Par(--4)xoxysm in Breast Cancer

Tripti Shrestha-Bhattarai,1,5 Nikhil Hebbar,1,5 and Vivek M. Rangnekar1,2,3,4,*
1Graduate Center for Toxicology
2Department of Radiation Medicine
3Department of Microbiology, Immunology and Molecular Genetics
4L.P. Markey Cancer Center
University of Kentucky, Lexington, KY 40508, USA
5These authors contributed equally to this work
*Correspondence: vmrang01@email.uky.edu
http://dx.doi.org/10.1016/j.ccr.2013.06.010

Women suffering from breast cancer often succumb to incurable recurrent disease resulting from therapy-resistant cancer cells. In this issue of Cancer Cell, Alvarez and colleagues identify downregulation of the tumor suppressor Par-4 as the key determinant in apoptosis evasion, which leads to tumor recurrence in breast cancer.

Breast cancer is the second leading cause of cancer deaths in women. It is estimated that in the US alone, more than 200,000 women will be diagnosed and more than 20% will die of breast cancer in 2013 (Siegel et al., 2013). Surgical intervention in conjunction with chemotherapeutic agents generally provides distinct benefits to patients with localized primary tumors, especially those that have been detected early. Breast cancer patients with positive estrogen receptor (ER), progesterone receptor (PR), and/or human epidermal growth factor receptor 2 (HER2) status are also responsive to targeted therapeutics given as monotherapy, such as trastuzumab, bevacizumab, or cetuximab, or in combination with chemotherapy (Alvarez et al., 2010). Moreover, polychemotherapy that includes cyclophosphamide, methotrexate, and fluorouracil or anthracycline-based regimens combined with taxanes has met with considerable success in prolonging survival and delaying breast cancer recurrence (Martin et al., 2013). However, one in five patients exhibits relapse of the disease within 10 years after treatment (Brewster et al., 2008). In addition, about 20% of newly diagnosed breast cancer cases present with triple-negative status, which is defined by loss of ER, PR, and HER2 (Metzger-Filho et al., 2012), and more than 50% of primary tumors change their hormone receptor status from ER-positive, PR-positive to ER-negative, PR-negative at the time of recurrence (Thompson et al., 2010). Breast cancers with triple-negative or basal-like characteristics are often associated with a high risk of metastasis and both local and distant recurrence compared to receptor positive tumors (Ahmad, 2013). Treatment of such recurrent breast tumors is challenging owing to their aggressive nature, as they tend to be resistant to the “standard of care” adjuvant or neo-adjuvant systemic therapies. Consequently, patients with recurrent disease have low median survival. Despite the alarming statistics on recurrent breast tumors, the development of effective treatment modalities has been severely hampered by the paucity of data on the therapy-resistant traits of such tumors. There exists an urgent need for more effective breast cancer prognosis to aid the judicious treatment of tumors that are most likely to recur.

The report by Alvarez et al. (2013) in this issue of Cancer Cell transcends many of these foregoing limitations and provides timely insight into the molecular basis of breast cancer recurrence. In this seminal paper, the authors present compelling evidence that downregulation of the pro-apoptotic tumor suppressor prostate apoptosis response-4 (Par-4) (Hebbar et al., 2012) is a major determinant underlying breast cancer recurrence. Although Par-4 was first described in prostate cancer, it is ubiquitously expressed and serves as a tumor suppressor in diverse tumor types (Hebbar et al., 2012). Based on a highly refined analysis of gene expression records from human breast cancer data sets and the I-SPY 1 trial, Alvarez et al. (2013) conclude that low levels of Par-4 expression result in significantly decreased recurrence-free survival. Elegantly designed cell culture and mouse tumor experiments by Alvarez et al. (2013) indicate that primary breast tumors consist of a heterogeneous population of cancer cells, especially with regard to Par-4 expression (Figure 1). Tumor cells expressing high levels of Par-4 are eliminated by apoptosis following oncogene inhibition or chemotherapy, whereas those expressing low levels of Par-4 show an increased propensity to recur at both local and distant metastatic sites. Par-4 downregulation was particularly associated with all breast cancer traits that confer poor prognosis, such as ER-negative status, basal-like subtype, and high grade (grade III). By using primary and recurrent murine tumors driven by HER2/neu, MYC, or WNT1 oncogenic signaling, the authors demonstrate that tumors that relapse after oncogene inhibition or chemotherapy exhibit significantly diminished levels of Par-4 RNA and protein. Moreover, using orthotopic tumors with either low levels of Par-4 caused by short hairpin-RNA knockdown or overexpressed Par-4 via retroviral transduction, the authors demonstrate that downregulation of Par-4 is essential for tumor recurrence. By contrast, Par-4 levels do not influence primary tumor formation. The observations on the three distinct clinically relevant mouse models therefore recapitulate the findings of the meta-analysis on human breast cancer gene expression profiles. Thus, oncogene inhibition or chemotherapeutic regimens select for pre-existing tumor
cells that express low levels of Par-4, which are below the critical threshold for induction of apoptosis, and such cells constitute the therapy-resistant population that emerges as a recurrent tumor.

It is noteworthy that the expression of endogenous Par-4 as well as ectopic Par-4 is suppressed in recurrent tumors. Similar observations were previously made in the prostate of mice coexpressing oncogenic SV40 antigens and the SAC transgene, which represents the killer domain of Par-4 (Zhao et al., 2007). Zhao et al. (2007) found that when the SAC transgene was spontaneously eliminated from benign areas of the prostate, such cells continued to express the SAC domain remaining normal or benign. These findings not only reiterate the tumor suppressor potential of Par-4, but they also reaffirm that Par-4 induces apoptosis specifically in malignant tumor cells, not normal or benign cells.

The findings of Alvarez et al. (2013) on tumor recurrence also shed light on the distinct roles for Par-4 and other key proteins WNT and SNAIL, which have been previously shown to regulate EMT and metastasis. Unlike SNAIL and WNT, which have been reported to be overexpressed or activated in breast cancer EMT, metastasis, and recurrence (Ahmad, 2013), low Par-4 levels do not cause EMT or metastasis per se, permitting breast cancer recurrence independent of these traits by evading apoptosis. Importantly, the authors show that downregulation of Par-4 is necessary and sufficient for recurrence and occurs independently of SNAIL. Future studies on the mechanism by which Par-4 is downregulated in breast tumors may further unravel the molecular network involved in Par-4 regulation and breast cancer recurrence. Mechanistically, increased Par-4 expression in tumor cells in response to oncogene inhibition or treatment with chemotherapeutic agents was shown to enhance ZIP kinase-induced phosphorylation of MLC2 and multinucleation, ultimately leading to tumor cell apoptosis. Because the induction of tetraploidy is tumorigenic in a p53-deficient background, an intact p53 pathway is most likely required for this response. Although ZIP kinase has been previously shown to interact with Par-4, it is plausible that direct binding to Par-4 may not be necessary to provoke the ZIP kinase-dependent MLC2 phosphorylation and cytokinesis failure in breast cancer. Consequently, unlike tumor cells expressing basal levels of Par-4 that are readily eliminated by chemotherapeutic or oncogene inhibition, tumors expressing low Par-4 levels fail to elicit the ZIP kinase-driven apoptotic response and therefore constitute local or distant recurrent disease. Although the role of Par-4 in inducing apoptosis has been well characterized in the broader cellular context (Hebbar et al., 2012), this manuscript demonstrates an atypical mechanism for apoptosis by Par-4 in oncogene-addicted cells. These findings offer a new mechanistic link between oncogene addiction, Par-4 regulation, and tumor recurrence.

In summary, Alvarez et al. (2013) provide crucial insights into the heterogeneity of breast cancer and suggest that Par-4 is a viable prognostic marker for breast cancer recurrence. Their findings may provide the basis for the development of novel treatment strategies for breast cancer, such as nanotechnology, to deliver recombinant Par-4 to tumors in order to replenish intracellular Par-4 levels and sensitize the tumors to the action of therapeutics, which may prolong disease-free survival in breast cancer patients.

ACKNOWLEDGMENTS

This work was supported by a KLCR grant and NIH/NCI grant CA060872 (to V.M.R.).

REFERENCES

Acute myeloid leukemia (AML) is a heterogeneous clonal disorder of hematopoietic stem and progenitor cells characterized by the presence of recurring chimeric transcription factors and/or mutations affecting key components of the transcription machinery. With the exception of acute promyelocytic leukemia, which is uniquely sensitive to targeted therapies, which is largely dispensable for normal hematopoiesis, plays an important role and is a potential therapeutic target in mixed lineage leukemia.

Identification of tractable signaling molecules essential for leukemogenesis facilitates the development of effective targeted therapies. In this issue of Cancer Cell, Miller and colleagues report that Integrin Beta 3, which is largely dispensable for normal hematopoiesis, plays an important role and is a potential therapeutic target in mixed lineage leukemia.

MLL (Figure 1A). In spite of their promise in preclinical disease models, the development of specific inhibitors targeting the transcription machinery is still in a very early phase and a bottleneck for translating these results into the clinics. Approaches in identifying more tractable signaling molecules in which small-molecule inhibitors are readily available therefore appear as attractive alternatives.

In this issue of Cancer Cell, Miller et al. (2013) reported their in vivo shRNA screening approach to identify novel regulators required for MLL-AF9 leukemia in a mouse model with a focused library targeting 268 known or candidate cancer-associated genes. Using massively parallel sequencing to determine the relative distribution of each shRNA at different time points, 60 candidate genes targeted by at least two different shRNAs were found to be significantly depleted in vivo. Among them were Ctnnb1, Myb, and Met2c, which have been previously shown as critical targets for MLL. In addition, Itgb3 was also among the most highly depleted, suggesting that Itgb3 could represent a novel target for MLL-AF9 leukemia. Its functional significance was further validated with individual shRNAs targeting Itgb3, which resulted in a significant delay of the AML disease onset in recipient mice. Integrins are known to mediate many cellular processes and interact with the microenvironment. Consistently, the authors showed that leukemic cells with a reduced level of Itgb3 had a compromised ability to home and engraft in the bone marrow upon transplantation in both mouse and humanized xenograft models. On the other hand, successfully engrafted MLL cells carrying Itgb3 shRNA progressively declined over time, indicating a critical function of Itgb3 in mediating leukemic proliferation in addition to homing. The pathological importance of Itgb3 signaling in MLL-AF9 leukemia was further demonstrated by the depletion of leukemic clone with shRNAs targeting its heterodimeric partner, Itgav. Interestingly, the loss of Itgb3 is largely dispensable for normal hematopoiesis as Itgb3 germline knockout mice show no obvious hematopoietic defect and human patients with biallelic ITGB3 mutation do not have a bone marrow failure phenotype. In terms of homing, Itgb3 knockout did not impair hematopoietic reconstitution of normal LSK cells over a 24-week period after transplantation, although confirmation of persistent Itgb3 knockdown in the transplanted cells was not demonstrated. Nevertheless, these data together reveal Itgb3 as a novel therapeutic target for MLL (Figure 1B).

To gain further mechanistic insight into Itgb3 signaling in AML, a secondary in vivo...