Chapter 2
Current Status and Perspectives in Stem Cell Research: The Concept of Normal Stem (NSC) and Cancer Stem Cell (CSC)

Science is organized knowledge. Wisdom is organized life.
Immanuel Kant

Abstract This chapter intends to give the audience a basic idea on normal and cancer stem cells, as two essential types of cells in stem cell phenomenology connected by the same feature—"stemness." This is an introductory conceptual consideration of what these cells are and where are their similarities and differences. The chapter discusses normal and abnormal mechanisms that are working in both type of these cells and make them different. General features of both cell types are given in condensed manner. The development of methodology for their isolation, purification, and segregation from other cell types in normal and cancerous tissues is presented. New methods such as magnetic beads separation, magnetic levitation, detection of microspheres in CSC, the confirmation of CSC entity through injection of the cells in NOD/SCID mice, are described. The impact of the concepts upon development of a new movement in cancer therapy, cancer stem cell targeted therapy is explained.

2.1 Introduction

Today, stem cell research is dedicated to better understanding of the fundamentals of normal stem cells (NSC) from different sources as well as cancer stem cell (CSC) concept. In order to understand CSC concept it is necessary to look into fundamental features of NSC [1–6].

Both types of cells are connected with the feature of "stemness," which in essence covers existence of one primitive cell with potential for renewal and trans-differentiation into advanced progenitors. And, while in a normal stem cell these progenitors mature into final, morphologically and functionally determined products, in CSC (due to activity of other mechanisms) we have resulting, immature cells of the tissue from which the cancer evolves, as a direct consequence of its tumorigenic functionality.

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2.2 Stemness, Migration, Circulation, and Seeding

In animals, the signaling molecules used within stem cell niches are often the same used to organize growth and tissue patterning during embryogenesis, such as homologues of the Notch, Wingless, and Hedgehog proteins from Drosophila [1, 2]. However, normal stem cell is seen by the eye of stem cell expert as the state of stemness, which assumes renewal, proliferation, and differentiation into final mature state [3]. The property of pluripotency is believed to depend at least in part on the way the chromatin is organized, that is, how the DNA is packed in the nucleus and how this affects the access of regulatory proteins to genes required for cell differentiation. As will be mentioned at the other place, polycomb proteins play an important role in regulating the chromatin to repress differentiation genes and therefore maintain the pluripotency of animal stem cells [2]. Cancer cell is also renewing, but does not differentiate completely, and therefore, never reaches mature state neither according to morphological nor functional criteria. Both types of cells give heterogeneous populations with different potential of stemness [4–7]. It seems that phenomenon of stemness is very well conserved through evolution within the range from *planaria* across *protozoa* and *ancestral prokaryotes* to *mammals* in animal world and in the plants between meristems (stem) cells of plants [8]. Thus, both sorts of humane stem cells: normal and cancerous, express phenomena of "resistency to drugs and radioactive irradiation" in the context of early evolutionary origin of stemness. Migration, circulation, and seeding into distant locations are also the features of both types of these cells.

2.3 Mobilization

Let us take, for example, normal, adult very small embryonic-like stem cell (VSEL), originated from hypoxic niches of many organs in mammals including bone marrow (BM). Morphologically, VSEL cell is smaller several times than hematopoietic stem cell (HSC), has high N/C ratio and embryonic body with the cells of all three embryonic leaflets within cytoplasm [9]. Specifically stained with fluorescent dyes, it shows all markers of stemness and phenotypic difference (with respect to markers) according to HSC, as well as according to other adult stem cells. These cells can be mobilized from their "niches" through binding with growth factor (G-CSF/Neupogen/Filgrastim) upon dosage-dependent manner, and thus dragged into peripheral blood that can be collected by apheresis. Once dragged into the blood, these cells can be selected and quantified, by Flow Cytometry (FC), Fluorescence Acquired—sorting, (FACS) which allows for segregation of "good mobilizers" and bad ones, thus serving as one of the criteria for stem cell therapy [9, 10]. That feature (mobility) have also CSCs which, even without growth factor added, migrate/metastasize into distant organs carried by blood as circulating tumor cells (CTC) [11]. The most probably among CTC, there are CSCs as a minor fraction within entire population, but with very high tumorigenicity.

2.4 Similarities and Differences Between Embryonic and Adult Stem Cells

Both types are non-specialized cells, therapeutically efficient, and they can cure some diseases if adequately applied. One of the essential features that distinct adult from embryonic stem cells is the lack of pluripotent stem cells with unlimited transformation into cells and organs in the repertoire of adult stem cells, which disables organism to fully regenerate the organs, but only participate in repair and repopulation with cellular elements in the case when regeneration would be desirable [11, 12]. The invention of induced pluripotent stem cell (iPSC) from somatic cells thus has replaced the need for embryonic stem cell in adult diseases scenarios [13]. The pluripotent stem cells are mostly characteristics of amphibia and plant meristem, where they can regenerate in first case limb and in the latter, leaf.

2.5 Similarities and Differences Between Normal and Cancer Stem Cell

Cancer stem cells (CSCs) are tumor cells that have the principal properties of self-renewal, clonal tumor initiation capacity, and clonal long-term repopulation potential [12–22]. CSCs reside in niches, which are anatomically distinct regions within the tumor microenvironment. These niches maintain the principle properties of CSCs, preserve their phenotypic plasticity, protect them from the immune system, and facilitate their metastatic potential [18, 21]. Since CSCs survive many commonly employed cancer therapies, we examine the prospects of targeting different components of CSC as preferable therapeutic targets. CSC has similarities with, and it is also fundamentally different than normal stem cell. Thus, Table 2.1, summarizes similarities and differences between normal and CSCs. It is clear that beside the similarities which have been described, there are also fundamental differences between them, especially with respect to:

- Homeostatic regulation of surrounding tissue regulation (lost in CSC)
- Growth regulation which is controlled by cellular and molecular components of the niche in normal stem cells and by internal mechanisms in CSC
- Signal responses upon growth factors that exist in normal and do not exist in CSC
- Apoptotic responses (existing in NSC and non-existing in CSC)
- Limited replication in NSCs and lack of limitations in CSC)
- Angiogenic supportive network—solid Tu (non-existing in NSCs and a present in CSC)
- Tissue invasion (noncharacteristic for NSCs and typical for CSCs)
- Differentiation of resulting daughter cells (present in NSC and decreased or nonexisting in CSCs)
- Aberrant methylation (lacking in NSCs and present in CSCs).

Table 2.1 Normal stem cells versus cancer stem cells

Endogenous & Exogenous Cues	Normal Stem Cells	Cancer Stem Cells
Homeostatic Regulation of Tissue Regeneration Signals	Maintained	Lost
Genetic Plasticity	High	High
Growth Regulation	Niche-driven	Self-sustained
Antigrowth Signal Response	Yes	No
Apoptosis Signal Response	Yes	No
Limitation to Replication	Yes	No
Angiogenic Sustainability	No	Yes
Tissue Invasion & Metastasis	No	Yes
Differentiation of Resultant Daughter Cells	Yes	Impaired or None
Aberrant DNA Methylation	No	Yes
Anaerobic Respiration	Yes	Yes
Heterogeneous population of cells	Yes	Yes
Different Sets of miRNA involved	Yes	Yes

Further comparison indicates:

- hypoxic nature (anaerobic respiration) of both types of stem cells,
- heterogenous populations, and
- different sets of miRNA with participation in epigenetic regulation.

2.6 Resume Based upon Similarities and Differences Between NSCs and CSCs

CSC of mammals including humans, shares a certain number of characteristics with the adult normal stem cells (NSC). Although CSCs are present with participation of only 0.1% of the whole tumor, they can regenerate original tumor and migrate through blood vessels spreading the cancer into secondary locations [20]. It is very difficult to localize this thin cell fraction within the tumor, since for a long time the scientists did not know about their existence, and therefore, there were no methods developed to the work with them. It was looking like a needle in a hay stuck.

However, with the application of magnetic beads today the isolation of purified CSCs samples especially after specific markers were determined and is a relatively easy procedure.

2.7 What and Which Molecular Markers of CSCs We Know Today?

Beside genetic, epigenetic, and biochemical markers, in the past decade almost all phenotypic markers of protein nature, are identified—mostly of the cluster of designation type (CD), which do not change during cancerogenesis and perpetuate through clonal expansion, building up characteristic phenotype and funding the platform for precise isolation, examination, and targeted treatment (Table 2.1).

2.8 Isolation of CSCs Using Magnetic Beads

Isolation is now possible by magnetic beads coated with antibodies raised against specific CSC markers and by using the magnet which drags the cell/bead complex bound to the magnetic beads through antibodies. The cells dragged to the wall of the tube by the magnet from outside, are washed, and separated from antibodies with appropriate buffer, being now purified in the solution, ready for further expansion if needed or are instantly used, if planned so (Quiagen).

2.9 Theories of Origin of CSCs

In the studies related to cancer there are two fundamentally different theoretical explanations for emergence of the CSCs, none of which can explain all the features of the cancer: (1) *Stochastic theory* or the *Theory of clonal evolution* and *hierarchic theory* or the *Theory of cancer stem cell* [22]. According to the first theory, clonally expanded cells originate from one clone, are all equally changed/mutated and of the same tumorigenicity, while according to the Hierarchic Theory, only one cell—CSC is on the top and it is orchestrating further development of tumorigenicity. Surrounding cells are of different degrees of differentiation, but they do not have that tumorigenic strength to renew the cancer. Both theories are equally inspiring for further understanding of the concept of targeted therapy. However, in order to develop more efficient treatment of the cancer, it is of critical importance to determine which of the theories is correct [22–25]. If most of the cells can proliferate and metastasize, then, virtually, all the cells must be eradicated in order for

the disease to be cured, while the specific elimination of CSC would be enough if the theory of clonal evolution is only myth.

2.10 Current Tests for Determination of the Presence of CSCs

Today we have two types of tests for determination of the presence of CSCs: in vitro and in vivo. In vivo is already described as the repopulation of breast and pancreas tumor tissue built up from only CSCs in the body of immunodeficient NOD/SCID mice, while in vitro tests detect occurrence of microspheres during the growth of CSCs in colonies [23–28].

2.11 Sorting and Isolation of CTC Using the Method of Magnetic Levitation

Before we continue with CSCs, let us see what are circulating tumor cells (CTC) and what their significance is. CTCs originate from a primordial tumor mass and they are entering the peripheral circulation. CTCs are crucial for understanding the biology of metastasis, and they are also playing a vital role in diagnosis, prognosis, follow-up of the disease and individual therapy [22]. However, they are also rare in blood and due to that, problematic for isolation. Besides, viability of CTC can be easily compromised under high stress while we are deliberating them from the surface. Their heterogeneity regarding expression of the biomarkers makes their isolation very challenging; efficacy of isolation and specificity of contemporary applications is in need for improvement. Nanostructured substrates appeared as promising biosensor platform since they produce better isolation sensitivity with regards to the price of isolation of CTCs. Method of magnetic levitation has, however, emerged as one of the newest approaches to isolation of these cells [22]. The immediate question: are among those cells also CSCs, is not yet answered. Magnetic levitation, or magnetic suspension is the method by which the object is in suspension without any other support except the support of the magnetic force [22]. Magnetic force is used to contradict the effect of gravitational acceleration and any kind of acceleration. Cells have components of micro- and nanoscale as well as material contributing to their fundamental features of density and magneticity. Both types of cells, eukaryotic and prokaryotic, can levitate and every cell will have its unique levitation profile [22]. That is how CTCs differ from other cells by density and magnetic features, which helps their segregation from overall mass. This is sorting of cancerous cells without labeling and eventual centrifugation since during centrifugation there is the release of ROS and fragmentation of DNA,

consecutively. The costly aspect of labeled antibodies today is not such a problem, since there are many methods for expansion of normal and cancer stem cells one of which is natural—hypoxia [1].

2.12 The Emergence/Origin and Development of CSC: Mechanisms

What mechanisms are leading to establishment of CSC?

a. ***Genetic***

Definitely, it is the first that we would think about, but here we shall not talk about genetic mechanisms since they are field per se and require another book. Retinoblastoma gene, genes of Lynch syndrome, the genes changed in the presence of specific viruses such as Ebstein-Barr and Varicella Virus, are all genetic mechanisms working on the design of the CSC [28–33].

b. ***Epigenetic***

Mammalian embryonic development is tightly regulated process that, from a single zygote, produces a large number of cell types with widely divergent functions. Distinct cellular differentiation programmes are facilitated by tight transcriptional and epigenetic regulation [28]. However, the contribution of epigenetic regulation to tissue homeostasis after the completion of development is less well understood. The research on the effects of epigenetic dysregulation on adult stem cell function is in progress. Current evidence indicates that depending on the tissue type and the epigenetic regulator affected, the alterations range from minor to stem cell malfunction and disruption of tissue homeostasis, which may predispose to diseases such as cancer. Therefore, maybe more intriguing then genetic are these epigenetic mechanisms in the range from aberrant methylation of DNA to the histone modifications [28–33]. Epigenetic phenomenology is a broad term and these mechanisms broadly contribute to emergence of CSC [33–49].

2.13 Aberrant Methylation

DNA methylation has an important role in epigenetic regulation of genes during development and during different illnesses. DNA methylation is the process by which methyl groups are binding to DNA. We know that aberrant methylation is most frequently described as hyper-or hypomethylation. Methylation is also possible in the normal non-methylated CpG islands of the genes, which are so becoming dysfunctional and can influence formation of CSC. Methyl group is on the wrong site: on cytosine instead of thymine.

2.14 What Do We Need to Do with the Sum of Information?

Computer is a great help in organizing information of this kind and here is a conceptual proposal of the work on sorting of information which would help prediction, and planning of the work on detection and discoveries of cancerous lesions [49]. Computational modeling is the key factor in the understanding of biological systems. The interplay of computational modeling and experimental data already exist for the plants. Some effort is necessary to elevate the knowledge on animal SC to that extant in order to be able to answer deep questions such as: which signals are involved in the maintaining of stem cell network organization, how are asymmetric stem cell division and renewal achieved, which are underlying molecular mechanisms related to stem cell progeny differentiation?

2.15 How Was the Concept of CSC Therapy Designed?

Analyzing research during past few years, it is estimated that situation is mature enough for establishment of new concept, e.g., targeted cancer stem cell therapy [28–49]. While many investigators in the field of tumor therapy continue to upgrade the existing models of chemotherapy and radiation, in an effort to improve their efficacy by increasing their specificity, a particular cadar of investigators is taking a new road—directed toward CSC [40–42]. The starting contribution by Dick (1997) has established evident criteria of the CSC concept, by using NOD/SCID mouse model). Dick has successfully transplanted stem cells of acute myeloid leukemia (AML) into NOD/SCID mouse model where the AML human cells were regenerated (1994). Classical experiments of Al Hajj (breast carcinoma) and Li (pancreatic cancer) have supported concept even more [26–28]. Fluorescent labeling has detected clonally propagated, stable markers, and high tumorigenicity with resistance to therapy—typical signs of functionality of those, otherwise, rare cells (0.1% total population). Rarity of CSCs—requires development of therapeutic strategies different than conventional [49].

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