Stem Cell Therapeutics for Cancer

KHALID SHAH

WILEY Blackwell
Stem Cell Therapeutics for Cancer
Stem Cell Therapeutics for Cancer

Editor
Khalid Shah
Massachusetts General Hospital
Harvard Medical School
Boston, Massachusetts, USA
# Contents

*Contributors*  
*Preface*  

## Section 1 Introduction

1. Stem Cell Sources and Their Potential for Cancer Therapeutics  
   *Khalid Shah*

## Section 2 Migration and Fate of Stem Cells

2. The Role of CXCR4 as a Mediator of Glioma-Tropic Neural Precursor Cell Migration  
   *Moneeb Ehtesham, Elliot Min, and Rebecca Kasl*

3. Tumor Tropism of Mesenchymal Stem Cells  
   *Paula Y. P. Lam and Ivy A. W. Ho*

## Section 3 Stem Cell Therapy in Brain Cancer

4. Stem Cell-Mediated TRAIL Therapy for Highly Aggressive Brain Tumors  
   *Khalid Shah*

5. Stem Cell-Mediated Prodrug Gene Therapy of High-Grade Brain Tumors  
   *Cestmir Altaner*

6. Role of Naïve Cord Blood Stem Cells in Glioma Therapy  
   *Venkata Ramesh Dasari, Kiran Kumar Velpula, and Jasti S. Rao*

7. Stem Cell-Based Antiangiogenic Therapies for Brain Tumors  
   *Navid Redjal and Khalid Shah*

8. Treatment of Metastatic Neuroblastoma with Mesenchymal Stem Cell-Based Oncolytic Virotherapy  
   *Manuel Ramírez and Javier García-Castro*

## Section 4 Stem Cell Therapy in Other Cancer Types

9. Umbilical Cord Matrix Stem Cells for Cytotherapy of Breast Cancer  
   *Naomi Ohta, Atsushi Kawabata, Deepthi Uppalapati, Susumu Ishiguro, Deryl Troyer, and Masaaki Tamura*

10. Mesenchymal Stromal Cells as Effective Tumor Antigen-Presenting Cells in Cancer Therapeutics  
    *Raphaëlle Romieu-Mourez and Jacques Galipeau*
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Diagnostic and Therapeutic Mesenchymal Stem Cells for Breast Cancer Treatment</td>
<td>145</td>
</tr>
<tr>
<td></td>
<td>Róisín Dwyer</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Genetically Engineered Stem Cell Therapies Targeting Gastrointestinal Malignancy</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>Emily Keung, Peter J. Nelson, and Claudius Conrad</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Mesenchymal Stem Cells in Prostate Cancer: Clinical Opportunities</td>
<td>171</td>
</tr>
<tr>
<td></td>
<td>Diptiman Chanda and Selvarangan Ponnazhagan</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Primed Mesenchymal Stromal Cells for Cancer Therapy</td>
<td>187</td>
</tr>
<tr>
<td></td>
<td>Augusto Pessina, Arianna Bonomi, Eugenio Parati, Roberto Pallini, and Giulio Alessandri</td>
<td></td>
</tr>
<tr>
<td>Section 5</td>
<td>Combinatorial Stem Cell Therapies</td>
<td>203</td>
</tr>
<tr>
<td>15</td>
<td>MicroRNA Adjuvants in Stem Cell-Based Cancer Therapy</td>
<td>205</td>
</tr>
<tr>
<td></td>
<td>Maarten C. J. Anderegg and Maarten F. Corsten</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Stem Cell-Based Combination Therapies for Cancer: Systemic Delivery of a PI3K/mTOR Inhibitor (PI-103) and Stem Cell-Mediated Delivery of TRAIL in Brain Tumors</td>
<td>219</td>
</tr>
<tr>
<td></td>
<td>Tugba Bagci-Onder</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>The Efficacy of Clinically Approved Agents with Stem Cell-Delivered Therapeutics for Cancer Therapy</td>
<td>235</td>
</tr>
<tr>
<td></td>
<td>Hiroaki Wakimoto and Khalid Shah</td>
<td></td>
</tr>
<tr>
<td>Section 6</td>
<td>Tracking Stem Cells and Stem Cell-Based Therapeutics</td>
<td>245</td>
</tr>
<tr>
<td>18</td>
<td>Imaging Migration and Fate of Stem Cells in Experimental Models of Cancer</td>
<td>247</td>
</tr>
<tr>
<td></td>
<td>Deepak Bhere and Khalid Shah</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Multifunctional Molecules for Interrogating Stem Cell-Based Therapeutics</td>
<td>257</td>
</tr>
<tr>
<td></td>
<td>Shawn Hingtgen</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Tracking Cancer-Targeted MSC with PET Imaging</td>
<td>273</td>
</tr>
<tr>
<td></td>
<td>Véronique Roelants and Jean-Louis Vanoverschelde</td>
<td></td>
</tr>
</tbody>
</table>

Index

289

Color plate is located between pages 244 and 245.
Contributors

Giulio Alessandri
Department of Cerebrovascular Diseases
Fondazione IRCCS Neurological Institute Carlo Besta
Milan, Italy

Cestmir Altaner
Cancer Research Institute
Slovak Academy of Sciences
Bratislava, Slovakia
and
Cell Transplantation Centre
St. Elisabeth Oncological Institute
Bratislava, Slovakia

Maarten C.J. Anderegg
Academic Medical Center
Department of Surgery
Amsterdam, The Netherlands

Tugba Bagci-Onder
Koç University
School of Medicine
Istanbul, Turkey

Deepak Bhere
Department of Radiology
Massachusetts General Hospital
Harvard Medical School
Boston, Massachusetts, USA

Arianna Bonomi
Department of Biomedical, Surgical, and Dental Sciences
University of Milan
Milan, Italy

Diptiman Chanda
Department of Pathology
The University of Alabama at Birmingham
Birmingham, Alabama, USA

Claudius Conrad
Department of Surgery/ Division of Surgical Oncology
Affiliated Faculty Harvard Stem Cell Institute
Massachusetts General Hospital
and Brigham and Women's Hospital
Harvard Medical School
Boston, Massachusetts, USA

Maarten F. Corsten
Meander Medical Center
Department of Internal Medicine
Amersfoort, The Netherlands

Venkata Ramesh Dasari
Department of Cancer Biology and Pharmacology
University of Illinois College of Medicine
at Peoria
Peoria, Illinois, USA

Róisín Dwyer
Discipline of Surgery
School of Medicine
National University of Ireland Galway
Galway, Ireland

Moneeb Ehtesham
Department of Neurological Surgery
Vanderbilt University Medical Center
Nashville, Tennessee, USA
Jacques Galipeau  
Department of Hematology/Oncology and Pediatrics  
Winship Cancer Institute of Emory University  
Atlanta, Georgia, USA

Javier García-Castro  
Instituto de Salud Carlos III  
Majadahonda, Spain

Shawn Hingtgen  
UNC Eshelman School of Pharmacy  
The University of North Carolina at Chapel Hill  
Chapel Hill, North Carolina, USA

Ivy A. W. Ho  
Humphrey Oei Institute of Cancer Research  
National Cancer Centre of Singapore  
Singapore

Susumu Ishiguro  
Department of Anatomy and Physiology  
Kansas State University  
Manhattan, Kansas, USA

Rebecca Kasl  
Department of Neurological Surgery  
Vanderbilt University Medical Center  
Nashville, Tennessee, USA

Atsushi Kawabata  
Department of Anatomy and Physiology  
Kansas State University  
Manhattan, Kansas, USA

Emily Keung  
Department of Surgery  
Brigham and Women’s Hospital  
Harvard Medical School  
Boston, Massachusetts, USA

Paula Y. P. Lam  
Humphrey Oei Institute of Cancer Research  
National Cancer Centre of Singapore  
Singapore

Elliot Min  
Department of Neurological Surgery  
Vanderbilt University Medical Center  
Nashville, Tennessee, USA

Peter J. Nelson  
Medizische Klinik und Poliklinik IV  
Munich, Germany

Naomi Ohta  
Department of Anatomy and Physiology  
Kansas State University  
Manhattan, Kansas, USA

Roberto Pallini  
Institute of Neurosurgery  
Catholic University School of Medicine  
Rome, Italy

Eugenio Parati  
Department of Cerebrovascular Diseases  
Fondazione IRCCS Neurological Institute Carlo Besta  
Milan, Italy

Augusto Pessina  
Department of Biomedical, Surgical, and Dental Sciences  
University of Milan  
Milan, Italy

Selvarangan Ponnazhagan  
Department of Pathology  
The University of Alabama at Birmingham  
Birmingham, Alabama, USA

Manuel Ramírez  
Pediatric Hematology and Oncology  
Hospital Universitario Niño Jesús  
Madrid, Spain

Jasti S. Rao  
Departments of Cancer Biology and Pharmacology and Neurosurgery  
University of Illinois College of Medicine at Peoria  
Peoria, Illinois, USA
Navid Redjal  
Department of Radiology and Neurosurgery  
Massachusetts General Hospital  
Harvard Medical School  
Boston, Massachusetts, USA

Véronique Roelants  
Université catholique de Louvain  
Institut de Recherche Expérimentale et Clinique  
Pôle de Recherche Cardiovasculaire et Pôle d’Imagerie Moléculaire  
Radiothérapie et Oncologie  
Brussels, Belgium

Raphaëlle Romieu-Mourez  
The Montreal Center for Experimental Therapeutics in Cancer  
Jewish General Hospital  
McGill University  
Montreal, Quebec, Canada

Khalid Shah  
Department of Radiology and Neurology  
Massachusetts General Hospital  
Harvard Medical School  
Boston, Massachusetts, USA

Masaaki Tamura  
Department of Anatomy and Physiology  
Kansas State University  
Manhattan, Kansas, USA

Deryl Troyer  
Department of Anatomy and Physiology  
Kansas State University  
Manhattan, Kansas, USA

Deepthi Uppalapati  
Department of Anatomy and Physiology  
Kansas State University  
Manhattan, Kansas, USA

Jean-Louis Vanoverschelde  
Université catholique de Louvain  
Institut de Recherche Expérimentale et Clinique  
Pôle de Recherche Cardiovasculaire et Pôle d’Imagerie Moléculaire  
Radiothérapie et Oncologie  
Brussels, Belgium

Kiran Kumar Velpula  
Department of Cancer Biology and Pharmacology  
University of Illinois College of Medicine at Peoria  
Peoria, Illinois, USA

Hiroaki Wakimoto  
Departments of Radiology and Neurosurgery  
Massachusetts General Hospital  
Harvard Medical School  
Boston, Massachusetts, USA
Although they are a relatively new approach of therapeutics, stem cell-based therapies offer a huge potential in the practice of medicine. With the thorough understanding of stem cell biology and the advent of targeted therapeutics for cancer, stem cell-based therapeutic strategies are being explored in the treatment of various cancer types. This volume is focused on the application of stem cells in various cancers with emphasis on a number of aspects that are critical to the success of future stem cell-based therapies for cancer. The sections in this volume have been submitted by a range of experts working at the leading edge of the field, including oncologists, neurosurgeons, physicians, and research scientists. They cover a formidable array of topics in a concise way and offer differing scientific perspectives on specific aspects of stem cell-based cancer treatment.

The overarching theme of this text is not only to convey the facts, but also to spread a sense of excitement with a hint of challenge in stem cell research. Different sections of this volume are devoted to developing stem cell-based therapies for cancer with the main focus on tumoritrophic properties of stem cells, engineering targeted therapeutics, utilization of imaging techniques, and the recent combination studies that use currently employed drugs with stem cells. These sections are put together with the aim to make this text intellectually satisfying and to enable the users to appreciate the outstanding unanswered questions in the ocean of stem cell research with the focus on cancer therapeutics. This volume includes sufficient theoretical and practical details for students, established practitioners, and research fellows from different fields to become familiar with the potential of stem cell therapeutics in different cancer types.

Khalid Shah
Section 1

Introduction
Chapter 1

Stem Cell Sources and Their Potential for Cancer Therapeutics

Khalid Shah

Department of Radiology and Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA

Introduction

Stem cells are the natural sources of embriogenetic tissue generation and continuous regeneration throughout adult life. In embryogenesis, cells from the inner cell mass (ICM) of the gastrula are known as embryonic stem cells, and their multilineage potential is generally referred to as pluripotent.1 The gastrular ICM cells commence formation of the three germ layers: endoderm, mesoderm, and ectoderm, each committed to generating specified tissues of the forming body, and thus containing stem cells with more restricted potential than pluripotent stem cells.2 Tissue-specific stem cells, such as mesenchymal stem cells (mesoderm), hematopoietic stem cells (mesoderm), and neural stem cells (ectoderm), have been identified as present and active for virtually every bodily tissue and are hierarchically situated between their germ layer progenitors and differentiated end-organ tissues.2

Stem cells can be isolated in three ways: from the ICM of the gastrula (embryonic stem cells), from fetal cord blood, and from adult tissues or blood (adult/somatic stem cells). It is not entirely clear whether adult stem cells harbor intrinsic differences from embryonic stem cells. Embryonic stem cells display indefinite self-renewal capacity due to high telomerase expression. In contrast, telomerase activity in adult stem cells seems to be lower, limiting their perpetuation capacity in the long run.3 Adult stem cells have been studied extensively and are already a successful source of FDA-approved treatments for nine human diseases, such as Parkinson's disease and juvenile diabetes, currently applied in clinical centers.4 Though not as highly pluripotent and self-renewing as their embryonic counterparts, adult stem cells are much safer with respect to postgrafting tumor formation. Further, whereas the isolation of adult stem cells from specific parts of the body—such as brain or heart—is complicated, the advent of transdifferentiation techniques and ongoing discovery of unexpectedly plastic and versatile stem cells might provide autologous stem cells resembling these clonal subtypes.5,6 Namely, the long held dogma of differentiation as a rigid and nonreversible process has been challenged over the past decade by a vast amount of studies claiming to show transdifferentiation or even de-differentiation of committed cells. Mesenchymal stem cells (MSC), muscle stem cells, and neural stem cells all seem to possess
the potential of converting to tissue types of other lineages, both within or across germ lines.7–9 The highest degree of lineage plasticity has been imputed to bone marrow–derived MSC, which appear capable of giving rise to virtually all cell types following implantation into early blastocysts and are relatively easy to handle in vitro.8,10 Recent reports have shown that pluripotent stem cells could be generated from murine fibroblasts11 as well as from several human organs, such as heart, skin,12 and bone marrow.5 Also, researchers seem progressively to be able to guide differentiation of pluripotent stem cells into cell types of interest.13,14 These studies indicate that controlled transformation of naïve or committed adult cells from dispensable tissue into desired cell types for autologous transplantation might become reality in the near future.

**Adult Stem Cells**

**Mesenchymal Stem Cells**

The ability of MSC to develop into various cell types, and the ease with which they can be expanded in culture, have led to a great deal of interest in their use as therapeutic agents to treat a wide range of diseases. They can be isolated from adult human tissues, have the capability for self-renewal, and can differentiate into mesenchymal lineages—osteocytic, chondrocytic, and adipogenic. They can be expanded and manipulated in vitro and subsequently regrafted. Following reimplantation, they have been found to suppress the immune system, reintegrate into tissue architecture, and give rise to progeny consisting of both stem cells and lineage-restricted daughter cell types.15 Most importantly, MSC exhibit potent pathotropic migratory properties, rendering them attractive for use as targeted delivery vectors in tumor therapy.15,16

MSC have been successfully isolated from a number of organs including brain, liver, kidney, lung, bone marrow, muscle, thymus, pancreas, skin, adipose tissue, fetal tissues, umbilical cord, Wharton’s jelly, and placenta.17–20 The highest degree of lineage plasticity has been imputed to bone marrow–derived MSC, which are capable of giving rise to virtually all cell types following implantation into early blastocysts and are relatively easy to handle in vitro.8,10 Most of the preclinical studies to date have been performed with bone marrow–derived MSC, which might not be the most practical source available for the clinical settings. The harvesting of bone marrow requires an invasive procedure that yields a small number of cells, and the number, differentiation potential, and lifespan of bone marrow–derived MSC decline with patient age.21–23 Two alternate sources for harvesting MSC that have received considerable attention in recent years are adipose tissue and umbilical cord blood. Adipose tissue obtained from subcutaneous tissue represents the most abundant potential source for harvesting MSC reliably using simple techniques. The expansion potential, differentiation capacity, and immunophenotype of MSC derived from adipose tissue are nearly identical to those isolated from bone marrow.22 Umbilical cord blood, obtained after removal of the placenta, is a rich source of hematopoietic stem cells24,25 and has been shown to be also a rich source of MSC.26 Mononuclear cells can be separated and cultured from the cord blood, and cells in the heterogenous adherent layer have been shown to have a fibroblastoid morphology and express the same markers as bone marrow–derived MSC, namely CD13, CD29, CD49e, CD54, CD90, but not CD14, CD31, CD34, CD45, CD49d, or CD106, among others.27 Umbilical cord blood–derived MSC expand at a higher rate as compared to bone marrow and adipose-derived MSC,22,28 which may be due in part to higher telomerase activity.29 All three type of cells differentiate into osteocytes and chondrocytes,22,27,30,31 which is consistent with the properties of MSC.
Neural Stem Cells (NSC)

NSC isolated from both embryonic and adult human tissues have emerged as attractive candidates for delivering therapeutic proteins that specifically target glioma cells. These cells can be expanded and manipulated in vitro, and re-engrafted following transplantation. NSC have shown the ability to migrate extensively to sites of different pathologies and reintegrate into tissue architecture to give rise to progeny consisting of both stem cells and lineage-restricted terminal cell types. For therapeutic purposes, NSC must be derived, in a substantial number, from safe, consistent, and reliable sources and must meet the criterion of plasticity. Both embryonic stem cells (ESC) and adult NSC can be obtained in substantial amounts and have the intrinsic ability to adapt their specification fate in response to different environmental cues. Recent advances in the in vitro expansion of human ESC culture involve the characterization of defined factors that negate the use of feeder layers (often of murine origin), thus eliminating the problems of xenogeneic cell contamination and possible viral transmission. Adult NSC are multipotent cells that can be obtained from embryonic, fetal, neonatal, or adult central nervous system (CNS) tissue. These cells are found in abundance during embryonic development and their numbers and developmental potential dwindle as development progresses and exist only in small numbers and in specialized niches in the adult organism. In the adult CNS, these cells are especially enriched in the subventricular zone and the subgranular zone of the hippocampal dentate gyrus. Also, NSC have been isolated from the human postnatal cerebellum and adult brain. In humans, fetal NSC were originally isolated from the germinal zones in the subventricular region of a fetal telencephalon. Difference in developmental plasticity between embryonic, fetal, and adult stem cells could be either due to intrinsic cellular difference or disparity in the surrounding microenvironment but is most likely a combination of the two. This abrogation of developmental plasticity could also explain for the limited ability for tissue repair seen in the adult organism. Non-CNS–derived multipotent somatic stem cells, such as mesenchymal stem cells, placental cord blood stem cells, skin stem cells, and adipose tissue stem cells have recently been shown to have the potential to become NSC.

Therapeutic applications of NSC require a substantial number of cells that can be propagated in vitro in serum-free condition in the presence of epidermal growth factor (EGF) and β-fibroblast growth factor (FGF) as multicellular free-floating spheres or neurospheres. Withdrawal of growth factors promotes the spontaneous differentiation into mature cells (astrocytes, oligodendrocytes, and neurons) within the neurospheres. Regular disaggregations of neurospheres ensure the healthy propagation of NSC in vitro and numerical expansion of NSC. This, however, is time consuming and does not yield the large numbers of cells required for most experimental and clinical trials. Immortalization of primary NSC offers a solution to the above problem and can be achieved via the transduction of oncogenes such as the simian virus 40 large T antigen or the v-myc gene. These cells behave similarly as nonimmortalized NSC with the capability to migrate extensively in the developing and mature CNS. Ectopic expression of telomerase has also been shown to prolong the undifferentiated stemlike property of the NT2 neural progenitor cells.

Apart from ethical considerations, the therapeutic use of ESC is constrained by some key issues—such as feeder-dependent growth expansion. As mentioned previously, this vexing problem, especially in the in vitro propagation of human ESC, is gradually being solved with the characterization of factors responsible for maintenance of the differentiated state of the ESC. In addition, better understanding of developmental kinetics of stem cells helps to increase the yield of ESC-derived NSC. However, additional guidelines need to be instituted, especially with respect to avoidance of in vivo teratocarcinoma formation associated with ESC. Practical issues pertaining to these matters are discussed in a review by Martino and Pluchino.
**Induced Pluripotent Stem Cells (iPSC)**

Induced pluripotent stem cells are created by causing differentiated cells to express genes that are specific to embryonic stem cells. iPSC share many characteristics of embryonic stem cells, including the ability to differentiate into cells of all organs and tissues. The idea of being able to restore pluripotency to somatic cells by coexpression of specific reprogramming factors has created powerful new opportunities for modeling human diseases and offers hope for personalized regenerative cell therapies.51,52 iPSC have been shown to have the capacity to redifferentiate into almost any human cell type.

iPSC are a novel and practical tool for human disease modeling and correction, and in theory could serve as a limitless stem cell source for patient-specific cellular therapies.53 Pluripotency refers to the ability of stem cells to grow indefinitely in culture while maintaining the potential to give rise to any of the three germ layers: endoderm, mesoderm, and ectoderm. Somatic cells can be reprogrammed to a stem cell–like state by transferring their nuclear content into oocytes or by fusion with ESC, indicating that unfertilized eggs and ESC contain factors that can confer pluripotency to somatic cells.52,53 Takahashi and Yamanaka hypothesized that the factors that play important roles in the maintenance of ESC identity also play pivotal roles in the induction of pluripotency in somatic cells.11 A screen of 24 candidate genes led to the triumphant description of a tetrad of transcription factors—Oct4, Sox2, Klf4, and cMyc—sufficient to reprogram tail-tip fibroblasts of mice into iPSC.52,53 This contribution stimulated an overwhelming number of follow-up studies, with successful reprogramming quickly translated to human fibroblasts12,54,55 and then to a wide variety of other cell types, including pancreatic β cells,56 neural stem cells,57,58 mature B cells,59 stomach and liver cells,60 melanocytes,61 adipose stem cells,62 and keratinocytes,63 demonstrating the seemingly universal capacity to alter cellular identity.

**Other Stem Cell Sources**

**Dental Pulp Stem Cells**

The potential use of adult dental pulp as a source of MSC has also been explored and validated. Dental pulp (DP) is a vascular connective tissue similar to mesenchymal tissue. The dental pulp–derived stem cells (DP-MSC) have a phenotype similar to the adult bone marrow–derived MSC (BM-MSC), and these cells also express mesenchymal progenitor-related antigens SH2, SH3, SH4, CD166, and CD29 with a cellular homogeneity of 90%–95%. Also, the DP-MSC and BM-MSC populations have a similar gene expression profile.64,65 In contrast to BM-MSC, DP-MSC have presented a higher proliferation pattern and lower differentiation ability. The most evident difference is the inability of DP-MSC to differentiate towards chondrogenesis. This may indicate either that BM- and DP-MSC are present at different stages of commitment and differentiation, not marked by phenotypical characteristics, or that different humoral networks are involved in each microenvironment.64

In short, the dental pulp–derived stem cells are obtained from a very accessible tissue resource, which is further expandable by using deciduous teeth, and possess stem cell–like qualities, including very good self-renewal and multilineage differentiation. Their capacity to induce osteogenesis64,66 could be of great clinical application in implantology. Moreover, these cells also could have potential clinical application in autologous *in vivo* stem cell transplantation for calcified tissue reconstruction. Their proven immunomodulatory activity makes them suitable for suppression of T-cell–mediated reaction in the setting of allogeneic bone marrow transplantation.64
Menstrual Blood Stem Cells

Menstrual blood from the uterine lining has been recognized as a novel source of stem cells with high regenerative capability after the menstrual cycle. Additionally, stromal cells derived from menstrual blood (MenSC) can be acquired without invasive procedures and avoid any ethical controversies. These cells display stem cell–like phenotypic markers, a propensity for self-renewal, high proliferative potential in vitro, and the ability to differentiate towards diverse cell lineages.

The utilization of human MenSC as a potential source for reprogramming into iPSC offers several advantages. First, MenSC may be more easily reprogrammed than terminally differentiated fibroblasts. Second, the procedure for isolating MenSC is relatively simple, fast, and safe, and does not pose any ethical concerns. Third, it is convenient to obtain a large quantity of MenSC as the starting population for reprogramming. Fourth, because the reprogramming process requires only two factors, opportunities for insertional mutagenesis are minimized. Furthermore, obviating the requirement for KLF4 and c-MYC reduces the risk of inducing tumorigenesis. However, there is one obvious limitation for MenSC in that they are only obtained from menstrual blood samples of women of reproductive age, which may narrow their applications. However, if iPSC indeed have memory of the donor tissue, MenSC-iPSC should be the best candidate for producing MenSC to treat uterus-related problems.

Prospects and Caveats on the Way to the Clinics

Stem cell research is one of the most rapidly developing areas of science and medicine. The ability of adult stem cells, to preferentially migrate towards local and disseminated malignant disease and to interact with different tissue environments, present them as most attractive candidates for cell-based therapies in humans. For translation of promising preclinical studies into clinics, it is critical to develop a greater understanding of stem and progenitor cell characteristics, single-cell heterogeneity and their fate in mouse models that recapitulate more closely clinical settings. The type of stem cells used for a particular type of cancer in clinics will depend on their isolation efficiency and their pre-requirement as an allogeneic transfer. For example, the clinical translation of umbilical cord blood–derived MSC will be limited by their unreliable and often low isolation efficiency and requires allogeneic transfer. In contrast, allogeneic transfer is not necessary for adipose or bone marrow-derived MSC, in which case an autograft can easily be harvested from any patient. The advantage of using autologous stem cells is mainly their immunological compatibility, which has been shown to have a profound effect on cell survival after transplantation. For most of the stem cell based therapeutics for cancer, genetic manipulation of cells to combat the disease process will be required prior to transplantation. Before modification of the stem cells with a tumor specific transgene, a thorough understanding of the altered signaling pathways in different cancer types is necessary. This will ensure the specificity of the stem cell based targeted therapeutics. The safety of the transplanted stem cells is a major concern in clinical setting. Importantly, nonimmortalized adult stem cells do not confer the same danger as immortalized adult stem cells and may be used without posing risk to the patient. A number of clinical trials utilizing stem cells for cancer have not reported any major adverse events to date [NCT 00027820, NCT 00392886, NCT 00005799; www.clinicaltrials.gov]. There are also a number of ongoing clinical trials that are utilizing stem cells for cancer therapy; and the results of any adverse effect from such trials are still awaited. When the malignant transformation of transplanted stem cells is suspected,
Introduction

it would be desirable to selectively eradicate MSC by incorporating activatable cellular suicide genes into transplanted MSC or to selectively turn off gene expression. Possible mechanisms that allow for such controls are stem cell–conferred prodrug converting enzymes and transgenes that require additional in vivo cues for expression and the use of tetracyclin-regulatable promoters to turn off gene expression.

References

Section 2
Migration and Fate of Stem Cells
Chapter 2
The Role of CXCR4 as a Mediator of Glioma-Tropic Neural Precursor Cell Migration

Moneeb Ehtesham, Elliot Min, and Rebecca Kasl
Department of Neurological Surgery, Vanderbilt University Medical Center, Nashville, Tennessee, USA

Introduction

Despite significant advances in surgical and adjuvant therapies, the prognosis for patients with high-grade gliomas remains dismal. For patients diagnosed with glioblastoma multiforme (GBM), the most common and aggressive subtype of glioma, median survival remains less than a year, while five-year survival hovers at less than 10%. Such statistics demonstrate the nature of gliomas as resilient and challenging therapeutic targets. Gliomas originate as primary invasive neoplasms from glia throughout the CNS and include astrocytomas, oligodendrogliomas, and ependymomas. Furthermore, the tendency of high-grade gliomas to metastasize via invasive microsatellites that infiltrate deeply into normal brain tissue makes most current therapies unlikely to significantly improve patient outcomes. However, the heterogeneity of cell populations that make up these tumors, specifically glioma progenitor cells, provides a potential target for more specialized treatment. Traditionally, tumors were considered grossly as a homogeneous cell population. More recent research has provided support for a cancer stem cell hypothesis in which a small subset of cells serve as progenitors to induce tumor growth and invasion.

The transition from benign to malignant lesion often includes increasingly aberrant modifications to chemotactic gradients, cytoskeletal changes, hyperplasia, increased vascularity, and survival in hypoxic environments. These changes are thought to be tied to mutations in a number of normal pathways that utilize G-protein coupled receptors (GPCR), one of which is chemokine receptor 4 (CXCR4). Research to enhance fundamental knowledge of the mechanisms behind glioma proliferation and invasiveness has contributed to the discovery that CXCR4 is overexpressed in high-grade gliomas, especially GBM.

CXCR4

CXCR4, an alpha chemokine receptor with seven transmembrane helices, is a known mediator of lymphocytic chemotaxis and hematopoietic cell trafficking. Its only known ligand, chemokine ligand 12 (CXCL12), also known as stromal-derived factor-1α (SDF-1α),
exhibits remarkable specificity for CXCR4 (Figure 2.1). Such behavior is unusual for chemokines since these molecules are known for their generalized, promiscuous behavior while mediating inflammatory and homeostatic responses. The system throughput typically conducts via a display of high-affinity, low-specificity signal transduction. The unique precision of the CXCR4-CXCL12 interaction is thought to stem from disulfide bond formation between the CXCL12 N-terminus and second extracellular loop of CXCR4, enabling CXCL12 to penetrate the CXCR4 binding pocket and activate signaling.8 Ligand

Figure 2.1. NSC are tropic for disseminating glioma in vivo. β-Galactosidase-expressing NSC were inoculated into established intracranial GL26 tumors in C57BL/6 mice. Histological brain sections were then processed with routine X-gal staining, resulting in the development of a blue to dark blue precipitate within NSC-LacZ. Sections were then counterstained with neutral red. Tumor tissue could be identified by intense red staining of neoplastic nuclei and visible dense aggregates of tumor cells. T designates tumor, and N represents normal tissue. (a) Low-power image illustrating the presence of nonmigratory NSC-LacZ within main tumor mass (T), demarcated by arrows. (b) Panel illustrates NSC-LacZ that have moved out of the main tumor mass and are moving into the proximity of tumor cell islets that are migrating along the grey matter/white matter boundary, likely along a white matter tract (inset box). Note that migratory NSC-LacZ are still aggregated in neurosphere-like accumulations. (c) Panel represents a high-power magnification of the inset box in (b). Dark blue NSC-LacZ aggregates are clearly visible in close proximity to a disseminating tumor satellite (T). (d) High-power image of an independent tumor satellite (demarcated by arrowheads) at significant distance from primary tumor site. Blue NSC-LacZ are visible within the tumor, clearly indicating that NSC-LacZ are capable of extensive migratory activity in vivo and can intercalate themselves into disseminated tumor islets. Reprinted from Ehtesham et al., 2004.33 For color detail, please see color plate section.
The Role of CXCR4 as a Mediator of Glioma-Tropic Neural Precursor Cell Migration

binding mediates CXCR4 receptor hetero- and homodimerization in different stoichiometries with chemokine receptors such as CCR2 or CCR5. Although the precise interaction is not well understood, the dimerization state is thought to induce a conformational change that dictates whether a cooperative output will potentiate positive or negative effects.

Though its function has been well-established in the setting of immunology, CXCR4 and its ligand CXCL12 have shown increasing importance in the development of the nervous system. A number of animal studies have demonstrated their role in a wide range of areas throughout the brain. CXCR4 expression has been shown in radial glial cells in both adult zebrafish and mice. These cells are known to play an important role in mammalian development by supporting the migration of nascent neurons to their final destinations within the different layers of cortex. Two separate studies examining CXCR4 and CXCL12 knock-out mice demonstrated clear abnormalities in development of the cerebellum. Specifically, granule cell progenitors in the external granule cell layer inappropriately migrate into the Purkinje cell layer as a result of abnormal timing. In addition to the cerebellum, hippocampal dentate gyrus neurons, Cajal-Retzius cells, and cortical GABAergic interneurons also show aberrant migration patterns in CXCR4 knock-out mice. In each of these situations, neural progenitors utilize CXCL12-mediated chemoattractive gradients to attain their final positions within the developing brain, and disruption of this pathway results in abnormal migration.

In addition to its role in progenitor cell migration, the CXCR4/CXCL12 axis is also thought to regulate the proliferation and possibly neurogenesis of neural stem cells (NSC). The hippocampal dentate gyrus is a site of active neurogenesis within the adult brain. CXCR4 receptors are expressed by both DG neural progenitor cells and immature DG granule cells, while CXCL12 is also expressed within the DG in adults. Bhattacharya et al. demonstrated that CXCL12 exerts its effects in the adult DG by modulating GABAergic inputs to CXCR4+ progenitor cells. Such studies indicate a possible role for the CXCR4/CXCL12 signaling pathway in the regulation of adult neurogenesis. Additionally, it has been shown that CXCL12 has the ability to promote neural progenitor cell proliferation in vitro, and that CXCR4 antagonists abolished this CXCL12-mediated proliferation.

CXCR4 and NSC

Because of their tendency to disseminate widely throughout the brain parenchyma, high-grade gliomas are particularly difficult to treat with conventional therapeutic modalities. Surgical resection can only target the primary tumor mass, while global treatment strategies such as external beam radiation and systemic chemotherapy are unable to completely treat disseminated tumor satellites and exert numerous adverse effects. Therefore, the ability of NSC to “home in” to areas of tumor growth makes them a favorable vehicle for treatment of invasive gliomas. Aboody et al. were the first to show that NSC migrate toward intracranial tumor sites in a specific manner. Using an immortalized murine NSC line, they demonstrated that these cells could track to areas of disseminated tumor when injected intracerebrally or intravenously into glioma mouse models. Additionally, they engineered NSC to express cytosine deaminase, an enzyme that can convert the nontoxic prodrug 5-fluorocytosine to the cytotoxic 5-fluorouracil, resulting in significant shrinking of treated tumors compared with controls. Since then a number of reports have been published confirming tumor-tropic migration of NSC in addition to potential treatment options utilizing transgenes. Specifically, NSC engineered to express tumor necrosis factor–related apoptosis–inducing ligand (TRAIL) or interleukin-12 (IL-12) were able to inhibit tumor growth in murine
glioma models both at the primary tumor mass as well as disseminated tumor satellites. We also confirmed that this directed migration of NSC to areas of tumor growth in vivo was a nonrandom phenomenon; control animals without tumors did not demonstrate any significant NSC migration. While these initial studies were able to demonstrate the ability of NSC to target glioma and their potential utility in a clinical setting, the mechanisms by which these cells generate their specificity has remained unclear.

Further study has revealed that the coordinated migration of NSC involves a number of secreted regulatory factors, cell adhesion molecules, and extracellular matrix components. Ziu et al. demonstrated that ECM produced by tumors was highly permissive for NSC migration. Growth factors have also been implicated in this process. Vascular endothelial growth factor, an important initiator of angiogenesis upregulated in a number of tumors including gliomas, has been shown to strongly induce the migration of injected human NSC. Of particular note is the finding that the cell surface chemokine receptor CXCR4 and its ligand CXCL12 are potent mediators of NSC migration toward brain tumors. The CXCR4/CXCL12 axis has previously been implicated in mediating the invasiveness of high-grade gliomas. Invasive glioma cell populations both in vitro and in vivo significantly overexpressed CXCR4 compared to their noninvasive counterparts. The same study also demonstrated that glioma cells could also produce and secrete CXCL12 into the local microenvironment, providing a potential mechanism for autocrine signaling. Such evidence further supports the idea that the CXCR4/CXCL12 signaling pathway is an important mechanism involved in the targeted migration of NSC toward both primary and disseminated tumor foci.

Specifically, human NSC injected into the brains of glioma-bearing mice demonstrated significant tumor-specific migration away from the site of inoculation, while a residual population of NSC remained at the initial injection site and did not migrate (Figure 2.1). For NSC that had migrated, most of the disseminated cells showed positive staining through immunohistochemistry for A2B5 and GFAP, markers indicative of differentiation toward astrocytic lineages. These cells were also negative for EAAT1 and EAAT2, glutamate transporter-related proteins known to be expressed in differentiated astrocytes. The aforementioned results, along with examination of cell morphology, suggest that the vast majority of tumor-tropic NSC consist of astrocytic progenitor cells that have initiated, but not yet completed, differentiation. Additionally, cells that had demonstrated tumor-tracking behavior showed significant staining for CXCR4. To test the importance of CXCR4/CXCL12 in the tumor-specific migration of NSC, we utilized both anti-CXCL12 and anti-CXCR4 antibodies in tumor-conditioned media designed to induce NSC migration (Figure 2.2). Addition of anti-CXCL12 neutralizing antibody induced a marked decrease in NSC migration, although this result was not statistically significant ($p = .09$; $t$-test). However, addition of anti-CXCR4 neutralizing antibody showed a significant decrease in migration for both murine and human fetal NSC ($p = .022$ and $p = .003$, respectively; $t$-test). This data show the importance of CXCR4 in the tumor-tropic migration of these cells.

While the CXCR4 pathway has been shown to play a significant role in NSC migration, it is unclear whether the source of the chemotactic signal responsible for attracting NSC is released from the tumor cells themselves or from local nonneoplastic parenchyma responding to injury. Current evidence in support of the latter include the finding that hypoxic-ischemic injury can induce upregulation of CXCL12 from nearby astrocytes and endothelial cells, resulting in the migration of CXCR4-positive NSC to infarcted areas. Additionally, CXCL12 expression within malignant gliomas has been shown to be most significant within vascular endothelium as well as nontumorous perivascular cells that may be either neurons or microglia.