human colorectal xenografts are treated with gemcitabine.

The major contributions of Olive et al. (2009) and Provenzano et al. (2012) are in demonstrating the importance of the microenvironment of PDA, and of solid tumors in general, in determining resistance to drug therapy. The majority of research on drug resistance has concentrated on molecular properties of individual cancer cells. Although intrinsic sensitivity is important, it is only part of the story—stated simply, if a drug does not get to some of the tumor cells, they will not be killed no matter how sensitive they might be to the drug in cell culture. More research should focus on strategies that recognize the importance of the tumor microenvironment and drug delivery in limiting therapeutic efficacy. There are several approaches to this problem, some of which are outlined in Figure 1. They include strategies to target the ECM with enzymes such as PEGPH20 or inhibition of Hedgehog signaling, strategies to decrease drug sequestration in cells proximal to blood vessels thereby allowing better distribution of drugs to distal cells, and combination treatment using "conventional" therapeutics together with drugs that both diffuse to and target specifically cells distant from blood vessels. Promising drugs for the latter include hypoxia-activated prodrugs and agents that attack the process of autophagy, a survival mechanism for stressed tumor cells (Trédan et al., 2007; Yang et al., 2011).

In summary, the efficiency of systemic chemotherapy for PDA in particular and for solid tumors in general is hindered by poor delivery of drugs to some tumor regions and by effects of the tumor microenvironment on drug activity. As Provenzano and coworkers show convincingly, agents that improve drug delivery by modifying factors relating to the tumor microenvironment represent an important future direction for cancer therapy.

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A Tell-Tail Sign of Chromatin: Histone Mutations Drive Pediatric Glioblastoma

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Recent genomic analyses of pediatric glioblastoma, a poorly understood tumor with dismal outcome, have identified mutations in histone H3 variants that affect critical amino acids in the tail. The findings extend discoveries of chromatin regulator inactivation and gain-of-function mutations by documenting alteration of a modifiable histone residue in human cancer.

Brain tumors are the most common solid neoplasms of childhood and the primary cause of cancer-related deaths in children. Although their pathological classification is complex, most high-grade brain tumors in children are categorized as either embryonal (such as medulloblastoma) or glial (such as the diffusely infiltrative glioblastoma [GBM]). An anatomical variant of high-grade glioma, diffuse intrinsic pontine glioma (DIPG), is a particularly vexing clinical challenge given its location in the neurologically critical brain stem. Over the past few decades, major progress has been made in the understanding and treatment of children with medulloblastomas, but little real progress has been made in the treatment of children with malignant diffuse gliomas. Consistent prognostic estimates have been difficult to establish, since the clinical behavior of childhood diffuse gliomas is not as stereotypical as that of their more common adult counterpart.

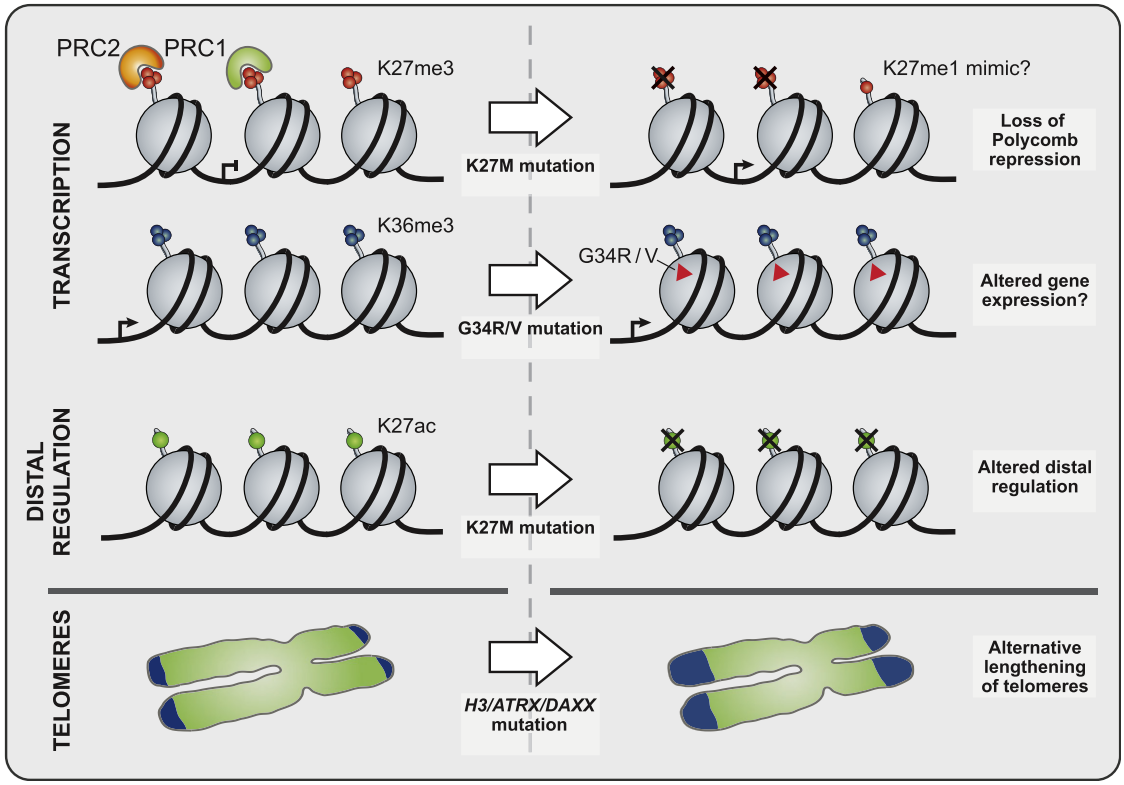

Figure 1. Model Depicts Possible Consequences of Histone and *ATRX/DAXX* Mutations
(Top) Histone H3 mutations may influence transcriptional regulation through loss of H3K27me3, which may impede repression by Polycomb-repressive complexes PRC1 and PRC2. K27M may also mimic H3K27me1. Structural changes through G34R/V mutation may indirectly affect H3K36 methylation. The K27M change may also affect distal elements, which are frequently marked by H3K27 acetylation.
(Bottom) Alternatively or in addition, *H3F3A* and *ATRX/DAXX* mutants may lead to alternative telomere lengthening.

Moreover, in recent years, it has become clear that although pediatric diffuse gliomas appear histologically identical to adult glioma, they have different underlying genetic compositions (Paugh et al., 2010). For example, whereas *IDH1* or *IDH2* mutations are found in the majority of grade II and III diffuse gliomas in adults and are thought to constitute early genetic events, they are rare in pre-adolescent children (Paugh et al., 2010). *TP53* mutations, a cardinal feature of diffuse astrocytic gliomas in adults, are far less common in diffuse gliomas in the first few years of life (Pollack et al., 2001), and combined loss of chromosome arms 1p and 19q, the classic genetic finding in diffuse oligodendrogliomas in adults, is exceedingly rare in pediatric oligodendrogliomas (Kreiger et al., 2005). Although it has been clear that pediatric diffuse gliomas are clinically and genetically different from their adult counterparts, no distinct genetic marker and key pathogenic mechanism has yet been discovered.

Two independent studies used whole-genome or whole-exome sequencing to interrogate the cancer genome of pediatric diffuse glioma (Schwartzentruber et al., 2012; Wu et al., 2012). In an extensive survey, Schwartzentruber et al. (2012) performed initial whole-exome sequencing of 48 pediatric GBMs, and 6 matched normal samples and discovered recurrent mutations in the *H3F3A* gene, encoding histone H3.3, in 31% of cases. These mutations were highly specific and comprised either K27M (9 of 48 cases), G34R (5 of 48 cases) or G34V (1 case). Interestingly, in an additional cohort of 784 adult and pediatric gliomas, *H3F3A* mutations were found in only 3% of adult GBMs but in 32% of pediatric GBMs and 18% of pediatric anaplastic astrocytomas. Wu et al. (2012) found *H3F3A* K27M mutations through whole-genome sequencing of 7 DIPGs and targeted sequencing of 43 additional DIPGs and 36 pediatric GBMs. Notably, K27M mutations were also found in the canonical histone H3.1 (*HIST1H3B*) in 18% of DIPGs. All histone alterations identified in the two studies are heterozygous, suggesting that these are gain-of-function mutations.

Histone H3.3 is deposited at active gene loci in the genome as well as at pericentromeres and telomeres. Unlike its canonical counterpart H3.1, H3.3 is incorporated into chromatin independently of the cell replication cycle (Talbert and Henikoff, 2010). The two mutations found in the two studies are located in the histone tail that is subject to extensive post-translational modification. In particular, the K27M mutation will block two widely studied modifications: K27 methylation, associated with Polycomb-mediated gene repression (Simon and Kingston, 2009), and K27 acetylation, present at active promoters and enhancers (Zhou et al., 2011). Although H3G34 is not itself subject to post-translational modification, it resides in close proximity to a lysine at position 36 (H3K36), whose methylation status is associated with transcriptional elongation (Zhou et al., 2011). In support of an indirect effect on

H3K36, Schwartzentruber et al. (2012) show that H3K36 methylation levels are increased in a tumor with G34R mutation. In addition, expression profiling of K27M and G34R/V mutant GBMs demonstrated differences in expression patterns of neural development genes, likely reflecting different effects of the two mutations on gene regulation.

Although H3.3 mutations conceivably affect gene regulatory functions, the selective advantage in pediatric GBM may actually relate to telomere maintenance and/or heterochromatin stability. Deposition of H3.3 within these genomic contexts is mediated by the ATRX-DAXX complex (Goldberg et al., 2010). Schwartzentruber et al. (2012) identified recurrent mutations in *ATRX* (14/48 cases) and its binding partner *DAXX* (2/48 cases). Overall, 21 of 48 (44%) pediatric GBMs had mutations in *H3F3A, ATRX*, or *DAXX*. Mutation of the ATRX-DAXX complex and either H3.3 tail mutation correlated with loss of ATRX expression in a pediatric GBM tissue array and an alternative lengthening of telomeres phenotype in GBM. Interestingly, although *IDH1* mutations were identified in 4 of 48 pediatric GBM, they were mutually exclusive with *H3F3A/ATRX/DAXX* mutations in this cohort.

Links between chromatin and cancer were initially drawn by oncogenic fusions containing chromatin proteins and by the anti-proliferative properties of chemical inhibitors of histone deacetylases (Baylin and Jones, 2011). These have been strengthened recently as an increasing number of whole-genome and exome cancer sequencing studies have identified prevalent mutations in histone modifying enzymes, nucleosome remodelers, and other regulatory proteins in chromatin. Although such findings are suggestive of direct roles for histone modifications, they fall short of definitive proof given that modifying enzymes also have nonhistone targets and frequently reside within large complexes with multi-faceted functions. Thus, the studies now presented by Schwartzentruber et al. (2012) and Wu et al. (2012) extend our understanding of how defects in chromatin contribute to cancer fitness by providing the first evidence of somatic mutations directly affecting a modifiable residue in histone proteins (Figure 1). Further study is essential to understand the mechanisms by which these and other alterations to the chromatin machinery contribute to malignant transformation and how they may be exploited for improved diagnosis and therapy.

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