Breast Cancer RESEARCH

VIEWPOINT

Top Notch cancer stem cells by paracrine NF-κB signaling in breast cancer

Weizhou Zhang[1*] and Sergei I Grivennikov[2*]

Abstract

Cancer stem cells are likely to play critical roles in metastasis, therapy resistance, and recurrence of hematological and solid malignancies. It is well known that the stem cell niche plays a key role for asymmetric division and homeostasis of normal stem cells, whereas cancer stem cells seem to use these niches. Among many pathways involved in self-renewal of cancer stem cells, nuclear factor-kappa B (NF-κB) signaling has been documented to promote their expansion in a cell-autonomous fashion. A recent study, however, suggests that paracrine NF-κB activation promotes the expansion of cancer stem cells through the activation of Notch in basal-type breast cancer cells.

Nuclear factor-kappa B (NF-κB) pathways, including canonical (activated by IκB kinase (IKK) α/β/γ complex and activation of RelA/p50) and non-canonical (activated by NF-κB-inducing kinase (NIK) and IKKα, activation of RelB/p52) pathways, link inflammation and cancer in a variety of animal models. NF-κB pathways are also important for normal mammary gland development as well as for breast cancer tumorigenesis and cancer stem cell biology, and previous studies specifically emphasize the cell-autonomous role of NF-κB activation in breast cancer stem cells (CSCs) [1]. Based on animal models, however, protumorigenic inflammatory signaling often works in a feed-forward manner. For example, the NF-κB activation in the tumor microenvironment induces the production of various cytokines which, in turn, activate NF-κB or other pro-carcinogenic pathways in cancer cells to stimulate cell survival and proliferation and to enhance the production of chemokines, leading to further recruitment of immune cells into the tumor [2]. Given the complexity of the tumor microenvironment, whether any paracrine signals activated by NF-κB contribute to self-renewal of CSCs *in trans* remains unknown.

An elegant study by Yamamoto and colleagues [3] recently identified a non-cell-autonomous NF-κB activation for the expansion of CSCs in basal-type breast cancer, one of the most malignant types associated with early relapse. Although CSCs can secrete factors regulating their own maintenance in an autocrine fashion, Yamamoto and colleagues demonstrated that non-CSC cancer cells, tumor-associated macrophages, and fibroblasts provide a supportive microenvironment for CSCs by producing Jagged 1 (JAG1), one of the five known ligands in the Notch pathway, which plays a critical role in CSC self-renewal. While NF-κB activation is not essential in CSCs themselves during this process, it is the TNFα-, NIK-, and IKKβ-dependent activation of NF-κB which drives JAG1 production from auxiliary cells. Somewhat surprisingly, IL-6 and −8 (well-known targets of the NF-κB pathway and suggested regulators of CSCs [4,5]) were not involved in this paracrine signaling and the expansion of CSCs. The authors further applied gene set enrichment analysis of extensive datasets to find a unique correlation between the NF-κB-JAG1 axis in 'basal' but not in any other subtypes of breast cancer.

Whereas Yamamoto and colleagues demonstrated the role of JAG1 in CSC homeostasis of the basal type of breast cancer, other reports identified that estrogen receptor $(ER)^{low/-}$ CSCs can be regulated by Notch activation in ER^+ breast cancer in an estrogen-dependent manner [6]. The evil connection between Notch and HER2 is also well documented in $HER2^+$ [7] and in ER^+ luminal breast cancer without HER2 amplification where Notch, in concert with receptor activator of NF-κB (RANK) signaling, upregulates HER2 transcription and regulates expansion of $HER2^+$ CSCs [8,9]. Although the importance of paracrine Notch signaling in $HER2^+$ CSCs has yet to be demonstrated, it is plausible that paracrine Notch activation may be a common regulatory theme

* Correspondence: weizhou-zhang@uiowa.edu; sergey.grivennikov@fccc.edu
[1]Department of Pathology, University of Iowa/Carver College of Medicine, 100 Medical Laboratories, Iowa City, IA 52241, USA
[2]Fox Chase Cancer Center, Cancer Prevention and Control Program, 333 Cottman Ave., Philadelphia, PA 19111, USA

for CSC renewal in breast cancer, thereby further reinforcing the notion that tumor microenvironment aids tumor progression in general and assists self-renewal of CSCs in particular. The mechanistic role of Notch in CSCs can be potentially explained by its ability to induce an epithelial-mesenchymal transition (EMT), a known biological process contributing to the development of CSCs [10]. While the requirement for Notch signaling may be uniform, it is quite possible that distinct pathways that induce the expression of Notch ligands differ for CSCs and non-CSCs in different types of cancer. For example, the IL-6/STAT3 pathway has been shown to induce JAG1 expression and promote malignant stem cell growth [11]. The link between NF-κB activation and JAG1 expression appears to be broken in claudin-low breast cancer, a recently identified molecular subtype with active NF-κB, EMT, and CSC signatures, but these parameters demonstrated no correlation with JAG1 expression [3]. Therefore, other pathways may be necessary for NF-κB-dependent and -independent JAG1 transcription. These data also explain the observation that TNFα failed to enhance JAG1 expression in luminal and claudin-low breast cancer cells, even though it strongly induced NF-κB activation. It will be interesting to find out the identity of additional signals, which cooperate with or substitute for NF-κB to induce JAG1 expression in basal breast cancer.

The crosstalk between NF-κB and Notch pathways is quite complex and depends on the exact pathophysiological context. Paracrine activation of Notch signaling by NF-κB-dependent JAG1 expression has been previously shown during B-cell activation [12].

Likewise, several cell types within the tumor microenvironment may activate NF-κB and produce JAG1, depending on the local context and exact identity of NF-κB-inducing stimuli. Unfortunately, the lack of simple markers makes it hard to detect CSCs *in situ* and find out their adjacent cells providing Notch ligands by current immunostaining techniques. Nonetheless, establishing the mechanistic connection between NF-κB and Notch pathways diversifies potential targets for therapeutic intervention. Long-term inhibition of canonical IKKβ/NF-κB signaling may be problematic because of severe immune complications [13]. There is no specific NIK inhibitor for pharmaceutical use yet. Because of its important role in both canonical and non-canonical NF-κB pathways in the immunity and the severe phenotype of NIK-deficient mice [14], inhibiting NIK may also cause severe side effects. Denosumab, a humanized monoclonal antibody neutralizing cytokine RANKL (receptor activator of nuclear factor kappa-B ligand) and inhibiting RANKL/RANK-mediated NIK/IKKα activation [15] and approved to treat breast cancer patients with bone metastasis, represents a potential agent for neoadjuvant therapy of RANK-positive basal breast cancer to target CSCs. Other selections include agents that target Notch signaling, such as γ-secretase inhibitors and monoclonal antibodies targeting ligands or receptors [16]. Toxicity has to be carefully evaluated because of their critical role in normal tissue homeostasis. Combinatory treatment with classic dose-dense chemotherapy should be considered when treating basal-type breast cancer to target both the bulk tumor cells and CSCs.

Abbreviations

CSC: Cancer stem cell; EMT: Epithelial-mesenchymal transition; ER: Estrogen receptor; IKK: IκB kinase; IL: Interleukin; JAG1: Jagged 1; NF-κB: Nuclear factor-kappa B; NIK: Nuclear factor-kappa B-inducing kinase; RANK: Receptor activator of nuclear factor kappa-B; RANKL: Receptor activator of nuclear factor kappa-B ligand; TNF: Tumor necrosis factor.

Competing interests

The authors declare that they have no competing interests.

Published: 30 Oct 2013

References

1. Shostak K, Chariot A: **NF-kappaB, stem cells and breast cancer: the links get stronger.** *Breast Cancer Res* 2011, **13:**214.
2. Grivennikov SI, Greten FR, Karin M: **Immunity, inflammation, and cancer.** *Cell* 2010, **140:**883–899.
3. Yamamoto M, Taguchi Y, Ito-Kureha T, Semba K, Yamaguchi N, Inoue J: **NF-kappaB non-cell-autonomously regulates cancer stem cell populations in the basal-like breast cancer subtype.** *Nat Commun* 2013, **4:**2299.
4. Charafe-Jauffret E, Ginestier C, Iovino F, Wicinski J, Cervera N, Finetti P, Hur MH, Diebel ME, Monville F, Dutcher J, Brown M, Viens P, Xerri L, Bertucci F, Stassi G, Dontu G, Birnbaum D, Wicha M: **Breast cancer cell lines contain functional cancer stem cells with metastatic capacity and a distinct molecular signature.** *Cancer Res* 2009, **69:**1302–1313.
5. Marotta LL, Almendro V, Marusyk A, Shipitsin M, Schemme J, Walker SR, Bloushtain-Qimron N, Kim JJ, Choudhury SA, Maruyama R, Wu Z, Gönen M, Mulvey LA, Bessarabova MO, Huh SJ, Silver SJ, Kim SY, Park SY, Lee HE, Anderson KS, Richardson AL, Nikolskaya T, Nikolsky Y, Liu XS, Root DE, Hahn WC, Frank DA, Polyak K: **The JAK2/STAT3 signaling pathway is required for growth of CD44(+)CD24(−) stem cell-like breast cancer cells in human tumors.** *J Clin Invest* 2011, **121:**2723–2735.
6. Harrison H, Simoes BM, Rogerson L, Howell SJ, Landberg G, Clarke RB: **Oestrogen increases the activity of oestrogen receptor negative breast cancer stem cells through paracrine EGFR and Notch signalling.** *Breast Cancer Res* 2013, **15:**R21.
7. Korkaya H, Wicha MS: **HER-2, notch, and breast cancer stem cells: targeting an axis of evil.** *Clin Cancer Res* 2009, **15:**1845–1847.
8. Magnifico A, Albano L, Campaner S, Delia D, Castiglioni F, Gasparini P, Sozzi G, Fontanella E, Menard S, Tagliabue E: **Tumor-initiating cells of HER2-positive carcinoma cell lines express the highest oncoprotein levels and are sensitive to trastuzumab.** *Clin Cancer Res* 2009, **15:**2010–2021.
9. Ithimakin S, Day KC, Malik F, Zen Q, Dawsey SJ, Bersano-Begey TF, Quraishi AA, Ignatoski KW, Daignault S, Davis A, Hall CL, Palanisamy N, Heath AN, Tawakkol N, Luther TK, Clouthier SG, Chadwick WA, Day ML, Kleer CG, Thomas DG, Hayes DF, Korkaya H, Wicha MS: **HER2 drives luminal breast cancer stem cells in the absence of HER2 amplification: implications for efficacy of adjuvant trastuzumab.** *Cancer Res* 2013, **73:**1635–1646.
10. Espinoza I, Miele L: **Deadly crosstalk: Notch signaling at the intersection of EMT and cancer stem cells.** *Cancer Lett* 2013.
11. Sansone P, Storci G, Tavolari S, Guarnieri T, Giovannini C, Taffurelli M, Ceccarelli C, Santini D, Paterini P, Marcu KB, Chieco P, Bonafè M: **IL-6 triggers malignant features in mammospheres from human ductal breast carcinoma and normal mammary gland.** *J Clin Invest* 2007, **117:**3988–4002.
12. Bash J, Zong WX, Banga S, Rivera A, Ballard DW, Ron Y, Gelinas C: **Rel/NF-kappaB can trigger the Notch signaling pathway by inducing the expression of Jagged1, a ligand for Notch receptors.** *EMBO J* 1999, **18:**2803–2811.

13. Greten FR, Arkan MC, Bollrath J, Hsu LC, Goode J, Miething C, Göktuna SI, Neuenhahn M, Fierer J, Paxian S, Van Rooijen N, Xu Y, O'Cain T, Jaffee BB, Busch DH, Duyster J, Schmid RM, Eckmann L, Karin M: **NF-kappaB is a negative regulator of IL-1beta secretion as revealed by genetic and pharmacological inhibition of IKKbeta.** *Cell* 2007, **130:**918–931.
14. Hacker H, Chi L, Rehg JE, Redecke V: **NIK prevents the development of hypereosinophilic syndrome-like disease in mice independent of IKKalpha activation.** *J Immunol* 2012, **188:**4602–4610.
15. Tan W, Zhang W, Strasner A, Grivennikov S, Cheng JQ, Hoffman RM, Karin M: **Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL-RANK signalling.** *Nature* 2011, **470:**548–553.
16. Wicha MS: **Targeting breast cancer stem cells.** *Breast* 2009, **18:**S56–S58.

10.1186/bcr3565
Cite this article as: Zhang and Grivennikov: **Top Notch cancer stem cells by paracrine NF-κB signaling in breast cancer.** *Breast Cancer Research* 2013, 15:316