have to eagerly wait for further studies of DTP3 in other NF-κB-dependent hematological malignancies and solid tumors that overexpress GADD45β. Hopefully, these studies will also pave the way for the development of other approaches for curtailing the survival activity of NF-κB in GADD45β–negative cancers.

REFERENCES


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DNA cytosine methylation is an ancient regulatory mechanism present in diverse phylogenies, including vertebrates, plants, and some fungi (Jones, 2012). Cytosine methylation of CG dinucleotides by DNA methyltransferases (DNMTs) in promoters is associated with gene silencing, e.g., in X chromosome inactivation and imprinting (Jones, 2012), and promoter hypermethylation is thought to contribute to aberrant regulatory programs in cancer (Shenker and Flanagan, 2012). Within the last decade, so-called “epigenetic” drugs have come to the fore with U.S. Food and Drug Administration approval of cytidine analog DNMT inhibitors such as 5-aza-2-deoxycytidine (5-Aza-CdR) for myelodysplastic syndrome and acute myeloid leukemia. Excitingly, studies have also hinted at the efficacy of this mode of intervention in solid tumors (Shenker and Flanagan, 2012), expanding the utility of these chemotherapeutics beyond hematologic malignancies.

Although promoter methylation, which maintains a “closed” chromatin state that impairs transcriptional initiation (Jones, 2012), has been well studied, less is known about the role of methylation in gene bodies. Intriguingly, unlike at promoters where methylation is associated with gene repression, genic methylation is positively correlated with gene expression (Figure 1A) (Maunakea et al., 2010; Varley et al., 2013). It is thought that DNA methyl-ation inhibitors cause promoter hypome-thylation and subsequent gene reactivation (Shenker and Flanagan, 2012). However, the effect of these drugs on gene body methylation has not been extensively studied.

In this issue of Cancer Cell, Yang et al. describe a causal relationship between gene body methylation and gene expression and a role for genic methylation in response to clinical DNA methylation inhibitors, which suggests that the mechanism of action of these inhibitors includes gene body hypomethylation-induced downregulation of cancer-associated genes.

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Figure 1. Gene Body Methylation, Gene Expression, and DNA Methylation Inhibitors

(A) Promoter methylation is associated with gene silencing, while gene body methylation is correlated with gene expression. Measurement of methylation at various time points after treatment with the DNA methyltransferase (DNMT) inhibitor 5-aza-2-deoxycytidine (5-Aza-CdR) reveals that promoters and gene bodies can be differentially remethylated. Rapid remethylation of genomic regions is dependent on DNMT3B. Slowly remethylated regions tend to be located in gene bodies and are enriched for c-MYC-regulated genes. Upon demethylation, rapidly remethylated gene bodies acquire a chromatin signature that may modulate transcription by altering nucleosome stability.

(B) Methylation of gene bodies may attenuate cryptic initiation from alternative promoters.

(C) Methylation of gene bodies may attenuate cryptic initiation from alternative promoters.

There are many hypotheses regarding the function of gene body methylation. For example, genic methylation might regulate alternative promoters (Maunakea et al., 2010). Alternatively, gene body methylation might inhibit cryptic transcription initiation events. In plants, cryptic initiation was proposed to generate antisense transcripts that base pair with mRNA transcribed from the canonical transcription start site (TSS), leading to double-stranded RNA-mediated methylation and silencing of the cryptic TSS (Tran et al., 2005) (Figure 1C). Consistent with this model, genic methylation is anticorrelated with transcriptional noise in vertebrates (Huh et al., 2013). It is attractive to hypothesize that an increase in cryptic initiation in active genes upon nucleosome destabilization by methylation inhibitors leads to DNMT3B recruitment and suppression of cryptic transcription by rapid remethylation.

This work by Yang, et al. (2014) also provides important insights into the pharmacology of DNA methylation inhibitors. Despite the apparent lack of specificity of methylation inhibitors, remethylation kinetics after drug withdrawal are dependent on genomic and chromatin contexts and driven by DNMT3B. Promoter demethylation appears to be sustained after drug withdrawal, while genic demethylation is comparatively short-lived, which may have implications for understanding clinical responses to methylation inhibitors. Mechanistically, DNA methylation inhibitors may normalize gene expression in cancer by reactivating silenced tumor suppressors through their action at promoters and buffer overexpression of oncogenes and metabolic genes by hypomethylating gene bodies.

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REFERENCES


Reprogramming the Tumor Stroma: A New Paradigm

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A recent article in Cell shows that vitamin D receptor activation reprograms reactive stroma in the tumor microenvironment to a less inflammatory, quiescent state and is associated with increased drug retention, tumor response, and survival in pancreatic cancer models. Stroma reprogramming, as opposed to ablation, may emerge as a new treatment paradigm.

It has been known for some time that carcinomas are associated with a reactive stroma microenvironment (Ronnov-Jessen et al., 1996). Reactive stroma usually initiates early in cancer progression, co-evolves with the cancer, and represents a host response to disrupted epithelial homeostasis. In effect, the reactive stroma response is a rather generic response, ostensibly to serve a repair-centric function. The persistence of this response is what is observed in fibrosis disorders and during cancer progression. Less clear are the specific cell types, their origins, and how the biology of reactive stroma affects tumor progression. Collectively, this reactive stroma has been referred to as carcinoma-associated fibroblasts, myofibroblasts, or stellate cells (Apte et al., 2004; Orimo and Weinberg, 2006; vonlaufen et al., 2008). The majority of studies have shown that reactive stroma generally promotes tumors, yet specific mechanisms are not understood. The biology affected by the stromal compartment in cancer is likely to be quite complex and involve a balance among tumor-promoting and tumor-inhibiting mechanisms. Nevertheless, the notion of targeting the reactive stroma within the tumor microenvironment as a means of inhibiting cancer progression is an attractive one.

Perhaps one of the most important perspectives regarding reactive stroma was noted by Dvorak years ago, that cancers are like “wounds that do not heal” (Dvorak, 1986). The biology of wound repair is very complicated and is characterized by pro-growth conditions that require reactive stromal cells, followed by a return to a normal differentiation state. This process involves a resolution of the reactive, pro-growth repair state to one of more normal tissue quiescence and biology. Hence, stromal reprogramming is a part of normal wound repair biology. If, as Dvorak pointed out, cancers are like wounds that do not heal, then it can be surmised that the stromal reprogramming that instructs the stroma back to differentiation during wound repair simply does not normally occur in cancer. Considerable evidence in the literature supports this concept, which is well outlined by Sherman et al. (2014) in a recent issue of Cell. As aptly pointed out in this article, in addition to tumor-promoting functions, the persistent stromal response has also been shown to inhibit effective drug delivery and influence patterns of therapeutic resistance in pancreatic cancer.

Sherman et al. (2014) show that the vitamin D receptor (VDR) is a critical regulator of pancreatic stellate cells, the reactive stroma observed in pancreatic cancer. Importantly, this study shows that VDR activation results in a reprogramming of reactive stroma and reduced inflammatory markers typically associated with fibrosis. In pancreatic tumor models, this VDR-mediated stromal reprogramming resulted in increased drug (gemcitabine) availability and reduced tumor volume. Remarkably, use of the VDR ligand resulted in a 57% increase in animal survival as compared to gemcitabine treatment only. Effectively, this study suggests that VDR activation resolves the reactive stroma phenotype to one that is noninflammatory and quiescent. In essence, a reprogramming of the stroma to a state more common of normal homeostasis, such as would occur naturally during completion of normal wound healing. In this regard, it would seem that VDR activation in pancreatic cancer changes the tumor’s status from being a wound that does not heal, as cited by Dvorak, to a wound that is partially healed in the important stromal compartment.

The overall importance of the Sherman et al. (2014) study is underscored by the...