Independence of epigenetic and genetic diversity in AML

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There is a growing realization that tumors are individual, dynamic ecosystems, which consist of heterogeneous cell populations that differ at the genetic and molecular level, and that this diversity facilitates their evolutionary 'fitness' and ability to weather selective pressures such as chemotherapy or radiotherapy[1]. The genetic heterogeneity of tumors has been known for decades from cytogenetic studies and, more recently, our understanding has been further refined by multiple population-based[2] studies and a handful of single-cell-sequencing studies[3] across a number of tumors. However, the degree and contribution of other measures of cellular heterogeneity, such as epigenetic variance, are poorly understood[4].

Acute myeloid leukemia (AML) is an aggressive hematological malignancy associated with a dismal outcome: usually, initial response to therapy is followed by relapse and resistance to therapy. Both genetic and clinical heterogeneity are evident between patients with AML[2], and genetic heterogeneity within individual leukemias has been demonstrated both at single time points and longitudinally after relapse[5]. By contrast, the role of epigenetic variation in AML is unclear, although several disease characteristics suggest that it might be important. First, AML is a relatively simple cancer genetically, with only 2–5 driver mutations per patient coding genome identified by whole-genome sequencing (WGS). In addition, multiple epigenetic regulators are targeted by mutation, deletion and chromosomal rearrangements in AML. Finally, altered epigenetic states and patterning, such as DNA methylation and patterns of histone modifications, are cardinal features of AML[6]. In this issue of *Nature Medicine*, Li *et al*.[7] address the role of epigenetic variation in cancer prognosis, demonstrating that epigenetic diversity is an important hallmark of AML, and that it seems to evolve independently of the genetic landscape.

The authors carried out large-scale analysis of epigenomic patterning by using enhanced reduced representation bisulfite sequencing (ERRBS) to detail DNA methylation in a cohort of 138 individuals with AML, for whom paired diagnostic and relapse leukemic bone marrow samples were available. They used the recently described methclone compositional entropy equation approach[8], which analyzes differences in combinatorial methylation patterns in four adjacent CpG dinucleotides (termed epialleles, Fig. 1) to identify variable regions (eloci) and which also quantitates the degree of variation or epigenetic allele burden (EPM, eloci per million loci) at these loci between samples. Samples obtained at diagnosis and at relapse were compared with normal bone marrow (NBM) and, for each patient pair, with each other. The methclone technique differs from other measures of methylation heterogeneity (MH), such as epipolymorphism analysis[9], because it measures dynamic changes rather than capturing a static measure. In addition, whole-exome sequencing (WES) and RNA-seq data were also available for subsets of patients (WES, n = 48; RNA-seq, n = 19), to enable a

direct comparison of epigenetic diversity with genetic diversity and transcriptional outcome in the same individual.

The authors' major finding was that higher epigenetic variance was correlated with a shorter time to disease relapse when patients were divided into groups on the basis of high and low EPM, particularly when EPM analysis was limited to promoter eloci. In addition, this association was independent of other potentially confounding variables, including age and crude estimates of tumor burden, such as the peripheral white cell count. Importantly, when the subgroup of patients with available WES data was analyzed similarly, dependent on mutation burden, no difference was seen in time to relapse between the two groups of high and low mutation burden. Epigenetic variability was increased in both diagnostic and relapsed AML, as compared to NBM, but the degree was itself variable upon disease progression. There was, however, an apparent redistribution of eloci from established transcriptional regulatory elements, such as CpG islands, promoters and enhancers, at diagnosis, toward intronic and intergenic regions at relapse. This observation raises the intriguing possibility that these novel regions might acquire regulatory function with disease progression.

The authors were then able to cluster the patients into three groups according to predominance of eloci clusters: unique to diagnosis, unique to relapse or shared between both relapse and diagnosis. No link was found between these groups and the presence of specific mutations within these groups; nor was any association found with clonal structure or complexity. However, individuals with a large number of eloci at diagnosis had fewer mutations evident at this time point. In addition, individuals with a higher mutational burden at diagnosis developed substantial numbers of eloci at relapse, which further suggests that epigenetic and genetic processes have independent trajectories during progression. The authors further found that gene-expression patterns differed between the clusters, wherein individuals with high levels of eloci at diagnosis demonstrated an upregulation of genes, including those encoding signaling proteins, whereas individuals with elevated eloci at relapse upregulated inflammation and immune-response-related genes.

The authors then focused their studies on longitudinal analysis of an exemplar case at five separate time points (diagnosis and four subsequent relapses), which further demonstrated a lack of concordance between genetic and epigenetic variation in the samples at the same time point. The most substantial increase in epiallele burden was noted at first relapse in this individual, long before the most striking change in mutational burden, which occurred at third relapse. This case not only further supported the idea that genetic and epigenetic diversity may be independent, but also suggests that they may be combinatorial in maintaining the tumor over the continuum of disease progression. Finally, the authors linked epigenetic variation to concordant changes in transcription. They found from bulk analysis of all samples that genes associated with eloci at diagnosis had increased differential expression between diagnosis and relapse when compared to those without eloci, and that genes associated with eloci had increased transcriptional heterogeneity in single-cell RNA-seq analysis.

This study has a number of implications for the role of heterogeneity in tumor biology. The independence of epigenetic and genetic heterogeneity in AML would be predicted to further increase clonal diversity and evolutionary fitness, and thus makes evolutionary sense. By contrast, however, interdependency of genetic and epigenetic events has been shown in

glioblastoma10, and it will be important to determine any similar relationships in other malignancies. Furthermore, it is possible that other mediators of the malignant phenotype, such as altered metabolism, also demonstrate cellular heterogeneity; investigation of this and any correlation with genetic and epigenetic variation are warranted. In addition, given that this study focused on individuals who relapse, would the epigenetic heterogeneity of patients with AML, but with a good prognosis, be less? Additionally, could the EPM measure at selected eloci be used as a predictive biomarker at disease diagnosis, for instance?

Finally, the mechanism(s) that drive epigenetic variation and the downstream consequences of this variation are largely unknown and require elucidation. Although the authors' data suggest that specific mutations—even those in modifiers of DNA methylation such as DNMT3A, TET2, IDH1 and IDH2—are not correlated with epigenetic variation, they did not investigate further what actually drives epigenetic diversity. Similarly, the loose correlation between epiallele burden, specific eloci and alterations in transcription warrants further investigation, and single-cell analysis is likely to be particularly helpful in determining how this epigenetic variation alters cellular phenotype. This study therefore paves the way for further work in larger series of AML samples and in prospective experimental systems to address these questions.

References

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Figure Legend

Figure 1. Independence of epigenetic and genetic heterogeneity during the progression of AML.

Li *et al*.[7] analyzed the genetic and epigenetic heterogeneity of AML at diagnosis and relapse after treatment; an example here typifies their findings. Differently colored cells represent genetic diversity and the small open and closed circles, DNA methylation. Their epigenetic analysis identified strings of four adjacent CpG dinucleotides that were dynamically methylated during disease progression. At diagnosis, in the six cells shown, there are only two patterns of combinatorial methylation at the two alleles represented, resulting in low epigenetic diversity. However, there is a more marked genetic heterogeneity at the same time point. By contrast, after treatment, there is an increase in epigenetic heterogeneity at relapse, as demonstrated by the more varied combinatorial methylation pattern, but a relative decrease in genetic diversity, that is independent of these epigenetic changes.