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Potential Signaling Pathways Activated in Cancer Stem Cells in Breast Cancer

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1. Introduction

Accumulating evidence suggests that cancer stem cells—which make up only a small proportion of heterogeneous tumor cells—possess a greater ability to maintain tumor formation than other tumor cell types. It has been proposed that cancer stem cells have characteristics in common with normal stem cells from tumor-prone tissue. For instance, cancer stem cells can self-renew and simultaneously produce differentiated daughter cells that proliferate strongly until they reach their final differentiated state. Apparent differences also exist between cancer stem cells and normal stem cells. The latter are maintained under tight homeostatic regulation and are passively protected in the surrounding microenvironment or stem cell niche in adult tissues. However, the former may actively contribute to tumor formation. This concept was first proposed from the research of hematological malignancy, however, it is now believed that many solid tumors also have cancer stem-like cells. Although the concept of cancer stem cells greatly impacts cancer biology and evokes a reconsideration of cancer treatment, the molecular mechanisms involved in the contribution of cancer stem cells to tumorigenesis remain to be obscure. There have been many attempts to identify signaling pathways specifically activated in cancer stem cells. For example, it has been proposed that transforming growth factor (TGF)- β pathway, the epithelial-mesenchymal transition (EMT) pathway or nuclear factor- κ B (NF- κ B) pathway may be activated in cancer stem cells. These potential pathways may contribute to self-renewal activity of cancer stem cells or have an influence on cancer stem cell niche. In this review, I would like to summarize our present understanding about potential signaling pathways activated in cancer stem cells in solid tumors, especially focusing on breast cancer, and then describe our recent findings about potential signaling pathways in breast cancer. Finally I would like to discuss how this increasing knowledge is utilized for developing novel molecularly targeting drugs for cancer treatment.

2. Definition and characteristics of cancer stem cells

The consensus definition of a cancer stem cell is a cell within a tumor that possess the capacity to self-renew and to cause the heterogenous lineages of cancer cells that comprise the tumor. Cancer stem cells can thus only be defined experimentally by their ability to recapitulate the generation of a continuously growing tumors. The implementation of this

approach explains the use of alternative terms in the literature, such as “tumor-initiating cell (TIC)” to describe putative cancer stem cells (Clarke, 2006).

Stem cells are defined by both their ability to make more stem cells, a property known as ‘self-renewal’, and their ability to produce cells that differentiate (Fig. 1) (Morrison and Kimble, 2006). One strategy by which stem cells can accomplish these two tasks is asymmetric cell division, whereby each stem cell divides to generate one daughter cell with a stem-cell fate and one daughter cell that differentiates. Stem cells can also use symmetric divisions to self-renew and to generate differentiated progeny. Symmetric divisions are defined as the generation of daughter cells that are destined to acquire the same fate. It is thought that stem cells use combination of both asymmetric and symmetric cell divisions to self-renew, proliferate, and differentiate. Both cancer stem cells and normal stem cells have the such similar characteristics.

Cancer stem cell shares many other properties with the normal stem cell. Normal stem cells exist properties that provide for a long lifespan such as relative quiescence, resistance to drugs and toxins through the expression of several ATP-binding cassette transporters, an active DNA-repair capacity, and a resistance to apoptosis. Many of the characteristics are shared also by cancer stem cells. Cancer stem cells have a long lifespan, and self-renewal capacity enabling them to maintain and expand the cancer cell population, although they themselves are quiescent and rarely proliferation.

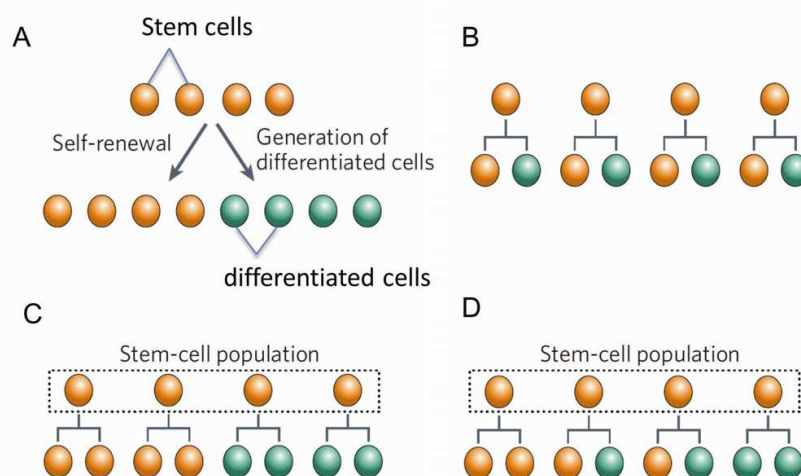


Fig. 1. Asymmetric and symmetric cell division of stem cells.
A, Stem cells self-renew and differentiate into progenitor cells.

B, Asymmetric cell division.

C, Symmetric cell division.

D, Combination of asymmetric and symmetric cell division.

An obvious question is where cancer stem cells arise; are they derived from normal stem cells or not? In hematological malignancy, it has been documented the existence of malignant stem cells in AML and CML. It has been thought that leukemic stem cells arise by mutation from normal stem cells or by mutation from progenitor cells, evoked by genomic instability in malignant cells. The mutation-prone property of malignant cells may even gives a self-renewing ability to the progenitor cells that do not have such ability originally.

Apparent differences also exist between cancer stem cells and normal stem cells. The latter are maintained under tight homeostatic regulation and are passively protected in the

surrounding microenvironment or stem cell niche in adult tissues. However, the former may actively contribute to tumor formation and may use cancer stem cell niche for their own survival.

3. The impact of the cancer stem cell hypothesis on the cancer therapy

To develop more effective cancer therapies, it is critical to determine which cancer cells have the potential to contribute to tumor progression. Because it was thought that most cancer cells proliferate extensively, traditional cancer therapies aim to eliminate as many cancer cells as possible by targeting cells with increased proliferation activity. However, relapse occurs in a significant number of patients even after complete tumor resection and systemic treatment involving chemotherapy and/or radiotherapy. In these circumstances, a recently proposed hypothesis involving cancer stem cells has drawn great attention. It is hypothesized that heterogeneous tumor tissue is maintained in a hierarchical organization of rare, slowly dividing cancer stem cells; rapidly dividing progenitor cells; and differentiated tumor cells. The growth and progression of tumors are thought to be driven by such subpopulations of cancer stem cells. Therefore, it is thought that cancer stem cells are relatively resistant to conventional chemotherapy and radiotherapy and might survive after systemic treatment. These cells may remain dormant for years but eventually cause relapse. Therefore, cancer therapy should target cancer stem cells that were not targeted by conventional therapy. Although the concept of cancer stem cells greatly impacts cancer biology and evokes a reconsideration of cancer treatment, the molecular mechanisms involved in the contribution of cancer stem cells to tumorigenesis remain obscure.

Potential cancer stem cells were first identified in hematological malignancies such as leukemia. Among solid tumors, breast cancer and brain tumors were firstly shown to have cancer stem cells. Subsequently, it has been shown that many types of cancer or tumors have cancer stem cells, such as colon cancer, pancreatic cancer, prostate cancer, lung cancer and melanoma.

4. Breast cancer stem cells

The development of biomarkers to identify breast cancer stem cells as well as the validation of in vitro and mouse models has facilitated the isolation and characterization of these cells from murine and human tumors. In human breast cancers, the CD24^{-/low}/CD44⁺ cell population was reported to be more highly enriched in breast cancer stem cells than was the CD24^{high}/CD44⁺ cell population (Al-Hajj et al., 2003). Several groups have also identified CD24^{-/low}/CD44⁺ cells as a breast cancer stem cell-enriched population in primary human breast carcinoma (Diehn et al., 2009; Shimono et al., 2009). In addition, aldehyde dehydrogenase (ALDH) expression has been used to isolate human breast cancer stem cell populations (Ginestier et al., 2007). More recently, highly pure breast cancer stem cell populations were obtained by using the lipophilic fluorescent dye PKH26, which labels relatively quiescent cells within a proliferating population (Cicalese et al., 2009). Just as primary tumors and xenografts contain cancer stem cell populations, established breast cancer cell lines may also contain cellular hierarchies driven by a population expressing cancer stem cell markers. In addition to involvement in tumor initiation, the cells also display increased metastatic potential.

5. Breast cancer cell lines as a model system of cancer stem cells

Although final proofs of cancer stem biology should be shown by experiments using tumor cells derived from human tumor tissues, it is convenient and useful if cancer cell lines are used as a model system for exploring biology. We and others found that CD24^{-/low}/CD44⁺ cell populations exist in various type of breast cancer cell lines and that each cell line had various expression levels of CD24 and CD44 (Fillmore and Kuperwasser, 2008; Murohashi et al., 2010). Three cell lines, HCC1954, MCF-7 and HCC70 cells, had small population (<10 %) of the CD24^{-/low}/CD44⁺ cells. This situation might be similar to the early stage breast cancer tissues in which the TIC population is assumed to be small. To determine the hierarchical organization of breast cancer cell lines, we analyzed the tumorigenic potential of the CD24^{-/low}/CD44⁺ and CD24⁺/CD44⁺ cell populations of HCC1954 cell line.

The *in vivo* tumorigenicity assay is the gold standard for identifying cancer stem cells or TIC. To improve the quality of the quantitative results, we used *in vivo* bioluminescence imaging (IVIS™) to measure tumor growth (Murohashi et al. 2010). We first transduced cells with a lentiviral vector encoding luciferase or d2Venus (an improved version of yellow fluorescent protein) cDNA. We measured transduction efficiency by expression levels of d2Venus using FACS and obtained high transduction efficiency in 92.60 % for HCC1954 cells. Next, we transduced a lentiviral vector expressing luciferase into these cells. Because we used similar MOI (multiplicity of infection) levels for transduction of the lentiviral vectors expressing luciferase and d2Venus, we expected similar levels of luciferase expression in the cell line (designated HCC1954-Luc). Cells in CD24^{-/low}/CD44⁺ populations were considered to be enriched for TICs and CD24⁺CD44⁺ populations were used as controls. Cells were implanted into mammary fat pads of NOD/SCID mice and tumor growth was measured by quantifying luciferase activity with the IVIS™ Imaging System (Fig. 2). Ten thousand HCC1954-Luc and MCF7-Luc cells of both populations were implanted. After 4 weeks, the analysis of luciferase activity indicated that cells in the CD24^{-/low}/CD44⁺ populations of HCC1954-Luc and MCF7-Luc generated significantly larger tumors than the control populations ($p < 0.05$) (Fig. 2A). Moreover, when we transplanted both populations of 1×10^2 HCC1954-Luc, tumors were generated only by the CD24^{-/low}/CD44⁺ population ($n=6$) (Fig. 2B).

These results indicate that CD24^{-/low}/CD44⁺ populations in breast cancer cell lines have higher tumorigenicity than the control populations. It is therefore likely that CD24^{-/low}/CD44⁺ cells in breast cancer cell lines may behave like TICs.

We examined the histology of tumors derived from HCC1954-Luc cells from both populations when 1×10^4 cells of each population were implanted. The hematoxylin-eosin (HE) staining revealed that tumors derived from CD24^{-/low}/CD44⁺ cells showed exclusively invasive patterns, with a variety of morphologies associated with the stromal component (Fig. 3A, B). However, tumors derived from control cells consisted of invasive and differentiated patterns, with tubular formations in association with the stromal component. The stromal component was larger in tumors derived from CD24^{-/low}/CD44⁺ cells than that derived from the control cells. The fact that differentiated patterns of histology were observed only in tumors derived from the controls suggests that differentiated tumors arose from non-TICs.

Next, we assessed the cell lineage and differentiation state of tumors derived from HCC1954-Luc cells by immunostaining for cytokeratin markers (Fig. 3C-F). The invasive lesions from CD24^{-/low}/CD44⁺ cells were mostly positive for the myoepithelial marker CK-14 but were

less positive for the luminal marker CK-18. On the other hand, the invasive lesions from the control cells were mostly negative for CK-14 but were positive for CK-18, suggesting that TICs contribute to the basal cell phenotype of transplanted tumors.

From these experiments, we demonstrated that cells derived from $CD24^{-/low}/CD44^{+}$ populations resulted in tumors larger than those of $CD24^{+}/CD44^{+}$ control populations. Importantly, when as few as 100 cells were implanted, only $CD24^{-/low}/CD44^{+}$ populations gave rise to tumors (Fig. 2B). This is an important criterion for TICs. Therefore,

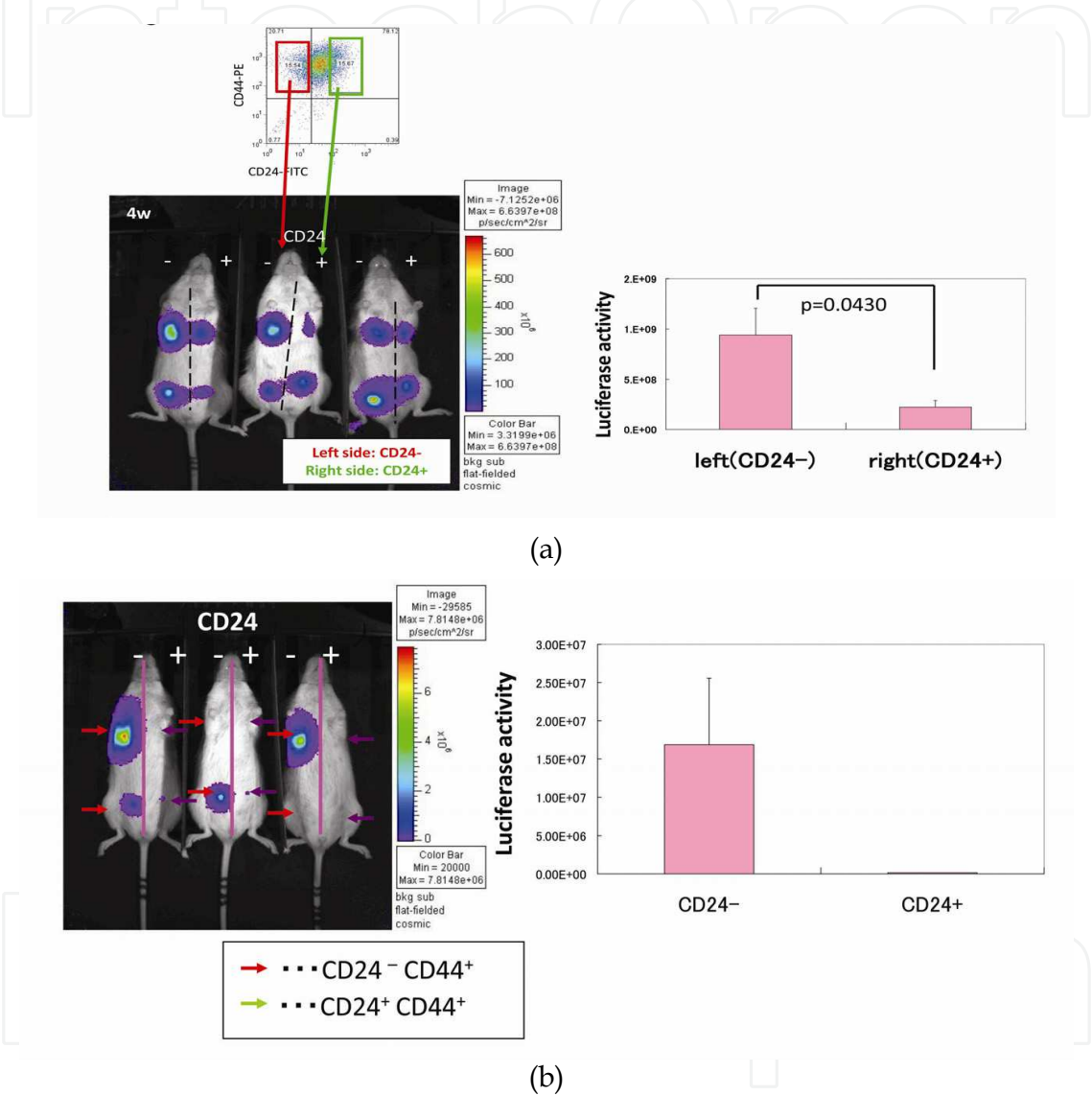


Fig. 2. Luciferase activities of $CD24^{-/low}/CD44^{+}$ cells in NOD/SCID mice. HCC1954 cells expressing luciferase were sorted by FACS. Ten percent of the entire population, belonging to $CD24^{-/low} CD44^{+}$, was selected as the TIC population ($CD24^{-}$). Ten percent of the whole population, belonging to $CD24^{+}/CD44^{+}$, was selected as the control ($CD24^{+}$). Ten thousand cells (A) or 100 cells (B) of the TIC population (left side of mice) or control population (right side of mice) cells were mixed with Matrigel and implanted in mammary fat pads of NOD/SCID mice. Luciferase activities were captured by IVIS™ after 4 weeks. Luciferase activities in implanted sites were quantified (n=6). Results are represented as the mean + SD. * $p<0.05$ (student t -test).

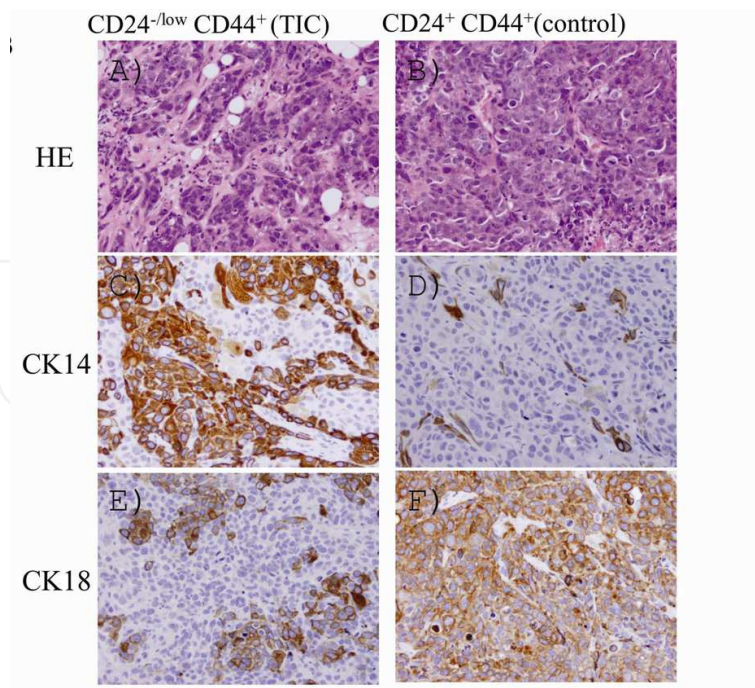


Fig. 3. Immunohistochemical analysis of tumors from HCC1954. A, HE-stained sections of the tumors derived from CD24^{-/low}/CD44⁺ cells (TICs) and CD24⁺/CD44⁺ (control) cells. B, Immunohistochemical analysis of CK-14 expression in the TIC and control populations. C, Immunohistochemical analysis of CK-18 expression in TIC and control cells. Brown staining represents a positive result.

CD24^{-/low}/CD44⁺ populations in the cell lines may be enriched with TIC-like cells. Our results revealed heterogeneity in cell populations divided into TIC-like cells and other cells. Therefore, it is reasonable to suppose that several breast cancer cell lines are heterogeneous and that they have distinct cell populations: TIC-like cells and other cells, with both cell types preserving the characteristics of TICs and other cells in primary cancer tissues, to some extent.

We further showed that tumors derived from TIC-like cells showed a more malignant histology and contained more cells positive for CK-18, in contrast with tumors derived from control cells, which exhibited more CK-14-positive cells. This suggests that TICs may not differentiate into cells with specialized or terminal patterns in this model and raises the possibility that TICs may not need to differentiate into all cell types in tumor tissues; though, normal stem cells can generate all cell types in a specific tissue. However, we cannot exclude the possibility that this transplantation model does not recapitulate the ability of TICs to differentiate into all cell types seen in breast cancer. In order to clarify this issue, other types of *in vivo* models should be analyzed.

6. In vitro assay of breast cancer stem cells

In recent years, the in vitro mammosphere formation assay has been established as a measure for the self-renewal of breast cancer stem cells. Mammospheres are floating cell aggregations, which include cancer stem-like cells, and can be serially passaged; they are obtained by culturing breast cancer stem cells in a defined medium containing growth

factors, including the epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) (Fig. 4). This medium is only a slightly modified version of the defined medium that includes the same growth factors, i.e., EGF and/or bFGF, and has been adapted for culturing neurospheres, which are aggregations of neural stem cells and their progenitors. This indicates EGF or bFGF involvement in the regulation of self-renewal of breast cancer stem cells in such in vitro culture conditions. However, little information is available regarding the regulatory mechanisms for self-renewal of breast cancer stem cells by EGF or FGF, and it is an open question whether EGF and/or FGF signaling is involved in the in vivo regulation of these cells.

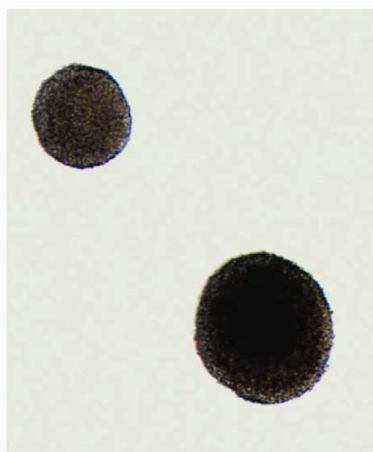


Fig. 4. Mammosphere cells derived from HCC1954 cells.

7. Inflammatory signaling pathways are potentially activated in breast cancer stem cells

Two gene expression profiling studies, comparing CD24^{-/low}/CD44⁺ cell populations with other populations in primary breast cancer cells or in normal tissue presented the CD24^{-/low}/CD44⁺ cell population-derived different signatures that seemed to predict poorer prognosis (Liu et al., 2007; Shipitsin et al., 2007). One study showed that TGF- β pathways appear to be activated in these cells (Shipitsin et al., 2007). It was subsequently reported that TGF- β induced the epithelial-mesenchymal transition (EMT) in mammary glands and stem-like cells in both normal mammary epithelial cells and breast cancer cells (Mani et al., 2008). Because TGF- β signaling can have positive or negative effects on tumorigenesis, additional signaling may still be needed to stimulate tumorigenesis (Massague, 2008).

The functional relationship between inflammation and cancer has been discussed since the 1860s (Coussens and Werb, 2002; Murohashi et al., 2010). Activation of several pathways involved in inflammatory responses has recently been detected in breast cancer stem cells. We and others recently discovered that NF- κ B, which is one of the main regulators of the transcription of inflammatory mediators, is activated in breast cancer stem-like cells.

We used gene set enrichment analysis (GSEA) which is a recently developed analytical method of gene-expression profiling. The results are easier to interpret biologically, and the method is more accurate and robust than individual gene analysis methods, such as fold change analysis of expression levels. To identify expressed genes that were highly enriched in CD24^{-/low}/CD44⁺ and control cells, we performed DNA microarray analysis using

HCC1954, MCF7, and HCC70 cell lines that have small populations of CD24⁻/CD44⁺ cells. As a control, we used CD24⁺/CD44⁺ cell populations.

We found that both TNF and IFN response gene signatures were markedly enriched in CD24^{-/low}/CD44⁺ populations (Murohashi et al.). Regarding individual genes, gene ontology (GO)-based classification revealed that genes involved in 'stemness', cell proliferation/maintenance, cell adhesion, cell motility, invasion, angiogenesis, growth factor/cytokine, immune response/suppression and metabolism were highly represented in CD24^{-/low}/CD44⁺ compared with the control cell populations. All of these genes may contribute to oncogenesis. For example, from the GSEA results, we found Notch2, a 'stemness'-related gene, LAMA3, a cell invasion- or adhesion-related gene and KLF5, EPAS1 and VEGF, angiogenesis-related genes. On the other hand, GSEA revealed that genes highly expressed in the control populations correlated with several cell-cycle-associated gene sets, which have large numbers of cell proliferation/maintenance-related genes.

One of the important effector molecules common to both TNF and IFN response pathways is NF-κB. NF-κB is a transcription factor complex and is typically a heterodimer of p50, p52, p65 (RelA), RelB and c-Rel. It is usually inactive and bound to IκB, an inhibitory protein, in the cytoplasm. Upon stimulation with signals such as TNF or IFN, IκB is first phosphorylated, then ubiquitinated and finally degraded. Released NF-κB translocates to the nucleus and binds to the κB sequence, where it promotes the transcription of various genes, including inflammatory cytokines. NF-κB has roles in inflammation, angiogenesis, inhibition of apoptosis, and tumorigenesis (Karin et al., 2002; Tabruyn and Griffioen, 2008).

We quantified NF-κB activities in nuclear extracts of CD24^{-/low}/CD44⁺ and control populations that were sorted by FACS. We found that the activity of NF-κB was significantly higher in CD24^{-/low}/CD44⁺ than in CD24⁺/CD44⁺ populations (n=4). We further examined the role of the activity of NF-κB in tumorigenesis using the mouse model. We transplanted 10⁴ cells of CD24^{-/low}/CD44⁺ populations into NOD/SCID mice, and treated them with DHMEQ, a specific inhibitor for NF-κB. In order to analyze the effects occurring during the course of tumorigenesis, we began inhibitor treatment two days after transplantation. We monitored tumor formation by *in vivo* imaging. We found that the luciferase activities of the tumors derived from CD24^{-/low}/CD44⁺ cell populations treated with DHMEQ were significantly decreased compared with that of untreated cell-derived tumors (Murohashi et al. 2010). These results suggest that NF-κB acts as a key effector of tumorigenesis derived from TIC-like cells.

Other reports have described that NF-κB-triggered inflammation is required for the maintenance of the epigenetic transformed phenotype and cancer stem-like cell population in the activated Src-driven breast cancer model (Iliopoulos et al., 2009). Although these observations suggest that NF-κB plays an important role in breast cancer stem cells, it is still unclear how NF-κB regulates 'stemness' of these cells. It is known that active NF-κB promotes expression of over 150 target genes. They may encode key molecules for self-renewing ability of breast cancer stem cells. Another possibility is that they encode key cytokines or chemokines, regulating the stem cell phenotype as described below.

8. Proinflammatory cytokines and chemokines and breast cancer stem cells

Several target genes of the NF-κB pathway, such as those encoding for proinflammatory cytokines and chemokines, have been identified as regulators of the breast cancer stem cell

phenotype. For example, we found high interleukin-8 (IL-8) and CC chemokine ligand-5 (CCL5) expression levels in CD24^{-/low}/CD44⁺ breast cancer stem-like cells, and the expression of these chemokines was inhibited by treatment with an inhibitor specific for NF- κ B in breast cancer stem-like cells (Murohashi et al. 2010). NF- κ B activation is involved in the expression of many inflammatory cytokines/chemokines, including vascular endothelial growth factor A (VEGFA), interleukin 8 (IL8) and chemokine (C-C motif) ligand 5 (CCL5), paracrine factors associated with stroma-like activities, which are among the list of highly ranked genes. In addition, VEGFA and IL8 are important factors for angiogenesis and tumorigenesis. Among the other highly ranked genes, we also noticed Toll-like receptor 1 (TLR1), another upstream activator for NF- κ B, and stromal cell-derived factor 2-like 1 (SDF2L1), which is reported to be upregulated through EMT, an important biological output of the TGF- β pathway.

Other reports showed that the IL-8 receptor CXCR1 is consistently expressed in breast cancer stem-like cell populations with high aldehyde dehydrogenase (ALDH) activity and that IL-8 increases the formation of primary and secondary mammospheres as well as that of breast cancer stem-like cell populations (Charafe-Jauffret et al., 2009). It appears that the IL-8/CXCR1 signaling pathway activates Akt and leads to nuclear translocation of β -catenin to induce complex formation with TCF for active transcription (Ginestier et al. 2010). Another report suggests the existence of a relationship between cancer stem-like cells and interleukin-6 (IL-6) expression (Sansone et al., 2007). The results of this study suggested that IL-6 may trigger a potential autocrine/paracrine Notch-3/Jagged-1 loop to boost the self-renewal of breast cancer stem cells. Likewise, it was shown that NF- κ B ensures high IL-6 levels both directly –by activation of IL-6 transcription– and indirectly –by inhibition of let-7 microRNA (Iliopoulos et al., 2009). The resulting high IL-6 levels activate NF- κ B, thereby completing the positive feedback loop that maintains mammosphere formation in vitro and tumorigenesis in nude mice in the breast cancer model. These observations suggest that IL-6 is an important key molecule in breast cancer stem cell biology.

Transforming growth factor- β (TGF- β) also plays a key role in immune homeostasis (Massague, 2008). It controls the initiation and resolution of inflammatory responses through the regulation of chemotaxis and activation of peripheral leukocytes, including lymphocytes, natural killer cells, dendritic cells, macrophages, mast cells, and granulocytes. These findings suggest that inflammatory cytokines and chemokines are critical components for the maintenance of breast cancer stem cells. However, it is still largely unknown how they maintain these cells; for example, it is equally possible that they regulate themselves in an autocrine manner or that they regulate a cancer stem cell niche in a paracrine manner.

In our findings, it is notable that genes related to stroma-like activities were highly enriched in CD24^{-/low}/CD44⁺ populations compared with control populations, such as inflammatory chemokines, angiogenic cytokines, SDF2L1, and TLR1. These stroma-like activities are thought to contribute to invasion, angiogenesis and immune response/suppression. Increasing evidence suggests that tumor stroma, consisting of ‘cancer-associated fibroblasts’ (CAF), play a major role in tumorigenesis (Kalluri and Zeisberg, 2006). CAFs secrete growth factors, cytokines, and chemokines. These, in turn, can induce inflammatory responses and angiogenesis by paracrine mechanisms. Tumor cells appear to use these activities for tumor progression. Our findings suggest that TICs behave like CAFs and contribute to tumorigenesis by producing growth factors, cytokines, and chemokines. In this sense, TICs may actively generate and maintain a microenvironment conducive to the progression of tumorigenesis, or in other words, a cancer stem cell niche (Fig. 5).

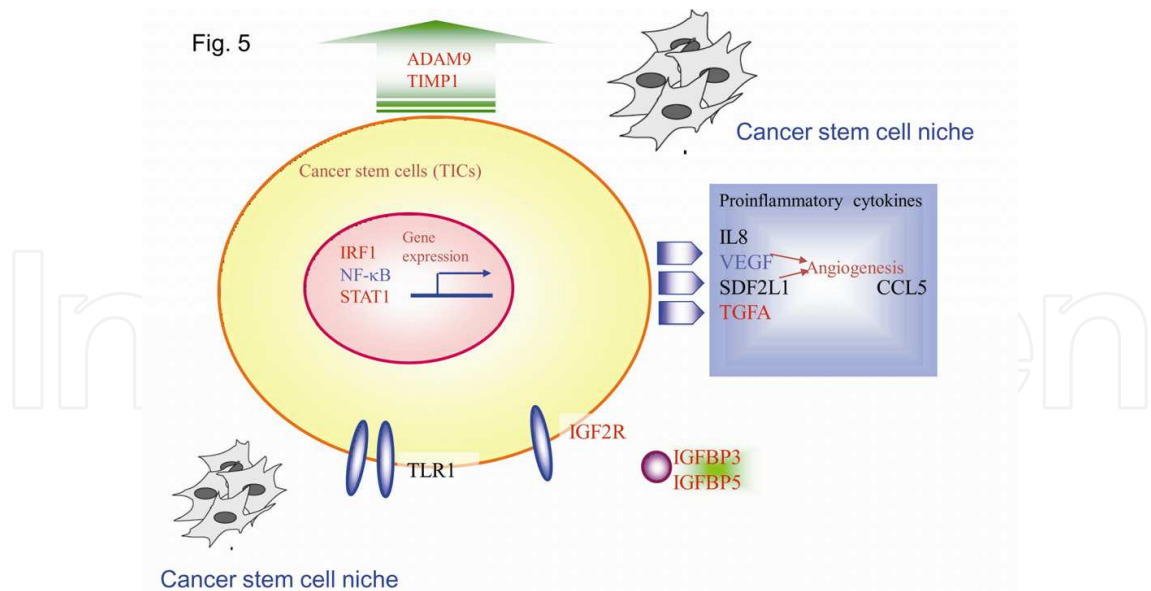


Fig. 5. Model of signaling pathways involving NF-κB in TICs.

We propose that TICs behave like CAFs, in that they actively generate and maintain the cancer stem cell niche in which NF-κB acts as a main effector that induces many secretory proteins, including cytokines and chemokines. Among GSEA-extracted genes, molecules having significantly high levels of mRNA expression or activity are shown in blue, and the others are shown in red. Molecules in black were confirmed to have significantly high levels of mRNA expression.

9. Anti-inflammatory drugs targeting cancer stem cells

Therapeutic targeting of cancer stem cells has the potential to eliminate residual disease and may become an important component of multimodality treatments. In clinical trials, it was found that several anti-inflammatory drugs reduce tumor incidence when used as prophylactics and slow down tumor progression and reduce mortality when used as therapeutics (Gupta and Dubois, 2001). These drugs include aspirin, which suppresses NF-κB transcriptional activity by preventing the binding of NF-κB to DNA(Zhang et al. 2010). Besides its well-documented preventive effects in colon cancer, several epidemiological studies have shown that aspirin reduces the incidence of breast cancer and that its use after breast cancer diagnosis is associated with a decreased risk of distant recurrence, breast cancer death, and death from any other cause (Holmes et al. 2010). Considering the recent advances in understanding inflammatory pathways in breast cancer stem cells, such findings support the possibility that the critical molecules involved in inflammatory pathways in cancer stem cells are appropriate targets for breast cancer treatment.

10. Conclusion

Our findings and others raise an intriguing possibility: TICs behave like CAFs and can actively generate and maintain the cancer stem cells and their niche, in which NF-κB acts as the main effector that can induce many secretory proteins, including cytokines and chemokines. An important avenue for future studies should be the extensive evaluation of our model, using clinical samples of breast cancer.

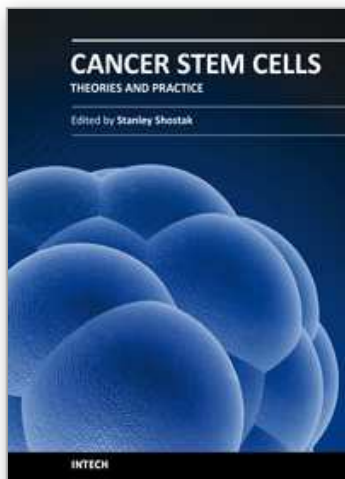
The discovery of the involvement of inflammatory signaling pathways in breast cancer stem cells has especially raised the possibility of developing drugs targeting molecules involved in these pathways in breast cancer stem cells. Further clarification of these mechanisms is important in order to identify critical components that could be targeted by cancer treatment. Examination of the functional roles of these molecules in normal stem cells is also important in order to avoid unnecessary side effects.

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