IN THE SPOTLIGHT

Tumor Twitter: Cellular Communication in the Breast Cancer Stem Cell Niche

Michael D. Brooks and Max S. Wicha

Summary: Communication between the diverse assortment of cells that constitute the tumor microenvironment plays an important role in tumor development. Using a p53-null mouse model, Zhang and colleagues describe a novel feedback loop involving breast cancer stem cells and their progeny mediated by WNT2, CXCL12, and IL6.
Cancer Discov; 5(5); 469–71. ©2015 AACR.

See related article by Zhang et al., p. 520 (6).

Although the heterogeneous cellular composition of tumors has been long appreciated, the biologic and clinical significance of this cellular heterogeneity is becoming increasingly evident. In addition to genetic heterogeneity generated by mutation and clonal selection, it is now clear that epigenetic regulation also contributes to tumor cellular heterogeneity (1). Epigenetic regulation mimicking normal differentiation contributes to the generation of hierarchically organized cellular clones that, although sharing common mutation profiles, express diverse gene expression patterns. At the apex of these hierarchies are populations of cells capable of self-renewal, as well as generating more differentiated cells constituting the tumor bulk. These "stem-like" cells are of clinical significance because they mediate tumor metastasis and contribute to treatment resistance. Recent research has suggested that tumor stem cells display the plasticity to transition between alternative states, including a relatively quiescent, invasive, mesenchymal-like state (EMT) and a more proliferative epithelial-like state (MET; ref. 2). Furthermore, transition between these states is regulated by the tumor microenvironment, which plays an important role in tumor metastasis.

The tumor microenvironment is constituted by a multitude of nontumorigenic cells, such as mesenchymal stem cells, stromal cells, and a host of immune cells, including myeloid-derived suppressor cells and macrophages (3). These cells form a specialized part of the tumor microenvironment termed the "cancer stem cell (CSC) niche." Communication between stromal cells and CSCs in the niche has been well studied and is mediated by a variety of secreted and cell–cell contact factors that are critical for CSC function (4).

Although most attention has been focused on the interaction between tumor cells and host cells in the tumor microenvironment, recent evidence suggests important roles for communication between heterogeneous populations of tumor cells. Marusyk and colleagues (5) demonstrated an important role for interclonal communication during mammary tumorigenesis. However, communication between CSCs and their progeny has been less well studied. In this issue, Zhang and colleagues (6) use a previously reported (7) syngeneic p53-null mouse model to examine the interactions between $CD29^{hi}CD24^{hi}$ cells, which mark a bipotent, strongly tumor-initiating cell population, and more mesenchymal $CD29^{hi}CD24^{lo}$ breast tumor cells. Microarray analysis of the $CD29^{hi}CD24^{lo}$ population showed a strong mesenchymal and claudin-low gene expression signature and indicated that these cells displayed high expression of a number of secreted signaling molecules, including WNT2, WNT9A, CXCL12, and IL6. Interestingly, as assessed by qRT-PCR analysis, the $CD29^{hi}CD24^{hi}$ population had significantly higher expression of a number of members of the WNT signaling pathway, including AXIN2, TCF7, and FZD7, while also expressing high levels of CXCR4, the receptor for CXCL12. This strongly suggested that the $CD29^{hi}CD24^{lo}$ population was secreting signaling molecules that were acting upon the $CD29^{hi}CD24^{hi}$ CSCs, promoting their self-renewal. Because the $CD29^{hi}CD24^{lo}$ mesenchymal cells were derived from the $CD29^{hi}CD24^{hi}$ population, this suggests that CSCs are able to generate their own niche.

In order to demonstrate the functional significance of these secreted factors, the various cell populations were grown in coculture in Transwell plates. $CD29^{hi}CD24^{hi}$ CSCs that were incubated with the mesenchymal $CD29^{hi}CD24^{lo}$ niche population showed an increase in both efficiency of mammosphere formation and sphere size and displayed an increased tumor-initiating capacity in mice. This increase in mammosphere formation efficiency could be significantly abrogated by shRNAs reducing expression of either WNT2 or CXCL12 in the $CD29^{hi}CD24^{lo}$ cell population or, alternatively, by knocking down their respective receptors FZD7 or CXCR4 in the $CD29^{hi}CD24^{hi}$ population. Finally, the results were validated in syngeneic mice by mammary fat pad injections of small numbers (2 to 20) of $CD29^{hi}CD24^{hi}$ cells alone or in combination with $CD29^{hi}CD24^{lo}$ cells. Coinjection of the mesenchymal cells with tumor-initiating cells (TIC) led to

Comprehensive Cancer Center, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan.

Corresponding Author: Max S. Wicha, University of Michigan, NCRC 26-335S, 2800 Plymouth Road, Ann Arbor, MI 48109-2800. Phone: 734-763-1744; Fax: 734-764-1288; E-mail: mwicha@med.umich.edu

doi: 10.1158/2159-8290.CD-15-0327

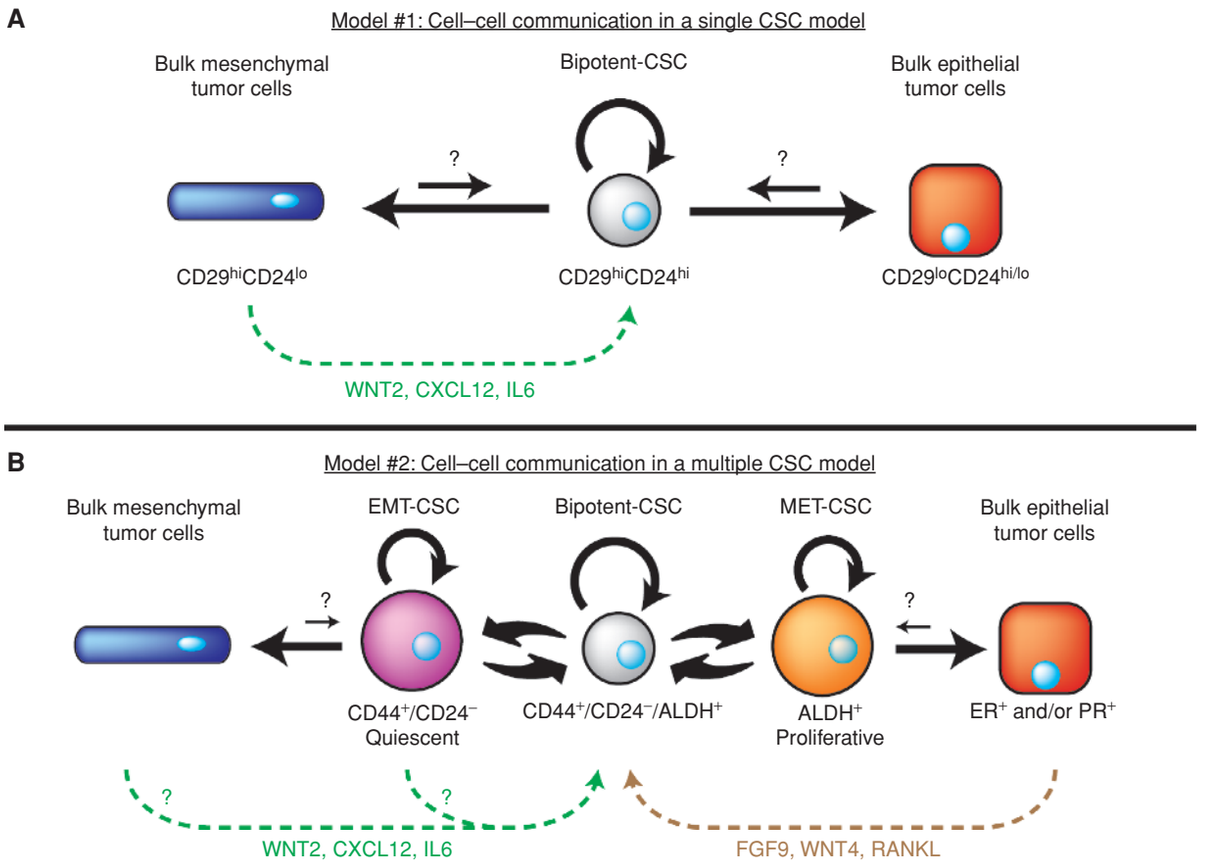

Figure 1. Two alternative models for interactions between tumor cells in the breast cancer stem cell niche. **A,** in the single CSC model, a differentiated mesenchymal cell population produces secreted factors that act to positively regulate a bipotent TIC population. **B,** an alternative model is that CSCs exist in alternative EMT and MET states that interact with each other as well as with more differentiated epithelial and mesenchymal tumor cells. In the presence of estrogen and progesterone, the more differentiated epithelial-like tumor cells produce FGF9, WNT4, and RANKL, whereas CXCL12 and WNT2 are generated by either EMT-like CSCs or other more differentiated fixed EMT-like tumor cells.

a much higher tumor initiation rate compared with similar numbers of TICs alone. Furthermore, the ability of the mesenchymal niche cells to increase the tumor-initiating capacity of CSCs was abrogated by knockdown of WNT2 in the mesenchymal niche cells, demonstrating an important role for this signaling pathway in tumor initiation.

A key element of the findings of Zhang and colleagues is the positive feedback loop between cancer stem cells and mesenchymal tumor populations derived from them. As a result, these tumors produce high levels of WNT2, CXCL12, and IL6, which in turn drives the self-renewal of tumor-initiating cells, which then produce more mesenchymal cells, and so forth (Fig. 1A). This might provide an explanation for the observation that basal and claudin-low breast tumors have the highest proportion of cancer stem cells (8) and are among the most aggressive and difficult to treat. This also emphasizes the importance of targeting both bulk and CSC populations to achieve maximum therapeutic effect. Moreover, signaling molecules involved in these feedback loops represent rational therapeutic targets. In fact, inhibitors of WNT, CXCR4, and IL6 have entered early-phase cancer clinical trials.

The work of Zhang and colleagues provides important new data on cell–cell communication in the cancer stem cell niche. However, a number of questions remain. These include further characterization of the $CD29^{hi}CD24^{lo}$ mesenchymal niche cell population. Although described as mesenchymal niche-like cells, they in fact have tumor-initiating capacity, albeit less than the $CD29^{hi}CD24^{hi}$ CSC population. Furthermore, tumors generated from this mesenchymal-like population are primarily epithelial rather than mesenchymal in nature. Together, this suggests that at least a fraction of the $CD29^{hi}CD24^{lo}$ population is better characterized as an EMT-like CSC, rather than as a fixed EMT-like cell. If this is the case, then it suggests that there may be interactions between different CSC populations that regulate their behavior, as illustrated in Fig. 1B. Further work will be required to determine the exact nature of these interacting cells.

The work of Zhang and colleagues adds to the growing body of literature demonstrating that CSCs are able to generate key components of their niche and that differentiated progeny may play important roles in CSC regulation. It has previously been demonstrated that the steroid hormones

estrogen and progesterone regulate normal and malignant mammary stem cells through feedback loops involving FGF9 (9), WNT4, and RANKL generated by differentiated mammary luminal cells (Fig. 1B; ref. 10). The work of Zhang and colleagues adds to these findings by demonstrating that mesenchymal-like progeny can also regulate breast stem cells through feedback loops. In total, these studies add to our understanding of the high degree of complexity that characterizes the tumor microenvironment and contributes to intratumor cellular heterogeneity. Although this heterogeneity represents a formidable therapeutic challenge, novel technologies, including single-cell DNA sequencing and RNA-seq, provide important tools to deconvolute this complexity. These studies thus may lead to the development of more effective therapeutic strategies.

Disclosure of Potential Conflicts of Interest

M.S. Wicha reports receiving a commercial research grant from Dompe, has ownership interest (including patents) in Oncomed Pharmaceuticals, is a consultant/advisory board member for Verastem, and has provided expert testimony for MedImmune. No potential conflicts of interest were disclosed by the other author.

Grant Support

The Wicha laboratory is supported, in part, by NIH grants R01-CA129765-06A1 and R01-CA101860-06.

Published online May 4, 2015.

REFERENCES

1. Easwaran H, Tsai HC, Baylin SB. Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance. Mol Cell 2014;54:716–27.
2. Liu S, Cong Y, Wang D, Sun Y, Deng L, Liu Y, et al. Breast cancer stem cells transition between epithelial and mesenchymal states reflective of their normal counterparts. Stem Cell Rep 2014;2:78–91.
3. Place AE, Jin Huh S, Polyak K. The microenvironment in breast cancer progression: biology and implications for treatment. Breast Cancer Res 2011;13:227.
4. Plaks V, Kong N, Werb Z. The cancer stem cell niche: how essential is the niche in regulating stemness of tumor cells? Cell Stem Cell 2015;16:225–38.
5. Marusyk A, Tabassum DP, Altrock PM, Almendro V, Michor F, Polyak K. Non–cell-autonomous driving of tumour growth supports subclonal heterogeneity. Nature 2014;514:54–8.
6. Zhang M, Tsimelzon A, Chang C-H, Fan C, Wolff A, Perou CM, et al. Intratumoral heterogeneity in a *Trp53*-null mouse model of human breast cancer. Cancer Discov 2015;5:520–33.
7. Zhang M, Behbod F, Atkinson RL, Landis MD, Kittrell F, Edwards D, et al. Identification of tumor-initiating cells in a p53-null mouse model of breast cancer. Cancer Res 2008;68:4674–82.
8. Prat A, Parker JS, Karginova O, Fan C, Livasy C, Herschkowitz JI, et al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res 2010;12:R68.
9. Fillmore CM, Gupta PB, Rudnick JA, Caballero S, Keller PJ, Lander ES, et al. Estrogen expands breast cancer stem-like cells through paracrine FGF/Tbx3 signaling. Proc Natl Acad Sci U S A 2010;107:21737–42.
10. Rajaram RD, Buric D, Caikovski M, Ayyanan A, Rougemont J, Shan J, et al. Progesterone and Wnt4 control mammary stem cells via myoepithelial crosstalk. EMBO J 2015;34:641–52.