
Cancer Stem Cells Derived from Mouse Induced Pluripotent Stem Cells

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Abstract

The malignancy of tumors is often explained by the presence of cancer stem cells, which are considered resistant to anti-cancer agents and radiation. Cancer stem cells are typically characterized by continuous proliferation and self-renewal as well as by potential of differentiation. Stem cells are considered to differentiate into tissue specific phenotype of mature cells being influenced by the microenvironment. In this context, cancer stem cells should be derived from the stem cells under the influence of a microenvironment, which induces malignant tumors. In this review, we propose this microenvironment as a ‘cancerous niche’ and discuss its importance on the formation and maintenance of cancer stem cells with the recent experimental results to establish cancer stem cell models from induced pluripotent stem cells. These models of cancer stem cell will provide the great advantages in cancer research and its applications in the future.

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Introduction

Cancer is a group of diseases, in which regulation of cell proliferation and growth is disrupted and cells are growing uncontrollably. The uncontrolled cell growth in cancer is caused commonly by genetic damages, including mutations of oncogenes and tumor suppressor genes. Thereby cancer cells are historically

considered driven from a single cell, that is, they are clonal. However, the individual cells that make up the cancer exhibit significant heterogeneity in their morphology, cell surface antigens, genetic alterations, pattern of gene expression profiles, epigenetic modifications and so on. One possible explanation of their heterogeneity is that cancer is a cellular hierarchy with cancer stem cells (CSC) at the apex, just like normal tissue development with their tissue stem cells (Wang and Dick 2005). The CSC concept derives from the fact that the unlimited growth of cancer tissues depends on a small number of distinct cells of which proliferation is unlimited. Nowadays, a CSC is defined as a cell within a tumor that possesses the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor. CSCs can thus only be defined experimentally by their ability to recapitulate the generation of a continuously growing tumor as reached to the consensus at the AACR Cancer Stem Cells Workshop (Clarke et al. 2006). However, characterization and analysis of these cells are limited due to the small number of CSCs in a tumor, and technical difficulty of isolation as a homogenous population of cancer stem cells from clinical samples. If it is possible to establish appropriate CSC lines, the recapitulation of CSC properties would be more precise and at the same time the development of new clinical cancer therapy would be accelerated. In this chapter, we introduce our recent work to establish cell lines with CSC properties *in vitro*. And also we will discuss the concept of CSC with the results obtained from our original cancer stem-like cells.

Microenvironment to Develop Malignant Tumors

In the field of regeneration therapy, the pluripotent stem cells such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are promising sources of differentiated cells for transplantation. When exposed to appropriate environment, the stem cells should

be directed to the progenitor cells such as hematopoietic cells, neural stem cells and so on, which are destined to differentiate into each mature cell such as macrophage, monocyte, neural cell, cardiac cell, and pancreatic β -cell. The behavior of stem cells is tightly regulated by the signals from surrounding microenvironment, so called 'niche' which supports the self-renewal of stem cells controlling stem cell number. Simultaneously, a niche regulates the differentiation, in turn, maintains tissue homeostasis (Moore and Lemischka 2006). Thus the cell fate is determined by the events and factors present in the range of a niche.

Taking the pluripotency of stem cells into consideration, it should be hypothesized that malignant neoplasm is one of the tissue types differentiated from stem cells. In this context, a CSC could be described as a progenitor cell that is destined to differentiate into a cancer cell. This might be called 'canceration' rather than tumor initiation. In this context, we propose the niche that directs stem cells into CSCs as 'cancerous niche' (Fig. 11.1). But cancerous niche could hardly be defined because normal tissue or body should have normal niche. How and where can we find the cancerous niche?

A Cancer Stem Cell Model

We designed unique experiments to convert pluripotent stem cells into the cells, which have the characteristic properties of CSCs, using established cancer cell lines (Fig. 11.2). As the result, we have reported that mouse iPSCs (miPSCs) could acquire characters of CSCs when miPSCs were cultured in the presence of conditioned medium prepared from various cancer cell lines (Chen et al. 2012). The established model of CSCs derived from miPSCs (miPS-CSCs) formed spheroids when they were cultured under non-adherent condition, implying they have capacity of self-renewal. The genes associated with stem cell properties and an undifferentiated state such as *Nanog*, *Rex1*, *Eras*, *Esg1* and *Cripto1*, were expressing in miPS-CSCs. Most importantly, miPS-CSCs

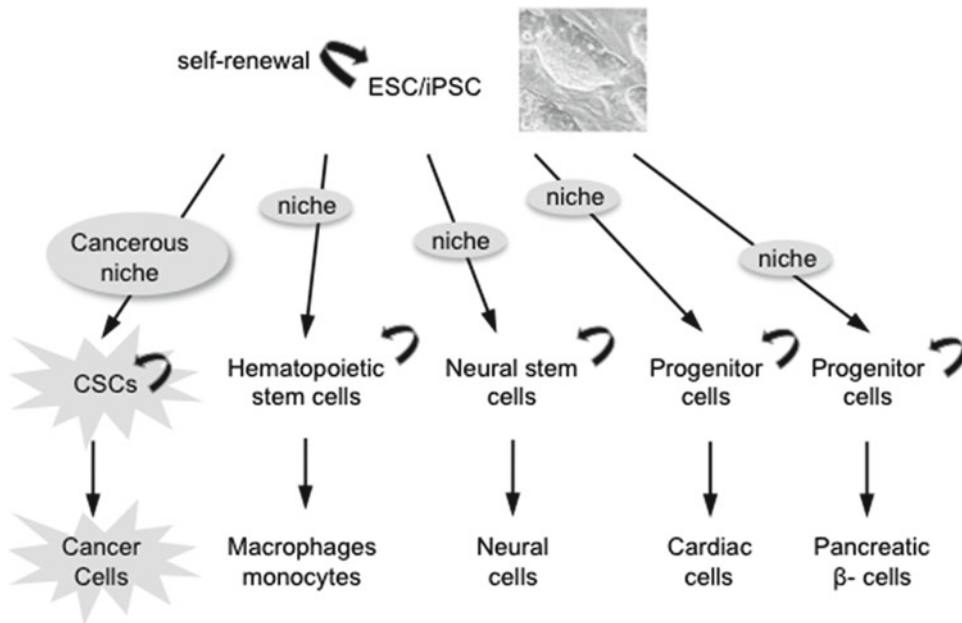


Fig. 11.1 ‘Cancerous niche’ developing malignancy. In the appropriate niche, the stem cells/iPSCs should differentiate into progenitor cells and terminally into each mature cell. When a malignant neoplasm is considered to

be a tissue differentiated from a stem cell, a cancer stem cell could be a progenitor cells giving rise to cancer cells. The niche affecting conversion of stem cells into cancer stem cells is hypothesized ‘cancerous niche’ in this review

exhibited high tumorigenicity with rapid growth in nude mice while parental miPSCs provided only benign tumors, teratomas. Among established miPS-CSCs, miPS-LLCcm cells, which were derived from miPSCs cultured in the media containing conditioned medium of Lewis lung carcinoma (LLC) cells, showed highly angiogenic and typically malignant phenotype after transplantation into nude mice. The procedure of establishment and assignment of this cell to a CSC is briefed below.

Based on our hypothesis that the ‘cancerous niche’ could generate CSCs by transforming or differentiating normal stem cells (Fig. 11.1), we cultured miPSCs with conditioned medium of LLC cells in the absence of mouse embryonic fibroblasts (MEF) as feeder cells and leukocyte inhibitory factor (LIF). After 4 weeks of culture, survived cells were expanded in the normal medium without MEF and LIF. Interestingly, in this condition, approximately 30–50% of cells retained GFP expression, which was under the control of *Nanog* promoter (Okita et al. 2007), indicating those cells should be undifferentiated.

This implies that the mechanism(s) for maintenance of undifferentiated state of miPS-LLCcm cells should not depend on MEF or exogenous LIF. To evaluate the self-renewal capacity of the cells, we examined the growth of the cells in suspension culture. The formation of spheroids expressing GFP was observed. When the spheroids were dissociated, individual cells formed new spheroids during serial passage in the suspension culture.

miPS-LLCcm cells in either adherent or suspension culture formed adenocarcinomas in nude mice exhibiting cells with high nuclear to cytoplasmic ratio, nuclear pleomorphism, aberrantly high mitotic rates, and multiple pathological mitotic figures. Furthermore, CD31 positive staining in the tumor showed multiple vascular vessels, indicative of angiogenesis (Fig. 11.2). Totally, the histology revealed that the tumors formed by miPS-LLCcm cells were malignant. It is noteworthy that 30–50% of the cells were GFP-positive in the tumors derived from miPS-LLCcm cells.

The characters of self-renewal capacity and tumorigenicity observed in miPS-LLCcm cells

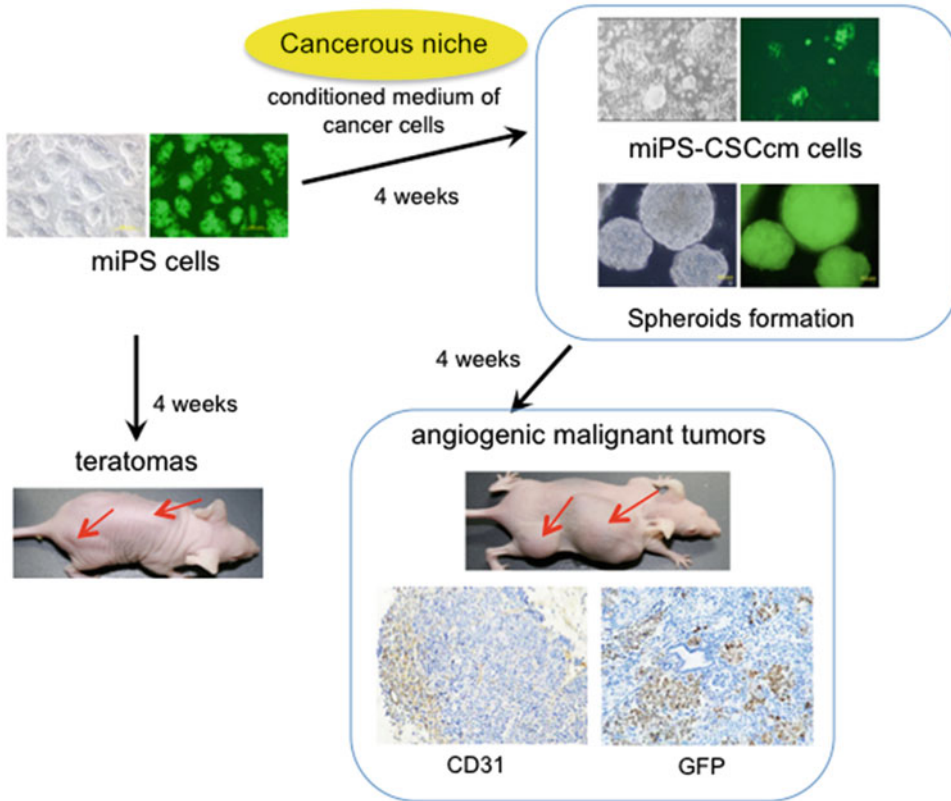


Fig. 11.2 Establishment of CSC model from miPS cells. miPS cells (Okita et al. 2007) were cultured in the media containing the conditioned medium of various mouse cancer

cell lines as the cancerous niche. Resultant cells showed CSC properties such as sphere formation and malignant tumorigenicity with extensive angiogenesis in vivo (Chen et al. 2012)

are consistent with the definition of CSCs (Clarke et al. 2006). Thus, we are proposing miPS-LLCcm cells as a model of CSC. Furthermore, the section from the tumor derived from miPS-LLCcm showed that more than half of the mesenchymal cells in the stroma of tumor turned out to be GFP negative. Also the gland-like structure was extensively stained with anti-cytokeratin antibodies (Chen et al. 2012). From these observations, we concluded miPS-LLCcm cells have the potential of differentiation, showing the heterogeneous lineage in the tumor. As for the differentiation potential of miPS-LLCcm cells, we will discuss again in the following section together with the previous reports on the differentiation of CSCs in glioblastomas (Ricci-Vitiani et al. 2010; Wang et al. 2010; Soda et al. 2011).

Potential of Differentiation and Induction of Angiogenesis

The capacity of differentiation in CSC should be considered with tumor angiogenesis. One of the definitive characters of CSC is the multipotency to create heterogeneous lineages in tumors. Three independent groups have recently reported the origin of blood vessels in tumors (Ricci-Vitiani et al. 2010; Wang et al. 2010; Soda et al. 2011). In the glioblastoma, a subpopulation of endothelial cells was found to carry the same somatic mutation as found in tumor cells, indicating the endothelial cells arose from the neoplastic origin. A series of analyses indicated that glioblastoma stem-like cells could differentiate into vascular endothelial cells in tumors. Because of the extensive

angiogenesis in the tumor derived from our miPS-LLCcm cells, the CSC model should be a critically important source to investigate precise mechanism of tumor angiogenesis. Our preliminary data show miPS-LLCcm cells could differentiate into endothelial cells forming tubular structure in vitro. The results from our study with miPS-CSCs will shed light on the molecular mechanisms of both differentiation of CSC and angiogenesis in tumors. Through the analyses, the physiological significance of the differentiation potency and self-renewal capacity in CSCs could be further clarified because endothelial cells are considered to create a stem cell niche promoting self-renewal of CSCs (Krishnamurthy et al. 2010; Zhu et al. 2011).

Future Applications of Cancer Stem Cell Model: Anti-Cancer Stem Cell Agents

It is widely known that CSCs show resistance toward the conventional chemo- and radiation-therapy. The characters of the resistance are considered to be one of the reasons for recurrence in patients after clinical treatments. The mechanisms involved in this resistance include expression of ABC drug pumps, expression of anti-apoptotic proteins, resistance to DNA damage, and so on (Zhou et al. 2009). The CSC model generated in vitro could be useful as tools to ask why and how they acquired those resistances and what kinds of molecules are critically responsible for them. They also should be useful to screen new anti-cancer agents that would eliminate CSCs by restricting their survival and/or differentiate CSCs to make them more sensitive to traditional drugs, because a large number of CSCs should be required to perform drug-screening in a high-throughput manner.

Future Applications of Cancer Stem Cell Model: Cancer Stem Cell Vaccine

Despite an attractive theory, cancer vaccination in clinical trials has not been satisfactory or successful. A reason of failure might be unexpected

presence of CSCs in tumors, which could not be characterized in detail at the diagnosis. Very recently, it has been shown that cancer vaccination induced the expression of *Nanog* in the tumor cells, and raised the relative quantity of immune-resistant stem-like cells in the tumor mass (Noh et al. 2012). Although further investigation is necessary to elicit the molecular mechanisms for selection of *Nanog* expressing cells, this report implies the contribution of CSCs to the acquisition of immuno-tolerance/escape of tumor. In the mean time, enriched CSCs were described immunogenically more effective than the whole cells in the tumor to induce protective antitumor immunity (Ning et al. 2012). Their results proposed the novel type of cancer immunotherapy against CSCs. Enough amounts of CSCs will be required as much as the drug screening process to generate vaccine in good quality and quantity. Thus, CSC models generated in vitro would have a great advantage as good sources of antigen.

Hypothetical Balance Between Stem Cells and Cancer Cells

Several studies have recently demonstrated that the ESC niche could have significant influence on the phenotype of aggressive cancer cells (Tzukerman et al. 2006; Postovit et al. 2008; Costa et al. 2009). These results indicate that the malignant phenotype of cancer cells could be suppressed in embryonic niche, accompanied by alternative expression of miRNAs and by change in epigenesis such as DNA methylation. The tumor microenvironment is supposed to play important roles in the initiation, progression and metastasis of cancer (Hu and Polyak 2008; Laconi 2007). It has been reported that tumor cells can inhibit p53 induction, one of the most famous tumor suppressor, in the fibroblasts adjacent to the tumor tissue. This suppression was considered to be dependent on the factor secreted from tumor cells (Bar et al. 2009), which raised the possibility that the factors secreted from the cancer cells might confer cancerous properties to the adjacent stem cells.

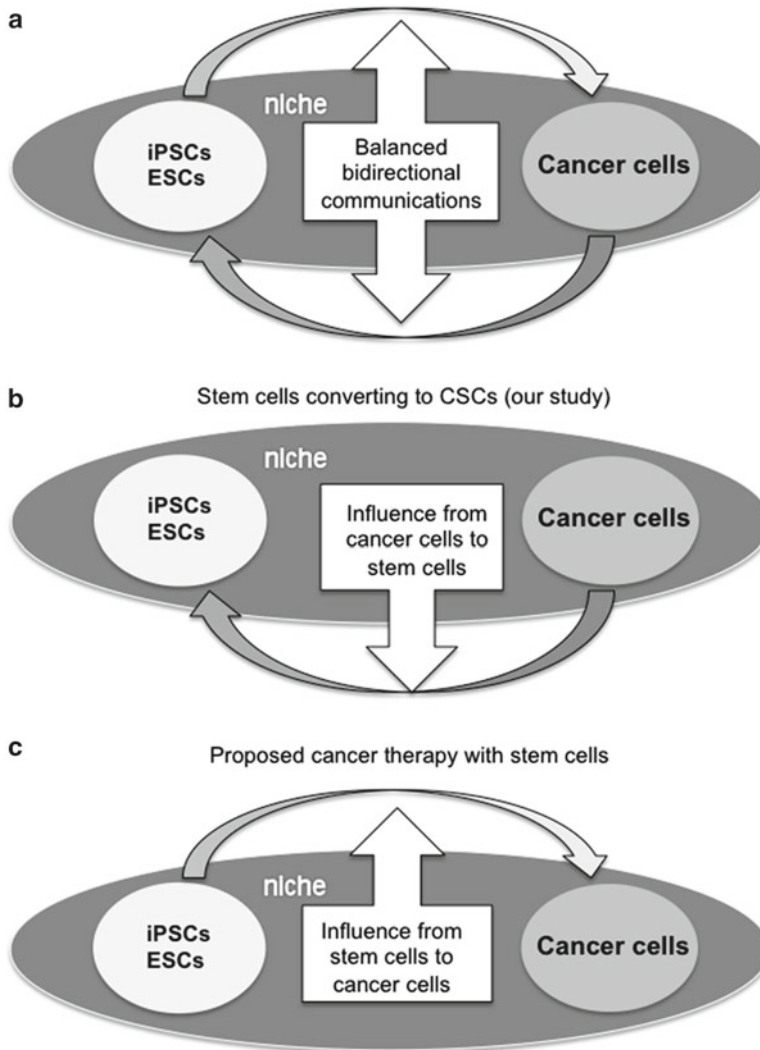


Fig. 11.3 Bidirectional communications should be present between stem cells and cancer cells. **(a)** Communications are balanced between stem cells and cancer cells. **(b)** Excessive communication from cancer cells to stem cells converts the

stem cells into cancer stem cells as shown in our study. **(c)** Excessive communication from stem cells to cancer cells suppresses the growth of cancer cells (Tzukerman et al. 2006; Postovit et al. 2008; Costa et al. 2009)

Our miPS-CSCs were obtained from the culture in the conditioned medium of cancer-derived cells but were hardly obtained in the coculture with cancer cells (Chen et al. 2012). Collectively, there appears bidirectional communications between cancer cells and ESCs (Fig. 11.3). Both communications should be regulating the activities of cancer cells and ESCs each other, so that the loss of either communication should make unbalanced regulations

that would result in converting stem cells into cancer stem cells, vice versa.

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