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REVIEW

Adenosine-to-inosine RNA editing meets cancer

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The role of epigenetics in tumor onset and progression has been extensively addressed. Discoveries in the last decade completely changed our view on RNA. We now realize that its diversity lies at the base of biological complexity. Adenosine-to-inosine (A-to-I) RNA editing emerges a central generator of transcriptome diversity and regulation in higher eukaryotes. It is the posttranscriptional deamination of adenosine to inosine in double-stranded RNA catalyzed by enzymes of the adenosine deaminase acting on RNA (ADAR) family. Thought at first to be restricted to coding regions of only a few genes, recent bioinformatic analyses fueled by high-throughput sequencing revealed that it is a widespread modification affecting mostly non-coding repetitive elements in thousands of genes. The rise in scope is accompanied by discovery of a growing repertoire of functions based on differential decoding of inosine by the various cellular machineries: when recognized as guanosine, it can lead to protein recoding, alternative splicing or altered microRNA specificity; when recognized by inosine-binding proteins, it can result in nuclear retention of the transcript or its degradation. An imbalance in expression of ADAR enzymes with consequent editing dysregulation is a characteristic of human cancers. These alterations may be responsible for activating proto-oncogenes or inactivating tumor suppressors. While unlikely to be an early initiating 'hit', editing dysregulation seems to contribute to tumor progression and thus should be considered a 'driver mutation'. In this review, we examine the contribution of A-to-I RNA editing to carcinogenesis.

Introduction

Epigenetic and post-transcriptional mechanisms are important for normal tissue development and gene expression. Numerous studies have documented global epigenetic abnormalities in cancer cells. Two such extensively dysregulated mechanisms are DNA methylation and covalent histone modifications, which affect tumor-specific gene expression through nucleosome remodeling (1,2). The large amount of data accumulated in the recent years transformed our perception of eukaryotic gene expression as it is now clear that enormous diversity can be generated at the RNA level. RNA molecules undergo elaborate processing and are subjected to a wide range of post-transcriptional modifications that affect their fate. These include splicing, 5′ capping, 3′ polyadenylation and RNA editing, to name a few. The most prevalent type of RNA editing in higher eukaryotes is the conversion of adenosine to inosine by hydrolytic deamination (A-to-I editing). Thought at first to be restricted to coding regions of only a few genes, recent bioinformatic analyses complemented by high-throughput sequencing revealed that it is a widespread modification affecting mostly non-coding repetitive elements in thousands of genes (3–5).

Abbreviations: ADAR, adenosine deaminase acting on RNA; dsRNA, double-stranded RNA; HSC, hematopoietic stem cell; LSC, leukemia stem cell; mRNA, messenger RNA; siRNA, small interfering RNA; UTR, untranslated region.

The basics of A-to-I RNA editing

A-to-I RNA editing is the irreversible deamination of adenosine to inosine in double-stranded RNA (dsRNA), conserved from sea anemones to Homo sapiens (6). The reaction is mediated by a family of adenosine deaminases acting on RNA (ADARs) and occurs co-transcriptionally at least in some cases (7–9). Three members of the ADAR family have been identified in mammals: ADAR1, 2 and 3. While ADAR1 and ADAR2 are expressed in most tissues, ADAR3 is exclusively detected in the central nervous system (reviewed in ref. 10).

All ADARs share a highly conserved C-terminal catalytic deaminase domain and contain several N-terminal dsRNA-binding motifs. ADAR1 has two major isoforms transcribed from alternative promoters: a shorter 110-kDa isoform localizes predominantly to the nucleus and a longer interferon-inducible, 150-kDa isoform shuttles between the nucleus and the cytoplasm (11–14). ADAR2 also has several isoforms, albeit less well characterized and localizes to the nucleus (15–17). ADAR1 and ADAR2 form homo- as well as heterodimers *in vivo* (18,19). In contrast, ADAR3 exists as a monomer, at least *in vitro*, which may explain why neither its enzymatic activity nor its editing substrates have been demonstrated (20).

The secondary structure of an RNA molecule largely determines which adenosines can be deaminated, with a minor preference for certain flanking nucleotides (21–24). Whereas long dsRNAs (>100 bp) are promiscuously and extensively hyperedited with up to 50% of adenosines deaminated, short (or long but imperfect) dsRNAs are more selectively edited, with only a few adenosines meeting the secondary structure criteria (24–27). The current model holds that dsRNA-binding motifs mediate editing selectivity by anchoring ADAR to a dsRNA region, whereas the catalytic domain lends specificity, that is preference for adenosines within a specific context of neighboring nucleotides (28,29). A recent study refined this model and found that the dsRBMs of ADAR2 also contribute to editing specificity by direct readout of the RNA sequence in the minor groove of a specific context (30). Non-selective hyperediting results in a different set of inosines in each molecule within the population of a given transcript. While ADAR1 is mainly responsible for hyperediting, ADAR2 is responsible for selective site-specific editing (24,27). However, some substrate overlap exists between the two (23).

RNA editing efficiency (the fraction of molecules in which an inosine appears instead of a genomically encoded adenosine) and patterns exhibit dynamic changes affected by environmental signals such as stress or internal signals such as metabolic state and cell cycle (31–38). Importantly, not every molecule that can adopt the required structure will eventually undergo editing. As a consequence, different messenger RNA (mRNA) variants of the same gene are generated, dramatically increasing the diversity of the transcriptome (39).

Initially, only a handful of selective editing sites were documented within coding regions, resulting in protein recoding and affecting protein properties and interactions. In recent years, large data sets obtained through high-throughput sequencing approaches integrated by bioinformatic analyses demonstrated a significantly wider extent of A-to-I editing affecting thousands of genes in tens of thousands of sites. These editing events occur mostly in non-coding repetitive sequences, such as *Alu* repeats, and tend to undergo non-selective hyperediting (3,4,40,41).

Inosines in the RNA molecule may serve different purposes, depending on the involved mechanism and interacting proteins.

Within coding sequences, inosines are decoded as guanosines by the translation machinery (as inosine preferentially base pairs with cytidine) (42), thus resulting in protein recoding. One well-studied example of editing-dependent protein recoding is the glutamate receptor subunit, GluR-B. Selective ADAR2-mediated editing of GluR-B leads to generation of an impermeable Ca^{+2}-ion channel due to a glutamine/arginine (Q/R) substitution (43). The splicing machinery also recognizes inosines as guanosines, enabling editing to generate and eliminate donor or acceptor splice site sequences. Editing-dependent alteration of splice sites in the ADAR2 transcript itself generates an alternative 3′-splice acceptor site (9) (see detailed discussion of editing and splicing in the context of cancer in a section below).

A-to-I editing also influences small interfering RNA (siRNA)- and microRNA-mediated gene silencing which depend on formation of dsRNA for their biogenesis and action. Inosines were shown to affect all major stages of microRNA biogenesis and function: (i) ADAR1 and ADAR2 edit specific adenosines in certain pri-microRNAs thereby suppressing subsequent processing by Drosha and Dicer (44,45), (ii) inosines present in mature microRNAs may alter binding to target sequences (46) or alternatively (iii) editing of 3′ untranslated regions (UTRs) harboring microRNA binding sites can have a comparable effect through modulation of base pairing or accessibility (47).

The significance of non-selective hyperediting of long dsRNAs occurring mostly in non-coding regions of transcripts harboring repetitive sequences is still largely unclear. Several lines of evidence support a role in regulation of gene expression through shortening of 3′ UTRs by nucleases that act on inosine-containing RNAs such as Tudor-SN (48,49) or alternatively through nuclear retention by a complex containing $p54^{nrb}$ which recognizes inosine-containing RNAs (50,51). In both cases, the proteins mediating the outcome specifically interact with inosine, which, unlike with the translation machinery, cannot be substituted for guanosine to receive the same effect.

Knockout mice that lack either ADAR1 or ADAR2 demonstrated that A-to-I RNA editing is essential for normal life and development. Homozygous disruption of ADAR1 in mice is embryonic lethal (at E11.5–12.5), most probably due to defective hematopoiesis and liver disintegration (52,53). $ADAR2^{-/-}$ mice are viable but prone to seizures and die shortly after birth. This phenotype is due to under-editing of the Q/R site in GluR-B pre-mRNA transcripts since $ADAR2^{-/-}$ mice were phenotypically rescued by insertion of genomically mutated $GluR\text{-}B^{R}$ alleles, which restored expression of the edited form of GluR-B at the Q/R site (54).

Dysregulation of RNA editing was linked to several human diseases. Reduced editing of GluR-B pre-mRNA at the Q/R site has been suggested to lead to motor neuron death in sporadic amyotrophic lateral sclerosis (55). Altered editing levels of serotonin receptor $5\text{-}HT_{2C}R$ transcripts were found in the prefrontal cortex of suicide victims and in neuropsychiatric disorders such as depression and schizophrenia (56). The direct involvement of A-to-I editing in cancer has not been extensively addressed experimentally. However, correlations as well as a limited number of experimental models indicate that A-to-I editing is severely dysregulated in cancer (57), allowing us to draw some important conclusions that are the subject of this review. Of note, not only ailment but also health was associated with editing: single-nucleotide polymorphisms in ADAR2 and ADAR3 were associated with exceptional longevity in different populations (58).

The broad involvement of A-to-I RNA editing in regulation of gene expression through different cellular mechanisms together with its cancer-correlated dysregulation raise the question of how these alterations in RNA molecules can lead to transformation and tumor progression.

A code within a code: a framework for understanding the editing code and its decoding

As mentioned above, A-to-I editing sites found in the transcriptome can be broadly divided into two groups: 'site-specific' editing events of select adenosine residues and non-selective 'hyperediting' clusters. These clusters primarily occur within *Alu* repeats due to their tendency to form the required dsRNA structures. It is instructive to further distinguish between those sites located in coding sequences and those in UTRs and introns. Editing events in coding regions are largely site-specific, while hyperediting characterizes UTRs and introns. How can we make sense of these categories specifying patterns and locations further compounded by their discovery in different functional classes of RNA molecules?

Looking around for additional hypothesized levels of information and regulation is surprisingly sending us time and again back to the 'inert' genetic material for answers, just to realize it contains more than meets the eye. It is becoming increasingly clearer that the same genetic sequence is sometimes required to simultaneously encode different types of information (59). A recent study demonstrated that the apparently redundant 'genetic code' is not so redundant after all: different codons for the same amino acid may affect the speed of translation and in turn specify posttranslational modifications (60). The meaning is simple yet striking: posttranslational modifications are encoded in the mRNA sequence. Does an inosine have more than one meaning?

The first of the 'editing codes' was in hindsight already apparent when A-to-I editing was initially recognized as only an RNA duplex unwinding activity in *Xenopus laevis* embryos (25,61,62). We later learned that editing can also result in stabilization of secondary structures (by targeting A–C basepair mismatches) (3,21,22,63,64). Even though the significance of this outcome is still unresolved, it is conceivable that by virtue of influence on secondary structure of UTRs, transcript translatability and interaction with RNA-binding proteins can be modulated. Inosine equals effect on secondary structure is the key to this code.

The earliest and prototypical examples of A-to-I RNA editing, namely GluRs and $5\text{-}HT_{2c}Rs$, taught us that the translational machinery decodes inosine as if it were guanosine (42) (because of its Watson–Crick base pairing with cytidine) leading to protein recoding affecting structure and function (43,65). This second editing code is integrated into the higher-order genetic code. It was later demonstrated to apply to other systems as well (such as splicing) (9). Inosine equals guanosine is the key to this code.

Our understanding of the biological meaning of *Alu* hyperediting is still in its infancy, albeit it constitutes the vast majority of editing sites. A recent study even estimated the contribution of *Alu* editing to transcriptome diversity is dramatically higher than that of alternative splicing (66). Is it indeed so? The discovery of proteins with a unique specificity toward inosine alone (that cannot be mimicked by guanosine), be it binding affinity able to retain edited transcripts in the nucleus ($p54^{nrb}$) (50) or endonucleolytic activity able to degrade them (Tudor-SN) (48), is a manifestation of an emerging third editing code employing yet other decoders, where evidently a different key applies: inosine equals inosine. The affinity of the $p54^{nrb}$ complex for inosines probably depends more on their quantity and density than on exact location and combination along the *Alu*. Estimating the impact of this code more likely requires use of measures other than nominal diversity.

Rising above the different codes, it should be stressed that the cellular outcome of A-to-I editing is ultimately dependent on the functions of the transcripts being edited. Therefore and bringing us closer to the focus of this article, the editing phenomenon as a whole does not fit the conceptual dichotomy between tumor suppressors and oncogenes. Acting by proxy, it can, however, be responsible for activating a specific proto-oncogene or inactivating a specific tumor suppressor. Whereas the net cellular phenotype of editing dysregulation is the sum of effects on individual transcripts—depending on cell type and circumstance, editing dysregulation of only one transcript may still account for the most notable phenotype (i.e. the edited version of GluR-B is able to solely rescue $ADAR2^{-/-}$ mice) (54).

When we detect the signature of A-to-I RNA editing in a transcript, we should ask ourselves: what is the code specified by inosines in this instance? Who decodes it? Noting what kind of transcript it is and where in the transcript editing occurs may assist us. We should also keep in mind that sometimes the same editing site can be written in two different codes to be read consecutively or competitively by two

decoders (67). The different codes adopted by the RNA editing machinery will guide our discussion. It will be interesting to find out what other codes inosines and ADARs have in store for us.

The cancer editome: initial attempts to understand the connection between editing and carcinogenesis

The considerable impact of A-to-I RNA editing on gene expression together with occurrence of editing events within tumor suppressors and oncogenes (68) warrant an in-depth investigation into possible involvement of editing in cancer. A partial survey of transcriptomes isolated from different solid tumors and matching controls to assess the frequency of editing events found in coding as well as in UTRs reveals a complex picture that can be best summarized as follows: the frequency of editing is significantly different in cancer compared with normal tissues; although clusters of editing events within repetitive Alu elements are mostly hypoedited in tumors, site-specific editing levels are consistently altered but lack a joint trend; brain tumors exhibit the most significant differences (57). Editing levels in hematological malignancies have not been comprehensively examined, except for a single study that reported a new editing event occurring at an intron branch point of protein tyrosine phosphatase, non-receptor type 6 (PTPN6) transcripts isolated from patients with acute myeloid leukemia (69). These altered editing patterns are reminiscent of the cancer-associated DNA methylation profile of global hypomethylation in repetitive sequences accompanied by site-specific hypermethylation in non-repeat DNA stretches, frequently associated with promoter regions (70). Maas *et al.* (71) were the first to demonstrate hypoediting of the GluR-B Q/R site in adult glioblastoma multiforme and in so doing provided a unique example in which the defined physiological outcome of hypoediting (assembly of Ca^{2+}-permeable alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate-type glutamate receptors) possibly explains some aspects of tumor behavior and symptomatology (epileptic seizures). Further experimental work in pediatric glioblastoma multiforme produced similar findings (72).

What is causing the change in editing levels? As regulatory mechanisms governing the editing machinery are largely unknown, research focused on assessing function and expression of ADAR enzymes. Once again, the findings do not lend themselves to easy interpretation. Experimental work on brain tumors demonstrated loss of ADAR2 activity (responsible for GluR-B editing) without reduction of its mRNA expression, side by side with elevation of ADAR1 and ADAR3 mRNA levels (72). In another study, mRNA levels of all three ADAR family members were reduced in gliomas of varying grades (57). Childhood acute leukemias, and especially newly diagnosed B-cell lymphoblastic leukemias (B-ALL), exhibit a significant overexpression of only the constitutive ADAR1 isofrom (p110), with a dramatic decrease in its level in patients achieving complete remission (73). Attempting to interpret the findings one must keep in mind that ADARs normally function predominantly as homodimers (18). ADAR1 overexpression, even in the setting of unchanged ADAR2 levels, probably tips the balance and leads to ADAR1/ADAR2 heterodimer formation, thus interfering with the specific editing activity of the latter (72). Alternatively, the observed down-regulation of all three enzymes—importantly not to the same degree—may theoretically cause simultaneous hypoediting of one transcript and hyperediting of another. As proposed by Gallo *et al.* (74), a model of progressively developing imbalance in expression of ADAR family members during tumor evolution has a power to reconcile the apparently incongruent expression of ADARs' in different tumors. One must keep in mind, though, that this proposed model relies on evidence limited to the expression of deaminases and hence that the perturbation of other, yet unknown, regulatory mechanisms might also be responsible for the observed changes in editing levels.

Naturally, the question is whether there are any hints that the change in editing levels has a causal relationship to malignant transformation (initiation); or alternatively—does the malignant phenotype depend to some extent on this change (progression), implying that restoring editing 'balance' would help curb cancer? The most compelling evidence suggesting the answer to the latter question is yes, at least in brain tumors, was provided using two main approaches: (i) rectifying a specific hypo-edited transcript: conversion of Ca^{2+}-permeable alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate-type glutamate receptors to Ca^{2+}-impermeable receptors through gene delivery increased apoptosis and suppressed proliferation of adult glioblastoma multiforme cells (75,76); (ii) reinstating ADAR 'balance': overexpression of ADAR2 in astrocytoma cell lines restored editing levels and regressed the malignant phenotype in terms of cell growth and migration (72). Moreover, correlations established between activity/expression of ADARs and tumor grade further imply editing dysregulation has a role in cancer progression (57,72). As for initiation, evidence implicating editing dysregulation as an early 'hit' along the slope ending in malignant transformation is lacking: no increase in cancer incidence was reported in either animal models of RNA editing dysregulation (54,77–83) or human subjects suffering from the pigmentary disorder dyschromatosis symmetrica hereditaria which carry heterozygous null mutations in ADAR1 (84,85). Judging by all above observations, while unlikely to be an early initiating hit, editing dysregulation—be it by way of mutation in ADAR genes or other epigenetic processes—seems to contribute to tumor progression and hence ought to be regarded as a 'driver mutation'.

Evidently, though RNA editing has a large impact on gene expression, we are still unable to draw a straight line connecting transcript-specific editing—leading for example to proto-oncogene activation or tumor suppressor inactivation—and carcinogenesis (Figure 1). So long as we are deducing from face value of deaminase expression we will not be able to decipher the network logic of editing dysregulation. The alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate-type glutamate receptor subunit GluR-B is the only editing target with an established bearing on cancer. A novel connection between RNA editing and modulation of DNA damage repair was recently made when an ADAR1-mediated editing event was identified in the DNA repair enzyme NEIL1, a base excision repair glycosylase (86,87). This site-specific event causes a lysine to arginine change in the lesion recognition loop of the protein, thus effectively changing its glycosylase activity and lesion specificity. It is tempting to speculate that this is a route through which RNA editing affects the number and types of mutations that accumulate in genomes of cancer cells. Notwithstanding, the analysis presented above, based on correlations between editing levels and cancerous states as well as on preliminary genetic manipulations of ADARs, still enables us to implicate editing dysregulation as a probable causative agent in cancer progression. We now turn to examine possible explanations at the molecular level.

Cancer stem cells

The enormous progress achieved in the general stem cell field in recent years has fueled efforts to find corollaries in cancer. According to the leanest interpretation of the cancer stem cell hypothesis, tumors are 'organs' composed of a heterogeneous hierarchy of evolving malignant cells derived from and maintained by a unique subpopulation of cells with 'stem cell' properties: namely, self-renewal and the ability to differentiate and create the repertoire making up the tumor, together endowing them with a uniquely sustained tumorigenic potential (88). Whereas the exact nature of this population (i.e. size, quiescence, markers and chemosensitivity), its homogeneity and relation to somatic tissue stem cells are still under debate, it is clear, from a clinical perspective, that tumor eradication will only be achieved through targeted therapy that addresses the unique properties of the cancer stem cell subpopulation (89).

In some hematological malignancies, evidence suggests that leukemia stem cells (LSCs) are derived from normal hematopoietic stem cells (HSCs), which acquired initial genetic hits (90–93). Recently, two studies in mice, elaborating on the initial observation of defective hematopoiesis in $ADAR1^{-/-}$ mice, have revealed the specific importance of ADAR1 for the maintenance of the HSC compartment, possibly by suppression of the interferon signaling pathway and

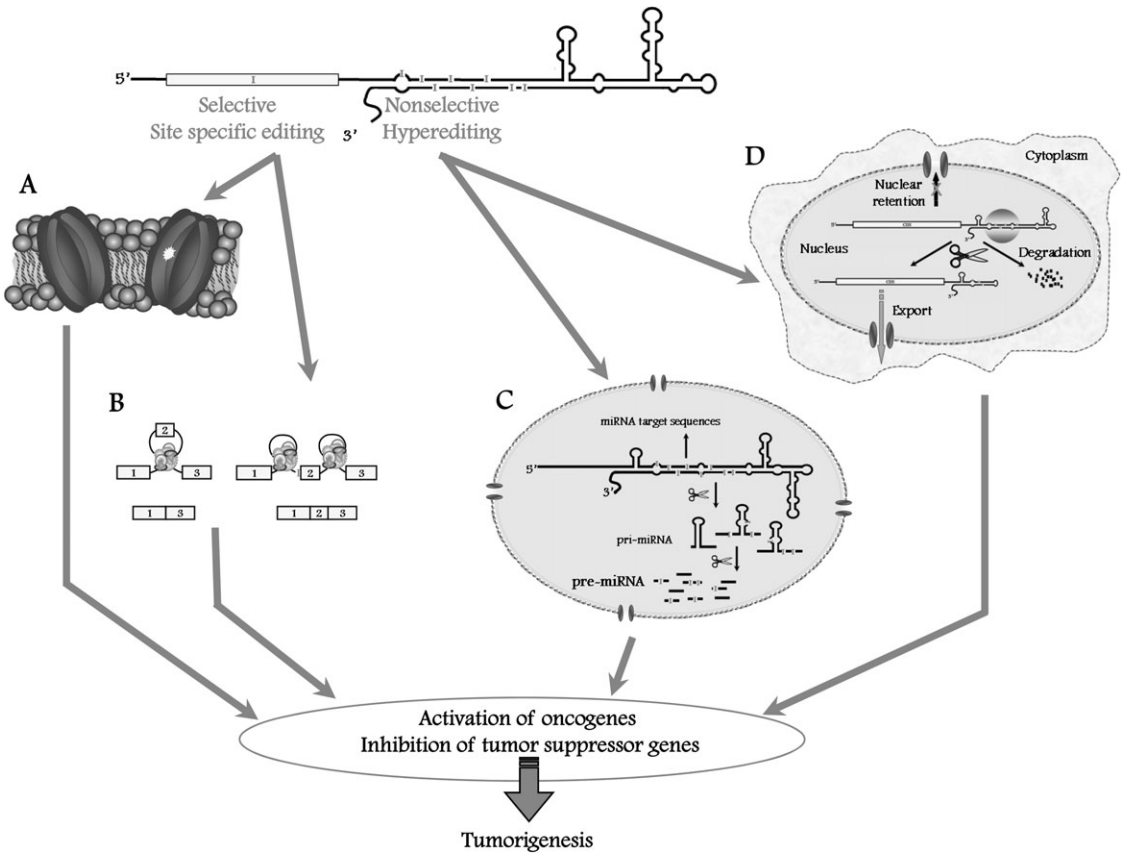

Fig. 1. A-to-I RNA editing sites can be divided into two groups: selective site-specific editing and non-selective hyperediting clusters that occur mainly in non-coding sequences. Selective editing may result in protein recoding when occurring within coding sequences (**A**) or alteration of splice sites (**B**). Non-selective hyperediting can influence gene expression through several mechanisms: altering microRNA binding sites (**C**); shortening of 3′ UTRs by nucleases that act on inosine-containing RNAs and nuclear retention by p54nrb (**D**). All these changes may result in activation of oncogenes or inactivation of tumor suppressor genes, thus leading to tumorigenesis.

consequent protection of HSCs (52,78,94). ADAR1 absence caused hematopoietic progenitor cells to undergo increased apoptosis and prevented them from forming differentiated colonies while exhausting HSCs due to continuous activation (94). It is plausible that the unique qualities ADAR1 confers to normal HSCs (for example: interferon signaling suppression, anti-apoptosis and quiescence) would also serve LSCs, as many markers are common to both HSCs and LSCs (91,93). The efficiency of interferon therapy in chronic myeloid leukemia might support this notion (95,96). It may be argued that the global hypoediting accompanied by ADAR1 down-regulation seen in various human tumors contradicts a possible role for ADAR1 in LSC maintenance (57). Whereas this observation was made in solid tumors, a recent study showed that ADAR1 is actually up-regulated in acute childhood leukemias (73), possibly reflecting a different role for ADAR1 in these two broad cancer categories. It brings to mind the apparent puzzling discrepancy between promyelocytic leukemia protein levels and function in solid tumors (an established tumor suppressor, where loss of promyelocytic leukemia protein predicts an unfavorable prognosis) versus in chronic myeloid leukemia stem cells (where it is responsible for maintaining the quiescence of LSCs, and its loss predicts a favorable outcome) to demonstrate our point (97,98). Taken together, we believe ADAR1 is probably important for the survival of some LSC compartments and therefore might serve as a valuable target in future therapy.

Editing and immunity

Chronic inflammation and cancer development are tightly connected (99). Chronic infections are chief exogenous inducers of inflammation, responsible for roughly 20% of all cancers. By now, it is firmly established that at least seven human viruses, namely Epstein-Barr virus, hepatitis B virus, human papillomavirus, human herpesvirus 8, hepatitis C virus, human T-lymphotropic virus type I and molluscum contagiosum virus are etiologic agents in 10–15% of human cancers worldwide (100). In the case of viral-induced cancer, the molecular basis of host susceptibility allowing for persistent viral infection is of obvious importance but is largely unknown. Infectious agents trigger an inflammatory response that precedes tumor development as part of the normal host defense directed at pathogen elimination. However, tumorigenic viruses devised ingenious ways to undermine host immunity and establish persistent infections causing low intensity but chronic inflammation (101,102). Host reaction to viral infection classically takes two forms: (i) innate immunity characterized by a rapid and generic response, (ii) adaptive immunity characterized by a highly specific response, which also confers future resistance. For clarity of our discussion, it is instructive to further distinguish between editing of host cellular RNAs and editing of viral RNAs. As will be described below, the editing machinery has a central role in both arms and influences cellular as well as viral RNAs in the setting of infection and inflammation (103).

Beginning with a role in immune cell development—as mentioned above in the ‘cancer stem cell’ section, ADAR1 is necessary for HSC maintenance and hence maturation of cells belonging to both innate and adaptive arms of the immune system is dependent on it. Interesting in this regard is the suggested underlying mechanism: suppression of the interferon signaling pathway and consequent protection of HSCs from apoptosis (94). Continuing with a role in immune cell function—up-regulation of ADAR1 in lymphocytes following stimulation with a variety of inflammatory mediators (tumor necrosis factor-α, interferon-γ) results in editing of host cellular RNAs, possibly contributing to proliferation and differentiation of lymphocytes (36).

As for editing as a direct antiviral mechanism—since most RNA viruses localize to the cytoplasm, the ADAR1p150 isoform, being interferon-inducible and cytoplasmic, is one of the several effectors belonging to the first line of innate immune defenses against viral infection (104,105). As such, it has proven roles in editing of the HCV RNA genome resulting in inhibition of its replication (106). It also edits the HHV-8 Kaposin transcript eliminating its transforming activity (107). As will be discussed in greater detail in the microRNA section below, editing of EBV-encoded microRNAs was shown to dictate viral latency (108).

In support of a direct antiviral function for ADAR1 are the specific inhibitors developed by adeno and vaccinia viruses against its enzymatic activity (109,110). Not unexpectedly, recent studies demonstrate some viruses (HIV and vesicular stomatitis virus, for example) evolved specific features taking advantage of the editing system and harnessing it to aid their replication, thereby making ADAR1 a viral 'accomplice' (111,112).

Surprising, and especially illuminating, is the proviral effect exerted by ADAR1 through direct inhibition of dsRNA-activated protein kinase (PKR), an interferon-inducible kinase with a central role in antiviral immunity (113,114). This response, counteracting another antiviral pathway to block apoptosis and assist viral replication, was demonstrated for measles virus, vesicular stomatitis virus and HIV-1. It compromises the ability of the host to fight infection, in effect promoting chronic inflammation, but through direct modulation of a host protein and not through a viral intermediate. Is it yet another example of viral manipulation? Taking into account that ADAR1 is also a suppressor of interferon signaling (78), potentially protecting the organism against its unleashed detrimental activation, and was also shown to negatively regulate immune response activated by cytosolic DNA (115), the answer is probably not. We believe that it manifests the role of ADAR1 in keeping antiviral response in check. This apparent contradiction was recently addressed by Gatignol *et al.* (113) who stressed the biased nature of our conception of cell response solely intended to counteract viral replication. Considering that every physiological response is the result of co-evolution of viruses with their hosts, it might be more suitable to view the situation as equilibrium between the need to avoid death due to viral replication and the need to avoid death due to hyperactive immune response.

The complex interactions outlined above suggest that ADAR1 is very pertinent to the basic host–viral interactions determining the persistency of viral infection. ADAR1 is not easily classified as proviral or antiviral, neither when considering the outcome of viral infection nor when regarding it as an effector of the interferon system. We propose that ADAR1 induction in viral infection essentially serves to combat viruses through direct editing of their transcripts (a mechanism sometimes outsmarted by viruses) and at the same time to modulate and limit the interferon inflammatory response. Therefore, it appears A-to-I RNA editing, and ADAR1 in particular, are key determinants of persistent infection, consequent chronic inflammation and resultant cancer risk.

Fighting retrotransposon-mediated 'natural mutagenesis'

'Insertional mutagenesis' of proto-oncogenes and tumor suppressors by mobile genetic elements can participate in carcinogenesis (116,117). Normal cells manage to protect themselves against adverse mutagenic effects inflicted by endogenous retrotransposons (*Alu*, long interspersed element 1) through the use of methylation, siRNAs and other approaches (118). Although the repressive effects of these mechanisms are partly relieved in cancer (global hypomethylation), it has been difficult to determine if retrotransposons are consequently 'jumping' in the genomes of human tumor cells and to what extent they are involved in genomic instability and cancer development (119). Iskow *et al.* (120) recently demonstrated such *de novo* somatic insertions are quite frequent in non-small-cell lung cancer and provided further evidence in favor of a causative relation between DNA demethylation and extensive retrotransposon mobilization in tumors.

Deaminating mostly retrotransposon-derived dsRNAs—is A-to-I editing another barrier against retrotransposition? Is the cancer-associated breach of editing defense lines somewhat responsible for the observed mobilization and insertion of retrotransposons? Several lines of preliminary evidence point to intriguing interactions of the editing system perhaps enabling it to ward off retrotransposons but are still too unripe to answer the posed questions in the affirmative.

Before proceeding to mention more intricate and indirect proposed mechanisms, it is worth mentioning probably the most straightforward one—direct editing of retrotransposon sequences may damage them and prevent their integration back into the genome.

Endogenous siRNAs formed out of retrotransposon-derived dsRNAs target their parent transcripts resulting in their suppression (121). ADAR2, equipped with the ability to hyperedit the parent transcripts, can prevent Dicer, at least *in vitro*, from cleaving them, thereby suppressing mature siRNA generation (49). In addition, ADARs avidly bind mature siRNAs, decreasing their effective concentration, thereby competing with RNA-induced silencing complex and reducing the efficiency of RNA interference (122). Alternatively, editing exposes the parent transcripts to the endonucleolytic activity of Tudor-SN directed specifically against inosine-containing dsRNAs (48,49). The outcome of these competing forces in terms of anti-retrotransposon activity is not clear and has not been put to experimental test.

There might be yet another route through which RNA editing participates in epigenetic silencing of retrotransposon activity as part of cell defense mechanisms. The discovery that vigilins can bind inosine-containing RNA and are also in a complex containing ADAR1 indicates a possible involvement in a heterochromatic silencing mechanism (123). According to a proposed scenario, ADAR1 hyperedits dsRNAs transcribed from genomic loci harboring repetitive elements; these, in turn, are recognized by vigilins, which recruit additional factors needed to transform the region into heterochromatin thereby suppressing the expression of retrotransposons.

Editing of microRNAs

MicroRNAs are small non-coding RNAs that act as posttranscriptional repressors of gene expression (124). Similar to the editing machinery, the effects of microRNAs on fundamental cellular processes are only understood when considering the downstream functions of the transcripts they target in a specific cell type and differentiation state. The involvement of microRNAs in processes driving cancer initiation and fueling progression and metastasis are by now well established (125). Distinctively aberrant microRNA expression profiles characterizing various cancers lie at the base of the prevailing hypothesis assigning tumor suppressor roles to down-regulated microRNAs (that normally down-regulate the expression of an oncogenes) and oncogene roles to up-regulated ones (that normally down-regulate the expression of a tumor-suppressor) (126). Various mechanisms help microRNAs escape strict expression regulation in cancer, ranging from structural genomic (translocation, amplification, deletion), through epigenetic (promoter hypermethylation, histone hypoacetylation) to defects in biogenesis (transcription repression/activation, Drosha/Dicer loss) (127).

The stem-loop secondary structure adopted by primary microRNAs (pri-microRNAs) and microRNA precursors (pre-microRNAs) enables interaction between the A-to-I editing machinery and the microRNA biogenesis pathway. Nishikura *et al.* have convincingly demonstrated how editing events in pri- and pre-microRNAs block the processing cascade by interfering with Drosha or Dicer cleavage steps, resulting in reduced levels of mature microRNAs (44,45). Depending on the position, when editing events do not interfere with enzymatic cleavage, they can end up in mature microRNAs with a potential to redirect their target specificity (46). At the other end of the equation, extensive A-to-I editing of mRNA 3′ UTRs may add to regulation of microRNA activity through modulation, creation or elimination of binding sites (47). Additionally, the resulting stabilization/destabilization of the secondary structure of target regions may

control RNA-induced silencing complex accessibility to binding sites. Not only cellular microRNAs but also viral ones are edited, providing another avenue for editing to influence the outcome of viral infection and concomitant cancer risk. In a recent study, editing of EBV-encoded microRNAs was shown to dictate viral latency (108). According to current estimates, ∼20% of human pri-microRNAs are edited, suggesting a large impact on global gene expression through modulation of microRNA levels and specificity (128). Thus, it appears that the contribution of editing dysregulation to the malignant phenotype might also be mediated through effects on microRNA expression and target specificity. Such events await discovery.

Editing and splicing

The formidable challenges of splicing and alternative splicing are met by the spliceosome through utilization of the 'splicing code'—a set of *cis*-acting RNA sequence motifs and structural features (such as secondary structure and exon length)—with the aid of a complement of *trans*-acting factors (129). By introducing diversity into open reading frames as well as UTRs of almost all multi-exon human genes, alternative splicing controls not only the identity of protein isoforms but also their spatial and temporal expression levels. There is ample evidence to assign a causative role for dysregulation of alternative splicing in cancer initiation and progression (129–131). Dysregulation can result from mutations in *cis*-acting elements within the affected gene or brought about through alterations in the *trans*-acting splicing environment entailing broader effects on multiple genes, as exemplified by up-regulation of the splicing factor SF2/ASF in various human tumors (132,133). A-to-I RNA editing, taking place cotranscriptionally at times (7–9), can shape the splicing code directly by altering *cis*-acting RNA sequence motifs and secondary structure or indirectly by editing of *trans*-acting factors thereby affecting their expression and/or isoform identity. Given that the splicing machinery interprets inosine as if it were guanosine, editing is able to produce a splicing event by creating the canonical 5′ and 3′ dinucleotide recognition sequences (AUAG, GUAA → IUAG, GUAI = GUAG) or otherwise eliminate one by destroying the 3′ recognition sequence (AG → IG = GG) or the internal branch point adenosine (9,134,135). ADAR2 editing of its own pre-mRNA to generate a proximal 3′ splice site with consequent premature translation termination and production of a non-functional enzyme is a notable example of auto-regulation achieved through interplay between editing and splicing. Editing of the branch point adenosine leading to intron retention was suggested to account for the tumor suppressor PTPN6 functional haplo-insufficiency in patients with acute myeloid leukemia (69). Another way for editing to modulate alternative splicing is by targeting splicing enhancer or suppressor sequences, thereby weakening or strengthening them (67). The observation that most cancer-associated splicing changes are not associated with mutations in the affected genes was taken to indicate that alterations in the trans-acting splicing environment probably account for them (136). However, based on the multiple ways by which editing can exert its control over splicing, we anticipate a larger part of this 'gap' would come to be explained by editing dysregulation common in cancers.

Editing the 3′ UTR landscape

Stability, localization and translation of mRNAs are largely determined by sequences in the 3′ UTR through interaction with regulatory proteins and small RNAs (137–139). An increase in the expression of mRNAs with shorter 3′ UTRs, terminating at upstream alternative polyadenylation sites, was demonstrated in the transcriptome of murine CD4$^+$ T lymphocytes following their activation (140). The shorter transcripts typically produced more protein. It seems that the regulated use of proximal alternative polyadenylation sites in states of increased proliferation is aimed at reducing the regulatory capacity of 3′ UTRs, which is largely repressive in nature.

Whereas oncogene activation is frequently the result of genetic alterations, oncogene overexpression can still occur even in the absence of mutation. The notion that a shift toward shorter 3′ UTRs is required to escape their repressive effect—which would otherwise restrict proliferation—is further supported by evidence linking 3′ UTR shortening to proto-oncogene activation and carcinogenesis (141). Thus, Mayr and Bartel (141) demonstrated shorter isoforms are more stable than their full-length counterparts and are more efficiently translated due to loss of microRNA-binding sites. Interestingly, the binding sites harbored within the longer 3′ UTRs could only account for a fraction of the observed increase in protein translation, indicating other regulatory elements contribute to the net repressive effect of 3′ UTRs.

Most A-to-I substitutions in humans occur within dsRNA structures formed by two adjacent, reversely oriented Alu elements. More than 50% of these events are localized to 3′ UTRs, generating hyperedited transcripts that may be retained in the nucleus by a protein complex containing the inosine-specific RNA binding protein, p54^{nrb} (50). It is not to say that hyperedited transcripts are exclusively nuclear and not to be found in the cytoplasm. The global hypoediting of repetitive *Alu* elements in cancer may enable escape from p54^{nrb}-mediated nuclear retention, resulting in altered intracellular localization and increased expression.

Spector *et al.* have opened a new perspective on the way editing-dependent nuclear-retention might participate in the regulation of gene expression: they demonstrated that during stress the anchored mouse CTN-RNA transcript is somehow cleaved, generating a shorter inosine-free protein-coding mCAT2 transcript able to escape nuclear retention, be exported to the cytoplasm and translated (142). The concept of an anchored nuclear pool of transcripts ready for immediate release is appealing and could serve important purposes, especially in stress response. Bioinformatic analysis revealed many such 3′ UTR cleavage events, demonstrating that the novel regulatory scheme depicted by Spector *et al.* might be relevant for hundreds of human genes harboring inversely oriented edited *Alu* repeats (143).

Taken together, the common dysregulation of A-to-I editing in human cancers in combination with the complex influence of *Alu* hyperediting on 3′ end generation and transcript localization may contribute to the altered transcriptional program necessary to sustain carcinogenesis. The unraveling of various ways by which cancer cell gene expression is modulated through RNA editing may lead to identification of novel diagnostic and prognostic markers and to development of new therapeutic modalities.

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