Enhancer of Zeste 2 as a Marker of Preneoplastic Progression in the Breast

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Abstract

Amplification of the Polycomb group transcriptional repressor Enhancer of Zeste 2 (EZH2) occurs in various malignancies including breast cancer, where its overexpression is associated with poor outcome. We found that EZH2 is up-regulated in ductal carcinoma in situ, atypical ductal hyperplasia, and even morphologically normal breast epithelial cells from women who have an increased risk of breast cancer. This review discusses how EZH2 may promote neoplastic conversion and it surveys the evidence suggesting that EZH2 may offer a clinical tool to help identify patients at risk for developing breast cancer before precursor lesions are histologically evident. (Cancer Res 2006; 66(19): 9352-5)

Introduction

Breast cancer is the most common malignancy and a leading cause of cancer-related death in women in the Western world (1). However, the events leading to the transition from normal to cancerous epithelium are still not fully understood. As our understanding of cancer genetics increases, the diagnostic and prognostic markers of breast cancer are limited. Thus, there is an urgent need for identification of novel molecular markers that can detect the neoplastic process at its earliest phases before cancer develops. Such markers would assist in predicting individual risk for breast cancer, as well as constitute the basis for preventative targeted therapies.

Perturbations of cellular transcriptional memory may lead to developmental defects and cancer (2, 3). Two groups of proteins, the Polycomb group (PcG) and the Trithorax group (TrxG), are primarily responsible for long-term maintenance of heritable transcription patterns (4). Both maintain the spatial patterns of homeotic box (Hox) gene expression that are established by segmentation genes early during embryonic development. In this process, TrxG proteins act as epigenetic activators whereas PcG proteins act as epigenetic repressors. PcG and TrxG proteins have been shown to be involved in regulating a wide variety of fundamental cellular processes such as stem cell maintenance, cell fate, cell division, and neoplastic cell transformation (5-8).

Enhancer of Zeste 2 is a Member of the Polycomb Repressive Complex

Biochemical studies have established that the PcG proteins form at least two multimeric complexes: the Polycomb repressive complex 1 (PRC1) and the EED-Enhancer of Zeste 2 (EZH2) complex [or Polycomb repressive complex 2 (PRC2)]. PRC1 contains multiple proteins including BMI-1, HPC proteins (CBX2, CBX4, CBX7, and CBX8), and RING proteins, whereas the PRC2 complex includes EZH2, EED, SUZ12, and RbAp48 (9). EZH2 is the human homologue of the Drosophila protein Enhancer of Zeste [E(z)]. EZH2 contains a SET domain, a highly conserved domain found in many proteins with histone methyltransferase activity (10). The functional conservation between Drosophila and human PcG proteins in methylaing histone H3 on Lys47 has resulted in the development of a model for PcG-mediated gene silencing (11). In this model, PRC2-mediated histone H3 Lys47 methylation facilitates the binding of the PRC1 complex through specific recognition of the H3-K27 mark by the chromo domain of the Polycomb protein within PRC1. The PRC1 complex has recently been shown to exhibit histone H2A-Lys119 (K119) ubiquitin E3 ligase activities, with Ring1B acting as the catalytic subunit (12). Thus, it is becoming clear that both activities, methylation and ubiquitination, are required for the regulation of silencing by the PcG complex. However, the relationship between ubiquitination on K119 of histone H2A and methylation on K27 of histone H3 and their exact molecular role in polycomb silencing are not clear. Another question that needs to be addressed is how PRC complexes are directed to their loci, because no site-specific DNA-binding factor has been isolated in the PRC complexes.

Considerable efforts are channeled to define the downstream targets of the PcG complexes in mammalian cells. Bracken et al. (13) identified >1,000 silenced genes by the PcG proteins in human embryonic fibroblasts, with a strong functional preference for genes involved in embryonic development and genes responsible for cell fate decisions, including genes in the Hox, Notch, Hedgehog, Wnt, transforming growth factor, and fibroblast growth factor signaling pathways. Boyer et al. found that PcG complexes repress a large cohort of developmental regulators in murine embryonic stem cells, and thus control differentiation. PRC1 and PRC2 repressed 512 genes, many of which encode transcription factors with key role in development. In addition to Hox genes, PcG proteins bound to transcriptional regulators in murine embryonic stem cells, as well as members of the Fox, Sox, Gata, and Tbx transcription factor families, which not only have roles in development but also in disease (14). For example, Gata3 has been found to play a role in breast cancer, with strong association with estrogen receptor expression, and seems to be a biomarker of prognosis for patients with breast cancer (15-17).

Overexpression of EZH2 in Human Cancers

In epithelial derived tumors, EZH2 was first observed strongly associated with the aggressiveness of prostate cancer (18). Three groups of investigators, including our group, have shown that EZH2 expression is significantly up-regulated in invasive carcinoma and breast cancer metastases and that overexpression of EZH2 is associated with poor outcome in breast cancer patients (19-21).

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It was also observed that EZH2 expression has a positive relationship with high cell proliferation rate in breast and other tumors including endometrium, prostate, and bladder (21–24). Although the precise mechanisms of EZH2-associated malignant transformation and progression have yet to be determined, it seems that EZH2 and its binding partner EED play an essential role in cell proliferation by regulating the pRB-E2F growth control pathway (25). The E2Fs are required in transcriptional regulation of genes that are important in cell cycle progression (26). Furthermore, we have recently found that overexpression of EZH2 in breast epithelial cells represses genes that function in the homologous recombination pathway of DNA repair, the dysregulation of which may cause aneuploidy and malignant transformation (27). Thus, based on our current knowledge of EZH2 functions, we postulate that the up-regulation of EZH2 protein results in transcriptional repression of key target genes, including genes involved in differentiation, proliferation, cell fate, and DNA repair mechanisms, which can drive the neoplastic process.

EZH2 as a Molecular Marker for Breast Cancer

The discovery of cancer biomarkers has become a major focus of cancer research. The widespread use of prostate-specific antigen in prostate cancer screening has motivated researchers to identify suitable markers for screening different types of cancer. Biomarkers are also useful for diagnosis, monitoring of disease progression, prediction of disease recurrence, and treatment efficacy. To date, only a few biomarkers are considered clinically useful for breast cancer progression. Estrogen receptor and progesterone receptor are two important components in the evaluation of breast cancer, of which the major clinical value is in predicting the response to endocrine therapy. The expression of HER2/neu, another known marker, in breast cancer cells is associated with clinical responsiveness to Herceptin and anthracycline-containing chemotherapy. New biomarkers of cancer diagnosis, prognosis, and prediction of treatment response, especially those that can be applied at early cancer development phases, are highly desired.

In 2003, we found that EZH2 is a promising prognostic marker for breast cancer patients (19). Its expression was significantly increased in invasive carcinomas that recurred and/or metastasized. EZH2 was able to independently predict outcome, adding prognostic information above tumor size and lymph node status, the two most powerful predictors of breast cancer outcome to date. Independent investigations from other laboratories supported our data, highlighting the potential clinical usefulness of EZH2 as a prognostic biomarker in breast cancer (20, 21).

Our recent study (28) delineates a hitherto unknown property of EZH2: the ability to detect preneoplastic lesions of the breast in vivo (ref. 28; Fig. 1). This study included samples from a wide range of breast tissues including normal breast from mammoplasty

![Figure 1. Model for EZH2 overexpression in facilitating breast cancer development and progression. We postulate that increased levels of EZH2 in morphologically unremarkable breast epithelial cells promote preneoplastic and neoplastic progression through transcriptional repression of key genes involved in cell proliferation, cell fate, cell differentiation, and DNA repair. EZH2 protein is elevated in atypical ductal hyperplasia and in DCIS, the precursor of invasive carcinoma.](image-url)
procedures, atypical ductal hyperplasia, and ductal carcinoma in situ (DCIS) of different histologic grades. A strong positive relationship was uncovered between EZH2 expression and the nuclear grade of the DCIS. The highest EZH2 expression was detected in high nuclear grade DCIS with comedonecrosis (derived from necrotic malignant cells that accumulate in the center of the ducts and commonly calcify), and the lowest EZH2 expression levels were observed in low nuclear grade DCIS lesions. This pattern is consistent with the clinical evolution of DCIS, as high nuclear grade lesions portent a higher recurrence rate and shorter disease-free survival interval than low-grade and intermediate-grade DCIS.

Significantly, we also documented up-regulation of EZH2 expression in morphologically normal breast epithelium adjacent to atypical ductal hyperplasia and DCIS when compared with normal breast samples obtained from patients who underwent breast reduction and who did not have a family history of breast cancer. In this context, it is possible that the observed EZH2 up-regulation in normal mammary epithelium in the vicinity of atypical ductal hyperplasia and DCIS might be a "field effect" resulting from the activation of signaling pathways triggered by adjacent atypical and cancerous cells in breast cancer progression. Alternatively, in light of other information (20, 21, 23), it is tempting to speculate that the increased expression of EZH2 in morphologically unremarkable breast epithelium may identify epithelium at increased risk for cancer development.

To directly test the latter hypothesis, we evaluated EZH2 protein expression in benign breast tissue samples from women who underwent a benign breast biopsy for a mammographic or palpable abnormality. These patients had no personal or family history of breast cancer. After a follow-up of 12 years, a subset of these patients developed either DCIS or invasive carcinoma of the breast, whereas the remaining patients stayed healthy. In the benign breast tissues of the patients who later developed carcinoma, there was a significant up-regulation of EZH2 compared with those who did not. These observations suggested that EZH2 protein up-regulation may be a marker of increased risk in early breast cancer development before histologic atypia becomes apparent.

We also investigated the levels of EZH2 protein in morphologically normal breast tissues from patients carrying a BRCA1 mutation who had undergone a prophylactic mastectomy. We chose this group of patients because women who inherit germ-line mutations in the BRCA1 gene have up to an 85% lifetime risk of developing breast cancer by the age of 70 years (29). Notably, EZH2 was up-regulated in morphologically normal breast epithelial cells from patients with BRCA1 mutations, when compared with normal breasts from patients with no family or personal history of breast cancer who had undergone a reduction mammoplasty.

Taken together, these data suggest that EZH2 up-regulation may mark breast epithelium at higher risk for neoplastic transformation years before morphologic abnormalities in the form of recognizable atypia become evident to the pathologist. At the same time, these data support the theory that precursors of breast cancer exist in histologically normal breast tissues and they offer a way to identify such precursors.

**Perspective**

To date, the earliest recognizable precursor of invasive carcinoma of the breast is atypical ductal hyperplasia, the histopathologic diagnosis of which is based on fairly subjective criteria. Emerging data support the hypothesis that histologically normal breast epithelial cells from healthy women already contain genetic and epigenetic alterations that render them more susceptible to neoplastic transformation. Despite advances in the discovery of prognostic and predictive biomarkers, there are no clinically useful markers of increased breast cancer risk. The discovery of molecular markers to detect the precancerous state would have profound implications in the prevention of breast cancer at the bench and at the bedside. EZH2 may be such a marker of which the overexpression in breast epithelial cells indicates an increased risk of neoplastic progression, and in the future it may also constitute a marker of response to novel preventative approaches that target the tumor cells. There is still work to be done in elucidating the mechanisms by which EZH2 contributes to oncogenesis, but it seems that assessment of EZH2 expression detection may assist clinicians in the management of women at increased risk for development of breast cancer, a promise that may be confirmed through prospective clinical studies.

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