COMMENTARY

How to Estimate Cancer Stem Cell Frequency Correctly

Hai G Liu, Xiao Hua Zhang*

Abstract

Cancer stem cell research is a focus for more and more cancer biologists and evidence of involvement in cancer devleopment is becoming more abundant. Earlier studies indicated cancer stem cells to be rare as determined by the standard xenotransplantation assay using SCID mice in vivo. However, recent studies have shown that syngeneic transplantation of mouse tumors or modifications to the xenotransplantation assay can effectively improve the accuracy of detection, with stem cells being more abundant than hitherto thought. Furthermore, to estimate frequency correctly, it is necessary to considerate cancer stem cell subsets with differing capacities for tumorigenesis.

Key Words: Cancer stem cells - tumorigenesis - frequency - xenotransplantation - syngeneic transplantation

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Introduction

There are two different models of cancer initiation and progression. One is the stochastic model: cancer is resulted from a cell mutated to a malignant one, and all of its offspring have the capacity of cancer formation and progression. Therefore, the best treatment of cancer is to get rid of every cancer cell. The other is the intrinsic model or stem cell model: cancer is initiated from a transformed stem cell or progenitor acquiring the stem cell property such as extensive self-renewal and multi-differentiation capacity, and not all of its offspring but only the daughters with the stem cell properties, which are termed cancer stem cells or cancer initiating cells, have the capacity for cancer formation and progression. Thus, according to the stem cell model, the best treatment of cancer is targeted removal of cancer stem cells (CSCs) or cancer initiating cells (CICs) which only occupy a small proportion in a cancer mass, as agreed at an American Association of Cancer Research (AACR) workshop on CSCs (Clarke et al., 2006). The treatment difference between the two models are the targets and their frequencies. Therefore, it is important to understand cancer stem cell's mechanism of cancer initiation and its frequency. Here, we discuss the cancer stem cell frequency and how to estimate it correctly.

Since Bonnet and Dick (1997) identified a rare population of leukemia initiating cells with CD34+CD38- and Al-Hajj et al (2003) discovered small numbers of breast CD44+CD24-/low tumorigenic cells, several types of cancer initiating cells or tumorigenic cells with specific markers have been sorted and identified, thought responsible for brain tumor s(Singh et al., 2003; 2004; Galli et al., 2004), prostate cancer (Collins et al., 2005), colon cancer (Dalerba et al., 2007; O'Brien et al., 2007; Ricci-Vitiani et al., 2007), pancreatic cancer (Hermann et al., 2007; Li et al., 2007), melanomas (Monzani et al., 2007; Schatton et al., 2008), lung cancer (Kim et al., 2005) and ovarian cancer (Zhang et al., 2008).

Cancer stem cell research is now a focus of more and more cancer biologists and stem cell researchers and the evidence of involvement in cancer development is becoming more abundant. Therefore, AACR convened a workshop with many experts to discuss some problems in cancer stem cell research in 2006 (Clarke et al., 2006). They reached an agreement that a cancer stem cell is a cell within a tumor that possesses the capacity to self-renew and cause the heterogeneous lineages of cancer cells that comprise the tumor. One characteristic of the cancer stem cell is its frequency: a minority of cancer cells in a cancer mass according to available evidence at that time (Table 1). This conclusion is mainly according to xenotransplantation study which is a standard functional assay recommended by AACR workshop on cancer stem cells.

Table 1. Cancer Initiating Cells with Specific Markers and their Frequency in Immune Deficient Mice before the AACR workshop on CSCs in 2006

Tumor site	CIC markers	% of cells expressing markers	Reference
AML	CD34++CD38-	0.02-2.0	Bonnet and Dick, 1997
Breast	CD44+CD24-/lowLin-	11-35	Al-Hajj et al., 2003
Brain	CD133+	6-29	Singh et al., 2004
Brain	CD133+	2-3	Bao et al., 2006
Prostate	$CD44^{+}2\beta1^{hi}CD133^{+}$	<0.1	Collins et al., 2005

*Department of Oncology, The First Affiliated Hospital of Wenzhou Medical College, Wenzhou, China *For correspondence: zhangxhwzmc@yahoo.com*

Xenotransplantation Assay Limitations

Some three years later, some scientists are raising questions as to whether cancer stem cells are rare or not. They challenge the xenotransplantation study as the standard functional assay for cancer stem cell identification. The xenotransplantation assay has a few of defects and limitations. First, rejection between human tumor and mice body will make mice body get rid of human tumor cells and lead human tumor cells to grow difficultly in mice. Second, tumor is a very complicate tissue and microenvironment is very important to support tumor cells grow with nutrition and lots of cytokines. Only transfer human tumor cells to mice body without suitable environment will lead tumor cells to grow slowly or die. Third, tumor growth needs a period of time which is different among different types of tumor. Therefore, the xenotransplantation assay may underestimate cancer stem cell frequency according to the above three limitations (Figure 1).

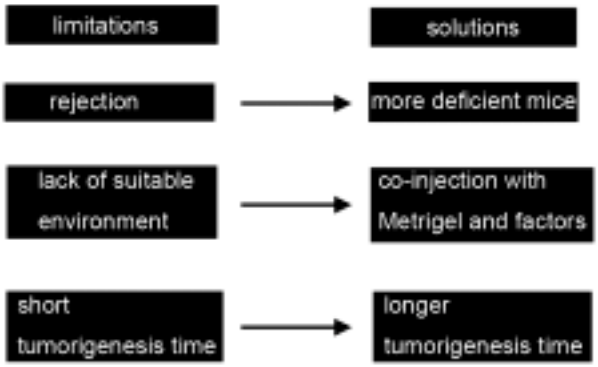

Figure 1. Limitations and Solutions of the Xenotransplantation Assay. The xenotransplantation assay has a few of defects and limitations. First, rejection between human tumor and mice body will make human tumor cells grow difficultly in mice. The solution to this limitation is to transplant human tumor cells to more deficient mice with less rejection. Second, tumor is a very complicate tissue and microenvironment is very important to support tumor cells grow with nutrition and lots of cytokines. Only transfer human tumor cells to mice body without suitable environment will lead tumor cells to grow slowly or die. Therefore, the solution to this limitation is to co-inject tumor cells with Metrigel and growth factors

Solutions to the Limitations

The first solution to the problem of the exnotransplantation study is syngeneic transfers of mice tumor. Kelly et al (2007) reported that they injected 10 to 105 cells of Eμ-myc B lymphoma, Eμ-N-RAS T lymphoma and PU.1-/- acute myeloid leukemia (all from on a mice C57BL/6 background) into non-irradiated congenic C57BL/6 recipient mice respectively. Recipients of 105 lymphoma cells have the capacity to develop tumors as well as 10 lymphoma cells. Furthermore, even injection of a single cell can cause a tumor in three of eight recipients within 33 to 76 days. Therefore, they concluded that the exnotransplantation assay may underestimate the cancer stem cell frequency and syngeneic transplantation of mice tumor may be a better assay to investigation of cancer stem cell characteristics (Figure 1).

The second solution to the problem of the xenotransplantation study is modification to the xenotransplantation assay. Quintana et al (2008) reported that modification to the xenotransplantation assay with three different ways could dramatically increase the detectable frequency of tumorigenic cell. The first modification way is replacement of non-obese diabetic/ severe combined immune deficiency (NOD/SCID) mice by NOD/SCID IL-2rg-/- which is lack the interleukin-2 gamma receptor and lack of natural-killer cell activity partly compared with NOD/SCID mice. 4000 human melanoma cells were injected into three NOD/SCID mice and NOD/SCID IL-2rg-/- mice, respectively. The first palpable time of NOD/SCID IL-2rg-/- mice was 40 days earlier than NOD/SCID mice and the tumorigenic frequency was higher than NOD/SCID mice (3/3 vs 1/3). The second modification way is co-injection with Matrigel which can increase tumor formation by cancer cell lines and enhance the engraftment of primary human epithelial cancer cells in immunocompromised mice. 400 human melanoma cells were injected into three NOD/SCID IL-2rg-/- mice with Metrigel or Vehicle, respectively. The first palpable time of NOD/SCID IL-2rg-/- mice with Metrigel was 70 days earlier than NOD/SCID mice with Vehicle and the tumorigenic frequency was higher than NOD/SCID mice with Vehicle (3/3 vs 1/3). The third modification way is combining the above two ways together. The melanoma initiating cell frequency of NOD/ SCID IL-2rg-/- mice co-injection with Metrigel was 5000-fold more than NOD/SCID mice with Vehicle (1/9 vs 1/ 46700). Therefore, modification to the xenotransplantation assay with more immunodeficiency mice and co-injection with suitable microenvironment can substantial increase the cancer stem cell frequency (Figure 1).

The third solution to the problem of the xenotransplantation study is to extend observation time of tumorigenesis. The melanoma initiating cell frequency was about 8-fold in 8 weeks more than in 32 weeks (1/ 111000 vs 837000) (Al-Hajj et al., 2003; Quintana et al., 2008). A similar phenomenon was discovered by Al-Hajj and his colleagues in identification of breast tumorigenic cells(Al-Hajj et al., 2003). They reported that CD44+CD24-/lowLineage- was the phenotype of tumorigenic breast cancer cells, which were able to initiate a tumor by xenotransplant into NOD/SCID mice, and CD24+ breast cancer cells were also able to form a tumor which is detected only upon necropsy after 12 weeks examination. Therefore, longer the observation time may discover more tumorigenic cells (Figure 1).

Cancer Stem Cells are not Rare

According to above evidence, syngeneic transplantation of mice tumor or modification to the xenotransplantation assay can effectively improve the cancer stem cell frequency. Therefore, some scientists raise the question that tumor growth need not be driven by rare cancer stem cells(Kelly et al., 2007). In comment on this problem, Kennedy et al (2007) emphasized that the

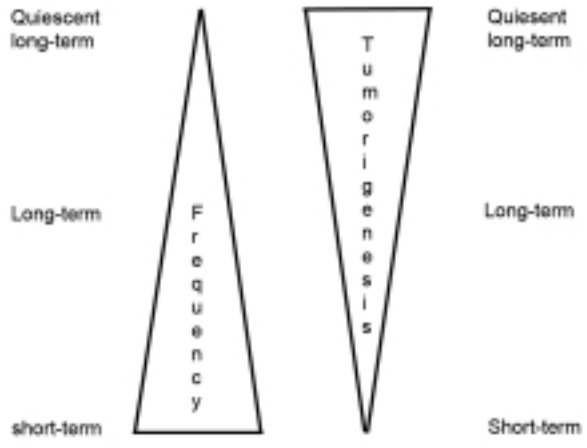

Figure 2. Frequencies and tumorigenesis capacity of cancer initiating cell subsets. Cancer initiating cells have three different subsets, quiescent long-term CICs, long-term CICs and short-term CICs. Quiescent long-term CICs occupy only a few percent in a cancer mass, but have the strongest tumorigenesis capacity; long-term CICs is more, but their tumorigenesis capacity is weaker than quiescent long-term CICs; short-term CICs is the most and their tumorigenesis capacity is the weakest in all of the three subsets.

absolute frequency is not an important characteristic of cancer stem cell, but the capacity of initiating a tumor in vivo is.

Cancer Stem Cell Subsets and Frequencies

However, the capacity of initiating a tumor is different among cancer initiating cells. Hope et al (2004) discovered that SCID leukemia initiating cells (SL-ILs) had three distinct classes, short-term, long-term and quiescent long-term with different capacity of tumorigenesis. Quiescent long-term SL-ILs give rise to both subclasses and long-term SL-ILs only give rise to short-term SL-ILs. It is a hierarchy like normal heamatogenesis. Hermann et al (2007) reported that CD133+ pancreatic cancer stem cells had different capacity of tumorigenesis in distant regions. Only CD133+CXCR4+ subset of cancer stem cells is able to transfer and build a metastasis. But CD133+CXCR4- subset only have the capacity of tumorigenes in primary region without metastasis. Diehn et al (2009) also found that cancer stem cells were not a group of cells with exactly the same characteristics. According to above experimental evidence, cancer stem cells or cancer initiating cells may have different capacity in tumorigenesis such as short-term, long-term and quiescent long-term subpopulations with different frequency in a cancer mass (Figure 2). In the case of that, different transplantation assays may detect different frequency of cancer initiating cells. The more deficient the transplantation host is, the more frequency of cancer initiating cells is detected. Therefore, we propose a hypothesis that the slightly deficient transplantation host may only detect quiescent long-term cancer initiating cells, and their frequency is the least; the more deficient transplantation one may detect both quiescent long-term and long-term cancer initiating cells, and their frequency is much more; the most deficient transplantation one can detect all of quiescent long-term, long-term and short-term cancer initiating cells, of course their frequency is the most.

Given the above considerations, syngeneic transplantation of mouse tumors or modification to the xenotransplantation assay can effectively improve the cancer stem cell frequency. Furthermore, it is necessary to investigate carefully different capacities for tumorigenesis corresponding to the different cancer stem cell subsets. Only if this is the case can we estimate cancer stem cell frequency correctly.

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