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*by* Pratika Y

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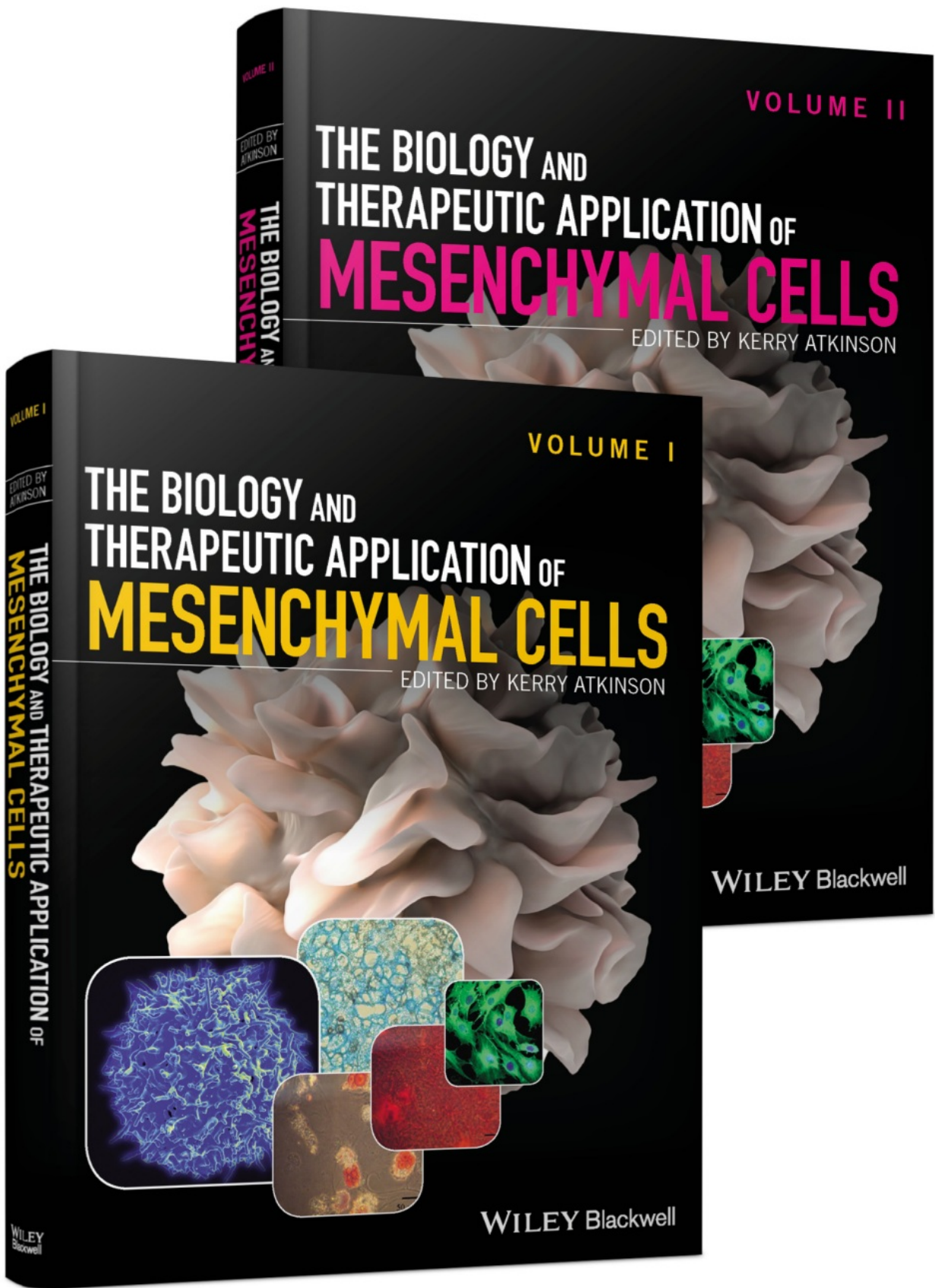
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# **The biology and therapeutic application of mesenchymal cells**

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**Kerry Atkinson**

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## 2 Contents

Contributors, xxvii

Editor's Preface, xxxv

### Section I: An overview of mesenchymal stem cells and mesenchymal stromal cells

#### 1 The mesenchymal stem cell, the mesenchymal stromal cell, and the mesenchymal stromal cell exosome, 3

*Kerry Atkinson*

##### 1.1 Nomenclature, 3

##### 1.2 The mesenchymal stem cell, 3

##### 1.3 The mesenchymal stromal cell, 4

##### 1.4 The mesenchymal stromal cell exosome and extracellular vesicles, 6

References, 7

#### 2 The nomenclature of mesenchymal stem cells and mesenchymal stromal cells, 8

*Armand Keating*

##### 2.1 Introduction, 8

##### 2.2 Historical perspective, 8

##### 2.3 The need for common terminology and definition: the International Society for Cellular Therapy white papers of the mid-2000s, 9

##### 2.4 Updating terminology, 9

References, 10

### Section II: The isolation and ex vivo expansion of mesenchymal stromal cells

#### 3 The isolation and expansion of mesenchymal stromal cells from bone marrow, 13

*Celena F. Heazlewood*

##### 3.1 Introduction, 13

##### 3.2 Stem cells, 14

##### 3.3 Isolation and characterization of bone marrow mesenchymal stromal cells, 14

##### 3.3.1 Cell surface markers, 15

##### 3.3.2 Chemokine receptor display, 15

##### 3.3.3 Mesodermal differentiation capability, 17

##### 3.4 The immunomodulatory properties of mesenchymal stromal cells, 17

##### 3.5 The transcriptome of mesenchymal stromal cells, 18

References, 20

#### 4 The biology and clinical applications of mesenchymal stromal cells derived from human gestational tissues, 24

*Celena F. Heazlewood*

##### 4.1 Introduction, 24

##### 4.2 Isolation of placental mesenchymal stromal cells, 25

##### 4.3 Characteristics of fetally derived mesenchymal stromal cells isolated from gestational tissues, 26

##### 4.3.1 Amniotic-membrane-derived mesenchymal stromal cells, 26

##### 4.3.2 Chorionic-membrane-derived mesenchymal stromal cells, 26

##### 4.4 Characteristics of maternally derived mesenchymal stromal cells isolated from gestational tissue (the decidua), 27

##### 4.5 Comparison of mesenchymal stromal cells from fetal and maternal tissues isolated from gestational tissues, 27

##### 4.6 Comparison of gene expression profiles between human term-placenta-derived mesenchymal stromal cells, human adult bone-marrow-derived mesenchymal stromal cells, and human umbilical-cord-derived unrestricted somatic stem cells, 28

##### 4.7 Preclinical mesenchymal stromal cell studies, 28

##### 4.8 Clinical applications of placental mesenchymal stromal cells, 29

##### 4.9 Manufacturing clinical-grade placenta-derived mesenchymal stromal cells, 29

##### 4.9.1 Phase 1 clinical trials using unrelated major-histocompatibility-unmatched placenta-derived mesenchymal stromal cells, 30

##### 4.10 Conclusions, 30

References, 30

#### 5 Human placenta-derived mesenchymal stem/stromal cells: fetal and maternal origins and critical parameters for ex vivo expansion, 32

*Rebecca A. Pelekanos and Varda S. Sardesai*

##### 5.1 Introduction, 32



- 5.2 Mesenchymal stem/stromal cells: a consensus definition?, 32
- 5.3 Prenatal and perinatal tissue sources of mesenchymal stem/stromal cells, 33
- 5.4 Fetal tissue-derived mesenchymal stem/stromal cells, 33
- 5.5 Placental and adnexal stem and progenitor cells, 33
- 5.6 Comparison of mesenchymal stem/stromal cells from different gestational sources, 33
- 5.7 Consensus classification of human placental mesenchymal stem/stromal cells, 34
- 5.8 Differentially isolating fetal or maternal mesenchymal stem/stromal cells from term placental villi, 34
- 5.9 Confounding factors for the isolation of fetal placental mesenchymal stem/stromal cells from chorionic villi, 34
- 5.10 Assumptions from the literature: lack of data, specific assays, and specific methodological detail, 35
- 5.11 Methods for determining fetal and maternal mesenchymal stem/stromal cells in a cultured cell population, 35
- 5.12 A novel method to isolate fetal and maternal placental mesenchymal stem/stromal cells, 36
- 5.13 Understanding the maternal origin of the placental mesenchymal stem/stromal cells: the septa, 36
- 5.14 Conclusions and future directions, 36
- Acknowledgments, 37
- References, 37

## 2 Section III: The cellular and molecular biology of mesenchymal stromal cells

### 6 Epigenetic regulation of mesenchymal stem/stromal cell growth and multipotentiality, 41

*Sarah Elizabeth Hemming, Dimitrios Cakouros, and Stan Gronthos*

- 6.1 Introduction, 41
- 6.2 Mesenchymal stromal/stem cells, 42
- 6.3 Epigenetics, 42
- 6.4 DNA methylation and histone modifications in mesenchymal stem/stromal cells, 45
- 6.5 Epigenetic regulation of osteogenic differentiation, 45
- 6.6 Epigenetic regulation of adipogenic differentiation, 47
- 6.7 Epigenetic regulation of myogenic differentiation, 48
- 6.8 Epigenetic regulation of chondrogenic differentiation, 48
- 6.9 Epigenetic regulation of mesenchymal stem/stromal cell lifespan and senescence, 52
- 6.10 Regulation of epigenetic modifications in mesenchymal stem/stromal cells for clinical use, 52
- 6.11 Conclusions, 52
- References, 53

### 7 Biological changes in human mesenchymal stromal cells during monolayer culture, 58

*Marietta Herrmann and Jennifer J. Bara*

- 7.1 Introduction, 58
- 7.2 Mesenchymal stromal cell isolation from bone marrow, 59
- 7.3 Mesenchymal stromal cell isolation from adipose tissue, 60
- 7.4 Biological characteristics, 60
  - 7.4.1 Morphology and colony formation, 60
  - 7.4.2 Growth kinetics, 61
  - 7.4.3 *In vitro* multipotency, 62
  - 7.4.4 Gene expression, 62
  - 7.4.5 Cell surface marker profile, 63
  - 7.4.6 Secretory profile, 66
- 7.5 Influences on tissue culture parameters, 66
  - 7.5.1 Seeding density, 66
  - 7.5.2 Culture medium and supplementation, 67
  - 7.5.3 Growth factors, 67
  - 7.5.4 Xeno-free media, 67
  - 7.5.5 Platelet-derived supplements, 67
  - 7.5.6 Serum-free media, 68
  - 7.5.7 Hypoxia, 68
- 7.6 Implications for basic and clinical research, 68
  - 7.6.1 Trial disparity, 68
  - 7.6.2 Alternative culture systems, 69
- 7.7 Conclusions and future directions, 70
- References, 70

### 8 The effect of three-dimensional aggregates on the biology of mesenchymal stromal cells, 75

*Yijun Liu, Ang-Chen Tsai, Xuegang Yuan, and Teng Ma*

- 8.1 Three-dimensional multicellular aggregates, 75
- 8.2 Three-dimensional aggregates of mesenchymal stromal cells, 76
- 8.3 Mechanism of mesenchymal stromal cells self-assembly into three-dimensional aggregates, 77
  - 8.3.1 Cell-cell contact, 77
  - 8.3.2 Extracellular matrix and the cytoskeleton, 77
  - 8.3.3 Mesenchymal stromal cells heterospheroids, 78
- 8.4 Mechanisms of aggregate-mediated mesenchymal stromal cell functional enhancement, 78
  - 8.4.1 Role of cell adhesion molecules in the fate decision of mesenchymal stromal cell three-dimensional aggregates, 79
  - 8.4.2 Effects of extracellular matrix, cytoskeleton, and morphology on mesenchymal stromal cell lineage commitment in three-dimensional aggregates, 80
  - 8.4.3 Role of molecular milieu and hypoxia-inducible factor activation, 80
  - 8.4.4 Metabolism changes in three-dimensional aggregates of mesenchymal stromal cells, 81

- 8.4.5 Enhanced anti-inflammatory properties of three-dimensional aggregates of mesenchymal stromal cells, 81
- 8.5 Bioreactor systems for three-dimensional aggregate production, 81
- 8.5.1 Scale-up and dynamics of culture, 81
- 8.5.2 Spinner flasks, 82
- 8.5.3 Rotary wall vessel, 82
- 8.5.4 Rotary orbital system, 83
- 8.5.5 Comparison of spinner flask and rotary wall vessel, 83
- 8.5.6 Other systems, 84
- 8.6 Transplantation of three-dimensional mesenchymal stromal cell aggregates in preclinical animal models of disease, 84
- 8.6.1 Enhanced secretory properties of mesenchymal stromal cells aggregates, 84
- 8.6.2 Immunomodulation by mesenchymal stromal cell aggregates, 84
- 8.6.3 Enhanced multilineage differentiation of three-dimensional mesenchymal stromal cells aggregates, 84
- 8.6.4 Recapitulation of mesenchymal condensation and osteochondral differentiation in bone and cartilage regeneration, 86
- References, 87
- 2**
- 9** Cell-cell signaling pathways that regulate mesenchymal stromal cell differentiation, 91  
*Leah Etheridge, Rebecca A. Mason, Fatima Saleh, and Paul Genever*
- 9.1 Introduction, 91
- 9.2 Mesenchymal stromal cell signaling is dependent on its type, 91
- 9.3 Identity of bone-marrow-derived mesenchymal stromal cells, 92
- 9.4 Mesenchymal stromal cell signaling in the stem cell niche, 93
- 9.5 Regulation of mesenchymal stromal cell differentiation by the TGF- $\beta$ /BMP signaling pathway, 95
- 9.6 Regulation of mesenchymal stromal cell differentiation by the Wnt signaling pathway, 97
- 9.7 Conclusions, 99
- References, 99
- 2**
- 10** Regulation of mitochondrial transport in mesenchymal stromal cells, 104  
*Shravani Mukherjee, Naveen K. Bhatraju, Tanveer Ahmad, and Anurag Agrawal*
- 10.1 Introduction, 104
- 10.2 Intercellular organelle transport, 105
- 10.2.1 Intercellular communication, 105
- 10.2.2 Mitochondrial biology, 105
- 10.2.3 Intercellular mitochondrial transport/mitochondrial donation, 105
- 10.3 Mesenchymal stromal cells as potential mitochondrial donors, 107
- 10.3.1 Mechanism of intercellular mitochondrial transport regulation, 108
- 10.4 Strategies to improve mitochondrial delivery to target cells, 110
- 10.5 The road ahead, 111
- References, 111
- 2**
- 11** The regulation of adipogenesis from adipose-derived stem/stromal cells, 114  
*Lin Chen and Lei Liu*
- 11.1 Introduction, 114
- 11.2 Adipose-derived stem/stromal cells, 115
- 11.2.1 Preparation and molecular characterization of adipose-derived stem/stromal cells, 115
- 11.2.2 Differentiation capacity of adipose-derived stem/stromal cells, 116
- 11.3 Process of adipogenic differentiation from adipose-derived stem/stromal cells, 116
- 11.3.1 Adipocyte development program, 116
- 11.3.2 Signaling pathways associated with adipogenic differentiation, 117
- 11.4 Regulation of adipogenic differentiation from adipose-derived stem/stromal cells, 118
- 11.4.1 Transcriptional regulation, 118
- 11.4.2 Epigenetic regulation, 119
- 11.4.3 Post-transcriptional regulation, 121
- 11.5 The future, 125
- References, 125
- 2**
- 12** Modulation of osteogenic differentiation in mesenchymal stromal cells, 131  
*Sean Gaynard, Jessica Hayes, and Mary Murphy*
- 12.1 Introduction, 131
- 12.2 Biology, 132
- 12.2.1 Sources of mesenchymal stromal cells, 132
- 12.2.2 Cellular regulation of osteogenic differentiation from mesenchymal stromal cells, 132
- 12.2.3 Molecular regulation of osteogenic differentiation from mesenchymal stromal cells, 134
- 12.2.4 Factors regulating homing of mesenchymal stromal cells to bone, 136
- 12.2.5 *In vivo* detection and contribution of mesenchymal stromal cells to osteogenesis, 137
- 12.2.6 Regulating the immune system for bone formation, 138
- 12.3 Clinical applications of mesenchymal stromal cells in bone disorders, 138
- 12.3.1 Bone regeneration, 138

- 12.3.2 Osteoarthritis, 139
- 12.3.3 Osteogenesis imperfecta, 140
- 12.4 Summary, 141
- References, 141
- 2** **13** The role of glycogen synthase kinase-3 inhibitors on bone modeling, 148  
*K. Jane Escott and Patrick J. O'Shea*
  - 13.1 Overview of glycogen synthase kinase-3, 148
  - 13.2 The response of skeletal cells to glycogen synthase kinase-3 inhibitors *in vitro*, 149
    - 13.2.1 Lithium chloride, 149
    - 13.2.2 SB-216763 and SB-415286, 151
    - 13.2.3 6-bromindirubin-3'-oxime, 152
    - 13.2.4 LY603281-31-8, 153
    - 13.2.5 CT99021/CHIR99021, 153
    - 13.2.6 AR28 (AZD2858), AR79, and AZ13282107, 154
  - 13.3 Bone anabolism through inhibition of glycogen synthase kinase-3 *in vivo*, 155
    - 13.3.1 Functional Wnt/ $\beta$ -catenin responses in *Xenopus laevis* model systems, 156
    - 13.3.2 Progenitor cell involvement in bone anabolism *in vivo*, 156
    - 13.3.3 Alteration in bone resorption *in vivo*, 158
  - 13.4 Impact of glycogen synthase kinase-3 inhibition in bone disease, 159
    - 13.4.1 Osteopenia and osteoporosis, 159
    - 13.4.2 Methotrexate-induced bone loss, 160
    - 13.4.3 Fracture healing, 160
    - 13.4.4 Multiple myeloma-associated bone disease, 161
    - 13.4.5 Periodontal disease, 161
    - 13.4.6 Clinical findings with lithium, 161
  - 13.5 Summary, 162
  - References, 163
- 2** **14** Early molecular events during *in vitro* chondrogenesis, 167  
*Tommy A. Karlsen, Rune B. Jakobsen, and Jan E. Brinchmann*
  - 14.1 Introduction, 167
  - 14.2 Adult articular cartilage, 168
  - 14.3 Developmental chondrogenesis, 168
  - 14.4 Molecular aspects of *in vivo* chondrogenesis, 169
  - 14.5 Determinants of *in vitro* chondrogenesis, 171
  - 14.6 Tissue source of mesenchymal stromal cells, 171
    - 14.6.1 *In vitro* cell culture, 171
  - 14.7 Three-dimensional culture systems and bioscaffolds, 172
  - 14.8 Epigenetic changes during early *in vitro* chondrogenesis, 172
    - 14.8.1 An introduction to epigenetics, 172
    - 14.8.2 DNA methylation of the *COL2A1* and *COL10A1* promoters, 173
    - 14.8.3 DNA methylation of promoters in other chondrogenesis candidate genes, 173
    - 14.8.4 Genome-wide map of quantified epigenetic changes during *in vitro* chondrogenesis of bone marrow mesenchymal stromal cells, 174
    - 14.8.5 Epigenetics: conclusions, 175
- 2** **14.9** Role of microRNAs during early *in vitro* chondrogenesis, 176
  - 14.9.1 An introduction to microRNAs, 176
  - 14.9.2 Role of miRNA-140 in developmental chondrogenesis, 177
  - 14.9.3 miR-140 targets identified *in vivo* and *in vitro*, 178
  - 14.9.4 Defining the role of miR-140 during chondrogenic differentiation of mesenchymal stromal cells and dedifferentiation of articular chondrocytes, 178
  - 14.9.5 Impact of microRNAs other than miR-140 on chondrogenic differentiation of mesenchymal stromal cells, 179
  - 14.9.6 MicroRNAs in chondrogenesis: conclusions, 181
- 2** **14.10** Early changes in gene expression during *in vitro* chondrogenesis, 182
  - 14.10.1 Genes involved in collagen fibrillogenesis, 183
  - 14.10.2 Genes involved in synthesis of proteoglycans and glycosaminoglycans, 183
  - 14.10.3 Transcription factor genes, 183
  - 14.10.4 Genes encoding other important cartilage molecules, 184
  - 14.10.5 Genes encoding unwanted molecules, 184
  - 14.10.6 Effect on gene expression of changes in the differentiation cocktail, 184
- 2** **14.11** Conclusions, 186
- References, 186
- 2** **15** The role of the extracellular matrix in the differentiation of mesenchymal stromal cells, 191  
*Peishun Shou, Qing Chen, and Yufang Shi*
  - 15.1 Summary, 191
  - 15.2 Multipotency of mesenchymal stromal cells, 191
  - 15.3 The extracellular matrix and mesenchymal stromal cell differentiation, 192
    - 15.3.1 The role of osteopontin in mesenchymal stromal cell differentiation, 193
    - 15.3.2 Geometric cues in mesenchymal stromal cell differentiation, 193
    - 15.3.3 Crosstalk between the extracellular matrix and mesenchymal stromal cells, 193
  - 15.4 Conclusions and future perspectives, 194
- Acknowledgments, 194
- References, 194

- 2**  
**16** Effects of hypoxic culture on bone marrow multipotent mesenchymal stromal cells: from bench to bedside, 196  
*Shih-Chieh Hung*
- 16.1 Introduction, 196
  - 16.2 Multipotent mesenchymal stromal cells, 196
  - 16.3 Criteria for defining human multipotent stromal cells, 197
  - 16.4 Problems encountered in the clinical application of multipotent mesenchymal stromal cells, 197
  - 16.5 The hypoxic niche of multipotent mesenchymal stromal cells, 197
  - 16.6 Involvement of hypoxia-inducible factor-1 $\alpha$  in hypoxia-mediated effects, 198
  - 16.7 Effects of hypoxic culture on glucose metabolism and oxidative stress of multipotent mesenchymal stromal cells, 198
  - 16.8 Effects of hypoxic culture on the apoptosis of multipotent mesenchymal stromal cells, 199
  - 16.9 Effects of hypoxic culture on expansion and life span of multipotent mesenchymal stromal cells, 199
  - 16.10 Effects of hypoxic culture on maintaining self-renewal and differentiation potential of multipotent mesenchymal stromal cells, 200
  - 16.11 Differentiation of multipotent mesenchymal stromal cells under hypoxic conditions, 200
  - 16.12 Effects of hypoxic culture on secretion of paracrine factors by multipotent mesenchymal stromal cells, 201
  - 16.13 Effects of hypoxic culture on engraftment of multipotent mesenchymal stromal cells, 202
  - 16.14 Effects of hypoxic culture on allogeneic transplantation of multipotent mesenchymal stromal cells, 202
  - 16.15 Conclusions, 203
  - Acknowledgments, 203
  - References, 203
- 2**  
**17** The role of cyclic tensile strain on osteogenesis and angiogenesis in human mesenchymal stem/stromal cells, 208  
*Adisri Charoenpanich, Josephine Bodle, and Elizabeth Lobo*
- 17.1 Introduction, 208
  - 17.2 Applications of tensile strain: an interpretation from physiological stimuli *in vivo* to bioreactors *in vitro*, 209
    - 17.2.1 Uniaxial tensile strain, 209
    - 17.2.2 Equi-/biaxial tensile strain, 210
  - 17.3 Mechanical sensing of mesenchymal stem/stromal cells, 211
    - 17.3.1 Integrins and the cytoskeleton, 211
    - 17.3.2 The nucleoskeleton and lamins, 212
    - 17.3.3 Primary cilia, 212
    - 17.3.4 Stretch-activated calcium channels, 213
    - 17.3.5 The glycocalyx, 213
  - 17.4 The molecular response of mesenchymal stem/stromal cells to cyclic tensile strain, 214
    - 17.4.1 Restructuring of mesenchymal stem/stromal cells and the surrounding extracellular matrix by mesenchymal stem/stromal cells in response to cyclic tensile strain, 215
    - 17.4.2 Mesenchymal stem/stromal cell secretomes that induce further responses from other cells, 216
  - 17.5 Summary, 217
  - Acknowledgments, 217
  - References, 217
- 2**  
**18** The evolving concept of mesenchymal stromal cells in regenerative medicine: from cell differentiation to secretome, 222  
*F.G. Teixeira, A. Pires, S.C. Serra, N. Sousa, and A.J. Salgado*
- 18.1 Mesenchymal stromal cells, 222
  - 18.2 The mesenchymal stromal cell secretome, 224
    - 18.2.1 Concept, 224
    - 18.2.2 Characterization techniques, 224
  - 18.3 The mesenchymal stromal cell secretome in transplantation and regenerative medicine, 225
    - 18.3.1 Graft-versus-host-disease, 225
    - 18.3.2 The central nervous system, 226
  - 18.4 The peripheral nervous system, 229
  - 18.5 Future perspectives, 230
  - References, 231
- 2**  
**19** The secretome of mesenchymal stem/stromal cells undergoing chondrogenic differentiation and those undergoing osteogenic or adipogenic differentiation, 236  
*Beatriz Rocha, Francisco J. Blanco, and Cristina Ruiz-Romero*
- 19.1 Introduction to protein secretion and the analysis of secretomes, 236
  - 19.2 Analysis of mesenchymal stem/stromal cell secretomes using proteomic approaches, 237
    - 19.2.1 Approaches to obtaining secretome samples, 237
    - 19.2.2 Experimental strategies for *in vitro* secretome analysis of mesenchymal stem/stromal cells, 237
  - 19.3 Analysis of the secretome of mesenchymal stem/stromal cells undergoing chondrogenesis, 242
  - 19.4 Characterization of chondrogenesis markers by secretome analysis, 242
  - 19.5 Characterization of osteogenesis markers by secretome analysis, 247
  - 19.6 Characterization of adipogenesis markers by secretome analysis, 247
  - 19.7 Conclusions and future perspectives, 247
  - References, 247



- 2**  
**20** Mesenchymal stromal cell extracellular vesicles/exosomes, 250  
*Ronne Wee Yeh Yeo, Ruenn Chai Lai, and Sai Kiang Lim*
  - 20.1 From cell to secretion to exosome, 250
    - 20.1.1 Mesenchymal stromal cells, 250
    - 20.1.2 Cell secretion, 251
    - 20.1.3 Mesenchymal stromal cell extracellular vesicles as the active therapeutic factor, 251
  - 20.2 Extracellular vesicles, 251
    - 20.2.1 Exosome biology and general functions, 252
  - 20.3 The therapeutic use of exosomes, 252
    - 20.3.1 Mesenchymal stromal cell exosomes, 253
    - 20.3.2 Characterization of mesenchymal stromal cell exosomes, 254
    - 20.3.3 The biochemical potential of mesenchymal stromal cell exosomes, 254
    - 20.3.4 Biochemical potency, 255
    - 20.3.5 Glycolysis, 256
    - 20.3.6 Proteasome activity, 256
    - 20.3.7 Signaling: adenosine signaling, 256
    - 20.3.8 Inhibition of complement activation, 256
    - 20.3.9 Restoring homeostasis, 256
    - 20.3.10 Bioenergetic homeostasis, 257
    - 20.3.11 Immune homeostasis, 257
  - 20.4 The clinical translation of mesenchymal stromal cell exosomes, 258
  - 20.5 Conclusions, 258

References, 258

- 2**  
**21** Role of tunneling nanotube crosstalk with distressed cardiomyocytes in controlling the heart repair potential of mesenchymal stromal cells, 264  
*Anne-Marie Rodriguez and Meriem Mahrouf-Yorgov*
  - 21.1 Introduction, 264
  - 21.2 Mesenchymal stromal cells as a promising tool to regenerate damaged heart tissue, 264
    - 21.2.1 Degenerative cardiac diseases: a major public health problem, 264
    - 21.2.2 Mesenchymal stromal cells: a promising tool to treat the effects of myocardial infarction, 265
    - 21.2.3 Mechanisms underlying the regenerative effects of mesenchymal stromal cells, 266
  - 21.3 Tunneling nanotubes: a universal route of intercellular communication between distant cells, 268
    - 21.3.1 Structural diversity of tunneling nanotubes, 269
    - 21.3.2 Mechanisms and factors involved in tunneling nanotube formation, 269
    - 21.3.3 The diversity of compounds transferred by tunneling nanotubes and their physiological relevance, 271
  - 21.4 Tunneling nanotubes: a novel cell-to-cell communication pathway improving the cardiac regenerative properties of mesenchymal stromal cells, 273
    - 21.4.1 Evidence of tunneling-nanotube-mediated communications between stromal cells and cardiomyocytes, 273
    - 21.4.2 Tunneling nanotube cell-to-cell communication with mesenchymal stromal cells rejuvenates distressed cardiomyocytes through a progenitor-like state, 275
    - 21.4.3 Tunneling nanotube cell-to-cell communication with distressed cardiomyocytes stimulates the paracrine repair function of mesenchymal stromal cells, 277
  - 21.5 Conclusions, 279

References, 280

- 2**  
**22** The preferential homing of mesenchymal stromal cells to sites of inflammation, 286  
*Catherine Sullivan*
  - 22.1 Introduction, 286
  - 22.2 Molecular mechanisms of migration, 287
    - 22.2.1 Chemokines, 287
    - 22.2.2 Integrins, 289
    - 22.2.3 Toll-like receptors, 289
    - 22.2.4 Matrix metalloproteinases, 290
    - 22.2.5 Growth factors, 291
  - 22.3 The inflammatory milieu, 291
    - 22.3.1 Passive migration, 291
    - 22.3.2 Hypoxia, 291
    - 22.3.3 Cytokines, 292
    - 22.3.4 Complement, 293
    - 22.3.5 Macrophages, 294
  - 22.4 Mesenchymal stromal cell extravasation, 294
  - 22.5 *In vivo* migration, 294
    - 22.5.1 *In vivo* migration studies, 294
    - 22.5.2 Controversies surrounding *in vivo* migration, 297
    - 22.5.3 Real-time *in vivo* imaging, 301
  - 22.6 Optimizing homing, 302
    - 22.6.1 Culture conditions, 302
    - 22.6.2 Pretreatment of mesenchymal stromal cells, 303
    - 22.6.3 Cell engineering, 303
    - 22.6.4 The host environment, 304
  - 22.7 Conclusions, 305

References, 306

- 2**  
**23** The role of chemokines in mesenchymal stromal cell homing to sites of inflammation, including infarcted myocardium, 314  
*Shan Wang and Yaojiong Wu*
  - 23.1 Summary, 314

- 23.2 Introduction, 314
- 23.3 Homing capacity of mesenchymal stromal cells, 315
- 23.4 Homing ability of mesenchymal stromal cells and their therapeutic effects, 316
- 23.5 Mechanisms of leukocyte trafficking to sites of inflammation, 316
- 23.6 Potential ligands/receptors for mesenchymal stromal cell homing, 317
- 23.7 Chemokine involvement in mesenchymal stromal cell homing, 317
  - 23.7.1 CCR1 and CCR2 involvement in mesenchymal stromal cell homing, 317
  - 23.7.2 The CXCR4–SDF-1 axis in mesenchymal stromal cell homing, 318
  - 23.7.3 Other chemokines, 319
- 23.8 Pretreatment of mesenchymal stromal cells with cytokines and growth factors, 319
- 23.9 Summary and future prospects, 319
- Acknowledgments, 319
- References, 320
- 2**  
**24 Live cell imaging and single cell tracking of mesenchymal stromal cells *in vitro*, 323**  
*James A. Cornwell, Maria Z. Gutierrez, Richard P. Harvey and Robert E. Nordon*
  - 24.1 Introduction, 323
  - 24.2 Technical considerations, 326
    - 24.2.1 Equipment, software, and hardware requirements, 326
    - 24.2.2 Image acquisition parameters, 327
    - 24.2.3 Image processing, 327
    - 24.2.4 Data storage, 328
  - 24.3 Single cell tracking and analysis, 329
    - 24.3.1 Cell tracking platforms, 329
    - 24.3.2 Recording live cell characteristics, 331
    - 24.3.3 Vital biomarkers for mesenchymal stromal cells, 332
    - 24.3.4 Mimicking *in vivo* microenvironments *in vitro*, 335
  - 24.4 Case study: tracking differentiation of endothelial cells from cardiac-derived mesenchymal stromal cells, 337
    - 24.4.1 Background and experimental aims, 337
    - 24.4.2 Methods, 337
    - 24.4.3 Results and discussion, 340
    - 24.4.4 Conclusion and future work, 342
  - 24.5 Future perspective on live cell imaging and single cell tracking, 342
  - References, 344
- 2**  
**25 The role of mesenchymal stem/stromal cells in angiogenesis, 347**  
*Annelies Bronckaers and Ivo Lambrichts*
  - 25.1 Introduction, 347
  - 25.2 The current concept of angiogenesis, 347
  - 25.3 Proangiogenic properties of mesenchymal stem/stromal cells, 350
    - 25.3.1 The mesenchymal stem/stromal cell secretome: a kaleidoscope of angiogenic molecules, 350
    - 25.3.2 The effect of mesenchymal stem/stromal cells on the behavior of endodermal cells *in vitro*, 352
    - 25.3.3 Mesenchymal stem/stromal cells induce angiogenesis *in vivo*, 354
  - 25.4 Mesenchymal stem/stromal cells as a therapeutic tool for diseases caused by insufficient angiogenesis, 355
    - 25.4.1 Peripheral ischemic arterial disease, 355
    - 25.4.2 Stroke, 355
    - 25.4.3 Myocardial infarction, 356
    - 25.4.4 Failure of surface wound healing, 357
    - 25.4.5 The dual role of mesenchymal stem/stromal cells in cancer biology, 357
  - 25.5 Enhancing the angiogenic efficacy of mesenchymal stem/stromal cells, 358
  - 25.6 Transdifferentiation of mesenchymal stem/stromal cells towards endothelial cells, 359
  - 25.7 Conclusions, therapeutic expectations, and challenges, 359
  - References, 361
- 2**  
**26 The relationship between mesenchymal stromal cells and endothelial cells, 366**  
*Seyed Mahdi Nassiri and Reza Rahbarghazi*
  - 26.1 Introduction, 366
  - 26.2 Transendothelial migration of mesenchymal stromal cells, 366
    - 26.2.1 Mesenchymal stromal cell adhesion to endothelial cells, 366
    - 26.2.2 Trans-endothelial migration, 369
  - 26.3 Mesenchymal stromal cell–endothelial cell crosstalk in angiogenesis, 370
    - 26.3.1 Juxtacrine interactions of mesenchymal stromal cells and endothelial cells, 370
    - 26.3.2 Paracrine interactions of mesenchymal stromal cells and endothelial cells, 372
  - 26.4 Mesenchymal stromal cell–endothelial cell crosstalk in tumor angiogenesis, 373
    - 26.4.1 Stimulation, 373
    - 26.4.2 Inhibition, 375
  - 26.5 Endothelial differentiation of mesenchymal stromal cells, 375
  - 26.6 Development of a biologically active niche through bidirectional endothelial cell–stromal cell crosstalk, 378
  - 26.7 Determination of stem cell fate through crosstalk with endothelial cells, 380

- 26.8 Beneficial effects of mesenchymal stromal cell–endothelial cell interactions in some tissue pathologies, 382
- References, 382
- 2**  
**27** The radioresistance of mesenchymal stromal cells and their potential role in the management of radiation injury, 391  
*Tara Sugrue, Irene Calvo-Asensio, and Rhodri Ceredig*
- 27.1 Mesenchymal stromal cells: modulators of hematopoiesis, 391
- 27.2 The response of mesenchymal stromal cells to ionizing radiation, 393
- 27.3 The DNA damage response, 394
- 27.3.1 Sensing damage: DNA damage response initiation, 396
- 27.3.2 Sending an SOS: DNA damage response signal transduction and amplification, 396
- 27.3.3 DNA damage checkpoints, 397
- 27.4 DNA double-strand break repair, 398
- 27.4.1 Nonhomologous end joining, 398
- 27.4.2 Homologous recombination, 400
- 27.4.3 DNA double-strand break repair pathway choice, 400
- 27.5 Apoptosis, 400
- 27.6 Cellular senescence, 401
- 27.7 Stem cells exhibit a mixed response to DNA damage, 401
- 27.8 The DNA damage response of mesenchymal stromal cells, 401
- 27.9 Effects of hypoxia on mesenchymal stromal cell radioresistance, 403
- 27.10 Clinical relevance of mesenchymal stromal cells in radiation injury: two sides to the coin, 405
- 27.10.1 Mesenchymal stromal cells and hematopoietic stem cell transplantation, 405
- 27.10.2 Mesenchymal stromal cells and the tumor microenvironment, 406
- References, 407
- 2**  
**28** The implications of multipotent mesenchymal stromal cells in tumor biology and therapy, 415  
*Pratika Y. Hernanda, Maikel P. Peppelenbosch, and Qiuwei Pan*
- 28.1 Introduction, 415
- 28.2 Origin and identification of mesenchymal stromal cells in the tumor microenvironment, 415
- 28.3 The migratory capacity of mesenchymal stromal cells, 416
- 28.3.1 Intrinsic migratory properties of mesenchymal stromal cells, 416
- 28.3.2 Stimuli produced by the tumor, 416
- 28.4 Context-dependent role of mesenchymal stromal cells in the tumor microenvironment, 417
- 28.4.1 Hypotheses on context-dependent roles of mesenchymal stromal cells in cancer, 417
- 28.4.2 The tumor-suppressing roles of mesenchymal stromal cells, 418
- 28.4.3 The tumor-promoting roles of mesenchymal stromal cells, 418
- 28.5 The potential immunomodulation by mesenchymal stromal cells in the tumor microenvironment, 419
- 28.5.1 Mesenchymal stromal cells inhibit natural killer cells and macrophages, 419
- 28.5.2 Mesenchymal stromal cells inhibit T cell proliferation, 420
- 28.5.3 Mesenchymal stromal cells promote the expansion and function of regulatory T cells, 420
- 28.5.4 Mesenchymal stromal cells inhibit the function of dendritic cells, 420
- 28.6 Therapeutic application of mesenchymal stromal cells in cancer, 420
- 28.6.1 Potential therapeutic application, 420
- 28.6.2 Reasons for caution, 420
- Acknowledgments, 421
- References, 421
- 2**  
**29** Mesenchymal stem/stromal cell therapy: mechanism of action and host response, 426  
*Aideen Ryan, Mary Murphy, and Frank Barry*
- 29.1 Mesenchymal stem/stromal cells, 426
- 29.2 Therapeutic application of mesenchymal stem/stromal cells, 427
- 29.3 Mechanism of action, 429
- 29.4 Host immune response to autologous mesenchymal stem/stromal cell transplantation, 430
- 29.5 Mesenchymal stromal cells in an inflammatory microenvironment, 430
- 29.6 Mesenchymal stem/stromal cells-mediated immunomodulation of the innate immune system, 432
- 29.7 Mesenchymal stem/stromal cells-mediated immune modulation of the adaptive immune system, 434
- 29.8 Host immune response to transplantation of allogeneic mesenchymal stem/stromal cells, 434
- 29.9 Summary, 435
- References, 436
- 2**  
**30** The differences between mesenchymal stromal cells and fibroblasts, 441  
*Luigi Balducci, Sharon Natasha Cox, and Giulio Alessandri*
- 30.1 Introduction, 441
- 30.2 Phenotypic similarities and differences between mesenchymal stromal cells and fibroblasts, 442
- 30.3 Cell surface membrane markers, 442



- 30.4 Gene expression profile of mesenchymal stromal cells and fibroblasts, 443
- 30.5 Differentiation potential of mesenchymal stromal cells and fibroblasts, 445
- 30.6 Immune modulation capability of mesenchymal stromal cells and fibroblasts, 446
- 30.7 Modulation of inflammation by mesenchymal stromal cells and fibroblasts, 448
- 30.8 Angiogenic properties of mesenchymal stromal cells and fibroblasts, 450
- 30.9 Conclusions, 451
- References, 451
- 2**  
**31** Derivation of mesenchymal stem/stromal cells from induced pluripotent stem cells, 456  
*Rebecca A. Pelekanos*
- 31.1 Introduction, 456
- 31.2 Mesenchymal stem/stromal cells as candidates for cellular therapy, 457
- 31.3 Mesenchymal stem/stromal cells, 457
- 31.4 Adult bone-marrow-derived mesenchymal stem/stromal cells, 457
- 31.5 Fetal tissue-derived mesenchymal stem/stromal cells, 457
- 31.6 Embryonic stem cells, 458
- 31.7 Embryonic stem-cell-derived mesenchymal stem/stromal cells, 458
- 31.8 Induced pluripotent stem cells, 458
- 31.9 Small-molecule methods for differentiating pluripotent stem cells into mesenchymal stem/stromal cells, 459
- 31.10 Derivation of induced pluripotent stem cell-mesenchymal stem/stromal cells through a novel transforming growth factor- $\beta$  inhibitor method, 459
- 31.11 Mesenchymal characterization of induced pluripotent stem cell-mesenchymal stem/stromal cells derived through the inhibitor method, 461
- 31.12 Immune tolerance to induced pluripotent stem cell-mesenchymal stem/stromal cells, 461
- 31.13 Kinetics of the proliferation of induced pluripotent stem cell-mesenchymal stem/stromal cells, 461
- 31.14 Tumorigenic potential of induced pluripotent stem cell-mesenchymal stem/stromal cells, 462
- 31.15 Critical parameters for future preclinical production of induced pluripotent stem cell-mesenchymal stem/stromal cells, 462
- 31.16 Plasticity of lineage commitment: reprogramming, deprogramming and dedifferentiation, 462
- 31.17 How to develop “young” mesenchymal stem/stromal cells: going backward to go forward?, 463
- 31.18 Small-molecules inhibitors for generating “young” stem cells, 463
- 31.19 Primitive stem cells and mesenchymal stem/stromal cell generation by physical factors, 463
- 31.20 Conclusions, 463
- 31.21 Future directions, 464
- Acknowledgments, 464
- References, 464
- 2**  
**32** The role of mesenchymal stem cells in hematopoiesis, 467  
*Jean-Pierre Levesque, Rebecca N. Jacobsen, and Ingrid G. Winkler*
- 32.1 Introduction, 467
- 32.2 Hematopoietic stem cells need a niche, 468
- 32.3 A mesenchymal hierarchy, 468
- 32.4 Identification of mesenchymal stem cells and their relationship with hematopoietic stem cells in the mouse, 469
- 32.5 More than one nestin<sup>+</sup> cell type and hematopoietic stem cell niche exist in the mouse bone marrow, 470
- 32.6 Controversies surrounding nestin<sup>+</sup> mesenchymal stem cells and other genetic models for alternative mesenchymal stem cells, 471
- 32.7 Other stromal cells regulate hematopoietic stem cells and additional tools to study their role in regulating hematopoiesis, 472
- 32.7.1 Osteoblastic lineage and osteoblasts, 473
- 32.7.2 Endothelial cells, 473
- 32.7.3 Megakaryocytes, Schwann cells, and the transforming growth factor- $\beta$  connection, 474
- 32.7.4 Adrenergic neurons, 475
- 32.7.5 Macrophages, 475
- 32.8 Human mesenchymal stem cells and human hematopoiesis, 476
- 32.9 Conclusions, 477
- References, 477
- 2**  
**33** The modulatory effects of mesenchymal stromal cells on the innate immune system, 481  
*Ko-Jiunn Liu, Men-Luh Yen, Li-Tzu Wang, and B. Linju Yen*
- 33.1 Introduction to the innate immune system, 481
- 33.2 Interactions with dendritic cells, 481
- 33.3 Interactions with monocytes, macrophages, and immature myeloid cells, 483
- 33.4 Interactions with natural killer lymphocytes, 484
- 33.5 Interactions with neutrophils, other granulocytes, and mast cells, 485
- 33.6 Interactions with complement, 485
- References, 486
- 2**  
**34** The modulatory effects of mesenchymal stromal cells on the adaptive immune system, 490  
*B. Linju Yen, Ko-Jiunn Liu, Men-Luh Yen, and Huey-Kang Sytwu*

- 34.1 Introduction to the adaptive immune system, 490
- 34.2 Interactions with T lymphocytes, 490
- 34.3 Interactions with B lymphocytes, 492
- References, 492

- 2** **35** The role of mesenchymal stromal cells in the repair of acute organ injury, 496  
*A.A. Temnov, A.V. Vagabov, A.N. Sklifas, V.I. Novoselov, and Y.A. Belyi*
  - 35.1 Effect of acute organ injury on the proliferative and functional activity of mesenchymal stromal cells, 496
    - 35.1.1 The effect of catecholamines on mesenchymal stromal cells, 496
    - 35.1.2 The impact of hypoxia as a factor of acute injury on mesenchymal stromal cell proliferation, 497
    - 35.1.3 Effect of hypoxia as a factor of acute injury on the paracrine function of mesenchymal stromal cells, 498
    - 35.1.4 Effect of tissue-specific proteins released after acute tissue injury on mesenchymal stromal cells, 501
  - 35.2 Paracrine effect of mesenchymal stromal cells in acute organ injury, 501
    - 35.2.1 Background, 501
    - 35.2.2 Paracrine factors secreted by mesenchymal stromal cells, 502
    - 35.2.3 Immunosuppressive and anti-inflammatory effects of mesenchymal stromal cells, 502
    - 35.2.4 The pro-angiogenic and tissue regenerative effects of mesenchymal stromal cells in acute organ injury, 505
    - 35.2.5 The antiapoptotic activity of mesenchymal stromal cells, 506
    - 35.2.6 Mesenchymal stromal-cell-derived microvesicles: an essential part of the paracrine mechanism, 507
  - 35.3 Mesenchymal-stromal cells in the treatment of acute ischemia-reperfusion injury, 508
    - 35.3.1 Ischemia-reperfusion injury pathogenesis, 509
    - 35.3.2 The use of mesenchymal stromal cells in kidney ischemia-reperfusion injury, 509
    - 35.3.3 Mesenchymal stromal cells and myocardial ischemia-perfusion injury, 510
    - 35.3.4 Mesenchymal stromal cells and ischemia-reperfusion injury of other organs, 511
    - 35.3.5 Conclusions, 511
  - 35.4 The use of mesenchymal stromal cells in acute lung and airway injury, 511
    - 35.4.1 Repair of the proximal regions of the airways after acute injury, 512
  - 35.5 Current approaches to controlled transplantation of mesenchymal stromal cells in acute organ injury, 515

- 35.6 Conclusions, 516
- Acknowledgment, 517
- References, 517

- 2** **36** The use of mesenchymal stromal cells in the treatment of diseases of the cornea, 524  
*Damien G. Harkin, Allison J. Sutherland, Laura J. Bray, Leanne Foy, Fiona J. Li, and Brendan G. Cronin*
  - 36.1 Introduction, 524
  - 36.2 Anatomy and physiology of the human cornea, 529
  - 36.3 Overview of corneal pathology, 530
    - 36.3.1 Ocular surface disease, 531
    - 36.3.2 Diseases of the corneal stroma and endothelium, 531
  - 36.4 Corneal transplantation and cultivated epithelial autografts, 532
  - 36.5 Evidence for mesenchymal stromal cells as modulators of corneal disease, 533
    - 36.5.1 Immunology of the cornea, 533
    - 36.5.2 Immunology of corneal transplantation, 534
    - 36.5.3 Immunomodulatory properties of mesenchymal stromal cells, 535
    - 36.5.4 Mesenchymal stromal cells as modulators of corneal wound healing and tissue regeneration, 535
    - 36.5.5 Mesenchymal stromal cells as modulators of corneal transplantation, 536
  - 36.6 Evidence for mesenchymal stromal cells as a source of new corneal cells, 537
    - 36.6.1 Mesenchymal stromal cell differentiation into corneal epithelium, 537
    - 36.6.2 Mesenchymal stromal cell differentiation into keratocytes, 538
    - 36.6.3 Mesenchymal stromal cell differentiation into corneal endothelium, 538
  - 36.7 The biology of cornea-derived mesenchymal stromal cells, 538
  - 36.8 Conclusions and future directions, 540
  - Acknowledgments, 540
  - References, 540

- 2** **37** The role of paracrine factors secreted by mesenchymal stromal cells in acute tissue injury, 544  
*Ying Wang, Tania Velletri, Chunxing Zheng, and Yufang Shi*
  - 37.1 Introduction, 544
  - 37.2 Cell replacement and cell empowerment, 544
  - 37.3 Paracrine factors produced by mesenchymal stromal cells, 545
    - 37.3.1 Growth factors and mesenchymal-stromal-cell-mediated tissue repair, 546

- 37.3.2 Soluble immunosuppressive factors and mesenchymal-stromal-cell-mediated tissue repair, 546
- 37.3.3 Inducible nitric oxide synthase/indoleamine 2,3-dioxygenase, 547
- 37.3.4 Prostaglandin E<sub>2</sub>, 548
- 37.3.5 Tumor-necrosis-factor-inducible gene 6 protein, 548
- 37.3.6 Chemokine (C–C motif) ligand 2, 548
- 37.3.7 Interleukin-6, 548
- 37.3.8 Interleukin-10, 548
- 37.3.9 Transforming growth factor- $\beta$ , 549
- 37.3.10 Human leukocyte antigen G, 549
- 37.3.11 Galectins, 549
- 37.3.12 Other soluble immunosuppressive factors secreted by MSCs, 549
- 37.4 Conclusions, 549
- Acknowledgments, 549
- References, 549

## **2** **38** Treatment of lung disease by mesenchymal stromal cell extracellular vesicles, 553

*Antoine Monsel, Ying-gang Zhu, Varun Gudapati, and Jae-Woo Lee*

- 38.1 Introduction, 553
- 38.2 Definitions and characterization of extracellular vesicles, 554
- 38.3 Nomenclature defined by size and morphology, 555
- 38.4 Common methods of collection of extracellular vesicles, 556
  - 38.4.1 Ultracentrifugation, 556
  - 38.4.2 Size exclusion, 556
  - 38.4.3 Immunoaffinity isolation, 556
  - 38.4.4 Polymeric precipitation, 556
- 38.5 Quantification of extracellular vesicles, 556
  - 38.5.1 Optical single-particle tracking: nanoparticle tracking analyses, 556
  - 38.5.2 Flow cytometry, 557
  - 38.5.3 Electron microscopy, 557
  - 38.5.4 Protein concentration, 557
  - 38.5.5 Cell count, 557
- 38.6 Interaction of extracellular vesicles with targeted cells, 557
- 38.7 Endogenous extracellular vesicles in lung disease, 558
  - 38.7.1 Endogenous extracellular vesicles in acute respiratory distress syndrome, 558
  - 38.7.2 Endogenous extracellular vesicles in chronic obstructive pulmonary disease, 560
  - 38.7.3 Endogenous extracellular vesicles in asthma, 561
  - 38.7.4 Endogenous extracellular vesicles as biomarkers in lung disease, 561

- 38.7.5 Endogenous extracellular vesicles as potential therapeutic targets for lung diseases, 562
- 38.8 Therapeutic properties of extracellular vesicles derived from mesenchymal stromal cells, 562
  - 38.8.1 Mesenchymal stromal cell vesicles for kidney injury, 562
  - 38.8.2 Mesenchymal stromal cell vesicles for cardiac injury, 564
  - 38.8.3 Mesenchymal stromal cell vesicles for liver injury, 565
  - 38.8.4 Mesenchymal stromal cell vesicles for neural injury, 565
  - 38.8.5 Mesenchymal stromal cell vesicles for lung diseases, 565
- 38.9 Remaining questions on the therapeutic use of mesenchymal stromal cell extracellular vesicles, 566
  - 38.9.1 Isolation and quantification techniques, 566
  - 38.9.2 Extracellular vesicle characterization, 566
  - 38.9.3 Feasibility of large-scale generation of extracellular vesicles, 566
- 38.10 Regulatory considerations for the clinical use of extracellular vesicles, 566
- 38.11 Conclusions, 566
- References, 567

## **2** **39** Evaluating mesenchymal stem/stromal cells for treatment of asthma and allergic rhinitis, 573

*Tatyana Gavrilova, Saritha Kartan, Lauren S. Sherman, Oleta A. Sandiford, and Pranela Rameshwar*

- 39.1 Summary, 573
- 39.2 Introduction, 573
- 39.3 Early and late asthma response, 573
- 39.4 Airway remodeling, 574
- 39.5 Innate immunity of the airway, 574
- 39.6 Adaptive immunity of the respiratory tract, 575
- 39.7 Toll-like receptors, 575
- 39.8 Allergic rhinitis and immunology, 575
- 39.9 Immune modulation by mesenchymal stem/stromal cells, 577
- 39.10 The future of mesenchymal stem/stromal cells as therapy for allergic diseases, 578
- References, 578

## **2** **40** Stem cell therapies for Huntington's disease, 581

*A.T. Crane, J. Rossignol, and G. L. Dunbar*

- 40.1 Introduction, 581
- 40.2 Huntington's disease, 581
  - 40.2.1 Prevalence and symptomology, 582
  - 40.2.2 Neuronal pathology, 582
  - 40.2.3 Mechanisms of neurodegeneration, 583
- 40.3 Animal models, 584
  - 40.3.1 Transgenic models, 584
- 40.4 *In vitro* models, 584

- 40.5 Experimental therapies, 585
- 40.6 Cell transplantation, 585
  - 40.6.1 Mesenchymal stem/stromal cells, 585
  - 40.6.2 Genetic engineering of mesenchymal stem/stromal cells, 587
  - 40.6.3 Embryonic and fetal stem cells, 588
  - 40.6.4 Neural stem cells, 589
  - 40.6.5 Induced pluripotent stem cells, 590
  - 40.6.6 Co-transplantation paradigm, 593
- 40.7 Conclusions, 593
- References, 594

## 2 Section IV: The role of bioengineering in the therapeutic applications of mesenchymal stromal cells

- 41 Endometrial mesenchymal stromal cell and tissue engineering for pelvic organ prolapse repair, 601  
*Shanti Gurung, Jerome A. Werkmeister, and Caroline E. Gargett*
  - 41.1 Introduction, 601
  - 41.2 Pelvic floor disorders, 601
  - 41.3 Pelvic organ prolapse, 602
    - 41.3.1 Surgical treatment for pelvic organ prolapse, 602
    - 41.3.2 New meshes for treatment of pelvic organ prolapse, 603
  - 41.4 Tissue engineering, 603
    - 41.4.1 Candidate cells for tissue engineering applications for pelvic organ disorders, 603
  - 41.5 Endometrium is highly regenerative and contains stem/stromal cells, 606
    - 41.5.1 Human endometrial mesenchymal stem/stromal cells, 606
  - 41.6 Culture expansion of endometrial mesenchymal stem/stromal cells toward current good manufacturing practice conditions, 608
  - 41.7 Tissue engineering for pelvic organ prolapse repair, 609
    - 41.7.1 A large animal preclinical model for pelvic organ prolapse, 611
  - 41.8 Conclusions, 612
  - Acknowledgments, 612
  - References, 612

- 2 42 Closed automated large-scale bioreactors for manufacturing mesenchymal stromal cells for clinical use, 616  
*Kerry Atkinson, Nicholas Timmins, G. Kiel, Celena Heazlewood, Michael Doran, and Gary Brooke*
  - 42.1 Introduction, 616
  - 42.2 Design of a semi-automated closed-system bioreactor capable of manufacturing mesenchymal stromal cells for clinical use, 616

- 2 42.3 A commercially available closed-system bioreactor for manufacturing mesenchymal stromal cells for clinical use, 617
- References, 618

## 2 Section V: GMP manufacturing of mesenchymal stromal cells for clinical use

- 43 Current good manufacturing practice for the isolation and *ex vivo* expansion of mesenchymal stromal cells derived from term human placenta for use in clinical trials, 621  
*Kerry Atkinson, Dahlia Khalil, Celena Heazlewood, and Nina Ilic*
  - 43.1 Source of mesenchymal stromal cells for use in clinical trials, 621
  - 43.2 Inclusion criteria for mothers wishing to donate their term placenta for isolation and expansion of mesenchymal stromal cells for use in clinical trials approved by a human research ethics committee, 622
  - 43.3 Exclusion criteria for mothers wishing to donate their term placenta for isolation and expansion of mesenchymal stromal cells for use in clinical trials approved by a human research ethics committee, 622
  - 43.4 Mesenchymal stromal cell manufacturing, 623
    - 43.4.1 The good manufacturing process facility, 623
    - 43.4.2 Quality control and quality assurance, 623
    - 43.4.3 Isolating and expanding mesenchymal stromal cells from human term placenta, 623
    - 43.4.4 Testing performed on mesenchymal stromal cells manufactured for clinical use, 623
  - 43.5 Phase 1 trials using placenta-derived mesenchymal stromal cells, 625
  - References, 627

- 2 44 A comparison of high-tier regulatory documents pertaining to biologic drugs including mesenchymal stromal cells in Australia, Europe, and the USA using a manual documentary analysis, 628  
*Nina Ilic*
  - 44.1 Introduction, 628
  - 44.2 Background, 628
  - 44.3 Definitions used by the Australian Therapeutic Goods Administration, the European Medicine Agency, and the US Food and Drug Administration for “biologicals”, 629
  - 44.4 Complexity of the area, 632
  - 44.5 Analysis of documents, 633



- 44.6 Regulatory science, 639
- 44.7 Interpretation of the analysis of the documents, 640
- 44.8 Conclusions, 641
- References, 641

## **Section VI: The therapeutic application of mesenchymal stromal cells**

### **45 The use of mesenchymal stromal cells in acute and chronic heart disease, 647**

*Ariel Wolf, Wayne Balkan, and Joshua Hare*

- 45.1 Introduction, 647
- 45.2 The biology of acute and chronic ischemic cardiomyopathy, 647
- 45.3 Characterization of mesenchymal stromal/stem cells, 648
  - 45.3.1 Immunomodulatory properties, 649
  - 45.3.2 Antifibrotic effects, 650
  - 45.3.3 Cardiomyogenesis *in vitro* and *in vivo*, 650
  - 45.3.4 Neovascularization, 651
  - 45.3.5 Paracrine effects, 652
  - 45.3.6 Exosomes, 652
  - 45.3.7 Mitochondrial transfer, 652
  - 45.3.8 Preconditioning, 652
  - 45.3.9 Genetic modification, 653
- 45.4 Cell combination therapy, 653
- 45.5 Clinical trials utilizing bone-marrow-derived mesenchymal stromal/stem cells, 654
  - 45.5.1 Acute myocardial infarction, 654
  - 45.5.2 Chronic myocardial infarction, 655
- 45.6 Clinical trials utilizing adipose-derived mesenchymal stromal/stem cells, 656
- 45.7 Preconditioning in the clinical setting, 657
- 45.8 Conclusions, 657
- References, 657

### **46 The role of mesenchymal stem/stromal cells in the management of critical limb ischemia, 661**

*P.K. Gupta, Chullikana Anoop, Balasubramanian Sudha, R Mathiazhagan, Raj Swathi Sundar, and Majumdar Anish Sen*

- 46.1 Introduction, 661
- 46.2 Mesenchymal stem/stromal cells and angiogenesis, 663
- 46.3 Potency assays for cells to be used in critical limb ischemia, 664
  - 46.3.1 Ixmylocel-T, 664
  - 46.3.2 Stempeucel<sup>®</sup>, 665
- 46.4 Preclinical studies, 665
  - 46.4.1 Preclinical safety studies, 665
  - 46.4.2 Preclinical efficacy studies, 667
- 46.5 Clinical trials in critical limb ischemia, 667

- 46.5.1 Safety of mesenchymal stromal cells in clinical trials, 667
- 46.5.2 Efficacy of mesenchymal stromal cells in clinical trials of critical limb ischemia, 668
- 46.5.3 Clinical trials in India, 671
- 46.5.4 Stempeutics research experience in critical limb ischemia, 671
- 46.5.5 Phase I/II study in patients with critical limb ischemia, 671
- 46.5.6 Phase II study in patients with Buerger's disease, 673

46.6 Conclusions, 673

References, 674

### **47 The role of mesenchymal stromal cells in the management of musculoskeletal disorders, 677**

*Stefan Zwingenberger, Ishaq Ojodu, Maik Stiehler, and Stuart B. Goodman*

- 47.1 Summary, 677
- 47.2 Introduction, 677
- 47.3 Stem cells for bone regeneration, 679
  - 47.3.1 Bone defects, 679
  - 47.3.2 Osteonecrosis, 680
  - 47.3.3 Wear-particle-related osteolysis, 681
  - 47.3.4 Systemic bone diseases, 681
- 47.4 Stem cells for cartilage regeneration, 682
  - 47.4.1 Osteoarthritis, 683
- 47.5 Stem cells for tendon regeneration, 683
- 47.6 Stem cells for skeletal muscle regeneration, 684
- 47.7 Stem cells for wound repair, 685
- 47.8 Conclusions, 685
- References, 685

### **48 The potential role of bone marrow mesenchymal stromal cells in the treatment of ischemic stroke, 690**

*Yujun Pan and Ruohan Sun*

- 48.1 Introduction, 690
  - 48.1.1 Stroke, 690
  - 48.1.2 Stem cells, 691
  - 48.1.3 Mesenchymal stromal cells, 691
- 48.2 Transplantation route and mechanisms of migration, 693
- 48.3 Tracking techniques for transplanted mesenchymal stromal cells, 698
- 48.4 Cytokines and neurotrophic factors, 699
- 48.5 Angiogenesis, 699
- 48.6 Neurogenesis, 700
- 48.7 Axonal sprouting and remyelination, 701
- 48.8 Antiapoptotic effects, 701
- 48.9 Immunomodulation, 702
- 48.10 Pretreatment of mesenchymal stromal cells prior to their administration in animal models of ischemic stroke, 702

- 2**  
**52** **The biology and potential clinical applications of mesenchymal stromal cells in diseases of the lung, 770**  
*Yuben P. Moodley, Jesse D. Armitage, and Dino B.A. Tan*
- 52.1 Introduction to lung disease, 770
    - 52.1.1 The global burden of lung disease, 770
    - 52.1.2 The pathogenesis of lung diseases, 770
    - 52.1.3 The range of lung diseases, 771
  - 52.2 What are stem cells?, 771
  - 52.3 What are mesenchymal stromal cells?, 771
  - 52.4 Lung-resident mesenchymal stromal cells, 772
  - 52.5 Tracking mesenchymal stromal cells in the body, 772
  - 52.6 The properties of mesenchymal stromal cells that favor repair, 772
    - 52.6.1 Avoidance of immune recognition, 772
    - 52.6.2 Mechanisms of mesenchymal stromal-cell-mediated immunomodulation, 772
    - 52.6.3 Mesenchymal stromal-cell-mediated repair via trophic factors, 775
  - 52.7 Mesenchymal stromal cells as delivery agents for drugs, 775
    - 52.7.1 Viral transduction, 775
    - 52.7.2 Genetic modulation, 776
    - 52.7.3 Nanoparticle incorporation, 776
    - 52.7.4 Surface modification, 776
    - 52.7.5 Preconditioned mesenchymal stromal cells, 776
  - 52.8 Preclinical and clinical studies of mesenchymal stromal cells in pulmonary diseases, 776
    - 52.8.1 Idiopathic pulmonary fibrosis, 776
    - 52.8.2 Chronic obstructive pulmonary disease, 779
    - 52.8.3 Acute lung injury and acute respiratory distress syndrome, 780
  - 52.9 Challenges in mesenchymal stromal cell administration in lung diseases, 781
    - 52.9.1 Optimal dosage of mesenchymal stromal cells, 781
    - 52.9.2 Timing of mesenchymal stromal cell administration, 782
  - 52.10 Summary and conclusions, 782
  - References, 782
- 2**  
**53** **The role of mesenchymal stromal cells in diseases of the lung, 787**  
*Kerry Atkinson*
- 53.1 Introduction, 787
  - 53.2 Pulmonary fibrosis, 787
    - 53.2.1 Animal models, 788
    - 53.2.2 Clinical trials of mesenchymal stromal cells, 788
  - 53.3 Asthma, 788
    - 53.3.1 Preclinical models, 790
    - 53.3.2 Clinical trials of mesenchymal stromal cells, 790
  - 53.4 Obliterative bronchiolitis, 790
    - 53.4.1 Preclinical animal models, 790
    - 53.4.2 Clinical trials of mesenchymal stromal cells, 791
  - 53.5 Chronic obstructive pulmonary disease and emphysema, 791
    - 53.5.1 Preclinical animal models, 792
    - 53.5.2 Clinical trials with mesenchymal stromal cells, 792
  - 53.6 Bronchopulmonary dysplasia, 792
    - 53.6.1 Preclinical animal models, 792
    - 53.6.2 Clinical trials using mesenchymal stromal cells, 792
  - 53.7 Acute respiratory distress syndrome and acute lung injury, 792
    - 53.7.1 Preclinical animal models, 793
    - 53.7.2 Clinical trials with mesenchymal stromal cells, 793
  - 53.8 Conclusions, 793
  - References, 793
- 54** **Mesenchymal stromal cells for the treatment of autoimmune diseases, 794**  
*Christopher N. Lewis and Jacques Galipeau*
- 54.1 Cell biology of endogenous mesenchymal stromal cells, 794
    - 54.1.1 Mesenchymal stromal cells coordinate hematopoietic stem cell development, 795
    - 54.1.2 Mesenchymal stromal cells and central tolerance in the bone marrow, 795
  - 54.2 Cell biology of mesenchymal stromal cells in culture, 796
    - 54.2.1 Mesenchymal stromal cells and B cell immunosuppression, 797
    - 54.2.2 Mesenchymal stromal cell and T cell co-culture assays, 797
  - 54.3 Immunosuppression: lessons from oncology, 797
    - 54.3.1 Programmed death ligand 1 and immunosuppression by tumors, 798
    - 54.3.2 Programmed death ligand 1 and immunosuppression by mesenchymal stromal cells, 798
    - 54.3.3 Indoleamine 2,3-dioxygenase and immunosuppression by tumors, 799
    - 54.3.4 Indoleamine 2,3-dioxygenase and immunosuppression by mesenchymal stromal cells, 799
  - 54.4 Mesenchymal stromal cell response to inflammatory signals: licensing and integration, 800
    - 54.4.1 Mesenchymal stromal cells and complement, 800
    - 54.4.2 Mesenchymal stromal cells and toll-like receptors, 801
    - 54.4.3 Interferon- $\gamma$  in the immune response, 802

- 54.4.4 Mesenchymal stromal cells and interferon- $\gamma$ , 803
- 54.4.5 Tumor necrosis factor- $\alpha$  in the immune response, 803
- 54.4.6 Synergy of interferon- $\gamma$  and tumor necrosis factor- $\alpha$  in mesenchymal stromal cells, 804
- 54.5 Strength of signal and integration, 804
- 54.6 Clinical applications of mesenchymal stromal cells for immune-mediated diseases, 806
  - 54.6.1 How *in vitro* data inform assessment of clinical efficacy, 806
  - 54.6.2 Random donor, industrial scale, 806
  - 54.6.3 Allogeneic mesenchymal stromal cells, low passage, 807
  - 54.6.4 Autologous mesenchymal stromal cells for autoimmune diseases, 808
- 54.7 Conclusions and next steps, 809
- References, 809
- 2**  
**55** The role of mesenchymal stromal cells in bacterial infection, 814  
*Sailaja Ghanta, Konstantin Tsoyi, and Mark A. Perrella*
  - 55.1 Introduction, 814
  - 55.2 Experimental models of bacterial infection and sepsis, 815
  - 55.3 Effects of mesenchymal stromal cells on the innate immune response, 816
  - 55.4 Effects of mesenchymal stromal cells on the adaptive immune response, 819
  - 55.5 Antimicrobial activity of mesenchymal stromal cells, 819
  - 55.6 Mesenchymal stromal cells and endothelial/epithelial dysfunction, 819
  - 55.7 Mesenchymal stromal cells and effect on organ injury in infection, 819
  - 55.8 Mesenchymal stromal cell cytokine and growth factor production, 820
  - 55.9 Toll-like receptors and mesenchymal stromal cells, 821
  - 55.10 Mesenchymal stromal cell homing, 821
  - 55.11 Mesenchymal stromal cell response to oxidative stress, 821
  - 55.12 Paracrine effects of mesenchymal stromal cells, 821
  - 55.13 Transcriptomic analysis of mesenchymal stromal cell therapy in sepsis, 822
  - 55.14 Summary, 822
  - References, 822
- 2**  
**56** The use of mesenchymal stromal cells in solid organ transplantation, 825  
*Céline Gregoire, Alexandra Briquet, François Jouret, Chantal Lechanteur, Etienne Baudoux, Olivier Giet, Olivier Delloye, Frédéric Baron, Olivier Detry, and Yves Beguin*
  - 56.1 Introduction, 825
  - 56.2 Potential effects of mesenchymal stromal cells in solid organ transplantation, 825
  - 56.3 Immunomodulation, 826
  - 56.4 Tissue and organ regeneration, 826
  - 56.5 Prevention of ischemia–reperfusion injury, 826
  - 56.6 Mesenchymal stromal cell administration in solid organ transplantation, 826
    - 56.6.1 Kidney transplantation, 826
    - 56.6.2 Liver transplantation, 829
    - 56.6.3 Heart transplantation, 831
    - 56.6.4 Lung transplantation, 831
    - 56.6.5 Pancreas and islet transplantation, 831
    - 56.6.6 Bowel transplantation, 832
  - 56.7 Conclusions, 832
  - References, 832
- 2**  
**57** The role of mesenchymal stromal cells in allogeneic hematopoietic stem cell transplantation, 836  
*Kerry Atkinson*
  - 57.1 The immunobiology of allogeneic hematopoietic stem cell transplantation, 836
    - 57.1.1 Graft rejection and late marrow failure, 836
    - 57.1.2 Graft-versus-host disease, 836
    - 57.1.3 The graft-versus-leukemia effect, 837
    - 57.1.4 The recipient's response to infection, 837
  - 57.2 The immunobiology of mesenchymal stromal cells, 837
  - 57.3 The role of mesenchymal stromal cells in the expansion of hematopoietic stem cells, 837
  - 57.4 The role of mesenchymal stromal cells in marrow graft rejection, 837
  - 57.5 The role of mesenchymal stromal cells in the prevention of acute graft-versus-host disease, 838
  - 57.6 The role of mesenchymal cells in the treatment of corticosteroid-refractory acute graft-versus-host disease, 838
  - 57.7 The mesenchymal stromal cell exosome: a substitute for the mesenchymal stromal cell?, 839
  - References, 839
- 2**  
**58** The role of mesenchymal stromal cells in the management of skin wounds, 841  
*Sung-Whan Kim*
  - 58.1 Introduction, 841
  - 58.2 The wound healing process, 841
  - 58.3 The role of mesenchymal stromal cells in the wound healing process, 842
    - 58.3.1 Immune modulation, 842
    - 58.3.2 Antimicrobial activity, 842
    - 58.3.3 Chemotactic and migratory activities, 842
    - 58.3.4 Paracrine activity, 842
    - 58.3.5 Differentiation, 842
  - 58.4 Conclusions and the future, 842
  - References, 843



## 2 59 The role of mesenchymal stromal cells in skin wound healing, 845

*Miao Teng and Hengshu Zhang*

- 59.1 Summary, 845
- 59.2 Introduction, 845
- 59.3 The role of bone-marrow-derived mesenchymal stromal cells in wound healing, 845
- 59.4 The role of adipose-tissue-derived mesenchymal stromal cells in wound healing, 849
- 59.5 The role of mesenchymal stromal cells from placental tissues in wound healing, 851
- 59.6 The role of mesenchymal stromal cells from dermal tissue in wound healing, 852
- 59.7 The role of mesenchymal stromal cells from blood in wound healing, 853
- 59.8 Questions and challenges regarding mesenchymal stromal cell administration in wound healing, 853
- 59.9 Conclusions, 853
- References, 853

## 2 Section VII: Mesenchymal stromal cells as delivery vehicles for therapeutic agents

### 60 The role of mesenchymal stromal cells in human brain tumors, 859

*Brittany C. Parker Kerrigan, Tal Shahar, Shinji Yamashita, and Frederick F. Lang*

- 60.1 Introduction, 859
- 60.2 Mesenchymal stromal cells in the therapy of human gliomas, 860
- 60.3 Cellular therapy for gliomas, 860
- 60.4 The advantages of mesenchymal stromal cells in clinical use, 861
- 60.5 The rationale for using mesenchymal stromal cells in glioma therapy, 861
- 60.6 Mechanisms underlying mesenchymal stromal cell tropism for gliomas, 863
- 60.7 Strategies to enhance mesenchymal stromal cell homing to gliomas, 864
- 60.8 Types of therapeutic cargo, 864
  - 60.8.1 Secreted proteins, 864
  - 60.8.2 Prodrug enzymes, 865
  - 60.8.3 Replication-competent oncolytic viruses, 865
  - 60.8.4 Antibodies, 866
  - 60.8.5 Nanoparticles, 866
- 60.9 Delivery routes of mesenchymal stromal cells in clinical applications, 866
- 60.10 Mesenchymal stem cells in the biology of gliomas, 867

- 60.11 Controversy over tumor-associated mesenchymal stromal cells in solid tumors and gliomas, 867
- 60.12 A model of mesenchymal stromal cells in glioma biology, 868
- 60.13 Prospects for clinical use of bone-marrow-derived mesenchymal stromal cells in glioma therapy, 868
- References, 869

### 2 61 Mesenchymal stromal cells as gene delivery vehicles to treat nonmalignant diseases, 873

*Julie R. Beegle, Jan A. Nolte, and Fernando A. Fierro*

- 61.1 Introduction, 873
- 61.2 What are mesenchymal stromal cells?, 873
- 61.3 Genetic modification of mesenchymal stromal cells, 874
  - 61.3.1 Safety concerns, 874
  - 61.3.2 Choice of vector system, 874
- 61.4 Preclinical models of gene-modified mesenchymal stromal cells: mesenchymal stromal cell migration and survival, 875
- 61.5 Gene-modified mesenchymal stromal cells as immune modulators, 877
- 61.6 Gene-modified mesenchymal stromal cells in skeletal disorders, 878
- 61.7 Gene-modified mesenchymal stromal cells in cardiovascular disease, 880
- 61.8 Gene-modified mesenchymal stromal cells in kidney disease, 881
- 61.9 Gene-modified mesenchymal stromal cells in neurological disease, 881
- 61.10 Gene-modified mesenchymal stromal cells in other nonmalignant diseases, 883
  - 61.10.1 Hemophilia, 883
  - 61.10.2 Metachromatic leukodystrophy, 883
  - 61.10.3 Mucopolysaccharidosis type VII, 883
  - 61.10.4 Diabetes mellitus, 883
- 61.11 Conclusions and future directions, 883
- References, 885

### 2 62 Gene therapy for cancer using mesenchymal stromal cells, 892

*Ryosuke Uchibori and Keiya Ozawa*

- 62.1 Introduction, 892
  - 62.1.1 Biological characteristics of mesenchymal stromal cells, 892
  - 62.1.2 Immunomodulatory effects of mesenchymal stromal cells on immune cells, 893
  - 62.1.3 Tumor homing of mesenchymal stromal cells, 893
- 62.2 Applications of genetically engineered mesenchymal stromal cells for cancer therapy, 893
  - 62.2.1 Interferons, 893
  - 62.2.2 Interleukins, 894

- 62.2.3 Chemokines, 894
- 62.2.4 Suicide genes, 894
- 62.2.5 Other approaches, 894
- 62.3 Molecular mechanisms of mesenchymal stromal cell accumulation at tumor sites, 894
  - 62.3.1 Migratory factors, 895
  - 62.3.2 Interactions between mesenchymal stromal cells and endothelial cells, 895
- 62.4 Considerations in the use of genetically engineered mesenchymal stromal cells in cancer therapy, 895
- 62.5 Summary and conclusions, 896
- References, 896

## 2 Section VIII: The present and the future

### 63 Breaking news, 901

*Kerry Atkinson*

- 63.1 *In vitro* laboratory studies, 901
- 63.2 Preclinical *in vivo* animal studies, 903
- 63.3 Clinical trials, 908
- 63.4 Regulatory approval for marketing mesenchymal stromal cell products, 909
- References, 909

### 2 64 Reconciling the stem cell and paracrine paradigms of mesenchymal stem cell function, 912

*Siddaraju V. Boregowda and Donald G. Phinney*

- 64.1 Summary, 912
- 64.2 Introduction, 912
- 64.3 The stem cell paradigm revisited, 913
- 64.4 The paracrine paradigm, 914
  - 64.4.1 “Mesenchymal stem cell pharmacology”: cells are not drug-like, 915
  - 64.4.2 Priming to enhance mesenchymal stem cell paracrine action also impacts cell growth and survival, 916
  - 64.4.3 Licensing of immunomodulatory activity biases cell differentiation, 916
- 64.5 Modeling mesenchymal stem cell function using lessons learned from immunology, 916
- 64.6 A stem-cell-centric view of mesenchymal stem cells, 919
- 64.7 Closing remarks, 920
- References, 920

Glossary, 927

Index, 949

## CHAPTER 28

# The implications of multipotent mesenchymal stromal cells in tumor biology and therapy

Pratika Y. Hernanda,<sup>a</sup> Maikel P. Peppelenbosch,<sup>b</sup> and Qiuwei Pan<sup>b,\*</sup>

<sup>a</sup> Medical Genetics Laboratory, Centre of Biomolecular Research, Wijaya Kusuma University Surabaya, Indonesia

<sup>b</sup> Department of Gastroenterology and Hepatology, Erasmus MC Cancer Institute, Erasmus University Medical Center, Rotterdam, The Netherlands

### Chapter menu

28.1 Introduction, 415	28.5 The potential immunomodulation by mesenchymal stromal cells in the tumor microenvironment, 419
28.2 Origin and identification of mesenchymal stromal cells in the tumor microenvironment, 415	28.5.1 Mesenchymal stromal cells inhibit natural killer cells and macrophages, 419
28.3 The migratory capacity of mesenchymal stromal cells, 416	28.5.2 Mesenchymal stromal cells inhibit T cell proliferation, 420
28.3.1 Intrinsic migratory properties of mesenchymal stromal cells, 416	28.5.3 Mesenchymal stromal cells promote the expansion and function of regulatory T cells, 420
28.3.2 Stimuli produced by the tumor, 416	28.5.4 Mesenchymal stromal cells inhibit the function of dendritic cells, 420
28.4 Context-dependent role of mesenchymal stromal cells in the tumor microenvironment, 417	28.6 Therapeutic application of mesenchymal stromal cells in cancer, 420
28.4.1 Hypotheses on context-dependent roles of mesenchymal stromal cells in cancer, 417	28.6.1 Potential therapeutic application, 420
28.4.2 The tumor-suppressing roles of mesenchymal stromal cells, 418	28.6.2 Reasons for caution, 420
28.4.3 The tumor-promoting roles of mesenchymal stromal cells, 418	Acknowledgments, 421
	References, 421

## 28.1 Introduction

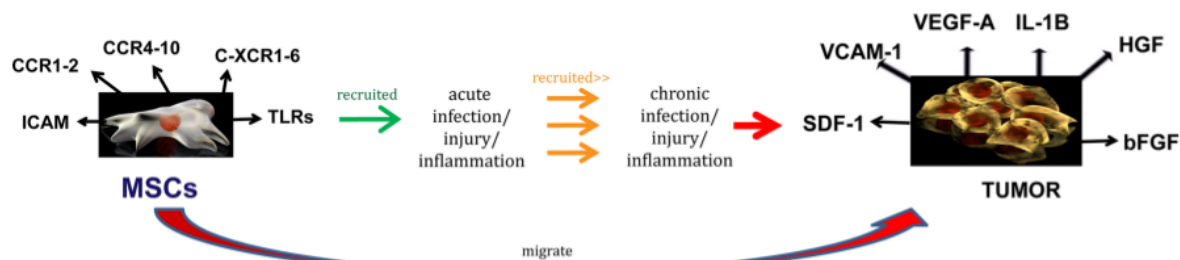
Multipotent mesenchymal stromal cells (MSCs) are able to differentiate *in vitro* into adipocytes, osteoblasts and chondrocytes [1] and into bone *in vivo*. MSCs have now been isolated from multiple organs and they form part of the endothelial wall of blood vessels [2,3]. In addition, they are a key component of tumor stroma and have been found to play important roles in various cancers [4]. Tumors, having many of the characteristics of injured tissue, may attract MSCs from their local tissue or from the circulation. The tumor tropism property of MSCs has suggested the possibility of their use as vehicles to deliver anticancer drugs or genes. However, extensive experimental research has demonstrated both anti- and procancer roles of MSCs in a context-

dependent manner. It is now generally assumed that within this microenvironment reciprocal tumor–stroma crosstalk influences the phenotype of tumor cells, their progression and their metastasis [5]. Various studies have reported the tumor-promoting effects of MSCs [6–8]. Others have provided evidence for an anti-oncogenic role of these cells [9–12]. MSCs are emerging as vehicles for anticancer drug/gene delivery [7]. This chapter aims to dissect this observed discrepancy in different experimental settings of human cancer so as to provide possible guidance to the appropriateness of clinical applications.

## 28.2 Origin and identification of mesenchymal stromal cells in the tumor microenvironment

MSCs were initially identified by placing whole bone marrow cells in plastic culture dishes and observing the subsequent

\* Corresponding author: Department of Gastroenterology and Hepatology, Erasmus MC, room Na-617, 'sGravendijkwal 230, NL-3015 CE Rotterdam, The Netherlands. E-mail: [q.pan@erasmusmc.nl](mailto:q.pan@erasmusmc.nl)



**Figure 28.1** Mechanisms for the recruitment of MSCs into tumors. MSCs express chemokine receptors, intercellular adhesion molecule (ICAM), C-C motif chemokine receptor (CCR)1–2, CCR4–10, C–X–C motif chemokine receptor (CXCR)1–6, and toll-like receptors (TLRs). Tumors release various cytokines, chemokines, and growth factors, including basic fibroblast growth factor (bFGF), stromal cell-derived factor 1 (SDF-1), hepatocyte growth factor (HGF), interleukin (IL)-1 $\beta$ , vascular cell adhesion molecule 1 (VCAM-1), and vascular endothelial growth factor (VEGF)-A, acting as chemoattractants for MSCs. MSCs in the local organ or tissue or circulating MSCs may be recruited into the tumor. MSCs may be constantly recruited in different stages from chronic infection, injury, and inflammation to cancer development.

expansion of a rare population of plastic-adherent cells [13,14]. The spatial distribution and properties of MSCs within their organ/tissue *in vivo* are relatively unclear [15]. MSCs have been isolated from most organs, including kidney, umbilical cord, brain, liver, lung, and bone marrow [16–18], and may have unique properties depending on their source. Recruited by the inflammatory milieu, MSCs migrate to the microenvironment of tumors and orchestrate the hallmarks of cancer cells [19]. The enrichment of MSCs in the tumor environment has been reported in both primary human cancers [20,21] and in metastases [22–24]. A resident population of MSCs has also been identified in the human adult liver and express a unique gene signature [25]. Additionally, MSCs share a molecular signature with mesenchymal tumor cells and favor early tumor growth in mice [26].

In response to injury or infection, MSCs can be released from the bone marrow and migrate toward sites of injury to promote tissue regeneration [27]. High frequencies of MSCs have been found in tumors with extensive inflammation, suggesting the recruitment of MSCs in response to inflammation [20]. Additionally, high circulating levels of endothelial progenitor cells have been observed in many cancer patients, which might also home to the site of tumors and promote tumor growth [28–30]. MSCs may be recruited locally and/or from the circulation to tumor sites (Figure 28.1). Future studies using somatic genomic signatures may provide a definite answer.

### 28.3 The migratory capacity of mesenchymal stromal cells

MSCs tend to be recruited by injured tissue, where they are thought to contribute to tissue repair and wound healing [31]. As tumors are often considered to have many characteristics of injured tissues, it is not surprising to find MSCs enriched within tumors. Various preclinical models have confirmed that MSCs

can migrate to certain types of tumors, and this is one of the rationales of using MSCs as vehicles for anticancer drug/gene delivery [7,32]. MSCs are also relatively resistant to ischemia, because in the absence of oxygen they can survive by anaerobic adenosine triphosphate production [33], which may give them a competitive advantage in a tumor microenvironment. The tumor-tropic migratory property of MSCs is attributed to two main mechanisms [34,35].

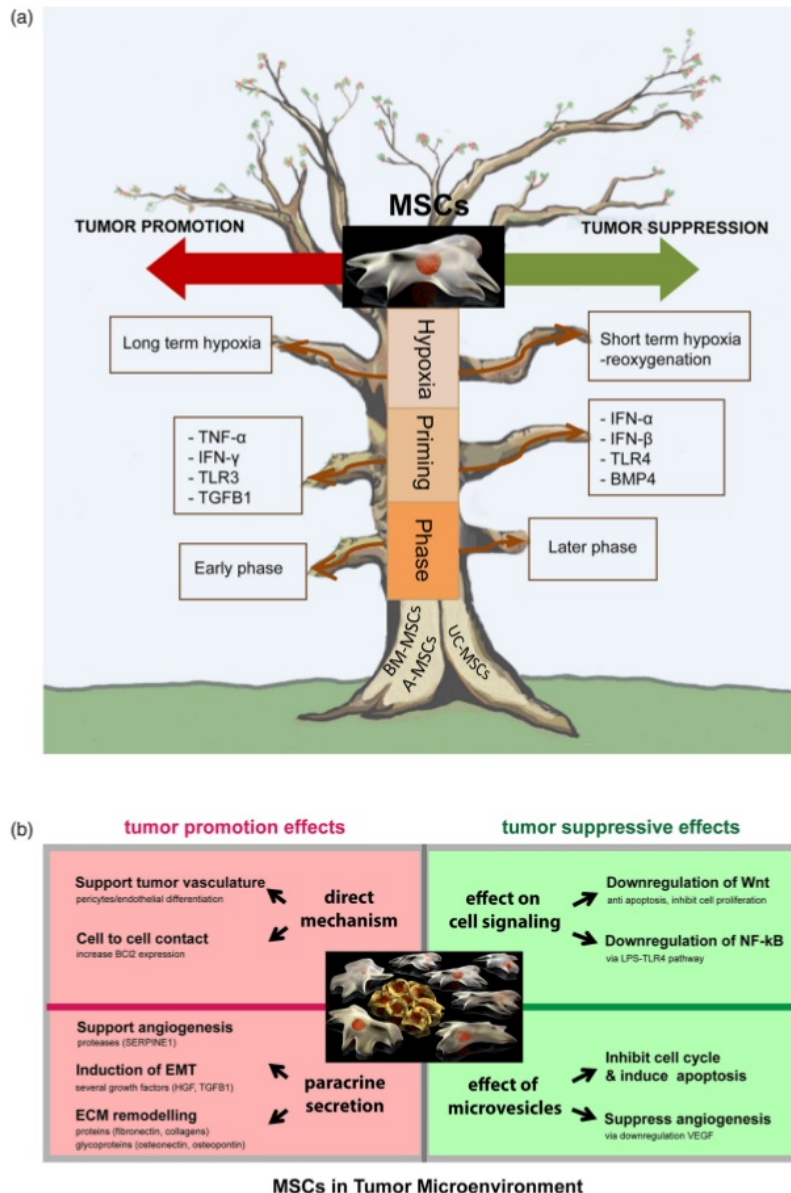
#### 28.3.1 Intrinsic migratory properties of mesenchymal stromal cells

Activated human MSCs express adhesion molecules such as integrins, ICAM-1, ICAM-2, and VCAM-1, which enable these cells to migrate [36]. MSCs also express chemokine receptors, including CCR1, CCR2, CCR4, CCR6, CCR7, CCR8, CCR9, CCR10, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, and CX3CR [31]. Production of their respective ligands is a characteristic of inflamed tissue and malignant tissue, and thus these receptors are likely involved in the specific accumulation of MSCs in both processes. Thus, the cognate ligands of these receptors are efficient chemotactic stimuli for MSCs. Additional receptors implicated in MSC migration are the TLRs. TLR1–6 have been identified in human MSCs and it has been reported that TLR stimulation enhanced the migratory function of MSCs [37].

#### 28.3.2 Stimuli produced by the tumor

Malignant cells have been shown to produce relatively high amounts of MSC chemoattractants, including HGF, SDF-1 (also known as CXCL12), bFGF, VEGF-A, and VCAM-1 [38,39]. Tumor-derived IL-1 $\beta$  has been found to be a mediator of the proinflammatory response in MSCs exposed to tumor conditioned media, a mechanism that is regulated by focal adhesion kinase and mitogen-activated protein kinase signaling [40].





**Figure 28.2** Context-dependent role of MSCs in the tumor microenvironment. MSCs can exert tumor-promoting or tumor-limiting effects, which depend on the context in which the MSCs are present. (a) Different sources of MSCs, the presence of ligands, cytokine-primed MSCs, and the hypoxic microenvironment may lead to different effects of MSCs in the tumor microenvironment. The properties of the MSCs and the particular tumor microenvironment may result in dual roles for MSCs that can either suppress or promote tumor progression. (b) The tumor-suppressive mechanisms are mainly mediated by MSC microvesicles and downregulation of the Wnt and nuclear factor kappa light chain-enhancer of activated B cells (NF-κB) pathway. The tumor-promoting mechanisms are mainly attributed to factors secreted by MSCs and direct effects such as support of tumor vasculature, epithelial to mesenchymal transition (EMT) transition, and extracellular matrix (ECM) remodeling. BM-MSCs: bone-marrow-derived MSCs; A-MSCs: adipose-derived MSCs; UC-MSCs: umbilical-cord-derived MSCs; BMP4: bone morphogenetic protein 4; IFN: interferon; LPS: lipopolysaccharide; TNF-α: tumor necrosis factor-α.

## 28.4 Context-dependent role of mesenchymal stromal cells in the tumor microenvironment

The tumor microenvironment is substantially different from that of normal organs and this is a mechanism in cancer development [41]. Depending on the experimental conditions, MSCs can exert tumor-promoting [20,24,42,43] or tumor-limiting effects [9,11,12,44,45]. Various hypotheses have been postulated

to explain the context-dependent role of MSCs in cancer (Figure 28.2a).

### 28.4.1 Hypotheses on context-dependent roles of mesenchymal stromal cells in cancer

#### 28.4.1.1 Phase-dependent hypothesis

MSCs appear to promote tumor growth when co-injected with tumor cells, but inhibit tumor progression when administered into established tumors [4]. Thus, the presence of MSCs during

the early phase of tumorigenesis may contribute to angiogenesis that is required for tumor initiation. An increase in blood vessel density was observed when MSCs were co-injected with tumor cell lines [8,46].

#### <sup>1</sup> 28.4.1.2 Priming-dependent hypothesis

MSCs express several TLRs, and their ability to migrate, invade, and secrete immune modulating factors is tightly regulated by specific TLR-agonist engagement [47]. TLR4-primed MSCs are polarized into a proinflammatory MSC type 1 (MSC1) phenotype, whereas TLR3-primed MSCs are polarized into the classical immunosuppressive MSC2 phenotype [47]. MSC1-based treatment of established tumors in an immune competent model attenuated tumor growth and metastasis, but MSC2-treated animals displayed increased tumor growth and metastasis [48]. The priming of all MSC types with inflammatory cytokines such as IFN- $\gamma$  and TNF- $\alpha$  results in higher levels of VEGF [19] and induction of inhibition of runt-related transcription factor 2, one of the pivotal factors driving osteoblast differentiation [49]. These effects, in turn, can enhance tumor progression. However, stimulation of MSCs with IFN- $\alpha$  and IFN- $\beta$  decreased tumor cell proliferation and induced tumor cell apoptosis in a mouse model of melanoma [50,51]. BMP4-differentiated bone marrow MSCs became less suppressive of T cell and natural killer (NK) cell proliferation and switched on their suppressive machinery by activating both indolamine 2,3-dioxygenase (IDO) and cyclooxygenase 2 (COX-2), promoting the differentiation of neighboring MSCs and triggering the anti-inflammatory effect [52]. In contrast, preconditioning of MSCs with TGF- $\beta$ 1 resulted in proinvasive MSCs in the progression of colon cancer [53].

#### 28.4.1.3 Hypoxic-dependent hypothesis

A long-term hypoxic microenvironment may lead to undifferentiated tumor cells and stromal cells, providing essential cellular interactions accompanied by the upregulation of the stemness genes [54,55]. Permanent hypoxia-stimulated MSCs proliferated and reduced their capacity to differentiate [56,57]. However, short-term oxygen limitation increased the number of apoptotic MSCs after 3–24 h of hypoxic treatment [58,59]. Moreover, oxygen limitation in hypoxia-reoxygenation-induced cell apoptosis was mediated in part by the reduction of phosphorylation of Akt and extracellular signal-regulated kinase 1/2 (ERK1/2) in MSCs [60]. ERK1/2 belongs to the class of protein kinase signal transduction pathways that are used to relay numerous extracellular signals within cells and have been reported to be involved in various cellular functions, including apoptosis and proliferation [61]. Hypoxia switches on various signaling pathways, and its context dependency determines the overall cell response and alterations in MSC functions.

Collectively, tumor cells and the tumor microenvironment will affect the ultimate function of recruited MSCs. However, there are some factors that drive MSCs to suppress or promote tumor growth (Figure 28.2b).

### 28.4.2 The tumor-suppressing roles of mesenchymal stromal cells

Extensive studies have reported tumor-suppressing effects of MSCs in various experimental cancer models. A variety of processes and mechanisms possibly implicated in MSC-dependent tumor suppression have been studied.

#### 28.4.2.1 Effect on cell signaling

Several signaling pathways have been reported to be associated with MSC suppression of tumor growth. Wnt signaling is aberrantly activated in many types of tumors. In chemically induced murine liver tumors the administration of MSCs has been demonstrated to have tumor suppressive effects associated with Wnt signaling. Its target genes were downregulated, especially those related to antiapoptosis, mitogenesis, cell proliferation, and cell cycle regulation [62]. MSCs can secrete Wnt inhibitors, such as Dickkopf-1 [52,63]. MSC-dependent inhibition of NF- $\kappa$ B signaling in cancer cells also occurs [64]. In addition, TLR signals can stimulate downstream effectors that may interfere with the LPS-TLR4 pathway and inhibit NF- $\kappa$ B activation during liver fibrosis [65].

#### 28.4.2.2 Effects of mesenchymal stromal cell microvesicles

Microvesicles are fragments of plasma membrane ranging from 100 to 1000 nm secreted by many cell types. They play an important role in intercellular communication and are capable of modifying the activity of target cells through surface receptor interactions and the transfer of proteins, mRNA, and microRNA (miRNA). Microvesicles have been implicated in tumor-stroma interactions [66]. Microvesicles released by MSCs have been associated with tumor inhibition in several preclinical studies [67] and have been shown to inhibit cell cycling and induce apoptosis or necrosis *in vitro* and to inhibit growth of established tumors *in vivo* [66,68,69], providing a further antioncogenic effect. Exosomes, a smaller type of intracellular vesicle derived from MSCs, suppress angiogenesis by downregulating VEGF expression in breast cancer cells [70], and MSCs pulsed with tumor-derived microvesicles exert an enhanced antitumor activity against hepatocellular cancer [71]. Thus, the secretome of MSCs appears to play an important role in their tumor suppressive function.

### 28.4.3 The tumor-promoting roles of mesenchymal stromal cells

The tumor-promoting role of MSCs has been attributed to direct mechanisms and paracrine secretion, including modulation of the immune response.

#### 28.4.3.1 Direct mechanisms

MSCs have been shown to directly differentiate into pericytes or possibly endothelial cells [72], thus supporting tumor angiogenesis, which in turn can promote tumor growth. MSC and

tumor cell contact is another important direct mechanism. In lymphoma models, direct cell–cell contact was the major mechanism of promoting tumor cell proliferation and survival rather than secretion of soluble factors by MSCs [73]. It is well recognized that adhesion to the bone marrow of the malignant cells of the B cell neoplasm, multiple myeloma, provides the myeloma cells with protection against chemotherapy. Bortezomib is a proteasome inhibitor used in the treatment of multiple myeloma and mantle cell lymphoma. MSCs can suppress bortezomib-induced multiple myeloma cell growth inhibition in a cell–cell contact-dependent manner by increasing Bcl2 expression in the myeloma cells [74]. Cell–cell contact with MSCs was reported to protect chronic lymphocytic leukemia cells, another B cell neoplasm, from spontaneous and drug-induced apoptosis [75]. Bone-marrow-derived MSCs have been reported to fuse with non-small cell lung cancer cells, resulting in highly malignant subpopulations with stem-cell-like properties [76].

#### 28.4.3.2 Paracrine mechanisms

MSC promotion of tumor growth via paracrine mechanisms is mainly attributed to supporting angiogenesis, promoting tumor growth, and metastasis. Secreted factors from patient tumor-derived MSCs have been shown to promote tumor growth in a xenograft mouse model associated with upregulation of cell growth and proliferation-related processes and downregulation of cell-death-related pathways in tumor cells [20]. Several growth factors secreted by MSCs, including HGF and TGF- $\beta$ 1, induce proinvasive signals in cancer [77,78]. A large number of proteases that have proangiogenic properties secreted by MSCs may inhibit apoptosis in vascular smooth muscle cells and endothelial cells [79]. The protease named serpin 1, a member of the serine proteinase inhibitor superfamily, is abundantly secreted by MSCs and has been shown to regulate proliferation, migration, and apoptosis of vascular smooth muscle cells and endothelial cells [80]. Transplantation of MSCs promoted microvascular growth in a mouse model [46,81], suggesting that angiogenesis plays an important role in support of tumor growth by MSCs.

A tumor-promoting effect attributed to remodeling of ECM via a paracrine secretion of MSCs also occurs. The ECM is a major component of the cellular microenvironment and is composed of diverse proteins such as collagens, elastins, fibronectin, proteoglycans, and glycoproteins [82]. Glycoproteins, such as osteonectin (also known as secreted protein acidic and rich in cysteine, SPARC), are highly expressed in stromal fibroblast cells that have been reported to promote tumor progression in several cancers [83–86]. Abundantly produced soluble fibronectin [83,87] by MSCs also plays an active role in the invasive process of human colon and liver cancer [88]. Matrix metalloproteinase proteases and hyaluronan, as well as various other factors secreted by MSCs, are capable of remodeling the ECM and facilitating tumor progression [20,89–91].

In contrast, human MSC-secreted microvesicles have been reported to have a striking antitumor effect in cancer [92] and

tumor immune suppression [93], in which they transport mRNA, miRNA, and proteins between cells.

A functional role in neoplastic development and metastases has been attributed to the presence of miRNAs, small non-coding RNA molecules composed of approximately 22 nucleotides. They participate in RNA silencing and gene regulation [94]. In breast cancer, miR-21 and miR-205 were associated with tumor development, while miR-126 and miR-335 were related to metastases [37,95]. The promotion of progression of hepatocellular cancer by MSCs was attributed to miR-155 [89].

The effect of MSCs on tumor cells has also been reported to be associated with the induction of EMT [96], an effect that is further enhanced by the inflammatory milieu that characterizes many cancers. Initiation of metastasis requires invasion, and this is enabled by EMT: carcinoma cells in the primary tumor lose cell–cell adhesion mediated by repression of E-cadherin and break through the basement membrane with increased invasive properties and enter the bloodstream by intravasation. Later, when these circulating tumor cells exit the bloodstream to form micrometastases, they undergo the reverse process – mesenchymal to epithelial transition. Evidence of a role for MSCs in EMT is the observation of increased expression of cancer-associated fibroblast and EMT markers in a co-culture model of hepatoma cells and MSCs [97]. There is also evidence that there are intricate links between EMT-type cells and drug resistance in tumors [98]. MSC-dependent EMT induction has been associated with shorter tumor-free survival and poorer overall survival, demonstrating the clinical relevance of this effect [96,99]. MSCs might also promote tumor progression or invasion via inducing regulation of secretion of IL-6 and secretion of SDF-1 $\alpha$  in EMT [53,100].

## 28.5 The potential immunomodulation by mesenchymal stromal cells in the tumor microenvironment

MSCs can influence both the innate and adaptive immune systems, including the function of antigen-presenting cells [79,101], natural killer cells [102], B cells, and T cells [103,104] (and see Chapters 33 and 34). Immune suppressive cells accumulate in some tumors, which can impede immune surveillance and facilitate tumor growth [105]. The number and function of anti-tumor immune cells are decreased [54,106].

### 28.5.1 Mesenchymal stromal cells inhibit natural killer cells and macrophages

MSCs can modulate the function of NK cells [107] and macrophages [108]. NK cells are a type of lymphocyte that plays a role in the rejection of both tumors and virally infected cells. MSCs can inhibit the proliferation, cytotoxicity, and cytokine production of NK cells through secretion of IDO and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) [107,109]. In addition to IDO and PGE<sub>2</sub>, cancer-



associated stromal cells produce other soluble factors, such as VEGF and platelet-derived growth factor, which enhance tumor progression by promoting or attracting M2 macrophages, which are characterized by their production of high levels of the suppressive cytokine IL-10 and low levels of the proinflammatory cytokines IFN- $\gamma$  and TNF- $\alpha$  [110]. M2 macrophages activate T helper 2 cell activity and also promote angiogenesis, tissue remodeling, and repair. Proinflammatory stimulation by IFN- $\gamma$ , TNF- $\alpha$ , or LPS increases the expression of COX-2 and IDO in MSCs and these enzymes promote further M2 macrophage polarization [108]. Macrophages also play an important role in the innate immune response to virus infections. Innate immune responses, including TLRs, are important for viral clearance [111].

### 28.5.2 Mesenchymal stromal cells inhibit T cell proliferation

T cells are the major player of the adaptive immune response and are important in controlling malignant disease, mediating both cytotoxicity of cancer cells and releasing anti-oncogenic cytokines [112,113]. MSCs can inhibit T cell function through multiple pathways [114,115]. MSC suppression of T cell responses can be mediated by cell contact and soluble factors, including TGF- $\beta$ , HGF, PGE<sub>2</sub>, soluble human leukocyte antigen (HLA)-G5, IDO, and inducible nitric oxide synthetase [112,113,116–118]. Inhibition of T cell function by MSCs affects T cell proliferation and IFN- $\gamma$  production and induces the production of IL-4, resulting in a shift from a proinflammatory T cells to anti-inflammatory T cells [114,115]. Cell–cell contact T cell inhibition by MSCs can be mediated by surface expression of HLA-G [119], a nonclassical major histocompatibility complex class I molecule with tolerogenic functions that contribute to fetal graft tolerance and human allograft acceptance [120]. Fas ligand and programmed death-ligand 1 also play significant roles in immunomodulation mediated by MSCs [121].

### 28.5.3 Mesenchymal stromal cells promote the expansion and function of regulatory T cells

Regulatory T cells (Tregs) are a subset of T cells that suppress activation of the immune system to maintain homeostasis and tolerance to self-antigens. An increased number of highly activated Tregs were found to infiltrate the tumor milieu of liver tumors in which they were mainly localized in the stromal compartment of the tumors [106]. The frequency of Tregs has been associated with poor prognosis [122–124]. MSCs can induce the generation and expansion of Tregs by the secretion of TGF- $\beta$  [125], IDO [126] and the release of soluble HLA-G5 [113]. In contrast to their suppression of cytotoxic T cells, MSCs can induce the generation and expansion of Tregs [127]. Additionally, MSCs have been reported to induce the production of IL-10 by plasmacytoid dendritic cells (DCs), which in turn triggered the generation of Tregs [114].

### 28.5.4 Mesenchymal stromal cells inhibit the function of dendritic cells

It has been found that MSCs also display immunosuppressive potential through inhibiting the differentiation of DCs. DCs are the most efficient cells in presenting antigen to T cells. They play a key role in the initiation of the primary immune responses and in tolerance, depending on their activation and maturation status [128]. MSCs are capable of modulating the differentiation, activation, and function of DCs [129]. MSCs reduce the production of several cytokines by DCs, including IL-12 and TNF- $\alpha$  [130]. MSCs isolated from different tissue sources present distinct immunomodulatory profiles [131], so it will be important to more closely study MSCs present in tumors.

## 28.6 Therapeutic application of mesenchymal stromal cells in cancer

### 28.6.1 Potential therapeutic application

Several studies have demonstrated that MSCs have the capacity to reverse acute and chronic injury in different experimental settings [132–135]. MSCs have been reported to attenuate inflammation [136–138] and ameliorate autoimmune diseases [10,139–141]. The fact that MSCs can migrate into certain types of tumors has led to their use as vehicles for tumor-specific delivery of anticancer drugs or genes.

Genetically modified MSCs have been used to deliver anticancer genes and inhibit cancer cell proliferation *in vitro* and *in vivo* [95,142–144]; and see Chapter 62. Several studies have demonstrated that MSCs have anticancer effects in different experimental settings [62,96,145–147]. MSCs have been extensively investigated in clinical trials as potential therapy for a number of different diseases [148,149]. Approximately 18 trials have been registered at [ClinicalTrials.gov](http://ClinicalTrials.gov) involving the use of MSCs in various cancers. These include ovarian cancer (NCT02068794), prostate cancer (NCT01983709), and hematologic malignancy (NCT01854567).

### 28.6.2 Reasons for caution

Given the context-dependent role of MSCs, it appears possible that MSCs in the tumor microenvironment could promote tumor growth [150]. MSCs may facilitate malignant development in patients at high risk of developing cancer, such as those with chronic hepatitis B or C patients or recipients of organ transplants [19,151,152]. In addition, MSCs have the potential for malignant transformation during *ex vivo* expansion [153].

Furthermore, although MSCs can be detected by magnetic resonance imaging or radioactive labeling [154] for up to 25 days [25], the cellular fate and distribution *in vivo* of transplanted MSCs remain unclear because techniques for tracking infused MSCs have low sensitivity [155]. Because of unclear clinical benefits of MSCs in patients with cancer [156–158] and because of the role of MSCs in the tumor microenvironment, it would be

wise to be cautious in the use of MSCs in patients with malignant diseases.

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