vacuoles and Lysosomes from Cell Cultures and Brain Tissue	135
6. Western Blot Analysis of Autophagy Components and Substrates	136
References	140

7. Live-Cell Imaging of Autophagy Induction and Autophagosome-Lysosome Fusion in Primary Cultured Neurons	145
Mona Bains and Kim A. Heidenreich	
1. Introduction	146
2. Cultured Cerebellar Purkinje Neurons as a Model to Study Neuronal Autophagy	148
3. Characterization of Autophagic Vacuole Size and Number in Purkinje Neurons	151
4. Using Colocalization of Fluorescent Tags to Measure Autophagosome-Lysosome Fusion	154
5. Concluding Remarks	156
Acknowledgments	157
References	157
8. Using Genetic Mouse Models to Study the Biology and Pathology of Autophagy in the Central Nervous System	159
Zhenyu Yue, Gay R. Holstein, Brian T. Chait, and Qing Jun Wang	
1. Introduction	160
2. Methods	164
3. Analysis of GFP-LC3 Expression and Subcellular Localization in the CNS	167
4. Analysis of p62/SQSTM1 and Ubiquitinated Protein Inclusions in the CNS	171
5. Transmission Electron Microscopy (TEM) Analysis of Autophagosomes	173
6. Conclusion	177
Acknowledgments	178
References	178
9. Biochemical and Morphological Detection of Inclusion Bodies in Autophagy-Deficient Mice	181
Satoshi Waguri and Masaaki Komatsu	
1. Introduction	182
2. Detection of Ubiquitinated Proteins and p62 in Autophagy-Deficient Mice by Western Blot Analysis	183
3. Detection of Ubiquitinated Proteins and p62 in Cultured Hepatocytes Derived from Autophagy-Deficient Mice	187

4. Detection of Ubiquitin- and p62-Positive Inclusions at the Light Microscopy Level	188
5. Detection of Ubiquitin- and p62-Positive Inclusions at the Electron Microscopy Level	192
6. Conclusion	194
Acknowledgments	195
References	195
10. Analyzing Autophagy in Clinical Tissues of Lung and Vascular Diseases	197
Hong Pyo Kim, Zhi-Hua Chen, Augustine M. K. Choi, and Stefan W. Ryter	
1. Introduction	198
2. Methods for Preparation of Lung and Vascular Cells	199
3. Analysis of Autophagy	205
4. Chromatin Immunoprecipitation	211
5. Conclusions	214
References	215
11. Autophagy in Neurite Injury and Neurodegeneration: <i>In Vitro</i> and <i>In Vivo</i> Models	217
Charleen T. Chu, Edward D. Plowey, Ruben K. Dagda, Robert W. Hickey, Salvatore J. Cherra III, and Robert S. B. Clark	
1. Introduction	218
2. Studying Neuronal Autophagy <i>In Vitro</i>	220
3. Studying Brain Autophagy <i>In Vivo</i>	238
4. Future Perspectives and Challenges	244
Acknowledgments	244
References	244
12. Monitoring the Autophagy Pathway in Cancer	251
Frank C. Dorsey, Meredith A. Steeves, Stephanie M. Prater, Thomas Schröter, and John L. Cleveland	
1. Introduction	252
2. LC3: A Phenotypic and Functional Marker of Autophagy	254
3. Assessing the Role of Autophagy in E μ -Myc-Driven Lymphoma	264
4. Concluding Remarks and Future Perspectives	269
Acknowledgments	269
References	269

13. Autophagy Pathways in Glioblastoma	273
Hong Jiang, Erin J. White, Charles Conrad, Candelaria Gomez-Manzano, and Juan Fueyo	
1. Introduction: Autophagy and Gliomas	274
2. Prioritization of Methods to Characterize Autophagy in Gliomas	275
3. <i>In Vitro</i> Cellular Markers	276
4. <i>In Vitro</i> Biochemical Markers	279
5. Electron Microscopy to Monitor the Autophagic Vacuoles	281
6. <i>In Vivo</i> Analysis of Biochemical Markers	281
7. Autophagy Indicators as Surrogate Markers of Treatment Effect in Clinical Trials	284
8. Future Directions	284
References	285
14. Autophagy in Lung Cancer	287
Jerry J. Jaboin, Misun Hwang, and Bo Lu	
1. Introduction	288
2. Methods	291
3. Conclusion	301
References	301
15. Signal-Dependent Control of Autophagy-Related Gene Expression	305
Fulvio Chiacchiera and Cristiano Simone	
1. Introduction	306
2. Overview: Signal Transduction and Chromatin-Associated Kinases	307
3. The p38 Pathway in Colorectal Cancer Cells	308
4. Methods to Test Kinase Activity	309
5. Profiling Gene Expression Pattern	311
6. Transcriptional Control of ATG Genes	312
7. Analysis of Transcriptional Multiprotein Complexes	318
8. Concluding Remarks	322
Acknowledgments	323
References	323
16. Novel Methods for Measuring Cardiac Autophagy <i>In Vivo</i>	325
Cynthia N. Perry, Shiori Kyo, Nirmala Hariharan, Hiromitsu Takagi, Junichi Sadoshima, and Roberta A. Gottlieb	
1. Introduction	326
2. <i>In Vivo</i> Models of Autophagy in the Myocardium	329

3. Discussion	339
Acknowledgments	341
References	341
17. Autophagy in Load-Induced Heart Disease	343
Hongxin Zhu, Beverly A. Rothermel, and Joseph A. Hill	
1. Introduction	344
2. Mouse Models of Load-Induced Heart Disease	345
3. Analysis of Ventricular Remodeling	346
4. <i>In Vitro</i> Models of Load-Induced Hypertrophy	349
5. Techniques to Analyze Cardiomyocyte Autophagy	350
6. Immunohistochemistry for LAMP-1 or Cathepsin D to Monitor Changes in Lysosomal Abundance	352
7. Isolation of LC3 Proteins from NRVM in Culture	356
8. Isolation of LC3 Protein from Heart or Skeletal Muscle Tissue	357
9. Soluble/Insoluble Fractionation of NRVM	359
10. Perspective	360
Acknowledgments	360
References	360
18. Evaluation of Cell Death Markers in Severe Calcified Aortic Valves	365
Wilhelm Mistiaen and Michiel Knaapen	
1. Clinical Importance of Degenerative Aortic Valve Disease	366
2. Pathological Appearances of AVD	366
3. Mechanism of Progression of AVD	367
4. Autophagy: Major Player in the Progression of Aortic Valve Disease?	368
5. Methods for Detection of Cell Death Markers in the Degenerated Aortic Valve	370
6. Methods for Quantification of Calcified Aortic Valves	374
7. Discussion	376
8. Prospects and Concluding Remarks	376
References	377
19. Monitoring Autophagy in Muscle Diseases	379
May Christine V. Malicdan, Satoru Noguchi, and Ichizo Nishino	
1. Introduction	380
2. Histological Observation of Skeletal Muscles	381
3. Measuring Autophagy by Protein Quantification	387
4. Monitoring Autophagy in Cultured Skeletal Myocytes	388

5. Electron Microscopy Observation of Skeletal Muscles	392
6. Immunoelectron Microscopy	394
7. Conclusion	395
References	395
20. Analyzing Macroautophagy in Hepatocytes and the Liver	397
Wen-Xing Ding and Xiao-Ming Yin	
1. The Pathophysiological Relevance of Macroautophagy in the Liver	398
2. Analysis of Autophagy in Isolated Hepatocytes	398
3. Analysis of Autophagy in the Liver	410
4. Summary	413
Acknowledgments	414
References	414
21. Monitoring Autophagy in Lysosomal Storage Disorders	417
Nina Raben, Lauren Shea, Victoria Hill, and Paul Plotz	
1. Introduction	418
2. General Techniques to Monitor Autophagy In LSDs	419
3. LSDs Analyzed for Autophagic Involvement	428
4. Conclusion	444
References	445
<i>Author Index</i>	451
<i>Subject Index</i>	471