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Targeted Epigenetic Therapy: EZH2 Inhibition In B-Cell Malignancies

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COLUMN ARTICLE

Deregulation of epigenetic mechanisms is a hallmark of cancer and has an important role in carcinogenesis. Epigenetic alterations in tumors have been suggested to represent potential biomarkers reaching from diagnosis, prognosis and even been suggested as potentially useful for prediction of therapy response [1,2]. The reports on aberrant profiles of DNA methylation and histone modifications have recently propelled the interest of using epigenetically silenced or activated cancer genes as new targets for epigenetic anticancer therapy [3,4]. Recently, the advancement in genetic and epigenetic technologies (e.g. whole-genome sequencing (WGS), chromatin Immunoprecipitation sequencing (ChIP-Seq), RNA-Seq and whole-exosome sequencing (WES)) has facilitated the detailed mapping of the genomic and epigenomic tumor landscapes [5-7]. Next-generation technologies unravelling these epigenetic signatures, in parallel with the development of small-molecule inhibitors targeting active components of epigenetic modifiers may now have the potential to fully unleash our understanding of epigenetic alterations in cancer biology and how inhibiting these components may be of use for future treatment of patients [8,9].

A target that has attracted recent attention is the Enhancer of Zeste homolog 2 (EZH2), a SET domain histone methyltransferase that catalyses the formation of mono, di and tri-methylation of histone H3 lysine 27 (H3K27me1, 2 and 3). The EZH2 is the enzymatic subunit of the polycomb repressive complex 2 (PRC2), an important regulator of both normal development as well as disease. PRC2 and EZH2 via the installation of H3K27me3 repressive mark regulate expression programs related to stem cell self-renewal, differentiation, but also cellular transformation [10-12]. The pathologic activation of EZH2 due to genetic mutations and overexpression has been reported in several cancers i.e. bladder, prostate, lymphoma, myeloma, colon and breast [13,14]. However, not until the development of highly potent small-molecule inhibitors of EZH2 [15] and PRC2, these components could be evaluated as a therapeutic target.

EZH2 has an important role in normal early B-cell development [16], germinal center (GC) formation and in lymphomagenesis [17]. GC B-cells are characterized by rapid proliferation accompanied by increased mutational rate, which allow them to generate high-affinity antibodies. This window of increased cell proliferation and mutation rate provides excellent conditions for cellular transformation. In GC-derived tumors, including 20% of diffuse large B cell lymphomas (DLBCLs) and 10% follicular lymphomas EZH2 was found to harbor gain of function mutations (involving Y641 or A677) in its catalytic SET domain (FLs) [18]. EZH2 inhibition using EPZ005687 [19], EPZ-6438 [20]; GSK126 [21] showed a potent anti-lymphoma activity. Interestingly, EZH2 inhibition was most effective in DLBCL cell lines harboring the EZH2 gain of function point mutation suggesting that B-cell lymphomas with EZH2 gain of function mutations were oncogenically addicted on EZH2 to maintain their proliferative capacity and survival. In contrast, DLBCL cell lines with wild type EZH2 show non-ocogenic dependency on EZH2, possibly reflecting the described role for EZH2 in selected windows during B cell development

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[21,22]. EZH2 was found to support GC hyperplasia and lymphomagenesis via repressing the expression of genes constituting negative regulators of the cell cycle CDKN1A/p21 and CDKN1B/p27, the anti-apoptotic BCL2 and differentiation-related genes, including PRDM1 and IRF4 [17,22]. As a consequence, pharmacological EZH2 inhibition reduced GC hyperplasia, cellular proliferation and tumor growth both *in vitro* and *in vivo* by inducing of cell cycle arrest and terminal differentiation [22]. The current status of the EZH2 inhibitor E7438 (a derivative of EPZ-6438) is in phase I/II clinical trials as a single agent in subjects with B-cell lymphomas (https://clinicaltrials.gov/ct2/show/NCT01897571).

Multiple Myeloma is a tumor of the antibody producing plasma cells (PCs), thus it represents the far end spectrum of differentiation reflected in B-cell malignancies. MM is a genetically heterogeneous tumor characterized by the clonal expansion of malignant PCs in the bone marrow, which leads to clinical features among others i.e. bone marrow destruction and failure [23,24,25]. Already in 2005, EZH2 was suggested to be a potential oncogene in MM based on the fact that EZH2 was found to be overexpressed in malignant PCs as compared to normal BM PCs. EZH2 demonstrated oncogenic activity *in vivo,* this activity being dependent on its methyltransferase activity [26]. Recently, we and others have provided experimental and functional proof- of- concept that pharmacological inhibition of EZH2 using small-molecule specific chemical inhibitors have anti-myeloma effects and may now be considered for further experiment to elucidate this as a potential therapeutic strategy in MM [27,28]. Using ChIP-seq we provided for the first time a unique epigenomic profile of H3K27me3, bivalent and H3K4me3 targets in CD138+ PCs derived from MM patients. Underpinning the clinical relevance of this finding, the expression pattern of H3K27me3-marked genes correlated with poor patient survival [27]. Pharmacological inhibition of EZH2 by small molecular inhibitors [29] showed a decrease of the global levels of H3K27me3 and a set of reactivated genes such as ID2, ID3, and SOX2 in parallel to induced apoptosis [27]. These findings highlight EZH2 and the PRC2 as a promising complex for selective targeting and pave the way for studies evaluating the use of EZH2 inhibitors as MM therapeutic agents by *in vivo* experiments with these inhibitors alone or in combination with current treatment regimens.

Of particular importance, both MM [27] and B-cell lymphoma [22] studies report on the increase in the number of bivalent chromatin domains in malignant cells as compared with normal counterpart cells. Bivalent chromatin domains are marked by the transcription repressive H3K27me3 and permissive H3K4me3 marks. The co-existence of both marks may make these domains poised to be transcriptionally activated upon inhibition by epigenetic modifiers. Thus, bivalent genes should be preferred targets for gene activation mediated by pharmaceutical compounds directed to epigenetic modifiers. Indeed, studies in MM as well as in B-cell lymphoma [22,27] have confirmed that EZH2 inhibition mainly reactivates the expression of a set of bivalent genes with anti-tumor activity (Figure 1).

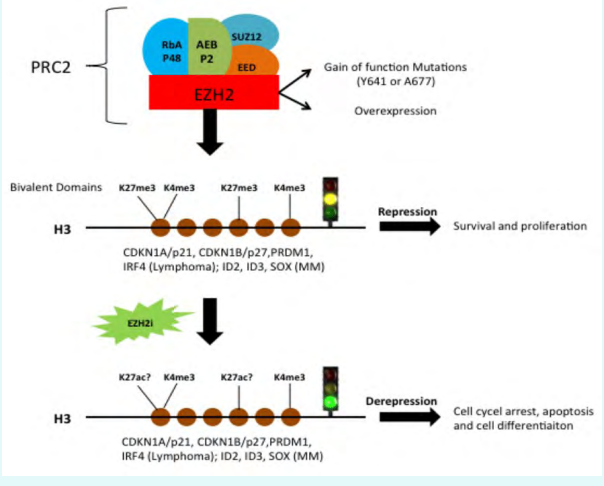

Figure 1: EZH2 enhances ceullar transformation via the formation of bivalent chromatin domains. EZH2 is the enzymatic subunit of PRC2 complex and its activity is dependent on other subunits. EZH2 installs H3K27me3 mark, which represses gene transcription alone or in combination with other histone marks. H3K27me3 and H3K4me3 form bivalent chromatin domains, which are repressed but ready to be transcribed upon stimulation. Whether H3K27me3 and H3K4me3 are located on the same H3 protein or on different H3 histones is not well understood. EZH2 activation due to genetic mutations or overexpression enhances gene repression via H3K27me3 mark and thus promotes transformation. EZH2 via the formation of bivalent domains represses genes with potential tumor suppressor functions, which get reactivated upon EZH2 inhibition. One possible hypothesis is that histone acetylation replaces trimethylation at Lys 27 at H3 upon EZH2 inhibition, which further support transcriptional activation of tumor suppressor genes.

Here we highlight the importance of new technologies unravelling epigenetic signatures and the development of small-molecule inhibitors targeting active components of epigenetic modifiers to unleash our understanding of the role of epigenetic alterations in cancer biology. Epigenetic modifiers and epigenetically silenced genes may be potential targets in cancer treatment and especially EZH2 has been reported as a potential target in B-cell lymphoma and multiple myeloma. EZH2 may be mutated for gain of function, but the upregulated expression has also been reported in tumors including MM [26,30] breast, bladder, melanoma, and prostate [31]. It will now be a challenging task to unravel the role of EZH2 and bivalent chromatin domains in carcinogenesis of tumors dependent on EZH2, and to evaluate the potential use of EZH2 inhibitors in models of these tumors. Still relatively unexplored, more studies are needed to translate the use of these inhibitors to clinical practice.

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