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Inflammatory signaling pathways in self-renewing breast cancer stem cells
Kunihioko Hinohara and Noriko Gotoh

Cancer stem cells (CSCs), which make up only a small proportion of heterogeneous tumor cells, may possess greater ability to maintain tumorigenesis than do other tumor cell types. Breast cancer tissue is reported to contain cancer stem-like cells. In order to eradicate tumor cells, various approaches have been taken to identify the critical molecules and signaling pathways in breast CSCs. Recent findings suggest that inflammatory signaling pathways are important for the maintenance of breast CSCs. Here, we review the current understanding of the role of inflammatory pathways in these cells and discuss future perspectives of the research on and the possibility of targeting the molecules involved in these pathways for developing treatments for breast cancer.

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Introduction
To develop more effective cancer therapies, it is critical to determine which cancer cells have the potential to contribute to tumor progression. Because 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. A recently proposed hypothesis involving cancer stem cells (CSCs) has drawn great attention. Potential CSCs were first identified in hematological malignancies such as leukemia. Subsequently, it was suggested that solid tumors such as breast cancers also contain such cells. It is hypothesized that heterogeneous tumor tissue is maintained in a hierarchical organization of rare, slowly dividing CSCs; rapidly dividing progenitor cells; and differentiated tumor cells. The growth and progression of tumors are thought to be driven by such subpopulations of CSCs [1–3]. Therefore, it is thought that CSCs 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 CSCs that were not targeted by conventional therapy.

Although the concept of CSCs greatly influences cancer biology and evokes reconsideration of cancer treatment, the molecular mechanisms underlying the contribution of these cells to tumorigenesis remain obscure. To clarify these mechanisms, it is important to identify the signaling pathways activated in CSCs. Recent data indicate that several potential signaling pathways are activated in these cells. This review discusses the involvement of growth factor and inflammatory cytokine/chemokine signaling pathways in breast CSCs and the possibilities for development of a novel strategy for the treatment of breast cancer by targeting these cells.

Breast CSCs
CSCs are thought to have characteristics in common with normal stem cells from tumor-prone tissue. For instance, CSCs can self-renew and simultaneously produce differentiated daughter cells that strongly proliferate until they reach their final differentiated state. Apparent differences also exist between CSCs and normal stem cells. The latter are maintained under tight homeostatic regulation and are passively protected in the surrounding microenvironment, that is, the stem cell niche, in adult tissue. However, the former may actively contribute to tumorigenesis and may use the CSC niche for this purpose.

The development of biomarkers to identify breast CSCs 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 [4–6]. In human breast cancers, the CD24 low/CD44 + cell population was reported to be more highly enriched in breast CSCs than was the CD24 high/CD44 + cell population [7]. Several groups have also identified CD24 low/CD44 + cells as a breast CSC-enriched population in primary human breast carcinoma [8**,9**]. In addition, aldehyde dehydrogenase (ALDH) expression has been used to isolate human breast CSC populations [10*,11]. More recently, highly pure breast CSC populations were obtained by using the lipophilic fluorescent dye PKH26, which labels relatively
quiescent cells within a proliferating population [12••,13••]. Just as primary tumors and xenografts contain CSC populations, established breast cancer cell lines may also contain cellular hierarchies driven by a population expressing CSC markers [10••,14••,15••]. In addition to involvement in tumor initiation, the cells also display increased metastatic potential [10••].

In recent years, the in vitro mammosphere formation assay has been established as a measure for the self-renewal of breast CSCs [16,17]. This assay has been validated by use of xenotransplantation models that are considered the gold standard for CSCs [18]. Mammospheres are floating cell aggregations, which include cancer stem-like cells, and can be serially passaged; they are obtained by culturing breast CSCs in a defined medium containing growth factors, including the epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) [17]. This medium is only a slightly modified version of the defined medium that includes the same growth factors, that is, 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 CSCs in such in vitro culture conditions. However, little information is available regarding the regulatory mechanisms for self-renewal of breast CSCs 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.

**Inflammatory signaling pathways activated in breast CSCs**

The functional relationship between inflammation and cancer has been discussed since the 1860s [19,20]. Activation of several pathways involved in inflammatory responses has recently been detected in breast CSCs (Figure 1, Table 1). We and others recently discovered that nuclear factor-kappa B (NF-κB), which is one of the main regulators of the transcription of inflammatory mediators [21], is activated in breast cancer stem-like cells. We used gene set enrichment analysis (GSEA), which is an analytical method for determining gene expression profiles on the basis of potential pathways. The results revealed that breast cancer cell lines contain a CD24•CD44−/CD44+ breast CSC population with a distinct

**Figure 1**

Potential inflammatory signaling pathways in breast CSCs. Breast CSCs may be regulated by chemokine- and/or cytokine-mediated inflammatory signaling in an autocrine or paracrine manner.
molecular profile, which includes inflammatory properties [15]. In particular, the genes involved in the NF-κB pathway as well as the tumor necrosis factor (TNF)/interferon (IFN) pathways were enriched in CD24−/low/CD44+ breast CSC populations than in CD24high/CD44+ cell populations. The NF-κB pathway is activated by the TNF and IFN pathways [22,23], suggesting that NF-κB is a key molecule in breast CSCs. In fact, suppression of NF-κB by using a low-molecular-weight compound such as DHMEQ resulted in decreased breast tumor growth [15,24]. In addition, NF-κB is a potential target for the inhibition of breast CSCs [25]. NF-κB inhibitors inhibit NF-κB activity to a greater extent in mammosphere cells than in bulk cancer cells, suggesting that the NF-κB pathway might be preferentially vulnerable in breast CSCs. 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 [26**]. Although these observations suggest that NF-κB plays an important role in breast CSCs, 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 [27]. They may encode key molecules for self-renewing ability of breast CSCs. Another possibility is that they encode key cytokines or chemokines, regulating the stem cell phenotype as described below.

**Proinflammatory cytokines and chemokines and breast CSCs**

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 CSC 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-κB in breast cancer stem-like cells [15]. It has been also reported that the IL-8 receptor CXCR1 is consistently expressed in breast cancer stem-like cell populations with high aldehyde dehydrogenase (ALDH1) activity and that IL-8 increases the formation of primary and secondary mammospheres as well as that of breast cancer stem-like cell populations [10]. 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 [28**].

Moreover, recent data suggest the existence of a relationship between cancer stem-like cells and interleukin-6 (IL-6) expression [29]. 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 CSCs. Likewise, Iliopoulos et al. revealed that NF-κB ensures high IL-6 levels both directly — by activation of IL-6 transcription — and indirectly — by inhibition of let-7 microRNA [26]. The resulting high IL-6 levels activate NF-κB, 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 CSC biology.

Transforming growth factor-β (TGF-β) also plays a key role in immune homeostasis [30–32]. 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 [33–35]. TGF-β has also been reported to have several functional roles in breast CSCs. Shipitsin et al. showed that the TGF-β pathway was specifically active in CD44+ cancer cells, where its inhibition induced a more epithelial phenotype [36]. In addition, induction of an epithelial–mesenchymal transition (EMT) in immortalized human mammary epithelial cells (HMLEs) by exposure to TGF-β resulted in the appearance of the EMT phenotype and acquisition of the CD24−/CD44high antigen phenotype [37**.38].

These findings suggest that inflammatory cytokines and chemokines are critical components for the maintenance of breast CSCs. However, it is still largely unknown how they maintain these cells; for example, it is equally possible that

### Table 1

**Inflammation-related genes involved in the regulation of breast CSCs.**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Characteristics</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF-κB</td>
<td>NF-κB is upregulated in breast CSCs, and its NF-κB activity is required for mammosphere formation.</td>
<td>[15*,25,26]**</td>
</tr>
<tr>
<td>IL-6</td>
<td>The IL-6 pathway is required for the self-renewal of breast CSCs.</td>
<td>[26,29]</td>
</tr>
<tr>
<td>IL-8</td>
<td>IL-8 treatment leads to enhanced mammosphere-forming efficiency, and IL-8 receptor CXCR1 blockade selectively depletes the breast CSC population.</td>
<td>[10*,15*,28]**</td>
</tr>
<tr>
<td>CCL5</td>
<td>CCL5 is expressed at high levels in breast CSC populations.</td>
<td>[15]</td>
</tr>
<tr>
<td>TGF-β</td>
<td>TGF-β is upregulated in breast CSCs, and its presence can induce EMT, resulting in the acquisition of the CSC phenotype.</td>
<td>[15*,26,37**]</td>
</tr>
<tr>
<td>TNF-α</td>
<td>The TNF-α pathway is upregulated in breast CSCs.</td>
<td>[15]</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>The IFN-γ pathway is upregulated in breast CSCs.</td>
<td>[15]</td>
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</table>
they regulate themselves in an autocrine manner or that they regulate a CSC niche in a paracrine manner.

**Anti-inflammatory drugs targeting CSCs**

Therapeutic targeting of CSCs has the potential to eliminate residual disease and may become an important component of multimodality treatments [39–41]. 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 [42]. These drugs include aspirin, which suppresses NF-κB transcriptional activity by preventing the binding of NF-κB to DNA [43]. Besides its well-documented preventive effects in colon cancer [42], 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 [44, 45]. Considering the recent advances in understanding inflammatory pathways in breast CSCs, such findings support the possibility that the critical molecules involved in inflammatory pathways in CSCs are appropriate targets for breast cancer treatment.

Recently, a promising strategy has been developed for selectively targeting breast CSCs by blocking the IL-8 receptor CXCR1 with the CXCR1 inhibitor, repertaxin [28**]. Repertaxin was originally developed to block IL-8 activity in order to reduce tissue damage after myocardial infarction or stroke [46]. Repertaxin has been evaluated in phase I clinical studies and found to be safe and well tolerated [47]. This suggests that strategies aimed at interfering with cytokine regulatory loops, such as those involving IL-8 and CXCR1, may represent a novel strategy for targeting breast CSCs. Because these cells may drive tumor progression and metastasis, such strategies may lead to improvement in the outcomes for patients with advanced breast cancer.

**Conclusions**

Recent extensive research has provided insights into the molecular mechanisms underlying breast CSC-driven tumorigenesis. The discovery of the involvement of inflammatory signaling pathways in breast CSCs has especially raised the possibility of developing drugs targeting molecules involved in these pathways in breast CSCs. 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. In addition, CSC-specific drug delivery systems are required to develop more effective therapy.

**Conflict of interest**

The authors have no conflicts of interest to disclose.

**Acknowledgements**

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**References and recommended reading**

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


This work provides functional roles of ROS in breast CSCs.


This study demonstrates that miR-200c provides a molecular link that connects breast CSCs with normal mammary stem cells.


This work reports the possible relationship between inflammatory genes and breast CSCs.


This study reported that p53 down-regulates the symmetric division of stem cells by using PKH-26 fluorescent dye as a marker of CSCs.


This work reveals that the heterogeneity of breast cancers could be explained, at least in part, by the number/proportion of cells displaying stem-like features contained within the tumor.

14. Fillmore CM, Kuperwasser C: Human breast cancer cell lines contain stem-like cells that self-renew, give rise to...

This work provides evidence that established breast cancer cell lines may also contain cellular hierarchies driven by a population.


This work suggests NF-kB activity is higher in breast cancer stem-like cells than in other cells.


This work suggests that blockade of IL8-CXCR1 axis provides a novel means of targeting and eliminating breast CSCs.


This work suggests EMT can induce phenotypes associated with CSCs.


