

VIEWPOINT

EZH2-mediated epigenetic repression of DNA repair in promoting breast tumor initiating cells

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Abstract

Members of the Polycomb-group (PcG) family of proteins, including EZH2 (enhancer of zeste homolog 2), are involved in establishing epigenetic silencing of developmental genes in adult and embryonic stem cells, and their deregulation has been implicated in cancer. In a recent report, EZH2-mediated epigenetic repression of DNA damage repair in breast tumor initiating cells (BTICs) was identified as a mechanism that could promote expansion of BTICs, and may contribute to cancer progression.

Background

EZH2 (enhancer of zeste homolog 2) is a member of the Polycomb-group (PcG) family and encodes a histone methyltransferase that is the catalytic component of the polycomb repressive complex (PRC)2 [1]. The PRC2 complex has a role in establishing methylation marks on histone H3 (triMe-K27 H3) to initiate chromatin condensation and silencing of genes involved in developmental processes [2,3]. PcG proteins are known to be essential in maintaining self-renewal and pluripotency of stem cells [4], and their deregulation has been linked to cancer [5].

The cancer stem cell (CSC) hypothesis proposes that a limited subpopulation of cancer cells has self-renewal capacity and is responsible for tumor growth [6]. According to the CSC hypothesis, progression towards aggressive behavior can ultimately be traced to changes that occur in the CSCs, or tumor initiating cells (TICs). However, experimental data on the molecular changes and the mechanism by which they could occur have been lacking. In breast cancer, stem cell-like properties associate with expression of CD44, and negativity/low CD24, that is CD44⁺/CD24^{-/low} [7]. Recently, Chang and

colleagues [8] studied EZH2 in CD44⁺/CD24^{-/low} cells, or breast tumor initiating cells (BTICs), and described an important link to genomic instability, activation of RAF1-β-catenin signaling, and expansion of the BTIC population.

The article

Chang and colleagues [8] demonstrate that increased EZH2 in CD44⁺/CD24^{-/low} cells correlates with their increased abundance, that is, the proportion of CD44⁺/CD24^{-/low} cells. Increased EZH2 in CD44⁺/CD24^{-/low} cells was correlated with decreased RAD51 levels. Using chromatin immunoprecipitation (ChIP), the authors show that EZH2, and other PRC1/PRC2 proteins, are recruited to a putative Polycomb response element on the RAD51 promoter. Here, hypoxia in the microenvironment was identified as a potential cause of EZH2 upregulation. The *RAD51* gene has a well established role in DNA repair through homologous recombination and, accordingly, EZH2-mediated epigenetic repression of *RAD51* associates with accumulation of DNA damage and chromosome abnormalities.

Using protein arrays, the authors identified increased expression of the *RAF1* gene as a potential change associated with EZH2-induced genomic instability. Importantly, this association was found to be a consequence of acquired DNA copy number gains over the *RAF1* gene at 3p25.2, resulting in activation of ERK and β-catenin, shown to confer survival and proliferative advantages for CD44⁺/CD24^{-/low} cells. Importantly, inhibition of RAF1-MEK-ERK-β-catenin activation by small molecule inhibitors (AZD6244, Sorafenib) leads to effective elimination of CD44⁺/CD24^{-/low} cells - that is, BTICs - suggesting potential implications with respect to clinical management of the disease.

Viewpoint

The most important insight from Chang and colleagues [8] is the observation that genomic instability induced by EZH2-mediated epigenetic repression of DNA repair, that is, RAD51, results in expansion of the BTIC population. Further, the order in which these changes occur did not influence the eventual outcome - that is, over-expression of EZH2 has the same effect as knock-down of

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RAD51. In this context, RAF1 amplification was described as a recurrent oncogenic consequence of down-regulated DNA repair leading to activation of ERK and β -catenin and the consequent promotion of the BTIC population. In breast cancer, EZH2 overexpression has previously been linked to aggressive and poorly differentiated carcinomas [9], and downregulated DNA repair [10]. The insights provided by Chang and colleagues relate to the identification of EZH2 in augmenting BTICs through epigenetic repression of DNA repair, leading to aggressive disease. These results are consistent with clonal evolution of CSCs, that is, the notion that aggressive secondary CSCs can arise from the primary population of CSCs. The clinical relevance relates to potential benefits from RAF1/MEK/ERK inhibitors in the treatment of breast cancer patients, and in preventing disease progression. Further, poly ADP ribose polymerase (PARP) inhibitors are highly effective in targeting cancer cells with homologous recombination defects and therefore hold unexplored potential in this regard, that is, in targeting secondary CSCs [11].

Although somatic mutations in DNA repair genes are rare in sporadic cancers, it is well established that susceptibility to cancer is commonly linked to inherited mutations in DNA repair genes, for example, *BRCA1*, *BRCA2*, *PALB2*, *WRN* and *MLH1* [12]. This has led some to speculate that defects in DNA repair genes cannot explain instability in sporadic cancers [12], whereas others have emphasized epigenetic mechanisms [13]. Chang and colleagues [8] describe results suggesting a causative link between epigenetic inactivation of DNA repair, the emergence of genomic instability, and cancer progression. In breast cancer, epigenetic inactivation of the *BRCA1* gene occurs through CpG island hypermethylation [14,15]. *BRCA1* is essential in DNA repair of double-strand breaks, with RAD51 as a crucial partner for error-free repair by homologous recombination [16]. Recently, Lim and colleagues [17] demonstrated that *BRCA1* defective breast cancers represent an expanded population of luminal progenitor cells. This is consistent with the results described in Chang and colleagues [8], demonstrating DNA repair defects and expansion of tumor cells with progenitor/stem-cell characteristics. In conclusion, an important task in future research will be to explore the relationship between epigenetic repression of DNA repair, the induction of genomic instability, and markers of CSCs.

Abbreviations

BTIC, breast tumor initiating cell; CSC, cancer stem cell; PcG, Polycomb-group; PCR, polycomb repressive complex.

Competing interests

The authors declare that they have no competing interests.

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