IN THE SPOTLIGHT

RAI2: Linking Retinoic Acid Signaling with Metastasis Suppression

Mark Esposito and Yibin Kang

Summary: Considerable evidence points to the importance of disseminated tumor cells, which are commonly detected in the bone marrow and display features of cellular plasticity, in predicting the clinical outcome of breast cancer. In this issue of *Cancer Discovery*, Werner and colleagues report on the discovery of retinoic acid–induced 2 (RAI2) as a differentiation factor that suppresses early metastatic spread of estrogen receptor–positive breast cancer. *Cancer Discov; 5(5); 466–8. ©2015 AACR.*

See related article by Werner et al., p. 506 (4).

In breast cancer, tumor cells disseminate to the bone marrow and form bone metastases in a large majority of late-stage patients. Although both denosumab (Xgeva) and bisphosphonates (Zometa) have been FDA approved to relieve the skeletal complications caused by bone metastasis (1), only therapies that prevent the hematogenous dissemination of primary tumor cells or eliminate early bone lesions can effectively avert bone metastasis–associated morbidity and mortality. Unfortunately, little is known about the genes that promote early metastasis of breast cancer cells to the bone, particularly in the estrogen receptor–positive (ER+) cancers that exhibit a higher propensity to develop bone metastasis (2).

Studies in both patients and animal models have shown that the presence of disseminated tumor cells (DTC) in the bone marrow is associated with an increased risk of bone metastasis. These cells exhibit a high degree of cellular plasticity related to features of the epithelial-to-mesenchymal transition (EMT), both of which facilitate metastatic spread (3). Furthermore, this EMT-induced cancer cell plasticity, combined with a supportive bone stromal environment, leads to the elevated resistance of DTCs to traditional cancer therapeutics (3). Therefore, identifying clinically relevant molecular pathways that lead to the early spread of DTCs and their associated cellular characteristics is key to the development of more effective treatments to prevent bone relapse.

In the study by Werner and colleagues (4), gene-expression profiling analysis was used to identify and validate predictors of DTC status in two independent sets of luminal breast cancer patients. Based on expression level changes, 28 genes were identified as candidate metastasis suppressors (i.e., lower expression in DTC-positive patients), together with 4 putative prometastatic genes. This candidate gene list was further analyzed in publicly available breast cancer genomic datasets to identify genes with consistent clinical prognostic powers. Three putative metastasis suppressors were found to negatively correlate with DTC status and predict longer survival in patients. Two of these genes, *RLN2* and *RERG*, maintained prognostic significance in two thirds of tested breast cancer datasets, whereas *RAI2* was consistently linked to good prognosis in all six tested breast cancer sets, as well as two lung cancer datasets, one colon cancer dataset, and one ovarian cancer dataset. Intriguingly, the role of RAI2 in cancer has never been previously investigated.

RAI2 was one of seven novel genes originally discovered to be induced by retinoic acid in embryonal carcinoma cells (5). In addition to these novel genes, many other genes associated with differentiation, such as Vimentin, N-cadherin, and HOX, were also induced by retinoic acid, causing the differentiation of mouse pluripotent stem cells into neural cell-like derivatives (5). Studies since have reported on the differentiation of numerous stem cell types as well as multiple cancer types by retinoic acid (6). However, although the effects of retinoic acid treatment have been analyzed in many different contexts, RAI2 has remained virtually uncharacterized.

To begin answering how RAI2 may function in breast cancer, the authors analyzed its expression in a panel of breast cancer lines (4). RAI2 expression was highest in the epithelial-like ER+ cell lines, whereas its expression was lost in the mesenchymal-like and highly metastatic cell lines. Interestingly, treatment with either ER antagonists or retinoic acid could induce RAI2 expression. The authors next asked if RAI2 was instructive in determining cellular traits such as morphologic plasticity or growth properties. Depletion of RAI2 through RNAi promoted dedifferentiation in epithelial breast cancer cells, leading to gross morphologic changes and a loss of E-cadherin staining. The expression of upstream differentiation factors GATA3, FOXOA1, and GRHL2 was also lost, accompanied by higher expression of classic mesenchymal markers, including Vimentin. These molecular changes are consistent with stronger migratory and invasive capabilities in the RAI2 knockdown cells. Interestingly, RAI2 depletion also increased phosphorylation of AKT at serine 473 and resistance to either AKT or mTOR inhibitors—both traits that have previously been identified in DTCs (3). Opposite

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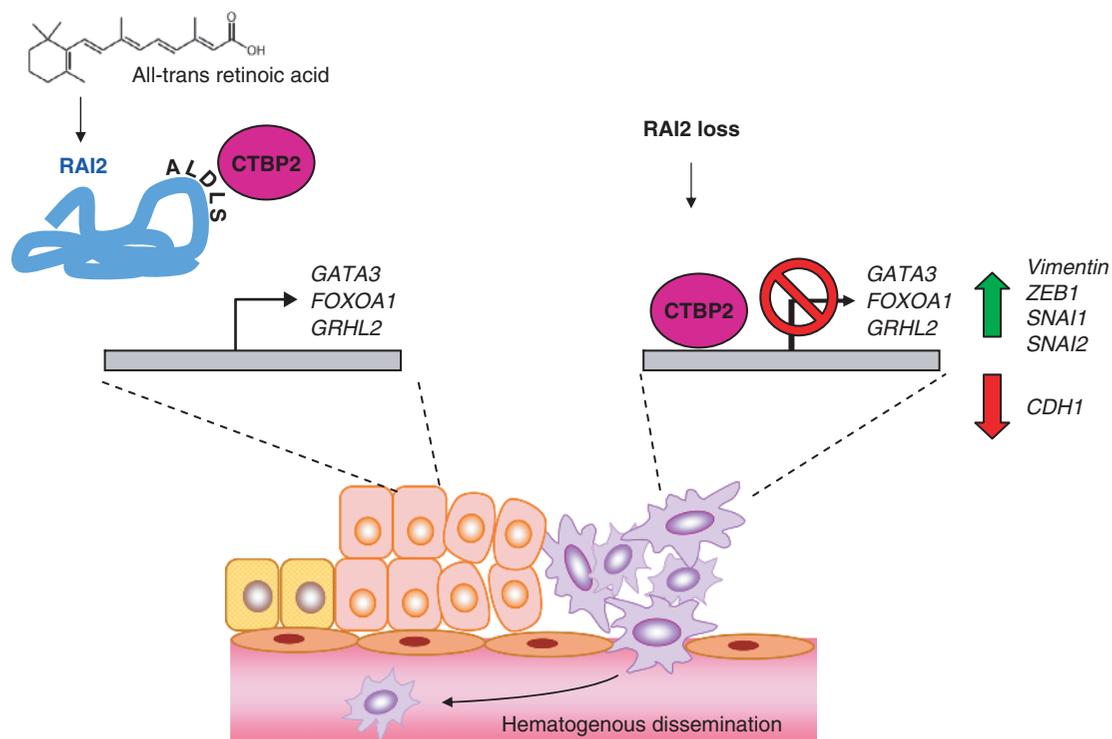
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Figure 1. The role of RAI2 in breast cancer metastasis. Differentiated breast cancer cells exhibit high levels of RAI2, which binds to the transcriptional corepressor C-terminal binding protein-2 (CTBP2) through a conserved ALDLS sequence, preventing it from suppressing the expression of breast cancer differentiation transcription factor genes *GATA3*, *FOXOA1*, and *GRHL2*. Metastatic cells lose expression of RAI2, allowing the suppression of differentiation transcription factors by CTBP2, and the development of EMT features that facilitate tumor dissemination.

results were found when RAI2 was ectopically expressed in the metastatic, mesenchymal-like cell line MDA-MB-231, as these cells lost both invasive and migratory capabilities.

Proteomic scale yeast two-hybrid assays have reported an interaction between C-terminal binding protein-2 (CTBP2) and RAI2 (7). Based on this observation, the authors identified two orthologically conserved ALDLS sites in RAI2 as important binding motifs for CTBP2. RAI2 knockdown reduced mRNA expression of the differentiation markers that are under the control of CTBP2 repression, including the direct targets *GRHL2*, *FOXOA1*, and *GATA3* (8). This result suggested that RAI2 may play a role in transcriptional regulation by preventing CTBP2-mediated repression of these genes (Fig. 1). Interestingly, RAI2 knockdown also reduced CTBP2 levels, indicating a more complex regulatory relationship between these two proteins. To more broadly identify RAI2-dependent CTBP2 targets, wild-type and mutant RAI2 were introduced into MDA-MB-231 cells. Microarray analysis revealed genome-wide alterations in many classic bone metastasis genes, such as *TGFB1* and *CCL2* (1), further suggesting a link between RAI2 and the bone metastasis gene network (4).

This study identified RAI2 as a clinically relevant regulator of tumor dissemination through enforcing the differentiated status of ER+ breast cancer cells. An important next step in the functional analysis of RAI2 will require testing its role in mouse models of breast cancer metastasis. Although the unstable knockdown of RAI2 by RNAi prevented its *in*

vivo functional analysis in the current study, the CRISPR genome editing technique can be used to ask whether genetic disruption of RAI2 can promote dedifferentiation, early dissemination, and metastasis. Furthermore, conditional overexpression of RAI2 can be used to dissect the putative metastasis-suppressive role of RAI2 in different stages of cancer progression and metastasis. At the molecular level, chromatin immunoprecipitation sequencing can be used to precisely define genes that are directly targeted by RAI2. Ultimately, it remains to be seen how RAI2 interaction with CTBP2 alters the gene regulatory program and promotes differentiation.

Another important observation made by this study is the seemingly complex relationship between retinoic acid, RAI2, and estrogen signaling. When cells were treated with either retinoic acid or an ER antagonist, ER expression was reduced, whereas RAI2 expression was increased. On the other hand, RAI2 knockdown also decreased ER expression. Therefore, a context-dependent regulatory relationship appears to exist between RAI2 and ER. Previous experiments have found a 39.3% degree of colocalization between estrogen response elements and retinoic acid response elements, which share 59.8% of target genes (9). It will therefore be important to further understand how retinoic acid and RAI2 influence estrogen signaling, and whether this can be therapeutically exploited.

The current study opens the door to using RAI2 and retinoic signaling in the clinical management of breast cancer.

RAI2 may be used as a prognostic biomarker for the likelihood of ER[+] tumor relapse to bone; ER[+] tumors with low RAI2 levels may be more likely to metastasize and should be treated with more aggressive adjuvant therapy or monitored more intensively. Therapeutic utilization of the retinoid signaling pathway should also be explored, as it plays a multifaceted role in tumor suppression by controlling cell growth, apoptosis, and differentiation (6). Highlighting its importance to cancer progression, retinoic acid treatment was able to cause complete remission in 95% of AML patients (10), and is used in clinical practice today for multiple blood cancers. Due to these suppressive effects of retinoic acid, many cancer types develop resistance to retinoid signaling. One hypothesized mechanism for this resistance is through the mutation or downregulation of the enzymes (ADH, ALDH, and CYP26) that convert vitamin A (retinol) to bioactive 9-*cis* retinoic acid or 13-*cis* retinoic acid (6). In tumors with mutations in any of these enzymes, providing an exogenous supply of bioactive retinoic acid may induce RAI2 and suppress metastatic progression. It remains to be tested whether treatment of breast cancer with retinoic acid can reduce metastatic relapse, and whether the mutational status of the retinoic acid synthesis pathway is linked to a different clinical outcome.

By identifying RAI2 as a putative metastasis suppressor associated with DTC status in ER[+] luminal breast cancer, Werner and colleagues provide novel insights into the process of early hematogenous dissemination. Yet, much research remains to derive a complete understanding of the role and molecular mechanism of RAI2 in the metastatic cascade. Given the broad clinical prognosis power of RAI2 in diverse cancer types, an in-depth understanding of RAI2 and its potential clinical applications will likely bring significant benefits to patients with cancer.

Disclosure of Potential Conflicts of Interest

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