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EZH2 takes the stage when BRCA1 loses

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BRCA1 is a hereditary breast cancer susceptibility gene. BRCA1 germ-line mutation carriers have greatly increased lifetime risks for development of breast cancer. Strikingly, BRCA1 mutations are synonymous with incidence of basal-like breast cancer, with characteristic expression of basal cell markers, including cytokeratins (CKs) 5/6 and CK17. This unique feature, or so-called "BRCAness," of hereditary breast cancers is commonly shared by sporadic basal-like breast tumors, many of which often have decreased BRCA1 expression as a result of gene methylation, suggesting a more common role for BRCA1 in regulation of the basal-like phenotype of breast cancer. An embryonic stem (ES) cell-like gene expression signature has been identified in breast cancers, which is primarily associated with the basal-like subtype but not others.[1] These findings imply that basal-like breast cancers share similar properties with ES cells, and that BRCA1 may play an important role in regulation of stem cell function.

Enhancer of zeste homolog 2 (EZH2) is the only catalytic subunit of the Polycomb repressive complex 2 (PRC2) that functions as a methyltransferase responsible for histone H3 lysine 27 trimethylation (H3K27me3) and epigenetic gene silencing. The Ploycomb-dependent function of EZH2 is essential for developmental patterning, X-chromosome inactivation, maintenance of ES cell pluripotency, repression of cell lineage differentiation, and generation of inducible pluripotent stem (iPS) cells. EZH2 is also implicated in tumorigenesis and progression, since its expression and activity are often deregulated in human cancers.

We recently demonstrate that BRCA1 directly binds to EZH2 but no other core components of PRC2, such as EED and SUZ12.[2] Co-immmunoprecipitation assay confirms that BRCA1 associates with the PRC2 complex in both human breast cancer and mouse ES cells. We further demonstrate that genetic deletion of one allele of BRCA1 or transient knockdown of BRCA1 in mouse ES cell lines promotes genome-wide EZH2 re-targeting and concordant increase in H3K27me3 levels at PRC2 target loci. Moreover, we show that decreased expression of BRCA1 increases EZH2 activity, resulting in inhibition of ES cell differentiation and an aggressive breast cancer phenotype, such as increased cell migration and invasion. Thus, our findings demonstrate BRCA1 as an essential negative modulator of EZH2 (**Fig. 1**).

Increasing evidence suggests that long non-coding RNAs (lncRNAs) have emerged as a critical aspect of cancer biology. HOTAIR lncRNA has been shown to be essential for the recruitment of EZH2 to its target gene loci.[3] Our recent findings show that BRCA1 binds to EZH2 in a region that is known to be bound by HOTAIR.[2] Mechanistically, we demonstrate that overexpression of BRCA1 blocks HOTAIR-mediated PRC2 targeting on chromatin while loss of BRCA1 enhances the interaction between EZH2 and HOTAIR, PRC2 occupancy and H3K27me3 levels at PRC2 target loci in human breast cancer cells (**Fig. 1**). Next-generation antisense oligonucleotides of cancer genes such as clusterin and IGF-IR are currently undergoing clinical or preclinical trials as an adjuvant therapy or a monotherapy for the treatment of human cancers. Our recent findings imply that antisense oligonucleotides against HOTAIR could be harnessed for the treatment of BRCA1-deficient breast cancer.

BRCA1 has been implicated in mammary luminal epithelial differentiation and basal-like breast cancer development.[4-6] However, which biochemical activities of BRCA1 mediate such a function remains poorly understood. Based upon our recent findings,[2] we envisage a model that reduced expression of BRCA1 due to gene mutation or promoter methylation augments EZH2 activity, which prevents committed cell lineage differentiation and/or favors undesired reprogramming of differentiated BRCA1-deficient luminal cells into basal-like, aggressive neoplastic cells. According to this model, specific inhibition of EZH2 could be exploited as a synthetic lethal option for treatment of BRCA1-deficient breast cancers. Notably, EZH2-specific inhibitors have been developed recently, and the antitumor effect of these inhibitors, such as GSK126, has been demonstrated in lymphoma mouse xenograft models.[7] It would logically be the next step to test the therapeutic effect of the EZH2 inhibitors on basal-like breast cancer, for which there is no cure at present (**Fig. 1**).

To date, BRCA1 has been shown to be involved in numerous biochemical processes including DNA damage response and repair, transcription regulation, and heterochromatin maintenance. It would be of great interest to elucidate how the Polycomb connection of BRCA1 affects its other functions or vice versa. We demonstrate that the binding of EZH2 by BRCA1 is substantially decreased following DNA damage,[2] suggesting that BRCA1-mediated inhibition of EZH2 can be attenuated in response to DNA damage. These results are not only relevant to the emerging link between PcG proteins and the DNA damage response, but also

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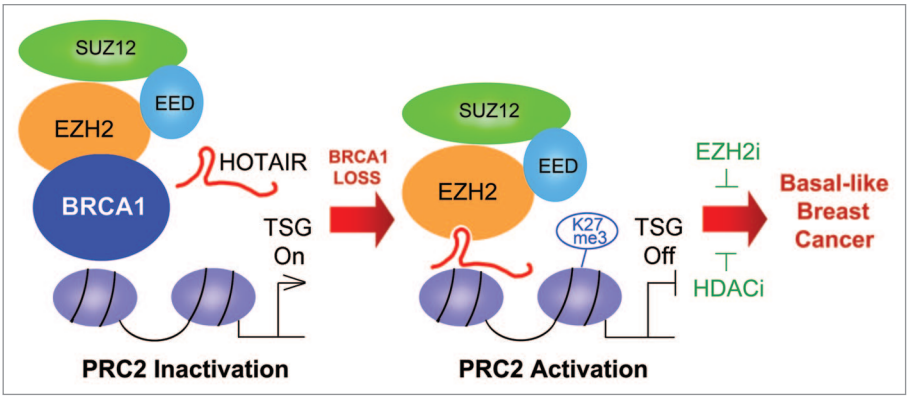

Figure 1. Cellular and molecular effects of BRCA1 loss on PRC2 function and development of basal-like breast cancer. BRCA1 binds directly to EZH2 in a region that is known to be bound by HOTAIR lncRNA, which, in turn, blocks HOTAIR-facilitated genome-wide occupancy of PRC2 on chromatin. However, loss of BRCA1 in mammary luminal epithelial cells may enhance the interaction of PRC2 with HOTAIR, thereby promoting PRC2 targeting on chromatin, increase in H3K27me3 levels at target loci, epigenetic silencing of tumor suppressor genes (TSGs), and ultimately development of basal-like breast cancer. Targeting of this deregulated pathway by the EZH2 inhibitors (EZH2i) or HDAC inhibitors (HDACi) may represent a viable therapeutic option for basal-like breast cancer.

provide further support to the notion that activated EZH2 might enhance DNA repair by repressing gene transcription at sites of damage.[8]

In summary, our recent work unravels a previously unrecognized molecular mechanism by which BRCA1 negatively regulates PRC2 functions and demonstrates that loss of BRCA1 augments EZH2 activity, thereby inhibiting ES cell differentiation and enhancing an aggressive breast cancer phenotype. Our findings imply that EZH2 could be a potential target for treatment of basal-like breast cancers with mutated or methylated BRCA1 gene.

References

1. Ben-Porath I, et al. Nat Genet 2008; 40:499-507; http://dx.doi.org/10.1038/ng.127; PMID:18443585
2. Wang L, et al. EMBO J 2013; 32:1584-97; http://dx.doi.org/10.1038/emboj.2013.95; PMID:23624935
3. Gupta RA, et al. Nature 2010; 464:1071-6; http://dx.doi.org/10.1038/nature08975; PMID:20393566
4. Liu S, et al. Proc Natl Acad Sci U S A 2008; 105:1680-5; http://dx.doi.org/10.1073/pnas.0711613105; PMID:18230721
5. Lim E, et al.; kConFab. Nat Med 2009; 15:907-13; http://dx.doi.org/10.1038/nm.2000; PMID:19648928
6. Molyneux G, et al. Cell Stem Cell 2010; 7:403-17; http://dx.doi.org/10.1016/j.stem.2010.07.010; PMID:20804975
7. McCabe MT, et al. Nature 2012; 492:108-12; http://dx.doi.org/10.1038/nature11606; PMID:23051747
8. Chou DM, et al. Proc Natl Acad Sci U S A 2010; 107:18475-80; http://dx.doi.org/10.1073/pnas.1012946107; PMID:20937877