tors (c-Jun kinase, ASK1, eIF2α) have been implicated in linking ER stress to the apoptotic machinery, no coherent picture has emerged, making it difficult to currently speculate as to the connection between ceramide signaling, Akt activation/inactivation, and these proapoptotic mediators (Boyce and Yuan, 2006).

In sum, the studies by Swanton et al. provide extensive new evidence, gleaned from diverse experimental strategies, that CERT, and its client ceramide, are integral to paclitaxel-mediated cell death. Further, these studies identify ER stress as a previously unrecognized source of signals leading to apoptotic cell death upon taxane exposure. A challenge posed by these studies is to identify which of the many biochemical events likely to be dysregulated by prolonged mitotic checkpoint activity yields the ER stress response. An additional challenge is to define the mechanism by which ER ceramide might regulate taxane sensitivity biochemically and/or pharmacologically. While inhibition of glucosidase attenuated the spindle checkpoint by conferring mitotic slippage, inserting sphingolipid metabolism into this process for the first time, there is insufficient data presented here to ascribe such regulation to ER ceramide levels. Although these studies probably bring up more questions than they answer, they do provide unequivocal data that signaling associated with ceramide metabolism regulates taxane-induced apoptosis, the fundamental event in taxane-mediated tumor response.

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Chromatin Modulation by Oncogenic Transcription Factors: New Complexity, New Therapeutic Targets

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Oncogenic transcription factors such as PML-RARα, RUNX1-MTG8, and others work in large part by the recruitment of inhibitors of gene transcription to target promoters leading to aberrant repression of gene expression. PML-RARα, an archetypal chimeric oncoprotein, was previously shown to bring complexes of histone deacetylases (HDACs), histone methyltransferases (HMTases), and DNA methyl transferases (DNMTs) to target genes. In this issue of *Cancer Cell*, Villa et al. show that the full complement of chromatin machinery can be commandeered by these transcription factors with the polycomb group of proteins representing the newest identified recruit.

The Polycomb Group (PcG) of proteins were initially discovered in *Drosophila* as epigenetic silencers of homeotic (HOX) genes. PcG proteins have since been shown to be required for the X chromosome inactivation, germline development, stem cell renewal, hematopoiesis, and cell proliferation.

Polycomb proteins form up to four different multiprotein Polycomb Repressive Complexes (PRCs) (Kuzmichev et al., 2005). The PRCs alter gene expression by binding to and covalently modifying chromatin of target genes. PRC1 consists of more than ten subunits including BMI-1, which is required for the proliferation and self-renewal of normal hematopoietic, leukemia, and neural stem cells. This complex possesses a histone H2A-K119 ubiquitin E3 ligase activity.

PRC2, 3, and 4 contain proteins Enhancer of Zeste protein-2 (EZH2), Embryonic Ectoderm Development (EED), Supressor of Zeste-12 (SUZ12), and the histone-binding proteins RbAP46 and RbAP48. PRC4, in addition, contains the NAD+-dependent HDAC Sirt1, which has been implicated in gene silencing. There are four different mammalian EED proteins that can interact with EZH2 and SUZ12 resulting in the different PRC2, 3 and 4 complexes with differential histone substrate specificity (Kuzmichev et al., 2005). EZH2, the catalytically active component of PRC2/3/4, is an HMTase specific for histone H3 K27 and histone H1 K26. This HMTase activity requires the presence of all the other components of PRC2/3/4.

PRC complexes may interplay with one another. For example, after trimethylation of H3K27 by PRC2, 3, or 4, PRC1 is recruited to chromatin through a chromodomain component that recognizes trimethylated H3K27 where it ubiquitylates H2A-K119 and blocks transcription. PRC2-mediated trimethylation of H1K26 can be recognized by the Heterochromatin Protein 1 (HP1). HP1 has an important role in heterochromatin organization, maintenance, and gene repression by influencing the global structure of the chromatin. PRC components also interact with other silencing machinery. EZH2 can recruit DNMTs to PcG repressed genes (Viré et al., 2006). These proteins hypermethylate the CpG islands of target genes, offering binding sites for methyl cytosine-binding proteins, which in turn recruit HDAC complexes. Through all of these interactions, Polycomb proteins cause condensation of chromatin into higher-order structures unfavorable to gene transcription.

The polycomb complexes have key roles in normal development. Components of PRC2/3/4 are normally expressed at high levels in embryonic tissues and stem cells. EZH2 prevents stem cell exhaustion and blocks the differentiation of muscle myoblast. SUZ12 is essential for embryonic stem cell differentiation; it has been implicated in the formation of constitutive heterochromatin through the action of HP1α and the H3K9 HMTase SUV39H1 in differentiated murine ES cells. In multiple malignancies, aberrant expression or recruitment of PcG proteins contributes to the reversion of normal cells to a more stem cell-like phenotype. BMI-1 upregulation is associated with leukemia, mantle cell lymphoma, neuroblastoma, and lung cancer. High levels of EZH2 are associated with metastatic melanoma and endometrium, prostate, and breast cancer, as well as lymphoid malignancies (Sparmann and van Lohuizen, 2006). SUZ12 mRNA is upregulated in colon, breast, and liver tumors, while in endometrial stromal tumors, SUZ12 is fused to the transcription factor JAZF1. The frequent upregulation of PRC proteins may be due to deregulation of the E2F/Rb pathway in malignancy as E2F transcription factors bind and activate the promoter of many PRC component encoding genes.

The critical targets and pathways regulated by PRC complexes remain to be fully elucidated. The PRC2 complex binds to the promoter and repress the expression of *homeobox A9 (HOXA9)*, a gene frequently overexpressed in leukemia. How the function of this complex might be compromised in leukemia is uncertain. Expression profiling and chromatin precipitation identified target genes regulated by PRC proteins in normal and malignant cells. The recruitment of PRC complexes to silenced genes is cell type specific (Squazzo et al., 2006). In embryonal cancer lines, PRC components SUZ12 and EZH2 bind genes implicated in transcriptional regulation, in particular homeodomain genes. PRC components and associated chromatin modifications can be deposited over long stretches of the HOX genes of up to 10 kb, suggesting a global role in regulation of the gene cluster. By contrast, in adult carcinoma cells, a distinct set of target genes were enriched for encoding glycoproteins, receptors, and cell surface proteins with Ig-related sequences. In these cells, PRC protein recruitment and H3K27 trimethylation was limited to discrete regions within the target promoters, implying a different mode of recruitment than embryonal cells. Polycomb overexpression may be linked to specific cancer mechanisms. PRC2 binds and inhibits expression of hDAB2IP, a Ras GTPase activating protein with growth inhibitory properties, in prostate cancer (Chen et al., 2005) whereas PRCs bind to the promoter of MYT1 gene, whose *Xenopus* ortholog induces neural differentiation, in colon cancer (Kirmizis et al., 2004).

While Drosophila PcG proteins are recruited through specific Polycomb response elements (PRE), mammalian equivalents to the PRE have not been found. PRCs may be tethered to promoters through the interaction with general transcription factors as well as through some specific DNA-binding proteins. By genome-wide location analysis, Bracken et al. (2006) found more than 1000 genes bound by PcG proteins in human embryonic fibroblasts, including ones implicated in developmental and signaling pathways (e.g. Wnt, TGFb, FGF, Notch, and Hedgehog).

Acute Promyelocytic Leukemia (APL), defined as the accumulation of malignant hematopoietic precursors blocked at the stage of the promyelocyte is associated in ~98% of cases with t(15;17) yielding the PML-RARα oncoprotein. PML-RARα has several essential properties that distinguish it from wild-type Retinoic Acid Receptor α (RARα) (Di Croce, 2005; Quina et al., 2006; Licht, 2006) including (1) the ability to form homodimers due to the dimerization domain of PML. This leads to higher affinity for corepressors and enhanced recruitment

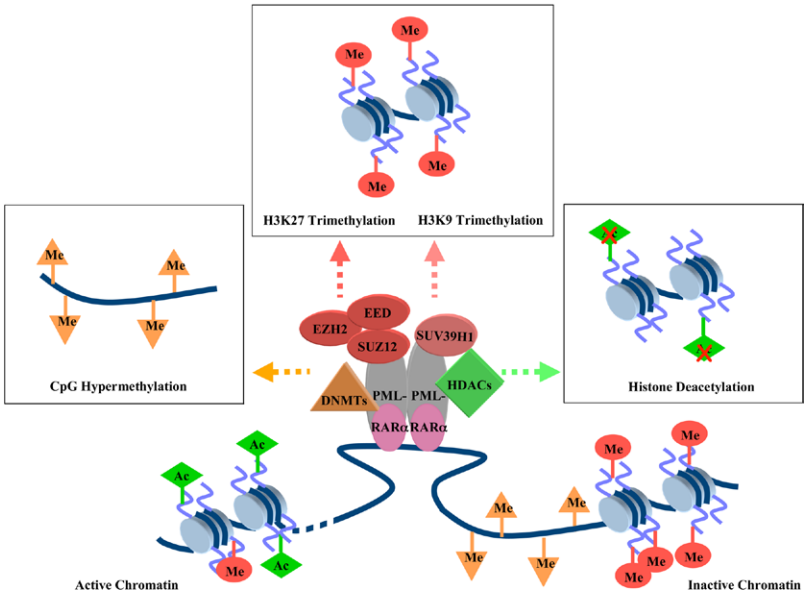

Figure 1. The Fusion Protein PML-RARα Inhibits the Expression of RARβ2 Gene through Different Epigenetic Modifications

The oligomerization of the fusion protein enhances the binding and recruitment of HDACs that remove the acetylated group in histones. This modification may be followed by the methylation of different histone residues by the HMTase SUV39H1 that methylates H3K9 and by the EZH2 component of the PRC2/3/4 that methylates H3K27. The polycomb complex recruits DNMTs that methylates CpG islands in the RARβ2 promoter. The Methyl-CpG Binding Protein 1 (MBD1) can bind to this methylated DNA and may recruit more transcriptional repressors to the chromatin. As a result of all these modifications, the transcriptional active chromatin (characterized by the presence of acetylated histones as well as methylated residues associated with gene activation, such as H3K4) becomes inactive. The red circles represent histone methylation, the green rhombuses represent histone acetylation, and the orange triangles represent DNA methylation.

of histone deacetylases. (2) The additional ability to recruit corepressors through interaction with the DAX protein, again mediated by the PML moiety. (3) The ability to bind to DNMT1 and DNMT3a and repressive HMTases (SUV39H1). (4) The ability to bind to widely spaced direct repeat sequences in addition to typical RAR sites. As a result, a multiprotein complex of PML-RARα may bind to typical RAR targets, such as the RARβ2 promoter, or to novel genes, repress, and even epigenetically silence such genes through the addition of histone and DNA methyl marks (Figure 1). This model remains incomplete as the target genes critical for the oncogenic properties of the fusion protein remain largely unidentified. Nevertheless, treatment of patients with the combination of all-*trans* retinoic acid (ATRA) and chemotherapy may cure up to 90% of these patients, due in part to the ability of retinoic acid to reverse repression by forcing the release of corepressor complexes from the fusion protein and stimulating PML-RARα degradation.

Villa et al. (2007), in this issue of *Cancer Cell*, now demonstrate the direct recruitment of PRC2/3/4 by PML-RARα protein to its target promoters. PML-RARα copurified with H3K27 methylation activity and the EED and SUZ12 proteins. Chromatin precipitations indicated that PML-RARα recruits these proteins, along with DNMTs, to alter chromatin configuration and silence expression of RARα target genes. Furthermore, siRNA-mediated knockdown of SUZ12 in NB4 APL cells reversed the epigenetic changes mediated by PRC2/3/4 (H3K27 di- and trimethylation as well as DNA methylation) and induced differentiation usually seen after ATRA treatment of such cells, confirming the biological importance of polycomb recruitment in the activity of PML-RARα.

The study of PML-RARα indicated the critical role of HDACs in hematological malignancy and led to a greater interest in the discovery of inhibitors against these enzymes, most recently leading to the approval of vorinostat for clinical use. However, HDAC inhibitors on their own have little activity in most malignancies. The study of the mode of action of PML-RARα gives some clue as to why: the repression machinery brought to genes by fusion oncoproteins is complex and, to some extent, redundant, using multiple mechanisms to silence gene expression. HMTases, key components of the polycomb complexes, represent another attractive enigmatic target for transcription therapy, and small molecules with relative selectivity for specific HMTases have now been reported (Kubicek et al., 2007). Furthermore, polycomb components can now be targeted by an S-adenosylhomocysteine hydrolase inhibitor 3-Deazaneplanocin A (DZNep) (Tan et al., 2007). This drug leads to the accumulation of adenosylhomocysteine, a metabolite that inhibits S-adenosyl-L-methionine-dependent methyltransferases, and leads to the degradation of the PRC2 components EZH2, SUZ12, and EED by an uncertain mechanism. This, in turn, causes a decrease in H3K27 methylation specifically in cancer cells and induces apoptosis. Intriguingly, the same PRC2 depletion effect and changes in H3K27 methylation was observed after treatment of human primary leukemia cells with the hydroxamate HDAC inhibitors LBH589 or LAQ824 (Fiskus et al., 2006), an effect correlated with the induction of apoptosis.

The study of APL and other relatively uncommon forms of hematological malignancy continues to yield insight into basic mechanisms of normal and aberrant gene regulation. Finding a single agent that can reverse these manifold changes, such as ATRA, has proven to be exceptional. Transcriptional therapy with a combination of agents that might affect histone acetylation, methylation, chromatin remodeling and DNA methylation remains an exciting possibility already being tested in clinical trials.

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The Potential of New Tumor Endothelium-Specific Markers for the Development of Antivascular Therapy

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Angiogenesis is a hallmark of solid tumors, and disruption of tumor vasculature is an active anticancer therapy in some cases. Several proteins expressed on the surface of tumor endothelium have been identified during the last decade. However, due to the expression in both physiological and tumor angiogenesis, only a few targets have been developed for clinical therapeutics. By thorough SAGE analysis of mouse endothelial cells isolated from various normal resting tissues, regenerating liver, and liver-metastasized tumor, Seaman and colleagues in this issue of *Cancer Cell* have demonstrated organ-specific endothelial markers, physiological angiogenesis endothelial markers, and tumor endothelial markers and revealed striking differences between physiological and pathological angiogenesis.

Angiogenesis is a hallmark of solid tumors. Disruption of tumor angiogenesis by blocking proangiogenic growth factors or shutdown of the established tumor blood vessels by vascular targeting agents has demonstrated therapeutic effects in human cancer. The vascular-disrupting effect can be mediated directly by toxic agents or selectively delivered by antibody or peptide targeting (Neri and Bicknell, 2005). The recent successful blockade of the VEGF pathway in several major cancers prolonged survival in phase III clinical trials and has encouraged the identification of new tumor endothelial markers (TEMs).

Early attempts to identify tumor vascular targets focused on the study of in vitro endothelial cell (EC)-isolates using a range of molecular, biochemical, and immunological techniques. These efforts have led to the identification of a limited number of molecular markers predominantly expressed on angiogenic vessels, but in both tumor and physiological angiogenesis. With the advent of new techniques, a great number of tumor endothelial molecules have been identified during the last decade. In silico methods have been used to define new angiogenesis genes such as Robo 4 (Huminiecki and Bicknell, 2000). In vivo phage display has been used to deliver peptides that selectively recognize organ-specific and tumor endothelium, leading to the