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● ● ● TRANSPLANTATION

Comment on He et al, page 1274

Control of GVHD: it's in our DNA!

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Selective depletion of the alloreactive T cells causing graft-versus-host disease (GVHD) without loss of the graft-versus-leukemia (GVL) effect is the holy grail of hematopoietic cell transplantation (HCT). In this issue of *Blood*, He et al demonstrate that inhibition of histone methylation leads to selective apoptosis of the alloreactive effector cells.¹ Moreover, they demonstrate that this inhibition of histone methylation remarkably stops ongoing GVHD.

Because our whole being is mostly dictated by our genetic code, its regulation is rightfully tightly controlled. This process at the DNA level resides with the addition or

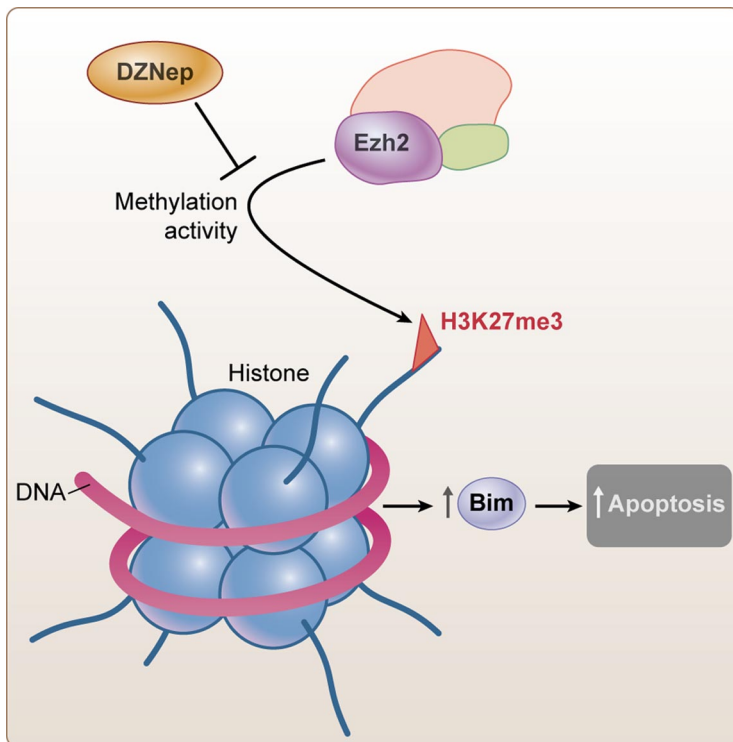
removal of methyl or acetyl groups to specific amino acids found on histones or on the DNA nucleotides. In the cell nucleus, DNA is wound around these histone proteins. Histone

methylation is the modification of certain amino acids in a histone protein by the addition of 1, 2, or 3 methyl groups. In general, methylation and demethylation are the “off” and “on” switches, respectively. This control is accomplished either by loosening the tails of the histone, thereby allowing transcription factors and other proteins to bind the DNA, or by winding their tails around the DNA, thereby restricting access to the DNA.² Histone methylation is normally associated with transcriptional repression. However, methylation of some lysine and arginine residues of histones results in transcriptional activation.

PolycombGroup (PcG) proteins maintain gene repression through histone modifications and are involved in cell regulation including cancers. Ezh2 is part of Polycomb Repressive Complex 2 (PRC2) and trimethylates the histone H3 at lysine 27 (H3K27me3). Among other functions, this histone silences *Bim*, a proapoptotic gene. Recent studies have shown that 3-Deazaneplanocin A (DZNep), a histone methyltransferase inhibitor, disrupts polycomb-repressive complex 2 (PRC2), and preferentially induces apoptosis in cancer cells, including acute myeloid leukemia (see figure).³

He and colleagues sought to study the impact of modulating histone methylation in GVHD specifically with DZNep. They demonstrate that inhibition of histone methylation with this molecule is associated with depletion of the Ezh2, decrease in the methylation of H3K27 leading to the increase in the mRNA and protein of the proapoptotic protein *Bim*, a major regulator of apoptosis in T-cell homeostasis and responses. He et al demonstrate that this effect is lost in *Bim* knockout mice. One of the most remarkable findings was that they were able to show that the effect on GVHD was present even after GVHD had occurred. The use of DZNep turned off ongoing GVHD with a decrease in the accumulation of allo-reactive effector T cells and inflammatory cells in the target organs of GVHD. In contrast to previous data in GVHD mediated by either DNA methylation inhibitors or histone deacetylase (HDAC) inhibitors, DZNep did not increase regulatory T cells or affect antigen-presenting cells.⁴⁻⁷

So, does this mean that GVHD versus GVL is solved? He and colleagues have taken us in an important direction but as with all



Proposed mechanism of action of DZNep. Inhibition of methylation by Ezh2 leads to increase in *Bim* and increase in apoptosis. Professional illustration by Kenneth X. Probst.

good papers, more questions arise. They do demonstrate that DZNep did not lead to a complete elimination of the alloreactive T cells. Thus the positive data reflect a balance between the stimulation needed to promote GVL versus GVHD. In other words, it may take either fewer T cells or less stimulation of T cells to effect GVL compared with GVHD. Our own data suggest that by simply changing the numbers of T cells without any further intervention, we can find a sweet spot where there is no clinical GVHD but with persistent GVL (Chen et al, Prevention of GVHD and preservation of GVL based on T-cell numbers, manuscript in preparation). Moreover, the hurdles of translation from murine models to humans are high. Ezh2 is involved in multiple signaling pathways such as Wnt/ β catenin, Ras, NF κ B, Notch, and so on, so off-target effects will need to be carefully understood. On the positive side, DZNep has activity against tumor cells and antiviral effects that could be beneficial. It will be exciting to see if this approach can be extended to other murine models of T cell–mediated inflammatory or autoimmune responses and then on to clinical studies.

● ● ● VASCULAR BIOLOGY

Comment on Kuo et al, page 1302

TM hidden treasure: lectin-like domain

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In this issue of *Blood*, Kuo et al have used recombinant lectin-like domain of thrombomodulin domain 1 (TMD1) to demonstrate the action of lectin-like domain in blocking Lewis Y antigen (LeY)–mediated angiogenesis and control of tumor growth.¹

Thrombomodulin (TM) anchors to vascular endothelial cell membrane with a single transmembranous domain.^{2,3} It has a large extracellular region that comprises a C-type lectin-like domain at the N-terminus, an epidermal growth factor (EGF)–homology domain that contains 6 EGF-like structures, and a Ser/Thr-rich domain linked to the membrane (see figure). Thrombin and protein C bind to the EGF-homology domain where thrombin cleaves protein C to generate activated protein C (aPC).² aPC is released into the blood where together with protein S it degrades coagulation factors VIIIa and Va and thereby prevents excessive blood clotting. aPC also possesses anti-inflammatory actions and is

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induced inflammatory responses.⁴ Here, Kuo et al provide evidence that recombinant TMD1 controls endothelial cell migration and tube formation through binding to LeY clustered at the endothelial cell membrane ruffles and protrusions.¹ Their results show that LeY mediates angiogenesis and TMD1 or LeY antibodies abrogate the angiogenic effect. Epidermal growth factor receptor (EGFR) is expressed on endothelial cell surface. It contains LeY that may be involved in angiogenesis. Investigations in vivo show that TMD1 blocks in vivo angiogenesis induced by EGF and suppresses tumor growth by inhibiting tumor angiogenesis. Taken together, the results indicate that the lectin-like domain of TM is a receptor for LeY that blocks angiogenesis and attenuates inflammatory tissue damage by binding and neutralizing LeY.

LeY refers to a tetrasaccharide moiety (Fucose α 1,2 Galactose β 1,4(Fucose α 1,3) N-acetylglucosamine), which is attached to proteins or lipids. LeY is related to blood group Lewis a and b. However, its biologic activities are distinct from the Lewis blood group. LeY has been shown to play an important role in inflammation and tissue damage induced by LPS of diverse microorganisms including *Helicobacter pylori*.⁵ LeY has recently been implicated in cancer cell proliferation and tumor growth in vivo that were considered to be mediated via EGF signaling.^{6,7} Findings from the report by Kuo et al provide new insights into the role of LeY-containing EGF receptors in angiogenesis, and based on the effect of recombinant TMD1 on suppressing tumor growth, the results imply that LeY promotes tumor growth in part via inducing angiogenesis.

Even with the information provided by Kuo et al, the physiologic role of TM in angiogenesis is far from clear. Because the intact TM possesses little activity on endothelial tube formation and angiogenesis, it is unlikely that the membrane-anchored TM is directly involved in control of angiogenesis. The reason why intact TM is devoid of antiangiogenic action is unclear. It may be speculated that the lectin-like domain is structurally blocked in the intact TM. Recombinant TMD1 represents a soluble form of TM fragments that are released from the structural block and the lectin domain is free to interact with LeY. This raises an intriguing question: are there lectin-like domains in circulating blood that may