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The Polycomb group protein Enhancer of Zeste 2: its links to DNA repair and breast cancer

Michael Zeidler · Celina G. Kleer

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Abstract The Polycomb group protein EZH2 is a transcriptional repressor involved in controlling cellular memory and has been linked to tumorigenesis in multiple organs. In this review we summarize the current knowledge on the function of EZH2 in cancer, with special focus on breast cancer, and propose a link between EZH2, the homologous recombination pathway of DNA repair, and breast tumorigenesis.

Keywords EZH2 · Breast cancer · DNA repair · Homologous recombination · Biomarker · Prognosis · Polycomb · RAD51 · RAD51 paralogs · RAD51L1

Introduction

Perturbations of the transcriptional memory of a cell may lead to developmental defects and cancer (Laible et al. 1999; Jacobs and van Lohuizen 1999). Two groups of proteins have long been found to be involved in the maintenance of heritable transcription patterns: The Polycomb Group (PcG) of proteins and their counterparts the Trithorax Group (TrxG) of proteins (Laible et al. 1997). Both maintain the spatial patterns of homeotic box (Hox) gene expression which develop early in embryonic development of *Drosophila* by expression of segmentation genes. TrxG proteins act as epigenetic activators whereas PcG proteins act as epigenetic repressors. Fifteen years ago, PcG and TrxG proteins have been already described to be dysregulated in cells of hematopoietic neoplasms (Haupt et al. 1991, 1992; Raaphorst et al. 2000; Visser et al. 2001). PcG proteins have been implicated in fundamental cellular processes such as cell fate and cell divisions, as well as in neoplastic transformation. This review will focus on the role of the PcG protein Enhancer of Zeste 2 (EZH2) on malignant transformation, with special emphasis on breast cancer development, and its effect on the homologous recombination mechanism of DNA repair.

The PcG protein EZH2 is a transcriptional regulator

Biochemical studies have established that the PcG proteins form multimeric complexes, called Polycomb-repressive complexes (PRCs) 1 and 2. PRC1 contains multiple proteins including BMI1, HPC proteins, and RING proteins, whereas the PRC2 complex comprises EZH2, EED, SUZ12, and RbAp48 (Levine et al. 2004). The histone methyltransferase (HMT) 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 HMT activity. In addition to the SET domain, EZH2 contains two closely spaced SANT domains, which are often found in chromatin remodeling enzymes (Yu et al. 2003). Interestingly, the neoplastic properties of EZH2 appear to be dependent on an intact SET domain (Varambally et al. 2002).

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M. Zeidler · C. G. Kleer (✉)
Department of Pathology and Comprehensive Cancer Center, University of Michigan, 3510C MSRB1, 1150 W. Medical Center Dr., Ann Arbor, MI 48109, USA
e-mail: kleer@umich.edu

PRC2 interacts directly with type 1 histone deacetylases (HDACs), and this has been suggested to be part of the silencing mechanism (van der Vlag and Otte 1999). PRC2 complexes methylate histone H3 at lysine 27 (H3-K27), with EZH2 being the catalytically active protein. In the currently accepted model, PRC2 methylates H3-K27, which then recruits PRC1 to specific genetic loci, while PRC2 is released (Plath et al. 2003; Cao et al. 2002). It is intriguing that the expression of EZH2's binding partner EED is neither dysregulated in prostate cancer nor in human mantle cell lymphoma suggesting that it might not be overexpression of EZH2 but the imbalance between EZH2 and EED causing the oncogenic effects (Visser et al. 2001).

Recently, Viré et al. described EZH2 being in direct control of DNA methylation, a mechanism of epigenetic silencing (Vire et al. 2006). The investigators showed that EZH2 interacts with DNA methyltransferases (DNMTs). However, this interaction is not restricted to EZH2 as EED and SUZ12 also co-immunoprecipitated with DNMTs supporting the idea that the whole PRC2 is involved in regulating DNMT activity.

Because PcG proteins act mainly as transcriptional repressors, a significant effort is being devoted to the identification of Polycomb target genes. Bracken et al. (2006) identified over 1000 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 Notch, Hox, Hedgehog, Wnt, TGF, and FGF signaling pathways. These data highlight the fundamental role that PcG proteins may play in controlling development, differentiation, stem cell biology, cell fate, and carcinogenesis.

EZH2 and human malignancies

There are several lines of evidence that PcG proteins have a role in cancer and cell cycle progression. For instance, disruption of the EZH2 gene in mice causes embryonic lethality suggesting a crucial role in development (O'Carroll et al. 2001). Interestingly, EZH2 is up-regulated in pre-implantation mouse embryos thus functioning as an early acting protein potentiating blastocyst growth (O'Carroll et al. 2001). Overexpression of EZH2 enhanced proliferation of Ramos cells, a B cell lymphoma cell line (Visser et al. 2001) and antisense oligonucleotides directed against EZH2 blocked the growth of HL60 cells (a granulomonocytic cell line) (Fukuyama et al. 2000). In human malignancies, both EZH2 and BMI1 are coordinately up-regulated in Reed–Sternberg cells of Hodgkin's disease (Raaphorst et al. 2000) and non-Hodgkin's lymphoma (Raaphorst et al. 2001) and are associated with cycling cells and degree of malignancy.

Varambally et al. found that EZH2 was up-regulated in metastatic prostate cancer providing the first line of evidence that EZH2 mis-expression is a key alteration in malignancies of epithelial origin (Varambally et al. 2002). Furthermore, they were able to distinguish patients with localized prostate cancer that are more likely to fail after prostatectomy by looking at the expression levels of EZH2 (Varambally et al. 2002). Our laboratory found that EZH2 is overexpressed in invasive carcinomas of the breast when compared to normal mammary epithelium, and that EZH2 overexpression is able to transform breast epithelial cells. Notably, we found that EZH2 is a promising novel biomarker of aggressive breast cancer, being elevated in invasive and metastatic tumors when compared to normal breast tissues (Kleer et al. 2003). Subsequently, several investigators have confirmed our findings on the prognostic value of EZH2 in breast cancer (Collett et al. 2006; Raaphorst et al. 2003; Bachmann et al. 2006), as well as discovered that EZH2 in elevated in other human malignancies including transitional cell carcinoma of the bladder (Raman et al. 2005; Arisan et al. 2005), aggressive and invasive urothelial carcinomas (Weikert et al. 2005), endometrial cancer (Bachmann et al. 2005), Wilms tumor (Zirn et al. 2006), and hepatocellular carcinoma (Sudo et al. 2005). Taken together, these data strongly suggest that EZH2 has a pivotal role in the tumorigenic process in multiple organs and tissues. To date, the precise mechanisms of EZH2-driven neoplastic transformation remain to be elucidated.

EZH2 as a prognostic biomarker for breast cancer

Breast cancer is the most common malignancy and a leading cause of cancer-related death in women in the Western world (Jemal et al. 2003). Despite advances in the early detection and treatment, once distant metastases develop the disease is incurable. Our laboratory is especially interested in the discovery and validation of biomarkers of breast cancer risk and prognosis that have potential clinical utility.

While studying the genetic determinants of aggressive breast cancer, we identified consistent overexpression of EZH2 in a subset of tumors with worse clinical outcome (Kleer et al. 2003). To investigate the expression levels of EZH2 in human breast tissues, we constructed high-density tissue microarrays. Overexpression of EZH2 protein became apparent in DCIS, suggesting that EZH2 levels increase before stromal

invasion occurs. The highest levels of EZH2 protein were detected in invasive carcinomas and metastases. EZH2 overexpressing tumors were of high histologic grade, and were negative for estrogen and progesterone receptors. Furthermore, EZH2 was able to identify patients with invasive carcinomas with a worse survival.

Next, we tested the hypothesis that EZH2 overexpression may play a role in promoting breast tumor development and growth. Overexpression of EZH2 in human mammary epithelial cells induced anchorage independent growth in soft agar, a hallmark of malignant transformation, and triggered the invasive abilities of human mammary epithelial cells (Kleer et al. 2003). Taken together, our studies and the studies from other investigators (Collett et al. 2006; Raaphorst et al. 2003; Buchmann et al. 2006) suggest that EZH2 is a promising prognostic biomarker in breast cancer.

Insights into the role of EZH2 in DNA repair mechanisms

The above sections highlight the advances in the knowledge of the effect of EZH2 overepression in cancer development. In this section, we will review the existing data linking EZH2 overexpression with defects in DNA repair as a potential mechanism of EZH2-driven breast carcinogenesis.

The mammalian genome is at constant risk of mutation as a result of DNA damage. Hampered DNA double-strand break (DSB) repair may lead to structural chromosomal abnormalities and aneuploidy causing cell death or neoplastic transformation. Two major pathways in charge of DSB-repair have evolved: non-homologous end joining (NHEJ) which is a homology-independent mechanism that rejoins broken ends, regardless or sequence, and homologous recombination (HR) which promotes accurate repair of DSB by copying intact information from an undamaged homologous DNA template (Mills et al. 2003).

The conservation of both pathways in most organisms underscores the threat unrepaired DSBs pose on the organism.

We hypothesized that EZH2 overexpression may lead to malignant transformation of mammary epithelial cells by hampering the function of the DNA repair machinery. We found several clues which implicate EZH2 overexpression on the regulation of the HR mechanism of DNA repair in breast epithelial cells (Zeidler et al. 2005).

Using cDNA microarray and real time quantitative PCR we found that EZH2 overexpression on breast epithelial cells resulted in a decrease in the mRNA levels of the RAD51 paralog genes. Protein levels of these genes were also decreased. The RAD51 paralogs (RAD51L1, RAD51L2, RAD51L3, XRCC2, and XRCC3) are required for proper HR DNA repair in vertebrates (Thacker 2005). They share up to 20% amino acid identity with human RAD51 and with each other. Their importance in HR is demonstrated by the fact that null mutations of any of the paralogs prevent RAD51 from assembling at damaged sites, resulting in decreased number of RAD51 repair foci, hampered HR, and increased sensitivity to DNA cross-linking agents.

Consistent with a decrease in the mRNA and protein levels of the RAD51 paralogs, EZH2 overexpression resulted in a significant decrease in the number of RAD51 repair nuclear foci after induction of DSBs using etoposide (Zeidler et al. 2005). Overexpression of EZH2 on several benign and malignant breast epithelial cells led to reduced survival after exposure to ionizing radiation and etoposide, both known to cause DSBs that are repaired predominantly by HR.

One of the consequences of ineffective HR DNA repair is the development of aneuploidy and chromosomal aberrations, which constitute genomic instability. Genomically unstable cells have increased sensitivity to DNA damaging agents such as radiation. We found that EZH2 overexpression in breast epithelial cells resulted in aneuploidization and chromosomal instability. Taken together, these data uncover a link between EZH2, a regulator of homeotic gene expression, and HR DNA repair. It is possible that the blockade of EZH2 specifically in the cancer cells may constitute a novel approach to treatment of breast cancers with EZH2 overexpression.

Concluding remarks and future directions

The contribution of EZH2 and the Polycomb group proteins to development and tumorigenesis is quickly being unraveled. The PcG proteins repress over one thousand genes in mammalian cells and seem to affect a variety of important cell decisions, for instance cell development, and HR repair. This variety of effects is not surprising considering that the PcG proteins repress genes by chromatin remodeling and therefore act on a broader scale than other repressors. It is remarkable that one of the PRC2 members, EZH2, is so prominently overexpressed in cancers whereas other members of the PRC2 complex are not. It thus seems plausible that EZH2's neoplastic properties may not be dependent on its function within the PRC2 complex but on novel, PRC2-independent roles. We suspect that EZH2's function as a regulator of HR repair may be independent of the other PcG proteins. Another

question that needs to be addressed by future research is the contribution of the SET and the SANT domains to the effects of EZH2 on DNA repair and tumorigenesis. Further studies will show whether uncomplexed EZH2 acts on HR repair by binding to target proteins like the RAD51 paralogs, by binding directly to DNA, or by acting as a key regulator in a whole network of proteins.

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