METHODS IN MOLECULAR BIOLOGY™

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Cancer Stem Cells

Methods and Protocols

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Humana Press

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ISSN 1064-3745 e-ISSN 1940-6029
ISBN 978-1-58829-938-3 e-ISBN 978-1-59745-280-9
DOI 10.1007/978-1-59745-280-9
Springer Dordrecht Heidelberg London New York

Library of Congress Control Number: 2009926169

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

This book is dedicated to Helena, Jeffrey, and Lauren whose love and devotion has illuminated my path and made each step joyful.

Foreword

Cancer is a devastating disease that affects millions of people in the world. Our therapies for tumors have mostly been based on classical chemotherapy and antiproliferative treatments. More recently, directed therapies against a causative oncogene have led to prominent reduction in tumor rates for some cancers. For instance, chronic myelogenous leukemia that is due to a BCR–ABL translocation can be targeted with Gleevec.

Despite these advances in individual tumors, a number of patients are treated for their primary tumors and ultimately relapse. Relapsing tumors can be due to resistance to chemotherapy or to antioncogene drugs. Another hypothesis is that heterogeneity in the tumor leads to an inability of classical chemotherapy to completely eradicate all cells of the tumor. This concept of heterogeneity has led some investigators to propose a cancer stem cell model.

As patients are treated with chemotherapy, most of the dividing cells are killed, but this leaves a small subset of cells that have the ability to remake the entire tumor. These are cancer stem cells. They possess the signals of self-renewal and yet can also differentiate. If one could understand more about the cells that remain after classical chemotherapy or the cells that can remake the tumor among the heterogeneous population, this information would have a huge impact on our treatment of cancer.

Over the past 3 years, a number of investigators have developed assays for stem cell populations within tumors. A small subset of cells within a tumor have the ability to be transplantable in mice. The work was initiated in the leukemia field mostly because the cell surface markers exist to purify progenitor and stem cell populations. Cancer stem cells have now been isolated from a number of solid tumors including tumors of the nervous system as well as breast cancer. Signaling events within this stem cell population is distinct from the other cells in the tumor. Pathways that would prevent self-renewal and perhaps further differentiate the stem cells are actively being investigated.

At the core of this field of cancer stem cells is a major question of the cell of origin of the cancer. It is possible that the oncogene transforms a particular stage of differentiated cell in the body and this leads to a cell that can be of acquired self-renewal, thereby allowing the tumor to always recur. Such is the case for the transformation of the hematopoietic stem cell with an oncogene leading to leukemia. It is also possible that a progenitor is transformed and acquires self-renewal in an aberrant fashion, dedifferentiates, or maintains itself in the presence of specific signals. Thus, the transplantable population of cells within a tumor or the self-renewing cell population of cells in a tumor may not equate to the cell that ultimately was transformed at the very beginning of the cancer.

There is much to be done for this field, including the understanding of signaling pathways that affect self-renewal in normal stem cell populations as well as cancer stem cell populations. In addition, each cancer stem cell should be purified to homogeneity and evaluated for such signaling pathways. This will involve the genomics and proteomics fields for finding surface molecules that can be used to purify these populations. Lastly, the field of cancer stem cells in human tumors still remains to be developed. Given that there are very few ways of actually propagating human tumors, this becomes a very difficult task. A number of studies have been done in immunodeficient mice in which tumors can be maintained and cell populations purified. This seems to be a promising approach, yet it still leaves us with placing tumors in an abnormal environment, and the tumors can

react and change their particular fate. It is also possible that culture conditions can alter the ability of cells to take on self-renewal programs and/or behave like cancer stem cells. Therefore, we need to have a better understanding of the tumor itself and its heterogeneity and what makes individual cells within a tumor undergo symmetric cell division, metastasize, or invade.

This is a very bright field, and this book has a substantial impact on our understanding of how tumors self-renew or differentiate.

Leonard Zon, Ph.D.

Preface

The concept of cancer stem cells has reinvigorated cancer research with a novel paradigm to study the cause and treatment of cancers. The role of putative cancer stem cells in initiating and supporting cancer has been described by several groups for a growing number of cancers. The characterization of the virulent cancer stem cell has focused the attention of clinician-scientists to this therapeutic target. With the interest of aiding cancer investigators to enter into the cancer stem cell field or to work with scientists who study cancer as a stem cell disease, we have compiled cancer stem cell research techniques and protocols from preeminent researchers in their respective fields. The methods involved in cancer stem cell research have all too often been shrouded in mystery, inhibiting healthy competition, discourse, and collaboration. It is my hope that this volume not only helps to aid a new investigator enter this fascinating field but also "levels the playing field" so that investigators may point to the same or comparable methods when discussing experiments and results.

To be considered as cancer stem cells, clonally derived cells from a tumor specimen must display the following characteristics: (1) they must self-renew and proliferate, (2) they must be able to differentiate and express markers typical of the end terminal cells of that organ (i.e. markers for astrocytes, oligodendrocytes, and neurons in the case of brain tumor stem cells), and (3) they must be able to generate tumors after in vivo transplantation in animal models that resemble the original tumor from patients. With this simplified definition as a point of departure, this volume opens with two animal models of cancer stem cells. Major categories of cancers from which cancer stem cells are derived are represented: leukemia stem cells, brain cancer stem cells, prostate cancer stem cells, pancreatic cancer stem cells, head and neck cancer stem cells, and pituitary adenoma stem cells. Methylation profiling to study cancer stem cells and the contribution of the niche in the regulation of cancer stem cells are explored. The presence and high expression of ABC transporter proteins, antiapoptosis protein, and DNA repair checkpoint protein in cancer stem cells could explain why common therapies, in particular chemotherapy and radiation, are not sufficient to eradicate the tumor. Immunologic targeting or specific targeting of self-renewal mechanisms of cancer stem cells may be an optimal means of targeting and clearing chemo- and radioresistant cancer stem cells. A method for immunologic targeting of cancers stem cells is described as a novel therapeutic strategy and, finally, the use of normal stem cells to treat its evil twin is proposed.

Prospective isolation of cancer stem cells through cell surface marker expression and isolation of cancer stem cells through the formation of spherical cell culture phenotypes are described. These methods are not without controversy. Neurosphere-derived cells are able to differentiate after the withdrawal of growth factors and give rise to a progeny of cells expressing markers of end terminal cells. However, concerns have been raised about the true reflection of "stemness" with the generation of neurospheres. The culturing of tumor cells until the formation of neurospheres precludes the direct comparison of cancer stem cells with the nonstem cell populations. However the use of cell surface markers is not without controversy, as cancer stem cells identified by cell surface markers inevitably have set the stage for the identification of a group of cancer stem cells that do not express the markers of interest. Methods to characterize self-renewal and differentiation are described as well as orthotopic transplantation in immunodeficient animal

models. A balanced introduction to the various methods of isolating, characterizing, and propagating cancer stem cells is made with the understanding that each method has its advantages and pitfalls.

Cancer stem cells are likely to share many of the properties of normal stem cells that provide for a long lifespan, including: relative quiescence, resistance to drugs and toxins through the expression of several ABC transporters, an active DNA-repair capacity, and resistance to apoptosis. Several groups have demonstrated that cells expressing stem cell markers from multiple cancer types exhibit resistance to conventional cancer therapies. Recent studies have indicated that the presence of a cancer stem cell population may be highly correlated with prognosis. Hence, therapeutic strategies to target cancer stem cells will be an important goal for clinician-scientists over the years to come.

The field of cancer stem cell research will create the foundation for the understanding of cancer initiation and propagation and the development of novel targets for cancer therapy over the next 10 years. It is our desire that this volume will aid in generating interest and support for this nascent field during this seminal time.

Los Angeles, CA ***John S. Yu***

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