Mutual dependence between cancer stem cells and their progenies: the niche created by the progenies is sustaining cancer stem cells

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The critical features of cancer stem cells (CSCs) are tumorigenicity, self-renewal and differentiation, which are considered to be responsible for tumor maintenance. It has been proposed that CSCs create a heterogenetic population in tumor by giving rise to diverse progenies in the apex of differentiation hierarchy. Our newest results indicate that differentiated cells from CSCs secreted soluble factors to keep the balance between self-renewal and differentiation of CSCs, partially via activation of Notch signaling pathway. The cellular population of tumor could be varied in response to the dynamic cellular communications in CSC niche, which is produced by CSCs themselves.

Keywords: cancer stem cell; niche; self-renewal; differentiation; endothelial cell

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It is widely accepted nowadays that cancer stem cells (CSCs) possess self-renewal capacity to maintain themselves as well as the potential to differentiate to numerous cancer cells thereby CSCs acquire tumorigenic properties. The cell populations with properties of CSC have been isolated from various types of malignant tumors and cancer cell lines, such as breast cancer [1], glioblastoma (GBM) [2], lung carcinoma [3], liver cancer [4], pancreatic cancer [5] and so on. CSCs are reported to be often resistant to clinical treatments for which the cancer relapse was attributed. Thus, understanding the mechanism(s) for maintenance of CSC population in tumor should be necessary to improve therapeutic strategies against cancer.

Giving the similar behavior between CSCs and normal stem cells, many researchers are focusing on the mechanism of the maintenance of CSC by the microenvironment “niche” [6, 7, 8], where CSCs reside and which is necessary for the maintenance of unique properties of CSCs [9]. The critical factors including soluble molecules, extracellular matrix proteins, stromal support cells and blood vessels [10] make up the unique normal/cancer stem niche. Normal tissue stem cells are responsible for organogenesis with their multi-potency, which should be regulated by niche. Similarly, CSCs as the origin of cancer cells can cause the heterogeneous population of cancer cell in tumor. In turn, the heterogeneous
population should be necessary for controlling the fate of cancer stem cells.

We have established CSC-like cell lines from mouse iPS cells\[^{11}\]. Using one of these cell line, miPS-LLCcm, we analyzed the relationship between CSC population and differentiated cancer cells in vitro, and found out that these two populations were dynamically balanced. The progenies of CSCs promote the self-renewal and modulate the differentiation of the CSCs\[^{12}\].

Taking advantages of the expressions of Green Fluorescent Protein (GFP) and puromycin resistant genes, which are cloned under the control of Nanog promoter in the miPSCs\[^{13}\], we could distinguish the stem-like cells from differentiated cells in the miPS-LLCcm bulk culture. The undifferentiated CSCs could be condensed in the presence of puromycin and spontaneous differentiation of CSCs could be allowed by removing puromycin from media. First, we observed that our miPS-LLCcm cell could differentiate into endothelial cells and form tube-like structure \textit{in vitro} on matrigel as in the case of glioblastoma reported previously\[^{14}\]. Intriguingly, the potential of endothelial differentiation was decreasing after repeated depletion of differentiated cells followed by spontaneous differentiation, implicating that this differentiation lineage was deprived in the absence of the secreted factor(s) from differentiated cells.

The self-renewal capacity of miPS-LLCcm was examined in suspension culture. The number of spheres significantly increased in the presence of conditioned medium, which had been prepared from bulk cell culture of miPS-LLCcm (CM-ad). This enhancement of sphere formation was partly blocked by DAPT, an inhibitor of Notch signaling pathway. Interestingly, the conditioned medium (CM-sp) prepared from CSCs (puromycin selected cells) had much less effects on the growth of CSCs than in CM-ad and the growth promoting activities were not inhibited by DAPT. These observations implicated that the activation of growth promoting Notch signaling in CSCs was stimulated by factor(s) secreted from the differentiated cancer cells, not from CSCs themselves. We also detected soluble form of typical Notch ligands, Dll1 and Dll4, both

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Figure 1. The model of CSC vascular niche created by CSCs themselves. CSCs can differentiate to endothelial cells and other cancer cells. The factors secreted from both endothelial cells and cancer cells can promote the self-renewal of CSCs. And the secreted factors from progenitor cells of cancer cells and CSCs themselves can promote the CSC self-renewal. Also, CSCs can secrete factor(s), such as VEGF, to promote the endothelial differentiation of CSCs themselves.
in CM-ad and CM-sp, noted that it was also present in CM-sp. None of typical ligands was specifically expressed in differentiated population. These observations suggested that the noncanonical mechanism(s) for Notch activation might be involved in the self-renewal of CSCs, and that the cancer cells differentiated from cells from CSCs should produce unrevealed factor(s) for Notch activation.

As mentioned above, after three rounds of cultivation in the presence or the absence of puromycin, the cells could no longer differentiate into endothelial cells. The conditioned medium prepared from the bulk cells after third round also promoted sphere formation of miPS-LLCcm cells. Since DAPT treatment has negligible effect on the sphere formation, notch activation was no more essential. These data suggested that cells differentiating/differentiated into vascular endothelial cells might be possible candidates for the source of Notch activator(s). Taken together, miPS-LLCcm can give rise to differentiated cancer cells and endothelial cells, which provide the CSC niche to promote self-renewal of CSCs themselves.

Several groups reported, the CSCs reside adjacent to the tumor blood vessels, which termed the vascular CSC niche to maintain the stemness stage. For example, through PI3K/Akt pathway, the stem-like medulloblastoma cells, which resided closely to the vascular niche, survived after radiation and underwent p53-dependent cell cycle arrest. Combined with anti-angiogenic agent, the anticancer treatment significantly reduced the tumor sphere formation. Both of these results suggest the vascular niche is important for CSCs maintenance. Thus, it has become an area of intense investigation to fully define the element of tumor vascular niche.

The classical model of tumor angiogenesis was widely accepted that the blood vessels formed from the pre-existing vessels by sprouting controlled by various growth factors, which were released by either the host cells or the tumor cells. However, investigators from our laboratory and others recently provided evidences that vascular endothelial cells are the members of the cells differentiated from the CSCs established from miPSCs, whereby the rapid growth of the solid tumors can be fueled as well as glioblastoma, ovarian, breast. The most famous model of this scenario is GBM stem cells. Besides the nutrition provision, vascular endothelial cells can be components of CSC niche to promote CSCs self-renewal. They have been shown to promote stem-like phenotype formation through Hedgehog signaling pathway. The perivascular expression of osteopontin, which is one of the ligand for CD44, can promote the stem cell-like properties and radiation resistance through enhancement of HIF-2α activity. Also, nitric oxide (NO) produced by endothelial cells can activate Notch signaling pathway, thereby reinforce the stem cell like character. Recent studies showed Notch ligands were expressed by endothelial cells and some tumor cells surrounding the Notch receptor positive CSCs in primary GBM tumors. Therefore, vascular endothelial cells are conceivable components of CSC niche to promote the self-renewal of CSCs.

However, increasing evidence indicates the CSCs and their vascular niche should make mutual communication (Fig. 1). Either under direct or indirect-culture with neural stem cells, endothelial cells isolated from brain were induced to form and maintain the vascular tubes. They showed the mutual contributions of neural stem cells and brain-derived endothelial cells in induction and maintenance of the neurovascular niche, underscoring their dynamic interactions. When compared to the more differentiated cell population, tumor derived stem cell-like glioma cells have been shown to secrete evaluated level of VEGF, which is well known as a key factor of angiogenesis, stimulated the tumor vascularization. As mentioned above, we found the differentiation capacity of CSCs towards endothelial cells is regulated by the factors secreted from the progenies of CSCs. This feedback regulation should be the result of heterogeneity maintained by their self-created niche. Our findings will contribute to comprehensively understand the maintenance of CSCs through interactions with their niche. This will lead to the novel clinical strategy against cancer stem cells in tumor therapy.

Conflicting interests

The authors have declared that no competing interests exist.

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