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Micro-editing mistake translates into a devastating brain tumor

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Commentary

RNA modifications are increasingly being recognized as critical players in cancer. While adenosine-to-inosine RNA editing is consistently deregulated in cancer, we are still unable to draw a straight line connecting transcript-specific editing and carcinogenesis. The findings by Choudhury et al. in this issue of the *JCI* bridge this gap by mechanistically implicating underediting of miR-376a* in promoting glioma invasiveness through redirection of its mRNA targets. Moreover, RAP2A and AMFR convincingly emerge as key regulators of glioma migration and invasion affected by deregulated microRNA editing. Being inherently malleable, epigenetic mechanisms may provide feasible targets for therapeutic benefit.

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Micro-editing mistake translates into a devastating brain tumor

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RNA modifications are increasingly being recognized as critical players in cancer. While adenosine-to-inosine RNA editing is consistently deregulated in cancer, we are still unable to draw a straight line connecting transcript-specific editing and carcinogenesis. The findings by Choudhury et al. in this issue of the *JCI* bridge this gap by mechanistically implicating underediting of miR-376a* in promoting glioma invasiveness through redirection of its mRNA targets. Moreover, RAP2A and AMFR convincingly emerge as key regulators of glioma migration and invasion affected by deregulated microRNA editing. Being inherently malleable, epigenetic mechanisms may provide feasible targets for therapeutic benefit.

Advances in the understanding of RNA-based regulatory pathways are revolutionizing biology and medicine. In this issue of the *JCI*, Choudhury et al. (1) describe the interplay of microRNAs (miRNAs), major players in cancer, with the adenosine-to-inosine (A-to-I) RNA editing machinery.

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MicroRNAs: small RNA molecules with a major role in cancer

miRNAs constitute a new type of regulators of gene expression. Thousands of miRNAs have been identified in plant and animal genomes, including the human genome (reviewed in ref. 2). miRNA genes are usually transcribed into primary stem-loop structure precursors (pri-miRNAs) that are processed in the nucleus by Drosha-containing complexes into approximately 70-nt hairpin precursors (pre-miRNAs). Pre-miRNAs are exported to the cytoplasm and further trimmed by Dicer complexes, with loop removal resulting in approximately 21- to 23-nt-long mature miRNAs. One strand of the miRNA duplex is bound by an Argonaute protein to form a miRNA-induced silencing complex (miRISC) that regulates the expression of target mRNAs containing complementary sequences. Pairing between miRNAs and their cognate mRNA targets usually results in decreased protein expression, owing to mRNA degradation and translational repression. Most target mRNAs are regulated by miRNA binding to the 3′ untranslated regions (UTRs). The resulting miRNA/mRNA duplex includes a perfect pairing segment between a "seed," comprising nt 2–7 at the 5′ end of the miRNA and the complementary target (2). This post-transcriptional regulatory mechanism is an integral part of most cellular pathways and gene networks. Aberrations of miRNAs biogenesis and function have

been found in various human diseases, and deregulation of miRNA expression in cancer is well documented (3). Sets of miRNAs were shown, based on their effect on target genes, to act as oncogenic or tumor suppressor regulators, suggesting that these might be novel targets for cancer therapies.

RNA editing: a global regulatory mechanism deranged in cancer

RNA editing is a posttranscriptional modification, altering the sequence of RNA from that encoded in the DNA (4–6). The most prevalent editing type in humans is A-to-I editing, catalyzed by the ADAR (double-stranded RNA-specific adenosine deaminase acting on RNA) enzymes. The splicing and translation cellular machineries recognize inosine as guanosine. Thus, genomically encoded adenosines can be interpreted as guanosines in the RNA sequence. A-to-I editing occurs in a tissue-specific manner, with brain tissue transcripts being the most edited (4). Some RNA editing targets play a central role in neurogenesis, and disruption of the editing machinery in lower organisms results in behavioral and neural defects. Moreover, deranged editing is associated with neuropathological disorders such as amyotrophic lateral sclerosis, epilepsy, and brain tumors. While some A-to-I RNA editing occurs in coding sequences and affects protein sequence and function, the majority of editing events in humans occurs in non-coding sequences, in particular within the primate-specific *Alu* repetitive elements. Hundreds of thousands of editing sites have been identified; most of them are located in introns and UTRs. Editing of noncoding sequences was suggested to be involved in RNA localization and stabilization, regulation of splicing and translation, as well as RNA interference (4, 5, 7, 8). Editing also targets foreign RNA and participates in host response to viruses (9). Notably, RNA editing has been shown to be involved in miRNAs biogenesis, degradation, and function (10).

Several studies have indicated that the global level of A-to-I editing is significantly decreased in human solid tumors, and since editing is very prevalent in the nervous system, editing in brain tumors has been studied extensively (11–13). *Alu* repeats are mostly hypoedited in glial tumors, and the level of expression of ADAR enzymes was found to be in inverse correlation with the grade and aggressiveness of the tumors. Similarly, editing of coding regions in glutamate receptor transcripts was also downregulated in brain tumors, with less editing in the more aggressive gliomas. Restoration of ADAR activity in glioblastoma cell lines decreases the proliferation rate and invasion/migration, implying a pathogenic role for crippled editing machinery in gliomas (11, 13).

Glioblastoma: multilevel genetic and epigenetic aberrations

Glioblastoma multiforme is the most common and deadliest primary brain cancer (14). It is the most invasive type of glial tumor, rapidly growing and commonly spreading to nearby brain tissue. Glioblastoma is very resistant to therapy, and there has only been limited improvement in prognosis over recent decades.

A multitude of genetic and genomic studies identified a variety of molecular aberrations relevant to gliomagenesis (14). Mutations, deletions, and epigenetic alterations were found to cripple key tumor suppressor genes and pathways such as *Rb1*, *p53*, and *CDKN2A* in the majority of gliomas. Co-deletion of chromosomes 1p and 19q, mutations of the *CIC* and *FUBP1* genes, and gain-of-function mutations of *IDH1* and *IDH2* were described recently in glial tumors and found to be associated with better prognosis. Amplification, overexpression, and mutations of receptor tyrosine kinases (RTKs) such as EGFR, PDGFR, and Met are linked to abnormal signaling and oncogenesis of high-grade gliomas. Deregulation of components downstream of RTKs such as the PI3K/Akt/mTOR and Ras/MAPK pathways further contribute to abnormal proliferation, survival, and invasiveness. FGFR/TACC fusion translocations (15) and STAG2 mutations (16) were shown recently to contribute to the degree of aneuploidy of glioblastoma.

In addition to the variety of genetic abnormalities, deranged epigenetic regulation has been described in glioblastoma (14). The best-studied example is silencing of expression of the DNA repair enzyme O^6-methylguanine-DNA methyltransferase by promoter CpG island methylation. Abnormal methylation of other genes and global hypermethylation also occur in glioblastoma.

RNA editing of microRNAs and cancer

The cellular outcome of RNA editing is ultimately dependent on the function of the transcripts being edited, but the key editing targets involved in carcinogenesis are largely unknown (17). One clear exception was provided a decade ago by Ishiuchi et al., who showed that restoring editing levels of the AMPA-type glutamate receptor subunit GluR2 increased apoptosis and suppressed proliferation of glioblastoma cells (18). The current work by Choudhury et al. (1) beautifully fills the void: the study builds upon past observations of global editing deregulation in cancer and goes on to skillfully dissect and prove the contribution of a specific molecular pathway.

miRNAs were shown to undergo A-to-I editing as early as 2004 (19). miRNA editing can occur within the seed sequence, thus altering target mRNA specificity (20). Moreover, editing can affect processing and maturation by disturbing Drosha- or Dicer-mediated cleavage (10, 21), or even affect RISC complex loading efficiency (22). Kawahara and colleagues provided a clear demonstration of the functional effect of A-to-I editing of miRNAs when they studied the tissue-specific editing of miR-376 cluster transcripts. They found that editing of a site in the seed of miRNA-376 led to silencing of phosphoribosyl pyrophosphate synthetase 1 (PRPS1), modulating uric acid metabolism (20).

A recent study based on massively parallel sequencing followed by careful bioinformatic analysis documented increased seed editing levels in several miRNAs, including two miR-376a isoforms, during mouse brain development (23). Editing at site +4 of mature miR-376a* was not detectable in early developmental stages but increased throughout development. There was a consistent correlation between the level of miR-376a* editing and the expression of TTK, which is suppressed by non-edited miR-376a* and with PRPS1, which is regulated by seed-edited miRNA. Another recent study demonstrated editing of several miRNAs in human brain, with the majority of verified edited sites in seed regions (24). The miR-376 cluster was found to be edited in this study as well.

The findings by Choudhury et al. further indicate that disruption of tight regulation of the miR-376 family has a significant effect on expression and function of their targets, with major consequences on gliomagenesis and clinical effects (1). They report on accumulation of an unedited form of miRNA-376a* in human glioblastoma cells that conferred increased migration and invasion capabilities. The authors provide not only comprehensive proof for the role of the unedited microRNA-376a*, employing both in vitro and xenografts models, but also compelling clinical correlations in terms of tumor volume and

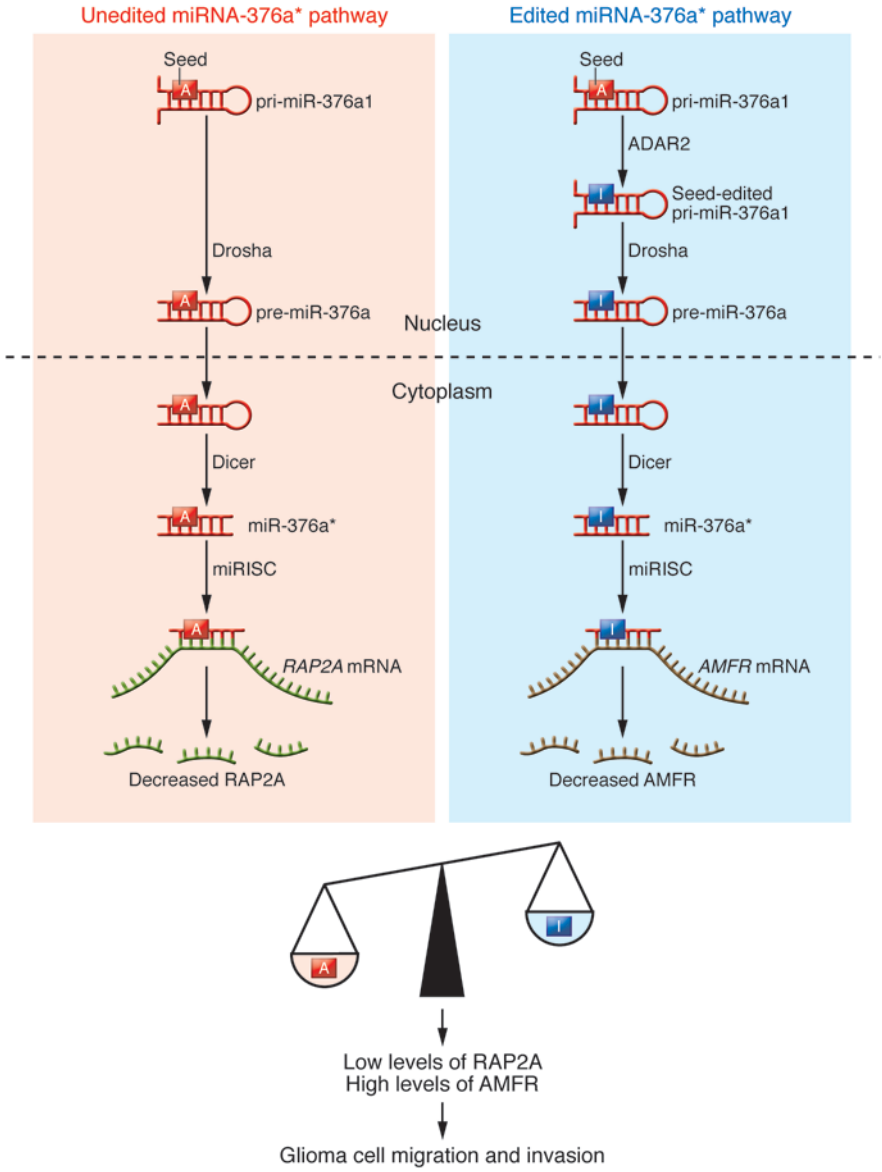

Figure 1
Mature miRNA-376a* seed sequence is unedited in high-grade gliomas, consequently promoting glioma cell migration and invasion through altered target specificity. RNA editing of miRNA seed sequences can potentially redirect their target specificity. While it is normally present in both edited an unedited forms in whole brain, dysregulated editing in high-grade gliomas results in accumulation of unedited miRNA-376a*. The capacity of unedited miRNA-376a* to repress RAP2A, a member of the Ras family of GTP-binding proteins, and its inability to target AMFR, a receptor for a tumor motility–stimulating secreted protein, promotes migration and invasion of glioma cells in vitro, and clinically correlates with the extent of invasive tumor spread.

patient survival. Furthermore, the authors go on to study two target proteins of this miRNA with opposite roles in regulating cell invasion, AMFR and RAP2A, each subject to regulation by either the edited or unedited form of miRNA-376a* (Figure 1).

RNA epigenetics: new opportunities for cancer diagnosis and therapy

The tenet that cancer is a disease of the epigenome as much as of the genome is by now well established (25). Research efforts in the 1990s focused on understanding the implications of DNA methylation abnormalities in cancer. During the last decade, efforts extended to encompass the role of chromatin modifications and have been rewarded by ongoing discoveries with proven diagnostic and therapeutic utility (26–28). RNA epigenetics is now the final frontier of exploration (29). With more than 100 characterized chemical modifications, many of them dynamic, we are now ready to map the topographies of the different RNA epigenomes with respect to the molecular origins of cancer. In parallel, epigenetic classification of tumors is expected to better define subcategories with implication for diagnosis, treatment, and prognosis. Hypotheses based on global profiles, like that of the A-to-I editome (30), are gradually being substantiated by detailed mechanistic work needed to translate the findings to the clinic. The inherent reversibility of most epigenetic mechanisms underscores their potential as targets for therapeutic intervention. The work by Choudhury et al. provides a fine example.

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Lung capillaries raise the hypoxia alarm

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When ventilation is blocked, the lung can protect against the loss of blood oxygenation by activating localized arterial vasoconstriction, reducing blood flow to underventilated regions, and redirecting flow to better-ventilated alveoli. This phenomenon, hypoxic pulmonary vasoconstriction (HPV), preserves the overall efficiency of blood oxygenation, but the mechanism by which the hypoxic signal is transmitted to the smooth muscle that contracts the arterioles has remained largely a mystery. In this issue of the *JCI*, Wang et al. reveal that the endothelial lining of the hypoxic alveoli plays a key role in sensing hypoxia and transmitting the signal to initiate HPV.

The equilibrium between ventilation to a lung region and the region's blood supply establishes blood oxygenation, the critical physiological outcome that ensures oxygen delivery to tissues. Multiple lung diseases threaten this equilibrium, potentially increasing the risk of systemic hypoxia. Hypoxic venous blood returning to the lungs gets oxygenated as it flows through alveolar capillaries, where oxygen diffuses into it from alveoli. Diseases that impair alveolar ventilation also impair blood oxygenation. This occurs, for example, in lung injury in which alveoli are inundated with plasma exudate and inflammatory cells and can therefore no longer be ventilated. Hypoxic blood streaming from diseased regions mixes with oxygenated blood streaming from well-ventilated regions. The admixture of the hypoxic streams potentially causes hypoxemia of the entire pulmonary outflow destined for systemic vascular beds. The lung protects against systemic hypoxemia by activating localized arterial vasoconstriction, which effectively turns off blood flow to underventilated regions, redirecting flow to better-ventilated alveoli and thereby restricting the formation of hypoxic streams. This phenomenon, commonly called hypoxic pulmonary vasoconstriction (HPV), preserves the overall efficacy of blood oxygenation. As Sylvester et al. discuss in a recent review (1), HPV has been long recognized, but is not entirely understood at the mechanistic level.

Signal sensing for HPV

In moderate hypoxia, defined as a fall in the oxygen tension of alveolar gas to 30–50 mmHg, HPV is seen within minutes, which indicates that the hypoxic signal rapidly traverses the spatial separation between the alveoli and the upstream arteriolar segment, the site of vasoconstriction. Since smooth muscle contraction is required for HPV, the bulk of the research in the field has focused on mechanisms of smooth muscle contractility. In general, these mechanisms are based on the notion that hypoxia of pulmonary arterial smooth

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