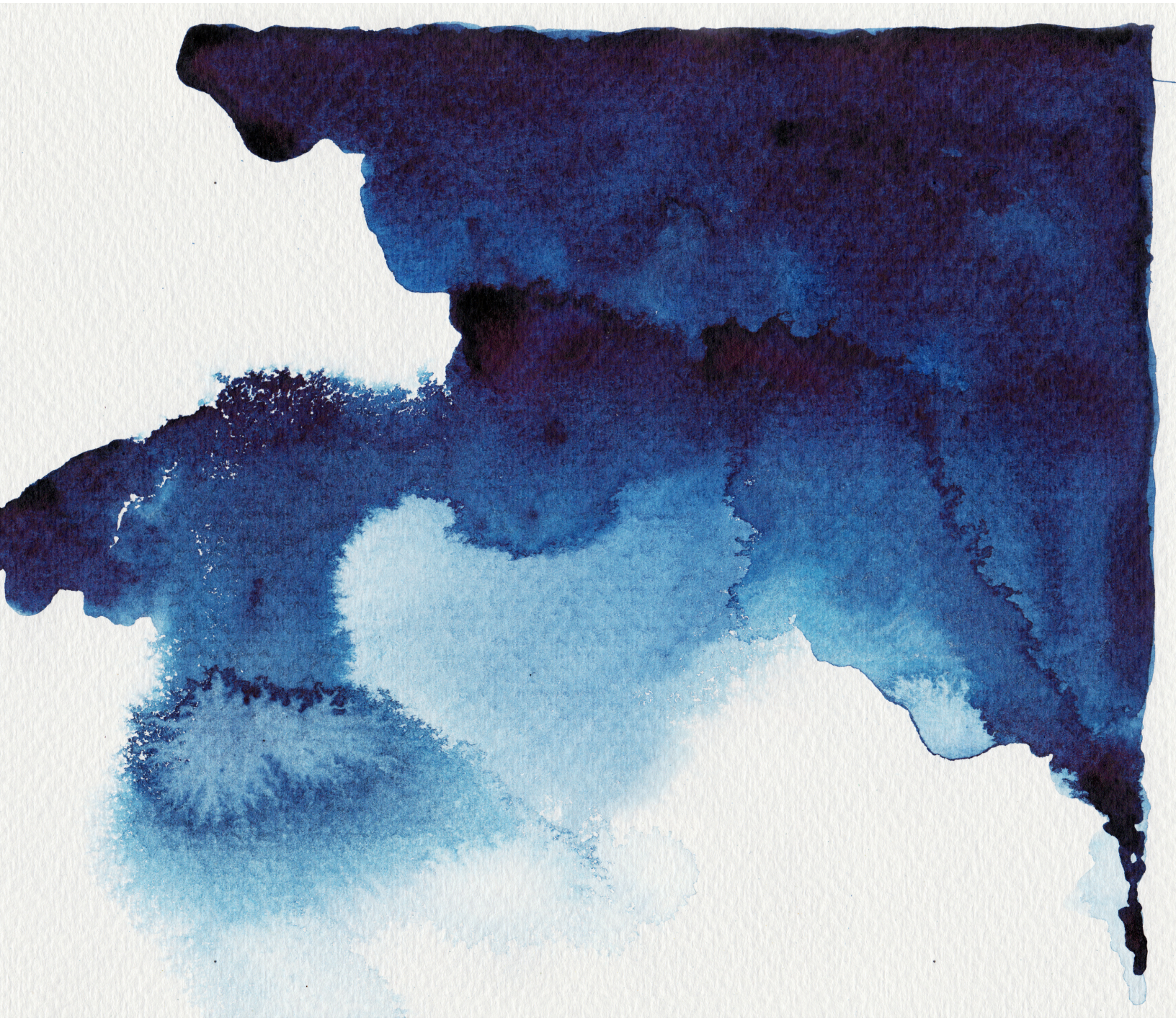
EPIGENETIC NOISE



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Epigenetic Noise Fuels Cancer Evolution

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Cancer is a disease of the genome and the epigenome. Previous studies have shown that genomic changes such as mutations, copy number variation, and genomic rearrangements drive cancer evolution. In this issue of *Cancer Cell*, Landau and colleagues add epigenomic changes, specifically locally disordered DNA methylation, to cancer's evolutionary trajectory.

Deep genomic sequencing technologies are beginning to resolve the underlying complexities of solid and hematologic cancers. Peter Nowell's proposed model for cancer evolution suggesting that cancers evolve through branched evolutionary trajectories fuelled by genomic instability is now increasingly being accepted as a basis for therapeutic failure and the increasing mismatch between cost and clinical benefit from current targeted therapeutic approaches (Nowell, 1976). As Nowell predicted in 1976, "One may ultimately have to consider each advanced malignancy as an individual therapeutic problem."

Work over the last three decades has revealed how diversity within tumors can be driven at many levels. Genomic instabilities can be initiated by deficiencies in DNA repair, DNA replication stress, telomere dysfunction, genome doubling events, and mitotic aberrations resulting in abnormal chromosome-spindle attachments precipitating whole chromosomal instabilities (reviewed in Burrell et al., 2013). Such genome instability processes may also be dynamic over space and time. As the resolution of cancer genomic analysis improves, so does an appreciation that the majority of solid tumors harbor at least one mechanism of genome instability that promotes further evolution and adaptation.

It has been appreciated for many years that diversity within individual tumors, manifested by chromosomal instability, is associated with poor outcome. Deep sequencing analyses have added to these observations. For example, through deep sequencing analysis of chronic lymphocytic leukemia (CLL), the presence of subclonal driver events was associated with poorer outcome (Landau et al., 2013). Emerging data suggest that diversity at any level is sufficient to influence clinical outcomes. Indeed, Carlo Maley and colleagues investigated this proposition in Barrett's esophagus, the preinvasive stage preceding the onset of esophageal carcinoma, examining multiple genetic and epigenetic drivers of diversity. The authors concluded that all diversity measures, both genetic and epigenetic, were strongly associated with clinical risk of progression (Merlo et al., 2010).

It is also appreciated that genetically similar cells may behave differently in the face of identical selection pressures. Altered epigenetic states are thought to be a mechanism through which these observations can be explained. For example, minority drug tolerant persistor cells result in drug resistance dependent on the histone demethylase JARID1A, which can be both dynamic and transient (Sharma et al., 2010). These data suggest the need for a comprehensive understanding of both the cancer genome and epigenome to predict tumor cell behavior. It is the understanding of the latter into which the study in this issue of *Cancer Cell* by Landau et al. (2014) sheds new light.

A defining feature of epigenetics is the ability to stably switch between different biological states. Typically, this can result in the expression of affected genes to be switched on or off or genome stability to be maintained or impaired. This process of switching allows for many different genetic programs to be run from the same genome, giving rise to many different epigenomes. The temporal and spatial regulation of these epigenomes are exquisitely controlled during normal development but severely disrupted in cancer. A common mechanism for epigenomic disruption is stochasticity, whereby changes are introduced randomly that lead to gain or loss of DNA methylation at certain CpG dinucleotides, the preferred sites of DNA methylation in mammalian cells including cancer cells. In addition, such seemingly stochastic changes may also be mediated by genetic variants as proposed in the inherited stochastic variation model, which provides a mechanism to explain an epigenetic role in selectable phenotypic variation (Feinberg and Irizarry, 2010).

Figure 1A illustrates three common scenarios of DNA methylation (DNAm) changes that have been observed in multiple cancers by comparison with normal tissue. As the name implies, variably methylated regions (VMRs) display oscillating gain and loss of DNAm. Their "noisy" appearance inspired a model whereby the epigenome can modulate cellular plasticity by regulating the effects of noise and thus explain the observed increase in VMRs and gene expression contributing to cancer heterogeneity (Pujadas and Feinberg, 2012). Regions of long-range epigenetic silencing and activation are defined by gain and loss of DNAm, respectively, and have been shown to remodel large domains of the cancer epigenome (reviewed in Stirzaker et al., 2014).

What differentiates the study by Landau et al. (2014), which focuses on CLL from previous studies investigating intratumor DNAm heterogeneity, is that they used deep bisulfite sequencing. This allowed them to assess DNAm heterogeneity at single molecules or reads derived from

individual cancer cells rather than aggregated values from populations of cancer cells. As illustrated in Figure 1B, some reads were found to be methylation concordant while others were found to be discordant, consistent with increased stochastic heterogeneity in the corresponding leukemic cells. To quantify this heterogeneity, the authors devised a new measure—proportion of discordant reads (PDR)–which provides an index for intratumor DNAm heterogeneity that is akin to the intratumor heterogeneity ratio index already in use for quantifying intratumor genetic heterogeneity from the TRACERx study (Jamal-Hanjani et al., 2014). By measuring the PDR index across promoters in a cohort of patients with CLL, Landau et al. (2014) established that increasing PDR levels are associated with adverse clinical outcome (Figure 1C).

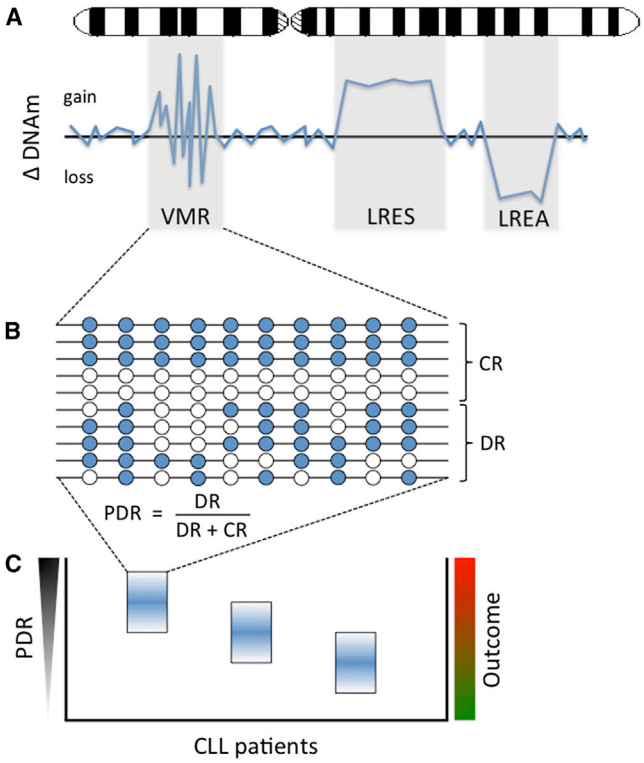

Figure 1. Illustration of Intratumor DNA Methylation Heterogeneity

(A) Major types of DNA methylation (DNAm) heterogeneity include variably methylated regions (VMR), long-range epigenetic silencing (LRES), and long-range epigenetic activation (LREA).

(B) Schematic bisulfite sequencing reads showing concordant (CR) and discordant (DR) DNAm and formula for calculation of the proportion of discordant reads (PDR).

(C) PDR differs in patients with chronic lymphocytic leukemia (CLL) and is associated with adverse clinical outcome.

Looking ahead, the hope is that this new approach will improve our ability to determine, and one day attenuate, the background DNAm in cancer. This would thereby allow for more accurate identification of positively selected methylation changes, the elusive epigenetic drivers of cancer progression and evolution. If successful, we will be one step closer to solving Nowell's individualized therapeutic problem by limiting the epigenetic fuel for cancer progression.

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