

Cancer stem cells contribute to tumor microenvironment.

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Abstraction

Tumor microenvironment is a key regulator of tumor initiation and progression consists of various types of cells including stromal cells, immune cells, blood vessels, neural cells and extracellular matrix. Previous studies focused on the reciprocal communications between cancer cells and functional cells in the tumor microenvironment. The functional cells in the tumor microenvironment were thought to be converted from normal cells recruited from tissues adjacent to tumors by cancer cells. Cancer stem cells are discovered in various cancers and are responsible for tumor initiation, growth, metastasis, resistance to therapies and relapse. Cancer stem cells facilitate cancer self-sustainability by continuous self-renewal and differentiation into cancer cells in the tumor mass. Recent works have demonstrated that cancer stem cells are capable to transdifferentiate and can directly give rise to components of tumor microenvironment. Here we discuss the capacity of cancer stem cells to produce neural cells and endothelial cells and pericytes that consist blood vessels in tumor microenvironment and the possibility that other functional cells of tumor microenvironment could originate from cancer stem cells.

Keywords: Tumor microenvironment, Cancer stem cell, Transdifferentiation.

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Introduction

Cancer is a self-sufficient system regulated by its microenvironment. Tumor microenvironment plays an important role in promoting all phases of cancer development including tumorigenesis, tumor growth and metastasis [1-3]. Over the last decades, intensive research has brought forth a great deal of knowledge about interactions between cancer cells and the tumor microenvironment. Tumor microenvironment can provide sustaining proliferative signal for cancer cells, induce angiogenesis, activate invasion and metastasis and help cancer cells to evade immune destruction. Up to recent years, most of the researches about tumor microenvironment focused on the reciprocal communication between cancer cells and the tumor microenvironment [4,5]. It is widely perceived that cancer cells recruit functional cells to infiltrate into tumor mass and the attracted cells in turn promote tumor growth and metastasis.

However, it is largely unknown how cancer cells, especially cancer stem cells take part in the process of the formation of tumor microenvironment. Recent works have brought about evidence showing that cancer stem cells can produce functional cells in tumor microenvironment. Targeting the process of cancer stem cells producing functional cells in tumor microenvironment may offer new therapeutic options for cancer treatment.

The Multi-Differentiation Potential of Cancer Stem Cell

Cancer stem cells (CSCs), also known as cancer stem-like cells, tumor-initiating cells or tumor-propagating cells, refer to a subpopulation of tumor cells that have the ability of self-renewal. CSCs were first identified in acute myeloid leukemia

[6] and then discovered in various solid tumors [7-9]. The main function of CSCs is to sustain and promote tumor growth by differentiate into cancer cells. In colon cancer, the stem-like population, which is CD44+/EpCAM+ or CD133+ initiates tumorigenesis and differentiates into colon cancer cells [8,10]. Not only can CSCs give rise to a new tumor that is very similar to the original tumor by self-renewal, but CSCs also share the potential of multi-lineage differentiation with normal stem cells. For example, glioma stem cells share some of the regulatory mechanisms with normal neural stem cells and have the potential to differentiate into progeny cells bearing neuronal or glial markers [11]. For glioma stem cells, it is reasonable to produce progeny cells with other neural markers under disordered regulation because of their neural origin. However, cancer stem cells also display the capability of trans-differentiation. The trans differentiation potential of CSCs is usually related with the function of tumor microenvironment.

Cancer Stem Cells Give Rise to Component of Stroma

Cancer associated fibroblast is one of the major types of stromal cells in tumor microenvironment [12]. Cancer associated fibroblasts are similar to normal fibroblast, therefore is considered to originated from resident normal fibroblast [13]. Pericytes can convert into fibroblast during acute tissue injury [14]. Cancer associated fibroblasts can also originate from cells that went through epithelial-mesenchymal transition (EMT) [15], a well-defined program in carcinoma. A study using CSC-like cell spheres from breast cancer cell lines has shown that CSCs can transdifferentiate in to myofibroblast-like cells *in vitro* [16]. Further study is needed to determine the capability of CSCs to transdifferentiate into cancer related fibroblasts *in vivo*.

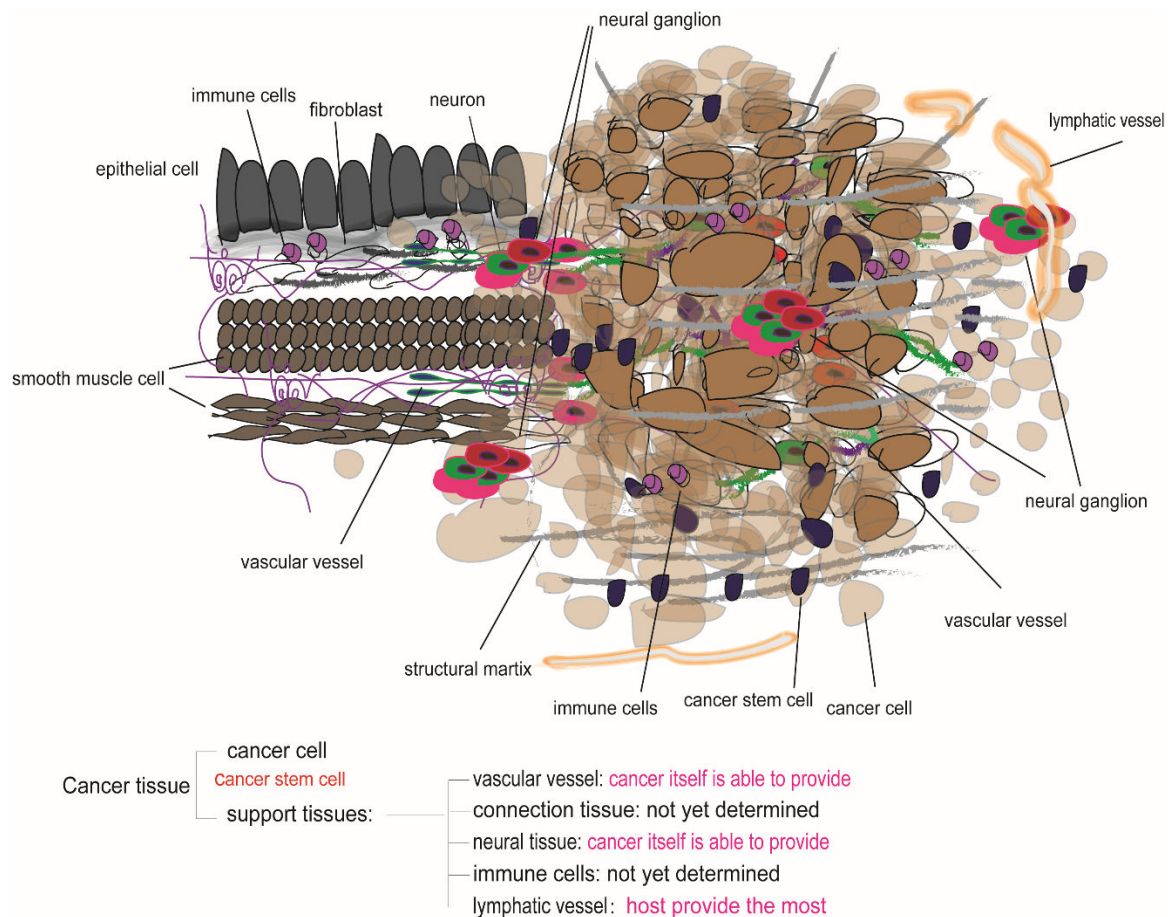


Figure 1. The constitution of cancer tissue. The cancer tissue consists of cancer cells including cancer stem cells, supporting tissues including stromal cells, immune cells, blood vessels, neural cells, extracellular matrix and lymphatic vessels.

Cancer Stem Cells and Immune Cells in Tumor Microenvironment

There are certain types of immune cells residing in tumor microenvironment, such as macrophages, dendritic cells and T cells. Tumor associated macrophages (TAMs), the major immune cells types in tumor microenvironment, play important roles in tumor growth and metastasis [17]. TAMs originate from progenitors in bone marrow derived from hematopoietic stem cell or progenitors in spleen [18]. Other immune cells in the tumor microenvironment generally originate from hematopoietic stem cell in the bone marrow. No direct evidence has proven that CSCs are capable of trans-differentiation into immune cells while some studies suggested the possibility. The colorectal cancer cells obtain higher metastatic ability and immune evasion capacity after fusing with macrophages and the accompanied nuclear reprogramming [19]. A haemato-epithelial cancer compartment in epithelial carcinoma resulted from the fusion of hematopoietic stem cell and cancer cells contribute to cancer cells stemness [20]. This lead to the speculation that cancer stem cells may fuse with cells derived from hematopoietic stem cells or directly transdifferentiate into tumor associated immune cells [21].

Cancer Stem Cells Contribute to Tumor Associated Blood Vessels

Among all the components of tumor microenvironment, blood vessels, especially endothelial cells, were the first to be considered suitable for drug and antibody development. Small molecules and antibodies targeting VEGF and PDGF and their receptors have become super stars in the pharmaceutical market. Neoangiogenesis antagonists are effective at first but the treatment is usually interrupted by recurrence and tumor progression [22]. It is possible that the failure of neoangiogenesis antagonist therapies are caused by the CSCs that are capable to contribute to tumor associated blood vessels.

Glioblastoma stem cells (GSCs) are the first to be found to have the ability to transdifferentiated into endothelial cells. It was previously reported that in the presence of endothelial cells, a small fraction of cultured neural stem cells could transform into cells that express endothelial markers rather than neuronal or glial markers and the converted cells have the capacity to form capillary networks [23]. Not only do glioblastoma stem cells share the ability of neural stem cells to differentiated neuronal and glial cells, but they also share the potential to transdifferentiate into endothelial cells [24,25]. When cultured in endothelial conditions, the CD133+ GSCs produce progeny cells expressing endothelial markers such as

CD31, CD144 and Tie2. Glioblastoma neurospheres could form capillary networks when cultured under endothelial conditions. Furthermore, functional endothelial cells and blood vessels with human origin are found in tumor xenograft produced from subcutaneous injection of GSCs in immunocompromised mice. The endothelial trans differentiation capacity of GSCs is critical to the tumor growth and cannot be impaired by anti-angiogenesis agent bevacizumab.

Vascular pericytes play critical roles in various physiological contexts, including support of vascular structure and function [26]. Previous studies suggested that pericytes in the tumor microenvironment derive from pericyte progenitors in surrounding normal tissues or from bone-marrow-derived cells (BMDCs) in tumors. Pericytes share some of the characteristics of fibroblast and can be produced by neural stem cell [27]. The hypothesis that cancer stem cells can produce pericytes was confirmed *in vitro* and *in vivo* using glioblastoma stem cells [28]. Taking advantages of fluorescent lineage-tracing reporter system, Bao and colleagues discovered that GSCs can transdifferentiate into pericytes *in vitro* and *in vivo* xenograft mouse model [28]. It was demonstrated that pericytes in primary human GBMs are commonly derived from neoplastic cells and inhibition of tumor growth and disruption of blood vessels happened when the pericyte potential of GSCs is impaired. The capacity of GSCs to generate vascular pericytes allows active vascularization in GBMs to support tumor growth, further indicating that cancer cells not only recruit and interact with but also contribute directly to blood vessel compartment of tumor microenvironment.

The endothelial potential is not restricted in neural cancer cells. It was recently reported that cancer stem cells of intestine carcinoma also have the capacity to generate functional endothelial cells [29]. As shown by our laboratory, human colorectal cancer stem cells are able to generate endothelial marker expressing cells both *in vitro* and in xenograft model *in vivo*. The functional blood vessels in tumor xenograft produced from subcutaneous injection of human cancer stem cells are constituted by endothelial cells with mouse origin together with human endothelial cells or endothelial cells with human origin exclusively. The co-existence of pure-human blood vessels and chimeric blood vessels indicates that cancer stem cells are able directly to contribute to the vasculature process and work in a flexible and economical way. The ability to take advantage of normal blood vessels while producing vascular structure on their own helps cancer to self-sustain and grow rapidly.

Cancer Stem Cells Contribute to Neural Component of Tumor Microenvironment

Nerves are a common component of normal tissue microenvironment which plays an important role in regulating tissue genesis and homeostasis [30]. Normal stem cells are closely regulated by autonomic nerves in their niche [31,32]. Stress related disorder is known to contribute to the development of various diseases for a long time [33]. The effect of stress on cancer is also noticed in early years.

Autonomic nerves are involved in the regulation of leukemia, lymphoma and solid tumors [34]. A special behaviour called perineural invasion happens in various tumors and is usually considered a signal of poor prognosis [35]. Apart from providing a way for cancer metastasis *via* cancer cell perineural invasion, cancer-nerve interactions also play important roles in the regulation of tumor genesis, growth and metastasis [36]. Therefore, nerves are considered to be a critical component of tumor microenvironment [37].

The interactions between cancer cells and nerves are similar to the cross-talk between cancer cells and blood vessels. Cancer cells could attract and recruit nerves to infiltrate into tumors by the secretion of neural growth factor or neurotrophic factor [38]. The nerves infiltrated into tumors in turn facilitate the growth and metastasis of cancer. In a milestone paper, Magon and colleagues reported that sympathetic nerves and parasympathetic nerves contribute to the development of prostate cancer [39]. The tumorigenesis is inhibited in mouse prostate cancer model after the ablation of sympathetic nerves. The tumor invasion of later stage of prostate cancer is regulated by cholinergic parasympathetic signals. Similar effect was found in the mouse model of gastric cancer that nerves play a critical role in the genesis and progression of cancer and even the resistance to therapeutic effect of chemotherapy [40].

Tumor associated nerves also participate in the regulation of genesis and homeostasis of tumor associated blood vessels. Sympathetic adrenergic nerve-derived noradrenaline in the prostate stroma is critical for the activation of angiogenesis. The catecholamine signal accepted by β 2-adrenergic receptor induced a switch from oxidative-metabolism to an aerobic glycolysis like metabolism and promote the angiogenesis effect of prostate epithelial cells [41].

The trans differentiation potential of cancer cells was first identified in glioblastoma stem cells. However, it would be impossible to assess the capacity of cancer stem cells to transdifferentiate into neural cells such as neurons and glial cells using glioblastoma stem cells due to their neural origin. Recently, we reported the neural potential of cancer stem cells of gastric and colorectal carcinoma [42]. Neural stem cells are induced to differentiate into neurons when cultured in the presence of retinol (vitamin A). When cultured in differentiation inducing medium containing low concentration of foetal bovine serum and vitamin A, a small fraction of gastric cancer stem cells (GCSCs) and colorectal cancer stem cells (CCSCs) are able to produce progeny cells with neuron markers such as microtubule associated protein 2 (MAP2) and tyrosine hydroxylase (TH). Some of the cancer stem cell-derived neuron-like cells even expressed synapse markers such as Synapsin and synaptic vesicle protein 2 (SV2) [42]. The expression of synaptic markers suggests that neurons originated from cancer stem cells could be functional and link to other neurons to form neural circuits. However, this hypothesis needs to be validated in future studies. What is interesting is that neurons originated from human cancer stem cells are observed in tumor xenograft produced by intraperitoneal injection of GCSCs and CCSCs rather than

tumor xenograft produced by subcutaneous transplantation of CSCs [42]. The distinct differentiation behaviour of CSCs displayed *in vivo* demonstrated the complexity of the regulation of the stemness and differentiation potential of CSCs. Furthermore, we discovered that the neural potential of different CSC mono-clones derived from a same patient varied a lot, which revealed the heterogeneity of cancer stem cells and indicated the existence of a hierarchical structure in the cancer stem cell population. GCSCs and CCSCs produced fewer neuron when the neural potential of CSCs is inhibited by the disruption of the expression of neuron marker MAP2. Knocking-down MAP2 also inhibited the tumor initiating and promoting capacity of GCSCs and CCSCs, indicating that neurons originated from CSCs play a critical role in the tumor microenvironment.

Future Perspectives

Tumor could be understood as a self-sustaining parasitic organ derived from disordered differentiation of stem like cells. Many of the cancer biological processes such as tumor growth and cancer cell metastasis are largely regulated by the tumor microenvironment. The need to achieve self-sustainability requires that cancer cells should not only be able to recruit functional cells from normal tissues and convert them into tumor associated cells to form tumor microenvironment but also be capable to produce progeny cells directly contribute to the component of tumor microenvironment. The capacity of CSCs to transdifferentiate into neural and endothelial cells which constitute tumor microenvironment is demonstrated *in vitro* and *in vivo*. Further studies are needed to address the possibility that CSCs directly give rise to stromal cells and immune cells of the tumor microenvironment. We initiated to measure lymphatic vessels in human gastric and colorectal carcinoma and identified that lymphatic vessels locate outside of the cancer tissues. In the murine intraperitoneal and subcutaneous xenografts produced by the gastric and colorectal CSCs, almost of all of lymphatic vessels are mouse origin, suggesting that the cancer cells could not contribute to lymphatic vessels.

CSCs displayed distinct differentiation potential when transplanted in different body positions of immunocompromised animal. When injected to a position closer to the original position where the primary tumor occurred, the CSCs are more likely to show their multipotential. Therefore, it is preferable to transplant CSCs orthotopically rather than inject CSCs subcutaneously when trying to analyze the differentiation potential of CSCs.

The stemness of stem cells could not be maintained permanently. The function and differentiation potential of stem cells gradually declines in the process of cell aging [43]. When cultured *in vitro*, the proliferation capacity of stem cells declines after repeated passaging [44]. *In vitro* and *in vivo* differentiation experiments have shown that CSCs would lose the capacity to transdifferentiate into endothelial cells and neurons after continuous passaging [29,42]. Taken together with the heterogeneity of cancer stem cells, the differentiation potential of CSCs revealed in different studies is likely to be

varied, even when the cancer type been studied is the same. When trying to induce glioblastoma stem cells to differentiate under similar culture condition, some researchers obtained endothelial cells while others obtained pericytes instead [7,24,28]. CSCs derived from cancer cell lines may not be the ideal model to study cancer stem cell biology because the heterogeneity and hierarchy of cancer stem cells may not be presented as well as in primary cancer stem cells derived from cancer patients. It should be better to conduct experiments on newly isolated CSCs. However, this could bring difficulties to researchers especially the ones who lack the access to a large amount of patient samples.

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