this may not be the answer. Administering higher doses of drug to patients is not an option because of off-target effects and toxicities. Due to its unique complexities, perhaps it is time to look beyond RAF? MEK inhibitors are showing promise in the clinic while we all eagerly await clinical data on inhibitors targeting ERK.

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EZH2: An Epigenetic Gatekeeper Promoting Lymphomagenesis

Holger Heyn[1] and Manel Esteller[1,2,3,*]
[1]Cancer Epigenetics and Biology Program (PEBC), Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, 08908 Barcelona, Catalonia, Spain
[2]Department of Physiological Sciences II, School of Medicine, University of Barcelona, 08036 Barcelona, Catalonia, Spain
[3]Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Catalonia, Spain
*Correspondence: mesteller@idibell.cat
http://dx.doi.org/10.1016/j.ccr.2013.04.028

In this issue of *Cancer Cell*, Béguelin and colleagues highlight EZH2 as an essential regulator for B cell activation and report an addiction of germinal center-derived neoplasms to EZH2 activity. This reversible process is specifically targetable and hence presents high translational value for lymphoma therapy.

The *enhancer of zeste homolog 2* (EZH2) is a SET domain containing methyltransferase catalyzing the methylation of histone H3, forming the transcriptional repressive epigenetic mark H3K27me3. EZH2 is a subunit of a multi-enzyme complex known as polycomb repressive complex 2 and is involved in chromatin compaction and gene repression. EZH2 is expressed in undifferentiated stem and progenitor cell types but predominantly silenced in somatic cells. Despite its repressive function through H3K27 tri-methylation, it frequently co-localizes with the activating histone modification H3K4me3. These bivalently marked genes present minimal expression level in undifferentiated cells, but upon differentiation initiation, lose H3K27me3 and are transcriptionally activated.

Lymphogenesis represents a special case wherein EZH2 is repressed in resting naive B cells, but is highly upregulated in primary lymphoid follicles during B cell activation and germinal center (GC) formation (Velichutina et al., 2010). Herein, EZH2 defines a GC-specific repression profile including silencing of cell cycle checkpoints and differentiation factors. This epigenetic setting allows rapid B cell proliferation, an important step during the maturation process in germinal centers. Consistently, EZH2 silencing results in cell cycle arrest at G_1/s transition (Velichutina et al., 2010). In line with its proliferation promoting function, EZH2 was shown to be highly expressed in GC-derived lymphomas, such as diffuse large B cell lymphomas (DLBCLs) (van Kemenade et al., 2001). Moreover, mutations in the SET domain, favoring the formation of trimethylated H3K27, have recently been reported as frequent events in DLBCL (Morin et al., 2011). EZH2 mutant tumor cells are almost exclusively detected in the GC-derived subtype, affecting about 20% of GCB-DLBCL patients and suggesting a subtype-specific function of the alteration. Clinically, mutant EZH2 can be specifically targeted using small molecule inhibitors, such as GSK126 (McCabe et al., 2012). Following drug application, EZH2 mutant lymphoma cells revealed reduced levels of H3K27me3 and, most importantly, presented a highly impaired proliferative potential in vitro and in mouse DLBCL xenograft models.

Although there is clear evidence for the contribution of EZH2 to B cell maturation and neoplastic transformation in GCB-DLBCLs, the underlying molecular

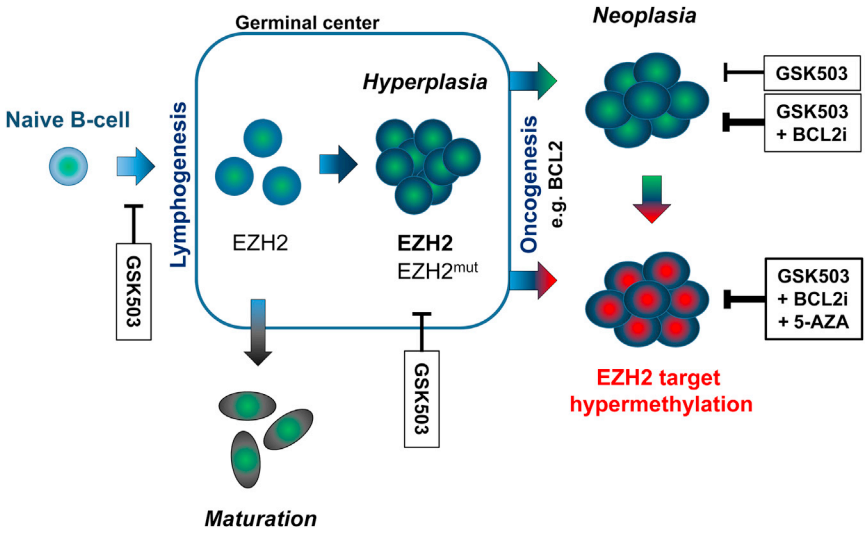

Figure 1. EZH2 Function Is an Important Component for B Cell Activation and Lymphomagenesis

EZH2 is actively participating in the formation of germinal centers in lymphoid follicles; however, it is repressed during later maturation steps. EZH2 hyperactivity (mutation or overexpression) further promotes proliferation, resulting in hyperplasia. Additional oncogenic events are required for neoplastic transformation, although germinal center-derived lymphomas remain addicted to EZH2 function. EZH2 inhibitors (e.g., GSK503) block hyperproliferation and transformation and are more effective against lymphomas in combination with other specific therapies (e.g., BCL2 inhibition [BCL2i]). The presence of EZH2 target gene hypermethylation in a subset of lymphomas suggests a potential positive implication of DNA demethylating agents (e.g., 5-azacytidine [5-AZA]) in combinational therapies.

mechanisms are not yet entirely identified. In this issue of *Cancer Cell*, Béguelin et al. (2013) performed a comprehensive functional analysis of the role of EZH2 in B cell biology and, in particular, during the B cell maturation process in germinal centers and associated lymphoma subtypes. The authors provide solid evidence that EZH2 expression is a crucial factor for germinal center formation in the spleen and that its repression in late GC B cells is necessary to permit an exit from the germinal center and allow subsequent differentiation steps. Functionally, EZH2 establishes a de novo-formed chromatin landscape, unique for GC B cells and involving repressive and bivalent loci. Mutant hyperactive EZH2 promotes the formation of germinal centers harboring highly proliferative B cells. Intriguingly, Béguelin et al. (2013) prove that, although favoring a hyper-proliferative state of GC B cells resulting in hyperplasia, mutant EZH2 is not sufficient for cell transformation (Figure 1). They provide evidence that additional oncogenic aberrations (e.g., BCL2) are required to turn GC B cells into diffuse large B cell lymphomas.

Important EZH2 downstream target genes have been described for epithelial tumors, but little is known about lymphomas. Béguelin et al. (2013) identified key EZH2 target genes in GC biology, including *CDKN1A*, which is critical for cell cycle checkpoint, and *IRF4* and *PRDM1*, transcription factors important for GC exit. The latter switch from a poised bivalent state to actively transcribed regulators during normal B cell maturation. *CDKN1A* and bivalent regulators were hyper-repressed in EZH2 mutant DLBCL cells, suggesting that transformed B cells are locked in their immature state by effective repression of anti-proliferative and differentiation-inducing genes.

Clinically important, the authors present evidence that GCB-DLBCLs are addicted to the oncogenic function of EZH2 independent of its mutational state, as impaired enzyme activity abolished tumorigenesis of mutant and wild-type cancer cells. To specifically target EZH2, the authors used a small-molecule inhibitor (GSK503), highly reducing the catalytic activity of mutant and wild-type EZH2 and enabling broad potential application in GCB-DLBCLs. Herein, the authors suggest GSK503 together with BCL-2 inhibitors, as a combinational treatment, outperform single-drug therapies in lymphoma models. Notably, GSK503 activity was restricted to GCB-DLBCL, without showing anticancer effects on ABC-DLBCL, a lymphoma subtype with repressed EZH2 derived from late-stage GC B cells. This is consistent with the observation that EZH2 mutations exclusively occur in GCB-DLBCLs, but not in the ABC-subtype (Morin et al., 2011).

Collectively, Béguelin et al. (2013) now prove that EZH2 is essential for the formation of germinal centers and that GC-derived lymphoma subtypes are addicted to the expression of wild-type or mutant EZH2. Most importantly, they displayed drug-induced reversibility of the transformation process with potential translational value for the clinic. Interestingly, previous studies from the group presented evidence of EZH2 participation in an epigenetic network involving DNA methylation. Herein, they described a gain of DNA methylation of EZH2 target genes during lymphomagenesis (Velichutina et al., 2010; De et al., 2013). Interestingly, hypermethylation of polycomb target genes (PcTGs) represents a frequent event in oncogenesis reported for leukemia and solid tumors (Schlesinger et al., 2007; Sandoval et al., 2013). Functionally, this is in contrast to the observation that H3K27me3 and DNA hypermethylation are generally mutually exclusive at gene promoters in a healthy context (Statham et al., 2012). In this regard, two scenarios can be considered when explaining the controversial epigenetic state of PcTGs in cancer contexts: an epigenetic switch from the repressive histone mark H3K27me3 toward DNA hypermethylation, herein reducing epigenetic plasticity and locking the target genes in a stable silent state. Alternatively, both epigenetic repressors might co-localize and thereby form a dual repressive state at CpG islands and transcription start sites in cancer (Statham et al., 2012). It should be noted that de novo DNA methylation of PcTGs mainly takes place at previously repressed and therefore silent genes. Thus, the switch toward DNA methylation or its co-localization with H3K27me3 is likely to reinforce the repressive state rather than induce major transcriptional changes.

In GC-derived lymphomas, both aforementioned scenarios might lock

proliferation checkpoint genes, such as *CDKN1A*, in their repressed state and, thereby, cancer cells even tighter in their undifferentiated phenotype. In this regard, mutant EZH2 exhibits a similar effect in GCB-DLBCL, with its hyperactivity increasing gene repression of B cell maturation-related factors. In both aberration types, a key position at the gate toward B cell differentiation is held by bivalent genes, whose hypermethylation or hyper-repression with H3K27me3 further blocks the B cells maturation process and locks the cells in a germinal center configuration. From a therapy perspective, it would be of great interest if GCB-DLBCL cases with a high degree of epigenetic switches and hence reduced reversibility were less sensitive to EZH2 inhibitor treatment. Herein, lymphoma patients with EZH2 hypermethylation phenotype might profit from combinational therapies of EZH2 inhibitors (McCabe et al., 2012) and DNA demethylating agents (Herranz et al., 2006) (Figure 1).

Unlike in GCB-DLBCL, biological consequences of PcTG hypermethylation in other cancer types are not easy to dissect. Here, PcTG silencing in cancer types derived from tissue progenitor cells might contribute to a transformation process toward cancer stem cells by effectively preventing cell differentiation. Likewise, somatically-derived cancer types might convert to a more undifferentiated state with support of the formation of a stem cell-like epigenetic setting. Accordingly, in addition to DLBCL, other tumor types might profit from therapeutic strategies involving EZH2 inhibitors and DNA demethylating agents.

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Luring BRCA1 to the Scene of the Crime

Richard Baer[1,*]
[1]Institute for Cancer Genetics, Department of Pathology and Cell Biology, Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY 10032, USA
*Correspondence: rb670@columbia.edu
http://dx.doi.org/10.1016/j.ccr.2013.04.013

To preserve genome stability, BRCA1 must be recruited to sites of DNA damage, where BRCA1 facilitates repair of double-strand DNA breaks (DSBs). In this issue of *Cancer Cell*, Li and Yu report that BRCA1 recruitment involves a novel interaction between its partner protein BARD1 and poly(ADP-ribose) chains at the DSB.

Germline mutations of *BRCA1* are a primary cause of hereditary breast and ovarian cancer. The central role of BRCA1 in the cellular response to double-strand DNA breaks (DSBs) is thought to be a key aspect of its tumor suppression activity (Li and Greenberg, 2012). The DSB response entails recruitment of various signaling and repair factors, including BRCA1, to nuclear sites of damaged DNA. These factors selectively accumulate at one or both of two cytologically distinct subcompartments: (1) the "DSB end" itself and (2) the flanking "DSB chromatin" marked by γH2AX, a phosphorylated form of the H2AX histone variant (Bekker-Jensen et al., 2006). Of note, BRCA1 can be recruited to both subcompartments, suggesting that it performs multiple, as yet undefined, functions in DSB repair. Its localization to DSB chromatin occurs through an elaborate pathway that entails H2AX phosphorylation, H2A polyubiquitination, and ubiquitin-mediated recruitment of the RAP80 complex (Li and Greenberg, 2012). Ultimately, γH2AX-dependent assembly of BRCA1 requires its direct interaction with Abraxas, a polypeptide within the RAP80 complex.

Early on, Celeste et al. (2003) reported that BRCA1 recruitment to DSBs is a kinetically biphasic process comprised of an early γH2AX-independent stage and a late γH2AX-dependent stage. Subsequently, Mailand et al. (2007) observed that BRCA1 localization to DSB flanking chromatin, but not the DSB end subcompartment, is ablated upon