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DNA Repair of Cancer Stem Cells

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Preface

In recent years, the strides made in understanding and elucidating both the origins and biological mechanisms responsible for driving cancer progression have been quite impressive. Specifically, the momentum that has coincided with the discovery and investigation of cancer stem cells (CSCs), tumor-initiating cells (TICs) or cancer-initiating cells (CICs) has been enormous. The investigation of every aspect of this deadly and lethal subpopulation has brought attention to its potential in a therapeutic light which we hope can translate into the clinic. The cancer stem cell hypothesis was first described with data from models of human leukemia by John E. Dick from the University of Toronto. The heterogeneity of human leukemia and the presence of stem cells in cancer was further translated into solid tumors by Al-Hajj et al. when they published a provocative paper in Proceedings of the National Academy of Sciences discussing the ability to distinguish tumorigenic (tumor-initiating) cancer cells from the nontumorigenic counterpart based on the expression of cell surface markers. The group reported that as little as 100 cells of this specific population were able to form a solid tumor when injected into the mammary fat pad of immunocompromised mice. The most critical aspect of this study was the data demonstrating that even tens of thousands of cells of the nontumorigenic cancer stem cell depleted fraction failed to produce a tumor.

Since this study, these cells have been heavily investigated and are now known to be the most aggressive cells within a solid tumor discovered to date. In recent years, many groups have demonstrated that in addition to being the most aggressive cells, they are highly resistant to current chemotherapy and radiation regimes employed in the clinic. The resistant nature of these cells has led many labs down the path of developing new therapies to eradicate them from patients. An interesting observation among our lab and others was that isolated CSCs express higher levels of DNA repair genes, and furthermore, lead to increased expression of crucial genes and pathways that contribute to their drug resistant characteristics. Thus, we have assembled a remarkable group of experts in both CSCs and DNA repair to discuss their research in light of the role of DNA repair genes and pathways in the CSC population. The common end goal is to contribute to the knowledge base and lead the field in investigating and studying additional mechanisms for potential therapies being designed to target this aggressive population of cells.

The concept of DNA repair conferring survival and progression is the overall theme of this book, and we believe provides a unique contribution to the CSC field in regards to developing new strategies to target this highly metastatic and resistant population. We hope this book can provide a foundation and support to future

scientists and clinicians working in the field of cancer resistance and cancer stem cells.

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Contents

Chapter 1
Introduction to Cancer Stem Cells

Chengzhuo Gao, Robert E. Hollingsworth and Elaine M. Hurt

Abstract A wealth of data points to the existence of a subset of tumor-initiating cells that have properties similar to stem cells, termed cancer stem cells (CSCs). CSCs are thought to be at the apex of a cellular hierarchy, where they are capable of differentiating into the other cells found within a tumor. They may also be responsible for both patient relapse due to their relative resistance to chemotherapy as well as metastasis. In recent years, much research has focused on these cells, their properties and potential targets within these cells for cancer treatment. This chapter will introduce the CSC theory, discuss important properties of these cells, and highlight the need to target them for improved patient outcome.

1.1 The Etiology of Cancer

Many hypotheses have been put forth through the years that attempt to explain the etiology of cancer. They have come from divergent fields of study; pathology, molecular biology and genetics but they all attempt to explain how a normal tissue can go from homeostatic equilibrium to something that grows without the checks and balances that govern normal biology. These theories include, but are not limited to, a viral basis of disease, clonal expansion, and the cancer stem cell hypothesis. While each of these theories attempts to explain the etiology of cancer, it is most likely that some of these independent theories work together to give rise to not only tumors, but tumors that are able to evade treatment strategies.

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1.2 The Cancer Stem Cell Hypothesis

Since the early pathologists could look at tissues microscopically the origins and progression of cancer has been pondered. It was noted that advanced tumors often presented with diverse areas of differentiation, proliferation, invasion and vascularity. It was during this time of early microscopic inspection that led Cohnheim, a student of Virchow, to propose his embryonal rest hypothesis ([1] and references therein). Cohnheim had noted that cancer tissues, teratomas in particular, had many of the same properties as embryonic tissue. This lead him to speculate that carcinogenesis occurs from dormant remnants of the embryo that are later reactivated. However, this theory was largely ignored. The spirit of this hypothesis reappeard in the 1970s when Barry Pierce et al. examined differentiation in teratomas (reviewed in [1]) and determined that a stem cell was responsible for initiation of the teratomas. They then furthered these observations into a theory that all epithelial cancers arise as a result of differentiation-paused adult tissue stem cells [2].

On the heels of this hypothesis, came some of the first evidence that leukemias may have a CSC origin. John Dick and colleagues showed that a sub-population of acute myelogenous leukemia (AML) cells, which shared a phenotype with normal hematopoietic stem cells, could confer cancer when transplanted into immunocompromised mice. Furthermore, the cells that did not have the stem cell-phenotype could not transfer AML to recipient mice [3, 4]. Several years later, CSCs were identified in breast cancer [5], followed by a myriad of solid malignancies (discussed in more detail below in "1.4 Identification of CSCs").

1.3 Properties and Cell of Origin of CSCs

Cancer stem cells are so named because they share many of the same properties as normal stem cells. They are capable of tumorigenesis, self-renewal and can differentiate to form the heterogeneous cell types present in tumors (Fig. 1.1a). These functional properties led to the CSC moniker; however, many argue that "tumor-initiating cells" would be a better description of these cells. For the purposes of this book, we will refer to these cells as CSCs, where a CSC has been defined as "a cell within a tumor that possess the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor" [6]. It has also been observed that CSCs are relatively resistant to chemotherapy and therefore may be responsible for patient relapse following treatment (Fig. 1.1b, discussed in detail in Chap. 3). Thus, the CSC hypothesis attempts to explain several observations of tumors, including the frequency at which tumor cells can give rise to new tumors, the generation of cells with multiple genetic alterations, and the heterogeneity of cell types present within tumors.

In theory, cancer arises from a single cell that has somehow subverted normal growth restrictions. However, experimental evidence has shown a requirement of many cells in order to seed a tumor. For example the growth of tumor cells in immunocompromised mice, typically requires that 1–10 million cells are implanted in

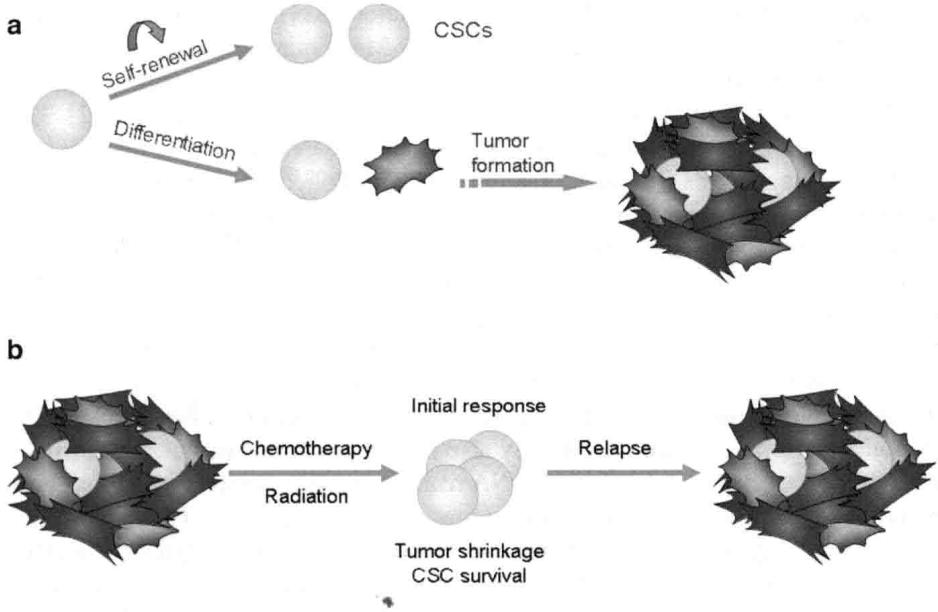

Fig. 1.1 Schematic representation of CSC-driven tumor formation. (**a**) CSCs can either divide symmetrically (self-renewal) to give rise to two CSCs or they can divide asymmetrically (differentiation) giving rise to one CSC and one differentiated progeny. The ability to give rise to a tumor, to self-renewal and differentiate into the heterogeneous cell populations found in the tumor are the defining characteristic of a CSC. The differentiated progeny of a CSC are often more proliferative than the CSC itself, but they have a finite replicative capacity. (**b**) CSCs are resistant to conventional therapies, including chemotherapy and radiation. These treatments can eliminate the more rapidly dividing differentiated cells but leave behind the CSC (discussed in more detail in Chap. 3). Once treatment is stopped, the remaining CSCs can begin to divide again and form a new tumor resulting in patient relapse

order to see tumor formation. This observation has many possible explanations, including injury to cells upon injection, the requirement for the right microenvironment (and the difference of this between human and mouse), as well as the requirement to have a variety of cell types present in order to efficiently induce tumor formation. However, even in experiments where human cancer patients were reinjected with their own tumor cells at different sites within their bodies, it took large numbers of cells in order for a new tumor to establish. Again, this could be consistent with the role of the tumor microenvironment, this time from one site to another; it is also consistent with the notion that only a small proportion of cells are capable of giving rise to a tumor. The idea that only a small proportion of the cells found within a tumor are capable of giving rise to a tumor is consistent with the CSC hypothesis. The frequency of CSCs in most cases has been reported to be low (typically less than 5 %) except in the case of melanoma where a variety of cells were shown to have equally high tumorigenicity [7, 8].

The origin of the cancer stem cell is still under debate. The requirement for multiple genetic insults in order to drive tumor formation has been recognized for a long time [9]. It is suggested that in order for a cell to live long enough to sustain

the genetic insults required to drive tumor formation, it is likely that a normal adult stem cell is the originator of a CSC phenotype. Mathematical models support this theory [10]. Furthermore, the leukemic stem cells (LSCs) of CML patients express BCR-ABL, the common translocation that drives cellular transformation [11]. This provides experimental evidence that CSCs sustain the genetic insults that are seen to drive carcinogenesis. The identification in CSCs of oncogenic driver mutations is also beginning to emerge for solid tumors. For example, the *TMPRSS:ERG* fusion has been found in prostate CSCs [12].

However, it is still possible that a more differentiated cell undergoes these genomic rearrangements and additionally picks up further mutations that impart the ability to self-renew as well as differentiate. Indeed fibroblasts can acquire the properties of pluripotent stem cells with the activation of just a few genes [13–15]. Moreover, it was recently shown that a catastrophic event in the cell can lead to many genetic alterations at a single time [16]. It may also be that environmental cues can trigger CSC properties even independent of DNA rearrangements. For example, it has been noted that cells undergoing epithelial-to-mesenchymal transition (EMT), a normal developmental process that promotes cancer invasion and metastasis, can acquire characteristics of CSCs [17]. These ilines of evidence suggest that a cell does not need to be long-lived in order to sustain many genetic alterations and that CSC properties can be bestowed by biological processes, and would therefore argue that any cell may be the fodder for CSCs.

1.4 Isolation of CSCs

Currently, there are several commonly used approaches for the isolation of cancer stem cells, including: (1) sorting of a side population (SP) by flow cytometry based on Hoechst dye efflux, (2) sorting of CSCs by flow cytometry based on cell surface marker expression, (3) enriching of CSCs by non-adherent sphere culture and (4) sorting of CSCs by flow cytometry based on aldehyde dehydrogenase (ALDH) activity (Fig. 1.2). All of these approaches enrich for CSCs to varying degrees, and each of them has its own advantages and limitations, which will be discussed below.

1.4.1 Side Populations

Goodell and colleagues, while analyzing murine bone marrow cells, discovered a small and distinct subset of whole bone marrow cells that were unstained by Hoechst 33342, a vital dye [18]. This Hoechst 33342 low population is termed SP. They found that the SP had the phenotypic markers of multipotential hematopoietic stem cells and were able to repopulate the bone marrow. Following their work, the SP has been extended to a variety of cancer types, including leukemia [19, 20], ovarian cancer [21], hepatocellular carcinoma [22], brain tumors [23–25], lung cancer [26, 27], thyroid cancer [28], nasopharyngeal carcinoma [29], mesenchymal tumors

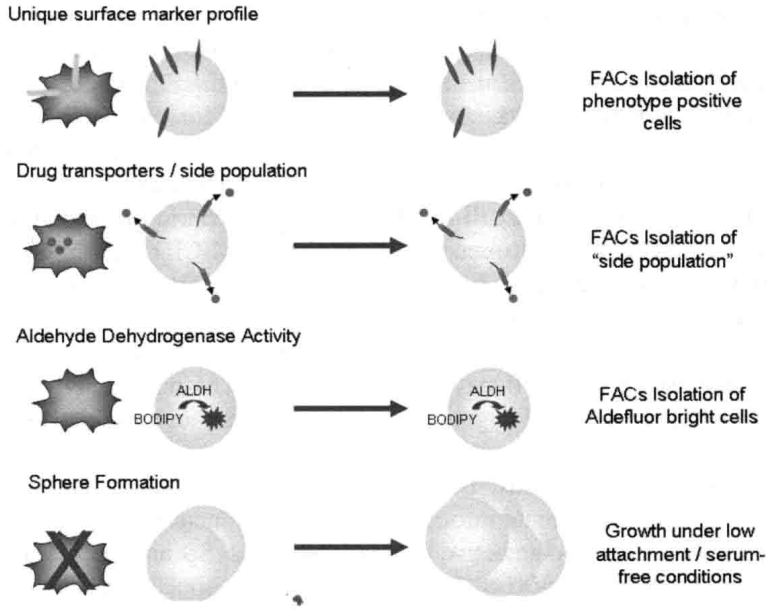

Fig. 1.2 Methods of CSC isolation. CSCs have been isolated using one of four methods. This is a pictorial representation of these isolation techniques and how they are used to identify the CSC versus the non-CSCs

[30], colon cancer [31], prostate cancer [32], breast cancer [33–35], head and neck cancer [36] and other cancers [37–40]. The SP cells purified from these tumor types harbor cancer stem cell-like cells with properties such as a "stemness" gene signature [23, 26, 28, 29, 35], self-renewal capacity [25, 26, 28, 29, 33, 35] and tumorigenicity [19, 21, 24–26, 28, 29, 35].

The ATP dependent transporter, ATP-binding cassette sub-family G member 2 (ABCG2, BRCP1), is generally believed to be responsible for Hoechst 33342 efflux by the SP. Several lines of evidence support this hypothesis. First, ABCG2 knockout mice show significantly decreased numbers of SP cells in both the bone marrow as well as in skeletal muscle [41]. Second, overexpression of ABCG2 dramatically increases the SP percentage of bone-marrow cells and reduces maturing progeny both *in vitro* and *in vivo* [42]. Third, ABCG2 is highly expressed in a wide variety of stem cells including the SP cells of neuroblastoma patients [23, 42]. Fourth, ABCG2 may be responsible for conferring drug resistance to the SP and CSCs. ABCG2 is a multidrug resistant pump expressed at variable levels in cancer cells, which can bind and expel cytotoxic drugs [43]. Thus, ABCG2 may lower intracellular levels of anticancer agents below the threshold for cell death in tumors, leaving resistant cells to repopulate the tumor. Indeed, inhibition of the ABC transporters sensitized SP cells of various cancer types to chemotherapeutic agents [44, 45].

However, there is not always a correlation between ABCG2 expression and the SP phenotype. For example, erythroblasts highly express the ABC transporter ABCG2 but do not have an SP phenotype [46]. Furthermore, the expression of ABCG2, and

the presence of the SP in general, do not always define the CSC population. For instance, in prostate cancer both purified ABCG2$^+$ and ABCG2$^-$ cancer cells have similar tumorigenicity to the SP cells *in vivo*, although the SP cells express higher level of ABCG2 mRNA than the non-SP cells [32]. Additionally, ABCG2 is not the only multi-drug resistance gene identified in SP stem-like cells. The expression of P-glycoprotein (ABCB1, MDR1) is also significantly up-regulated in SP cells of an oral squamous cell carcinoma cell line [47], although Feuring-Buske et al. found that there is no correlation between the expression of ABCB1 and the SP in acute myeloid leukemia [19]. Instead, Zhou et al. demonstrated that Hoechst 33342 efflux activity is compensated by ABCG2 in Abcb1 null mice [42], indicating that SP cells may utilize different drug transportation machineries in different environments. In addition, the presence of the SP may instead be the result of inefficient dye uptake as a reflection of the presence of largely quiescent cells, another characteristic of stem cells [26, 48].

Compared to other methods, isolation of the side population has two advantages. First, it carries additional information about the functional status of the cells, since this assay is based on an active metabolic process. Second, it is highly sensitive, with even rare SP events (<0.5 % of the total cell population) detected within heterogeneous samples [49].

But this method also has many disadvantages. Some of the limitations of this method have to do with the Hoechst 33342 dye itself. Owing to the fact that Hoechst 33342 is a DNA binding dye, it is toxic to cells. Shen and colleagues found that Hoechst 33342 staining for a prolonged periods of time increases apoptosis in C6 cells [25]. Furthermore, it is highly sensitive to slight variations in staining conditions. Hoechst concentration, the staining time, and the staining temperatures all are critical for the success of this approach.

Other disadvantages of SP isolation are due to the ability of this method to accurately define and purify CSCs. Importantly, the SP is not always necessary or sufficient for a CSC phenotype. In glioblastoma multiforme (GBM), the SP from the GBM lines did not enrich for stem-like activity *in vitro*, and tumorigenicity was lower in sorted SP compared with non-SP and parental cells [50]. Equally important, is that SP cells represent a heterogeneous cell population. Wan et al. demonstrated that SP cells purified from a laryngeal cancer cell line does harbor cancer stem cell-like properties, but they are heterogeneous indicating that SP cells are not identical to stem cells [51]. Combining SP detection with cell surface marker selection, may lead to a more efficient and reliable isolation of CSCs.

Despite the limitations existing in SP isolation, the presence of the SP population has some clinical relevance in certain disease indications. For example, the SP population can be identified in gastric cancer tissue and correlates with patient survival [37]. A limited clinical study in ovarian cancer also revealed a higher SP frequency in recurrent or metastatic tumors compared with primary tumors, suggesting a good correlation between the presence of SP and recurrence in ovarian cancer [52].

Besides the use in prognosis of cancer, the SP may also serve as a potential therapeutic target for cancer. Several studies have pioneered the possibility of specifically

targeting SP cells by exploiting pathways involved in drug resistance and differentiation [53]. For instance, Praveen et al. have isolated SP cells from multiple cancer cell lines and found that these SP cells are resistant to cytochrome C release and apoptosis. Based on this finding, they developed a high-throughput imaging assay, in which the cytochrome C-EGFP translocation is monitored in the sorted SP cells. Through this assay, the heat shock protein 90 inhibitors have been identified to sensitize the SP cells to some antitumor agents, such as cisplatin [54]. Moreover, an autologous vaccine for B-cell chronic lymphocytic leukemia (B-CLL) was made using a patient's SP cells. Following vaccination, the study showed an increase in (B-CLL)-reactive T-cells followed by a corresponding decline in circulating B-CLL SP cells [55]. This indicates that the SP may be a valid ground for cancer therapy.

1.4.2 Surface Markers

Initially used to identify and isolate normal stem cells, surface markers are now extensively used for the identification and isolation of CSCs in many malignances. Lapidot and colleagues were the first to isolate leukemia-initiating cells based on cell-surface marker expression and found $CD34^+$ $CD38^-$ cells, but not the $CD34^+$ $CD38^+$ and $CD34^-$ cells, harbored serial leukemic transplantation potential [3]. Following this initial prospective isolation of leukemia stem cells, breast CSCs were identified as $CD44^+$ $CD24^{-/low}$ by Al-Hajj and colleagues [5]. Later, based on their surface marker expression, CSCs have been isolated from various tumors (Table 1.1). Among all the surface markers used for CSCs isolation, CD133 and CD44 are the most commonly used in a variety of tumor types.

CD133 (Prominin 1), a five transmembrane glycoprotein, was originally identified both in the neuroepithelium and in various other epithelia of the mouse embryo [77]. Later, a novel monoclonal antibody recognizing the AC133 antigen, a glycosylation-dependent epitope of CD133, detected that CD133 is restricted in $CD34^+$ progenitor populations from adult blood, bone marrow and fetal liver cells [78]. In addition, CD133 expression is rapidly down regulated upon cell differentiation [79]. These characteristics of CD133 make it a unique cell surface marker for the identification and isolation of various CSCs (Table 1.1). The biological function of CD133 is still largely unknown. A single nucleotide deletion, which caused the truncation of CD133, is linked to an inherited form of human retinal degeneration [80]. A recent report has linked CD133 with endocytosis. In this study, CD133 knockdown improved Alexa488-transferrin (Tf) uptake in Caco-2 cells, while cell treatment with the AC133 antibody resulted in down regulated Tf uptake, [81]. Despite its utility as a marker of CSCs, CD133 does not appear to play a significant role in the maintenance of at least some CSCs. In colon cancer cells isolated from patients, CD133 knockdown did not affect their tumorigenicity *in vitro* and *in vivo* [82]. Instead, CD44 knockdown prevented tumor formation of the same cells.

CD44, a single transmembrane glycoprotein, is a major component of the extracellular matrix [83]. Besides acting as an adhesion molecule, it also functions as

Table 1.1 Cell surface markers used in the isolation of various CSCs

Tumor type	Phenotype	Reference
AML	$CD34^+CD38^-$	[3, 4]
Breast	$CD44^+CD24^-$	[5]
Brain	$CD133^+$	[56, 57]
	SSEA-1	[58]
Prostate	$CD44^+CD133^+\alpha_2\beta_1^{hi}$	[59]
	$CD44^+CD24^-$	[60]
Head and neck	$CD44^+$	[61]
Liver	$CD133^+$	[62]
	$CD90^+$	[63]
Colon	$CD133^+$	[64, 65]
	$EpCAM^{hi}CD44^+$	[66]
	$CD44^+/CD166^+$	[67]
Pancreatic	$CD44^+CD24^-EpCAM^+$	[68]
	$CD133^+$	[69]
Squamous cell carcinoma	Podoplanin	[70]
Lung	$CD133^+$	[71]
Melanoma	$ABCB5^+$	[72]
Gastric	$CD44^+$	[73]
Ovarian	$CD133^+$	[74]
	$CD44^+/CD177^+$	[75]
	$CD44^+$	[76]

a principle receptor for hyaluronan (HA) [84]. HA is enriched in the pericellular matrices of many malignant human tumors and plays an important role in tumor progression via regulation of receptor tyrosine kinases (RTKs), such as ERBB2 and EGFR [85]. Thus, as a critical receptor for HA, CD44 plays an important role in cell proliferation and survival via activation of the MAPK and PI3K/AKT pathways, respectively [85]. Furthermore, CD44 also plays an important role in the invasion of a variety of cancer cells, including breast [86], prostate [87], hepatoma [88], and mesotheliomas [89], and has been significantly correlated with the circulating prostate tumor cells [90]. Therefore, CD44 stands out as a surface marker for CSCs, as first shown by Al-hajj and colleagues in breast cancer [5]. Following their work, CD44 has been utilized as a surface marker to isolate CSCs from a variety of different tumors (Table 1.1).

CD44 was also explored as a potential diagnostic target for cancer detection as well as a drug target for cancer therapy [91–93]. For instance, in 2003, the humanized anti-CD44 antibody (bivatuzumab) labeled with rhenium-186 was used in phase I studies in patients with head and neck squamous cell carcinoma (HNSCC) [94, 95]. In this trial, these radiolabeled CD44 antibodies showed promising anti-tumor effects with low toxicity. Further, a different trial with the non-radiolabeled CD44 antibody (bivatuzumab mertansine) also had good patient response rates, although the development of this drug was terminated due to the death of a patient [96]. Recently, CD44 was also shown to target CSCs. In 2006, Jin et al. found that interruption of CSCs interaction with their microenvironment by monoclonal antibody directed against CD44 markedly decreased the number of the AML LSCs *in vivo*, indicating a key regulatory role of CD44 in AML LSCs [97].

While CD44 and CD133 appear to have broad tumor applicability, the choice of the cell surface markers tends to be tissue specific and is often based on previous knowledge of the development of that tissue. For instance, CD34 and CD38, the markers used for isolation of AML CSCs, are also the markers used to identify normal early hematopoietic progenitor cells [3, 4]. Another good example is CD138. CD138 is a marker for terminally-differentiated B cells (plasma cells). It has been shown that CSCs from multiple myeloma, a plasma cell malignancy, are $CD138^-$.

Compared to other methods of isolation, cell surface marker isolation has a major advantage of obtaining a precise population. However, the selection of which surface markers to use is one of the greatest pitfalls of this approach. As discussed earlier, many times markers selected are based on previous knowledge of the development of the tissue. For this reason, it may not be easy to find the right markers. For example, until recently there were no markers identified for CSCs of human primary gastric tumors [98]. Furthermore, the choice of markers made by researchers has not been unified, even within the same tumor type. As shown in Table 1.1, different markers have been used for the same tumor type. To further complicate the picture, many times there have not been careful comparisons done within the same study of all the proposed CSC markers in order to definitively test which marker combination is the best at identifying CSCs. A further complication is that the surface markers may be heterogeneous between patients even within the same tumor type. A recent study from 16 AML patients shows that the majority of LSCs are in the minor $CD34^+$ $CD38^-$ fraction in 50 % of the subjects, and in the $CD34^+$ $CD38^+$ fraction in the other 50 % [99]. Similar findings were also obtained from breast cancer patients. When Park et al. [92] evaluated the expression of stem cell-related markers at the cellular level in human breast tumors of different subtypes and histologic stages, they found that the cancer stem cell markers vary according to tumor subtype and histologic stage [100]. Ali et al. also demonstrated that breast CSC markers, such as CD44/CD24, ALDH and ITGA6, do not identify identical subpopulations in primary tumors [101].

Other limitations of choosing surface markers for the identification of CSCs are methodological. First, a large number of cells is required to sort and the number of CSCs identified by this approach is usually low ($<1 - 10$ %). Furthermore, when using tumor samples, the cells must first be dissociated typically with collagenase and/or other proteolytic enzymes. This dissociation step may damage the presentation of the cell surface antigens [102, 103].

1.4.3 Nonadherent Sphere Culture

Initially, nonadherent sphere culture was used to culture neural stem cells [104]. The cells isolated from the stratum of adult mouse by this method could generate both neurons and astrocytes. Later, it was shown that purified stem cells were capable of growing as spheres. For instance, $CD133^+$ cells isolated from normal human fetal

brain formed spheres *in vitro* [105]. These observations were then extended to cancerous tissues where further studies demonstrated that $CD133^+$ cells from the human brain tumors are also capable of forming neurospheres [57]. Since then, the ability of CSCs to form spheres in culture has been shown for most solid malignancies, including breast [106], melanoma [107], pancreatic [108], prostate [109], ovarian [75] and colon CSCs [110].

Most importantly, researchers found that the nonadherent sphere culture condition can enrich cells with CSC phenotypes [106]. They demonstrated that, in breast cancer, the CSC population with $CD44^+$ $CD24^-$ phenotype increased more than two-fold after culturing under nonadherent sphere culture conditions. In addition, the spheres were more tumorigenic. Since then, researchers have used sphere to formation to enrich CSCs from brain [56, 57, 111, 112], colon [110], pancreas [108], bone sarcomas [113] melanomas [107] and prostate [109]. The enrichment of CSCs by growing cells under sphere culture conditions has been confirmed by surface marker expression in all mentioned cases, except bone sarcoma, in which the surface marker remain to be determined.

Compared to the other methods of isolating CSCs, this method is easy to perform and allows researchers to obtain a larger number of CSCs. However, like all methods of isolation, this method also suffers from several disadvantages. The spheres are a heterogeneous population of cells containing both CSCs and non-CSCs. For example it has been shown that only a portion of the spheres are capable of self-renewal [106, 114]. Immunostaining of spheres from prostate cancer cell lines indicated that the spheres are heterogeneous for CSC markers [115]. Also, the conditions of sphere formation are critical to the overall success of CSC enrichment. In neurosphere cultures, it has been shown that the composition of spheres can be different due to the differences in sphere size, passage, culture medium, and technique [116].

1.4.4 ALDH Activity

In addition to the above three isolation methods, isolation of CSCs based on their aldehyde dehydrogenase (ALDH) activity is also commonly used. ALDH is a detoxifying enzyme responsible for the oxidation of intracellular aldehydes [117]. It functions in drug resistance, cell differentiation and oxidative stress response [118]. ALDH activity may be easily assessed in living cells using the ALDEFLUOR kit (Stem Cell Technologies). This kit utilizes an ALDH substrate BODIPY aminoacetaldehyde (BAAA), which is converted in the cytoplasm into a florescent molecule by ALDH enzymatic activity [119]. Recent studies have demonstrated a positive correlation between ALDH expression and overall survival of patients with different cancers [120]. To date, ALDH activity has been used to identify and isolate CSCs from AML [121], breast [122], melanoma [123], prostate [124], liver [71], ovarian [125], lung [126] and osteosarcoma [127].

Like other isolation methods, ALDH is also not a universal marker for cancer stem cells in any tumor type. In a CSC marker profiling study, the breast cancer