To *Infinium*, and Beyond!

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Glioblastoma (GBM) is a malignant brain tumor that kills most patients within 2 years. In this issue of *Cancer Cell*, Noushmehr et al., through The Cancer Genome Atlas (TCGA) project, provide one of the first integrated views of the GBM methylome, adding to our increasingly comprehensive understanding of this disease.

Epigenetic events are heritable changes in gene expression that are not accompanied by changes in DNA sequence. This epigenetic information—collectively termed the epigenome—helps to regulate the integrity and expression of genes in normal eukaryotic cells (Suzuki and Bird, 2008). In addition to its role in normal cells, there is increasing evidence that aberrant epigenetic events contribute to disease states, most notably cancer (Jones and Baylin, 2007). Several processes appear to mediate epigenetic control, including DNA methylation, histone modification, and nucleosome remodeling. Among these, hypermethylation of promoter regions has emerged as one of the best described epigenetic changes in tumors. These promoter regions contain cytosine phosphate guanine (CpG) islands, short regions of 0.5–4 kilobases rich in CpG content, that are generally unmethylated in normal cells (Jones and Baylin, 2007). Understanding the aberrant epigenetic changes in cancer has formulated around two key hypotheses: Promoter methylation can silence tumor suppressor genes, and aberrant epigenetic events occur across the genome, resulting in specific patterns of gene inactivation that contributes to the cancer state.

The appreciation that epigenetic events are a genome-wide phenomenon has led to the development of technologies that detect global patterns of methylation. In this issue of *Cancer Cell*, Noushmehr and colleagues present a comprehensive study of the methylome of human glioblastoma (GBM), the most common and malignant primary brain tumor (Noushmehr et al., 2010). Although DNA methylation alterations in GBM have been widely reported, the work by Noushmehr and colleagues is particularly significant because it focuses on the large cohort of GBM collected and studied through The Cancer Genome Atlas (TCGA) network (TCGA, 2008). The TCGA aims to characterize cancer genomes to identify means of improving cancer prevention, detection, and therapy. Previous TCGA efforts have identified subgroups of GBM by profiling patterns of mRNA expression, single-nucleotide polymorphism (SNP) copy-number change, and mutation across >200 tumors (Figure 1). These subgroups are defined predominantly by patterns of gene expression and include proneural, neural, classical, and mesenchymal types (Phillips et al., 2006; TCGA, 2008). Mutations and altered expression of *EGFR*, *NF1*, and *PDGFRA*/*IDH1* have further defined the classical, mesenchymal, and proneural subgroups, respectively (Verhaak et al., 2010). The work reported by Noushmehr and colleagues casts an additional layer of complexity across these emerging GBM subgroups, thereby providing exemplary evidence of the value of the TCGA (Figure 1).

Using the Illumina GoldenGate and Infinium platforms, Noushmehr and colleagues measured global patterns of methylation across a discovery set of 272 TCGA GBM samples. Similar to prior TCGA genomic studies, methylome profiling divided the GBM cohort into subgroups. Of the three methylome subgroups identified, one included tumors with a very robust pattern of DNA methylation, reminiscent of the CpG island methylator phenotype (CIMP) observed in colorectal cancer (Toyota et al., 1999). Like the colorectal-CIMP, the glioma (G)-CIMP was characterized by cancer-specific CpG island hypermethylation of a subset of genes.

Because the GBM subgroups have been best described by patterns of mRNA expression, the authors determined the degree to which these overlapped with those defined by methylome profiling. Interestingly, almost 90% of G-CIMP tumors (n = 21/24) were classified into the proneural subgroup by mRNA expression, suggesting that this pattern of methylation contributes to the development of proneural GBM. However, only 30% (n = 21/71) of all proneural GBM displayed the G-CIMP; thus G-CIMP probably contributes to the development of just a subset of proneural GBMs.

The authors then turned their attention to determining whether G-CIMP$^+$ proneural GBM represents a distinct disease subgroup. Notably, Euclidean statistics provided further evidence that G-CIMP$^+$ proneural GBMs are distinct from G-CIMP$^-$ tumors. In addition, patients with G-CIMP$^+$ proneural GBM were shown to be significantly younger at the time of diagnosis and had a significantly better prognosis than patients diagnosed with non-G-CIMP$^-$ proneural tumors. Although these findings certainly suggest that G-CIMP$^+$ and G-CIMP$^-$GBMs are distinct pathological entities, subsequent integrated genomic analyses performed by the authors support this notion most strongly. Using resequence data previously generated by the TCGA, the authors identified nine genes containing somatic mutations that were significantly associated with proneural G-CIMP$^+$ GBM. These included *IDH1*, *DST*, *EIF2AK4*, *EPHB4*, *FGFR4*, *LEMD3*, *MAPK7*, *TNFRSF10A*, and *TRPM3.* Remarkably, *IDH1* was mutated in 18 of 23 G-CIMP$^+$ GBMs, whereas 184 G-CIMP$^-$ tumors contained the wild-type allele. Prior DNA resequencing studies have shown *IDH1* mutations are enriched in secondary GBM cases and younger individuals and associated with increased patient survival (Yan et al., 2009). The positive association

between *IDH1* mutation and G-CIMP$^+$ suggests that they might cooperate in the transformation of cells within the glial lineage. Alternatively, subtypes of normal neural cells may exist that contain a CIMP and are uniquely susceptible to transformation by mutations in *IDH1*. Functional studies will be required to clarify these associations.

An obvious question posed by the discovery of the G-CIMP is whether it impacts global patterns of mRNA expression in GBM. Answering this question is not straightforward given that hypermethylation might have regional as well as global effects on gene expression that are complicated further by changes in DNA sequence and copy number. As a first step to determine the impact of the methylome on the transcriptome in GBM, the authors identified 1550 unique genes that were differentially hypermethylated (1520) or hypomethylated (30) between G-CIMP$^+$ and G-CIMP$^-$ proneural GBMs tumors and looked to see which of these also showed differential expression. Two hundred and sixty-three genes were downregulated and hypermethylated in G-CIMP$^+$ compared to G-CIMP$^-$ proneural tumors. Interestingly, these included *FABP5*, *PDPN*, *CHI3L1*, and *LGALS3*: elevated expression of these genes has been independently associated with poor outcome in GBM. Furthermore, the authors noted a 263 downregulated and hypermethylated G-CIMP$^+$ gene signature that has been associated previously with lower grades of glioma.

Finally, using MethyLight technology, the authors distilled their extensive G-CIMP methylome signature into an eight gene panel of seven hypermethylated loci (*ANKRD43*, *HFE*, *MAL*, *LGALS3*, *FAS-1*, *FAS-2*, and *RHO-F*) and one hypomethlyated locus (*DOCK5*). Using this panel and an extensive separate collection of tumors, the authors validated the frequency of the G-CIMP signature and its association with mutant *IDH1* in GBM and confirmed that the G-CIMP occurs with a much higher frequency in low-grade gliomas. Interestingly, among the lower-grade tumors, G-CIMP was twice as common in oligodendrogliomas as compared to astrocytomas and correlated with improved patient outcome.

This work by Noushmehr and colleagues represents an important step along the road toward understanding the biology of GBM. The correlation of G-CIMP status with other molecular and histological characteristics of the disease highlights the value of the unbiased and systematic approach to cancer genomics promoted by the TCGA. In particular, the finding that all grades of G-CIMP$^+$ gliomas across multiple tumor collections display a favorable outcome suggests that this methylome promotes a less aggressive tumor phenotype. These data contribute to the expanding legacy of TCGA that should lead us beyond correlative genomics to a real understanding of the biology and treatment of all types of GBM.

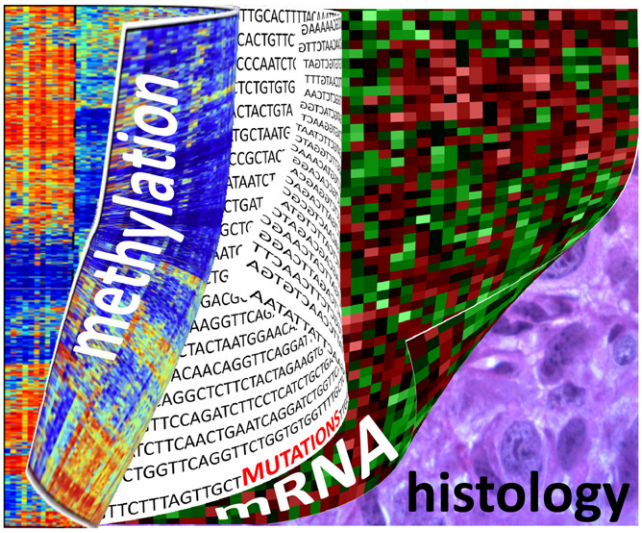

Figure 1. The Continued Efforts of the TCGA Project Are Advancing Our Understanding of Glioblastoma
With genomic technologies, the TCGA has layered detailed patterns of tumor gene expression, mutation and, in this issue of *Cancer Cell*, methylation over knowledge of histology and clinical characteristics to provide a comprehensive view of the disease.

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