The activity of RNA is controlled by different types of post-transcriptional modifications, such as the addition of methyl groups and other chemical and structural changes, that have been recently described in human cells by high-throughput sequencing. Herein, we will discuss how the so-called epitranscriptome is disrupted in cancer and what the contribution of its writers, readers, and erasers to the process of cellular transformation is, particularly focusing on the epigenetic modifications of ncRNAs.

Significance: Chemical modifications of RNA play a central role in the control of messenger and ncRNA activity and, thus, are tightly regulated in cells. In this review, we provide insight into how these marks are altered in cancer cells and how this knowledge can be translated to the clinical setting.

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AN INTRODUCTION TO RNA MODIFICATIONS

All life forms need mechanisms to regulate RNA levels and activities. An important part of these control belts for RNA occurs at the transcriptional level, but it is becoming apparent that a significant part of RNA homeostasis depends on RNA stability and degradation. This can be particularly true for common human diseases, such as cancer, where an aberrant RNA transcriptome constitutes a hallmark of transformed cells that cannot be fully attributed to alterations in the DNA-associated transcriptional start sites. In this respect, one interesting idea to be further explored is that molecular lesions in tumors occur not only in the original DNA molecule, but also in the RNA itself. More than 100 differentially modified nucleotides have been described and cataloged in RNA molecules (1). Although some of these have also been described in DNA, such as cytosine methylation, hydroxymethylcytosine, and, more recently, adenine methylation (2), DNA seems to have a smaller repertoire of modified nucleotides. RNA molecules can show a more diverse spectrum of modifications that includes, among others, pseudouridine or a hypermodified 7-deazaguanosine (queuosine).

From an academic standpoint, the RNA modifications can be divided into two main types: reversible and nonreversible. Among the first, one could count the different types of RNA methylation, such as cytosine and adenosine methylation, whereas in the most permanent RNA changes, one could cite editing and splicing [including the formation of circular RNAs (circRNA)]. However, this classic scenario is quickly evolving, and modifications previously presumed nonreversible are slowly becoming perceived as reversible.

Most importantly, the number and types of potential transcripts that are amenable to modification are growing almost exponentially, in part due to the emergence of transcriptomic techniques that use different strategies to provide RNA landscapes for 5-methylcytosine (m5C; refs. 3–5), 5-hydroxymethylcytosine (hm5C; ref. 6), N6-methyladenosine (m6A; refs. 7, 8), N4-methyladenosine (m4A; refs. 9, 10), pseudouridine (Ψ; refs. 11–13), or inosine-to-adenine editing (14). Many times, these chemical modifications are dynamic, adapting to the cell environment; however, at the same time, they are locally transmitted during mitosis and meiosis. Thus, in a similar manner as DNA and histone modifications, we can discuss an RNA epigenetic setting or an epitranscriptome. Herein, we will discuss how the ncRNA epitranscriptome is altered in cancer and the possible functional consequences of these alterations; the existence of defects in the writers (i.e., RNA-modifying enzymes), erasers (enzymes that remove these marks and modifications), and readers (proteins that decode these RNA modifications) of the ncRNA epigenetic code; and how this knowledge could be exploited for translational and therapeutic purposes. Figure 1 illustrates the potential of RNA modifications.
RNA modifications described in human cells; their writers, readers, and erasers; and the different effects on the transcript molecule.

CHEMICAL MODIFICATIONS OF ncRNAs AND THEIR FUNCTIONS

The role of chemical modifications of RNA has been extensively explored and discussed (1, 15, 16). However, with the exception of some post-transcriptional chemical marks on certain subtypes of ncRNAs, such as transfer RNAs (tRNA) and ribosomal RNAs (rRNA), that have been known for decades, the focus of research in this area has mainly been on the impact of these modifications on mRNA. By contrast, little attention has been devoted to their effects on other classes of ncRNAs. Part of the explanation for this current lack of knowledge relates, of course, to the most recent timing of ncRNA discovery and the very recent development of appropriate technologies. Indeed, in recent years, we have witnessed amazing exponential growth in the knowledge of other types of ncRNAs that exert essential roles in cellular homeostasis and contribute to the natural history of many human diseases, including cancer. These range from small ncRNAs (snRNA), such as miRNAs and piRNAs, to long ncRNAs (lncRNA), such as long intergenic ncRNAs (lincRNA), pseudogene transcripts, and circRNAs. Table 1 shows a summary of the various classes of ncRNAs, describing their characteristics in human cells and the role of selected RNA chemical modifications according to the transcript. The reader will note that major gaps remain regarding the chemical modifications of several ncRNA subclasses, and very few examples have been reported for those included in Table 1; however, we expect that this review will raise the interest of many researchers to act and fill in these unknown gaps. In the following paragraphs, we will discuss what is known about these post-transcriptional marks in ncRNAs and how the field could advance.
One of the most well-known chemical modifications of RNA affects its 5′-end, the so-called “5′ cap” (17). Therein, the most characterized cap modification is the addition of an N7-methylguanosine (m7G). Further changes frequently occur, and in some small nuclear RNAs (snRNA) and small nucleolar RNAs (snoRNA) a trimethylguanosine (m2,2,7G or TMG) cap can be added. snRNAs are additionally modified by ribose 2′-O-methylation on the first and second transcribed nucleosides to allow for correct splicing.

m7C was originally thought to be confined to two particular types of ncRNAs, tRNAs and rRNAs, but the use of bisulfite modification and deep sequencing has identified many ncRNAs to be similarly modified. Indeed, m7C is now known to be associated with many ncRNA types.

Table 1. Epitranscriptomics of ncRNAs

<table>
<thead>
<tr>
<th>ncRNA type</th>
<th>Size</th>
<th>Location</th>
<th>Number in humans</th>
<th>Functions</th>
<th>Described chemical modification</th>
<th>Examples of modified ncRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>miRNAs</td>
<td>19–24 bp</td>
<td>Widespread</td>
<td>&gt;1,500</td>
<td>Targeting mRNAs</td>
<td>Editing, m7C, m6A, U-tail, Poly(A), 5′-O-m</td>
<td>let-7, miR376a*, miR455-5p, miR381, miR26</td>
</tr>
<tr>
<td>piRNAs</td>
<td>23–31 bp</td>
<td>Clusters</td>
<td>&gt;20,000</td>
<td>Transposon silencing</td>
<td>2′-O-m, U-tail</td>
<td>piR30840, piR-L-163</td>
</tr>
<tr>
<td>snRNAs</td>
<td>150 bp</td>
<td>Different loci</td>
<td>Several</td>
<td>RNA splicing</td>
<td>CAP, TMG, 2′-O-m, Ψ, U-tail, m7C, m6A</td>
<td>U6 spliceosomal RNA</td>
</tr>
<tr>
<td>snoRNAs</td>
<td>60–300 bp</td>
<td>Mostly intronic</td>
<td>&gt;300</td>
<td>RNA modifications</td>
<td>CAP, TMG, 2′-O-m, Ψ</td>
<td>C/D Box and H/ACA Box snoRNAs</td>
</tr>
<tr>
<td>tRNAs</td>
<td>76–90 bp</td>
<td>Widespread</td>
<td>519</td>
<td>Amino acid carriers</td>
<td>m5C, m1A, Ψ, queuosine, mcm5U, editing</td>
<td>AspGUC, GlyGCC, AspGTC, GlyUCC, ValMAC</td>
</tr>
<tr>
<td>rRNAs</td>
<td>120–1874 bp</td>
<td>Clusters</td>
<td>6</td>
<td>mRNA reading/recoding</td>
<td>m5C, m1A, Ψ, editing, 2′-O-m, mΨ, m7G, m5A, ac4C</td>
<td>28S RNA, 12S RNA, 5S RNA</td>
</tr>
<tr>
<td>Vault RNAs</td>
<td>88–98 bp</td>
<td>Chr 5, Chr X</td>
<td>4</td>
<td>Vault particle regulation</td>
<td>m5C</td>
<td>vtRNA1.1, vtRNA1.2, and vtRNA1.3</td>
</tr>
<tr>
<td>IncRNAs*</td>
<td>&gt;200 bp</td>
<td>Widespread</td>
<td>&gt;30,000</td>
<td>Many</td>
<td>CAP, mC, mA, Ψ, splicing, editing, U-tail</td>
<td>HOTAIR, XIST, MALAT1, TERC, RPPH1</td>
</tr>
<tr>
<td>Circular RNAs</td>
<td>&gt;200 bp</td>
<td>Mostly intronic</td>
<td>&gt;15,000</td>
<td>RNA regulation</td>
<td>Splicing, editing</td>
<td>Within UBAC2, SMARCA, SPECC1, HIPK2</td>
</tr>
</tbody>
</table>

B. Role of selected RNA chemical modifications according to the transcript type

<table>
<thead>
<tr>
<th>Modification</th>
<th>Role in coding RNAs</th>
<th>Role in ncRNAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>m5C</td>
<td>Translation efficiency, mRNA structure, genetic recoding</td>
<td>Regulation of vault ncRNA processing into small RNAs, tRNA cleavage</td>
</tr>
<tr>
<td>hm5C</td>
<td>Translation efficiency</td>
<td>Unknown</td>
</tr>
<tr>
<td>m6A</td>
<td>Translation efficiency, mRNA structure, stability, export, and splicing</td>
<td>IncRNA silencing by XIST, tRNA selection</td>
</tr>
<tr>
<td>m1A</td>
<td>Translation efficiency</td>
<td>tRNA T-loop, rRNA regulation</td>
</tr>
<tr>
<td>Ψ</td>
<td>Translation efficiency, mRNA structure, genetic recoding</td>
<td>tRNA, rRNA, snoRNA, IncRNA regulation</td>
</tr>
</tbody>
</table>

*IncRNAs include, among others, lincRNAs, transcribed-ultraconserved regions (T-UCR), antisense RNAs, and pseudogene RNAs.

Abbreviations: CAP, 5′-end cap; m5C, 5-methylcytosine; m6A, N6-methyladenosine; m1A, N1-methyladenosine; Ψ, pseudouridine; 2′-O-m, ribose 2′-O-methylation; 5′-O-m, O-methylation of the 5′ monophosphate; TMG, trimethylguanosine; mcm5U, 5-methoxycarbonylmethyluridine; Ψ, 1-methylpseudouridine; m5G, 7-methylguanosine; m5A, N6-dimethyladenosine; ac4C, N4-acetylcytidine; hm7G, 5-hydroxymethylcytosine.
m^C in many other types of RNA transcripts (3–5). No strict motif for m^C has been described to date (3–5). m^C is common in mRNA 3′ untranslated regions (UTR), whereas Argonaute I–IV binding sites are enriched in the proximity of m^C sites, pointing to a role for m^C in miRNA targeting (3). Modification of IncRNAs by m^C regulates their processing in smaller regulatory ncRNAs (18), the positioning of 2′-O-methylation of snRNAs (19), and the production of tRNA precursors (3–5). The IncRNA XIST is also regulated by m^C where cytosine methylation has been shown to interfere with the binding of the histone modifier PRC2 (20), thus establishing a link between epigenetic modifications in RNAs and histones. Importantly, m^C is not a static mark of RNA. The same enzymes that demethylate DNA are also capable of catalyzing the oxidative demethylation of m^C to hm5C in RNA (21). hm5C is preferentially present in polyadenylated RNAs, where it may favor translation in the case of mRNAs (6). It has been reported as an over-represented motif that tends to be UC-rich and contains UCCUC repeats (6).

RNA can also be modified at adenosines in the form of m^A and m^I. The presence of m^A has also been discovered in DNA (2). m^A is the most abundant internal modification of mRNA (8, 22), but it is also relevant for miRNAs, controlling their maturation and expression levels. In this regard, there is an enrichment of the m^A consensus motif at the junction between the hairpin stem and the flanking single-stranded RNA regions of pri-miRNAs (23). The depletion of m^A diminished DGCR8 binding to pri-miRNAs, thus preventing the formation of the mature miRNAs, as has been shown in the case of the tumor-suppressor miRNA let-7 (24). m^A is also found in IncRNAs, although its functions in this type of transcript are still unclear. In the case of the extremely abundant IncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1; ref. 25), it has been shown that m^A destabilizes the hairpin stem structure (26). m^A is also required for the efficient transcriptional repression mediated by the IncRNA XIST (27). At the global RNA level, transcriptome-wide m^A mapping suggests the motif DRACH (D = A, G, or U; H = A, C, or U) as a consensus motif for m^A (7, 8, 28, 29). The other methylated adenosine present in RNA, m^I, is typically found in the tRNA T-loop. However, recent data demonstrate that m^A is not restricted to these abundant ncRNAs, but rather is also observed around the start codon upstream of the first splice site (9) and in the 5′ UTRs of mRNAs (10), in sharp contrast with the localization of m^A that is enriched in 3′ UTRs and near stop codons (8). The proposed motifs for m^A also differ greatly from those recognized for m^A (9, 10).

Two other RNA nucleotides are also worth mentioning: pseudouridine, the 5′-ribosyluracil isomer of uridine, and queuosine, a rare nucleoside that is structurally similar to guanine. Pseudouridylation is the most abundant modification in ncRNAs (30), not only found in tRNAs, tRNAs, and snoRNAs, but also observed in IncRNAs (11–13), such as the aforementioned XIST and MALAT1. Interestingly, queuosine substitution of guanosine at the wobble position of tRNAs may alter mRNA decoding (31). Overall, m^A is the most prevalent internal modification found in mRNA (15), with more than 20,000 unique sites estimated in IncRNAs (32), whereas m^C is most prevalent in tRNAs and snRNAs, with more than 30,000 unique sites in IncRNAs (32). Around 2,000 unique sites have been proposed for pseudouridine in IncRNAs (32), but, for this and for other RNA modifications, emerging epitranscriptomic data and further posterior validation experiments could provide different numbers.

Finally, RNAs can undergo specific modifications at their 3′-ends. Polyadenylation is the obvious modification that ncRNAs undergo to modify their stability, but uridylation can also occur. The best example is the precursor of let-7 (33–35); however, there is an increasing amount of data regarding uridylation and adenylation events affecting the activity of miRNAs, as recently reviewed (22). It is probable that the development of new high-throughput technologies will provide an improved landscape of the so-called “3′-terminome” of RNA.

Unlike the described RNA modifications, which are reversible and do not interfere with RNA sequence per se, there are more stable changes in RNAs, such as splicing and editing. These are irreversible changes to the RNAs that interfere with the stored information and with their role in cancer, which have been previously reviewed (36, 37). A few exciting recent discoveries in these areas are the cancer-associated alterations of the editing enzymes APOBEC3B (38–40) and ADAR1 (41, 42) and the role of circRNAs in tumorigenesis (43–47); however, they will not be discussed herein.

### CHEMICAL MODIFICATIONS OF ncRNAs IN CANCER AND THEIR MODIFIERS

The above-described scenario of balanced ncRNA modifications allows cellular homeostasis to undergo important alterations in human cancer that we are just now starting to understand. Without having a complete picture of the global epitranscriptome of transformed cells, we have to construct the puzzle from the small pieces that are emerging from each particular RNA modification. At the same time, we can try to go back upstream in the pathway and dissect what went wrong with the genes encoding the proteins responsible for adding, reading, or erasing a specific RNA modification.

For the processes of RNA capping and decapping modifications, such as m^G or m^2-G, and modifiers, such as the RNA cap methyltransferases RNTM, TGS1, CMTR1, CMTR2, and BCDIN3 (16) or the decapping enzymes CDP2 and NUDT16 (48), there is a lack of information in the transformed cell setting. More is known about m^C, particularly at the level of the involved RNA methyltransferases. The DNA methyltransferase 2 (DNMT2) turned out to be an RNA methyltransferase (TRDMT1) with activity first described for the methylation of the cytosine located in position 38 in tRNA^cap (49), a modification that affects its processing into tRNA segments (trF; ref. 50). Interestingly, DNMT2 mutations in cancer cells mostly lead to diminished RNA methyltransferase activity (51). The other type of cytosine-5 RNA methylation is constituted by the aforementioned XIST and MALAT1. Interestingly, queuosine substitution of guanosine at the wobble position of tRNAs may alter mRNA decoding (31). Overall, m^A is the most prevalent internal modification found in mRNA (15), with more than 20,000 unique sites estimated in IncRNAs (32), whereas m^C is most prevalent in tRNAs and snRNAs, with more than 30,000 unique sites in IncRNAs (32). Around 2,000 unique sites have been proposed for pseudouridine in IncRNAs (32), but, for this and for other RNA modifications, emerging epitranscriptomic data and further posterior validation experiments could provide different numbers.

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also in other ncRNAs including lncRNAs and vault-associated RNAs (4, 17). NSUN2 is upregulated in some tumor types by copy-number gains (52), and it is targeted by critical transforming proteins such as c-MYC (53) and Aurora kinase B (54). However, in other classes of malignancies, such as skin cancer, NSUN2 expression is depleted, causing a reduction in protein translation rates and increasing the tumor-initiating population (55). Other proteins in this family include NOP2 (NSUN1), NSUN3, NSUN5, and NSUN7. Interestingly, NSUN5 modulates life span and stress resistance (56), and NSUN3 deficiency, in addition to m^C reduction, results in diminished S-formylcytosine in tRNA (57).

In a manner similar to that of m^C in DNA, TET methylidioxygenases (TET1, TET2, and TET3) catalyze hydroxylation of m^C to hm^C in RNA (6, 20). Because TET1 mutations and deletions are observed in myeloid leukemias (in association with impaired hm^C in DNA; ref. 58), and because TET1 loss accompanies the presence of aberrant fusion proteins found in hematologic malignancies where it can act as a tumor-suppressor gene (59), one also wonders if the loss of TET proteins might have a greater impact in RNA demethylation than in DNA demethylation, a possibility that is yet to be explored.

In addition to its above-described presence in ncRNAs with a role in cancer, such as let-7 and MALAT1, m^A has been described to act in cell reprogramming by affecting mRNAs associated with different degrees of pluripotency, while at the same time miRNAs control m^A in these mRNAs by sequence pairing (60). Thus, m^A affects ncRNAs in multiple ways, and their related protein machinery could also potentially be involved in cellular transformation. The methylation of m^A is performed by methyltransferase-like 3 (METTL3), methyltransferase-like 14 (METTL14), and the cofactor Wilms’ tumor 1-associating protein (WTAP). For the latter, the connection with cancer is clear; it acts as a splicing binding partner for the Wilms’ tumor 1 (WT1) protein (61) and regulates cell-cycle progression (62). From a functional standpoint, WTAP and METTL3, upon their recruitment mediated by the RBM15 and RBM15B proteins, promote the methylation of XIST and its transcriptional repression activity (27).

Although readers of m^C or hm^C in RNA have not yet been identified, these proteins have been characterized for m^A: the YTS21-B homology (YTHD) domain-containing proteins that, in humans, are composed of the YTHDF family (YTHDF1, YTHDF2, YTHDF3; refs. 7, 63), YTHDC1, and YTHDC2. The YTHDC1 protein binds m^A residues of XIST and is required for the transcriptional silencing mediated by this ncRNA (27). Interestingly, the Drosophila nuclear protein YTS21-B also binds m^A residues in the sex determination factor Sex-lethal (Sxl; ref. 64) and contributes to sex determination (64, 65). The role of YTS21-B in the potentiation of Sxl alternative pre-mRNA splicing (64) has correspondence in human cells where the YTHDC1 protein has been described as a global regulator of mRNA splicing by its binding to m^A RNA residues (66). Interestingly, even if these proteins exert similar functions among different species, m^A modifications may nevertheless show different patterns, as has been recently shown in distinct primates (67). These variations could provide another explanation for the phenotypic differences between closely related species. Additional m^A RNA binders are the RNA-binding proteins HuR and HNRNP2B1, which modulate alternative splicing and enhance miRNA production (23, 24).

The m^A modification is not a final fixture of the RNA molecule, but it can be oxidatively reversed by two members of the ALKB family of Fe(II) and α-ketoglutarate–dependent dioxygenases, FTO (68) and ALKBH5 (69). FTO germline polymorphisms have been associated with melanoma risk (70), and loss-of-function mutations of FTO in a monogenic disorder impair impair proliferation and promote senescence (71). Conversely, FTO promotes leukemogenesis by reducing m^A levels of mRNA transcripts involved in cell differentiation (72). FTO was also found to be overexpressed in human breast cancer samples. Among the different breast cancer subtypes, the aggressive HER2-positive subtype is the one in which FTO is primarily overexpressed, suggesting a critical role for FTO in carcinogenesis and aggressiveness of breast cancer (73). Interestingly, FTO overexpression triggers an aberrant metabolic state that allows the breast cancer cell line SUM149 to survive glutamine deprivation stress (74). FTO-overexpressing cells are resistant to chemotherapeutic drugs and show higher metastatic potential. Further, a link to cancer is also found for ALKBH5, where Alkbh5 knockout mice undergo aberrant expression of the p53 functional interaction network (69), and ALKBH5 overexpression stabilizes NANOG levels by decreasing m^A and increasing the number of breast cancer stem cells (75).

For m^A in RNA, neither the corresponding methyltransferases nor the readers for this modification have been characterized, but RNA can be demethylated at this position by another member of the ALKB family of Fe(II) and α-ketoglutarate–dependent dioxygenases, ALKBH3 (10). It is worth pointing out that ALKBH3 has been recognized as a DNA-repair enzyme guarding the genome against alkylation damage (76), but the identified function of ALKBH3 as an RNA demethylase for m^A could open new lines of research in the area of chemotherapy-response prediction. In this regard, ALKBH3 repair function is dependent on the DNA helicase ASCC3 (77), and other helicase-binding partners have been associated with chemosensitivity profiles for DNA-damaging agents (78); thus, a link might exist between the m^A mark, the described helicase activities of its binding partners, and the observed chemosensitivity signatures, which warrants further research.

Regarding the other modified nucleotides that are less frequently investigated, pseudouridine formation in RNA involves the pseudouridine synthase (PUS) family, where most of the pseudouridine sites correspond to sequence motifs associated with these enzymes (11, 12), and dyskerin (DKC1; ref. 30). DKC1, which modifies rRNA, is mutated in X-linked dyskeratosis congenita, where an increased susceptibility to cancer exists and an impairment in rRNA pseudouridylation is observed before the onset of the disease in hypomorphic Dkc1-mutant mice (79). A specific defect of the internal ribosome entry site also occurs upon DKC1 loss, affecting the translation of tumor-suppressor proteins (80). DKC1 mutations also affect the stability of H/ACA small RNAs and are associated with impaired hematopoietic stem
Neither the readers nor the erasers of pseudouridine have been described to date. Although still poorly characterized, modified nucleoside and queuosine provide a new perspective on RNA modifications in cancer. In this case, although queuosine is incorporated into eukaryotic tRNAs by the tRNA-guanine transglycosylase, only bacteria can synthesize its precursor, queuine, de novo (82). Therefore, human cells must rely on nutrition and our gut bacteria to obtain the necessary queuine to substitute guanosine at the wobble position of tRNAs. Thus, two factors of increasing interest in the cancer arena, diet and the microbiome, could be linked by an RNA modification.

Last but not least, alterations in the chemical modification at the 3′-end of ncRNAs and abnormalities in the associated genes can also contribute to the natural history of cancer. For example, the precursors of the tumor-suppressor miRNA let-7 are monouridylated by the enzymes ZCCHC11 (TUT4), ZCCHC6 (TUT7), and PAPD4 (TUT2) to facilitate Dicer processing and miRNA maturation (35). Tumoral cells can express the oncogenic protein LIN28 that induces a long U-tail in pre–let-7 oligouridylation, which prevents the formation of the mature molecule (33, 35, 83). Interestingly, the poly(U) motifs are enriched close to poly(A) sites, both are regulated by heterogeneous ribonucleoprotein C (HNRNPC) and ELAV-like RBP1 (ELAV1; ref. 84), and alternative polyadenylation signals can clearly affect competing endogenous RNA networks. The degradation of the poly(U) tail is also tightly regulated by exoribonucleases from the RNase II/RNB family that is represented by the enzymes DIS3, DIS3L1, and DIS3L2 in humans. Therein, the link to cancer can also be invoked because DIS3L2 mutations are associated with the development of Wilms’ tumors (85). Coincidentally or not, the m6A RNA methyltransferase WTAP cooperates with WT1 protein to regulate proliferation (61, 62), suggesting that this tumor type (which is also characterized by the aberrant imprinting of ncRNAs) could be particularly associated with a disrupted pattern of RNA chemical modifications.

Table 2. Selected RNA modifiers involved in cancer

<table>
<thead>
<tr>
<th>Gene</th>
<th>Activity</th>
<th>Link to cancer (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNMT2</td>
<td>m5C RNA methyltransferase</td>
<td>Diminished activity associated with candidate mutations (51)</td>
</tr>
<tr>
<td>NSUN2</td>
<td>m5C RNA methyltransferase</td>
<td>Copy-number gain (52), oncogene targeting (53, 54), depletion increases tumor-initiating cells (5)</td>
</tr>
<tr>
<td>TET1</td>
<td>DNA and RNA m5C demethylase</td>
<td>Mutations and deletions in leukemia (58), tumor-suppressor role (59)</td>
</tr>
<tr>
<td>WTAP</td>
<td>Cofactor for m5A RNA methyltransferase</td>
<td>Binding partner of the tumor-suppressor WT1 (61), regulates cell-cycle progression (62)</td>
</tr>
<tr>
<td>FTO</td>
<td>m5A RNA demethylase</td>
<td>Variants associated with melanoma risk (70), promotes leukemia (72) and breast cancer (73, 74)</td>
</tr>
<tr>
<td>ALKBH5</td>
<td>m5A RNA demethylase</td>
<td>Knockout affects the p53 network (69), overexpression increases breast cancer stem cells (75)</td>
</tr>
<tr>
<td>ALKBH3</td>
<td>m1A RNA demethylase</td>
<td>Protection against alkylation damage (76)</td>
</tr>
<tr>
<td>DKC1</td>
<td>PUS</td>
<td>Mutated in dyskeratosis congenita (30, 79), regulates translation of tumor-suppressor proteins (80)</td>
</tr>
<tr>
<td>DIS3L2</td>
<td>Exoribonuclease for the poly (U) tail</td>
<td>Mutated in association with the development of Wilms’ tumors (85)</td>
</tr>
</tbody>
</table>

PERSPECTIVES AND CHALLENGES

The study of the cancer epitranscriptome is in its early stages, even more so if we devote our attention only to the chemical modifications of ncRNAs in transformed cells. However, knowledge in this area has recently made a quantum leap, as we have described above. Not only do we now have more complete deep-sequencing coverage of several of the described RNA marks, such as m5C, hm5C, m6A, m1A, and pseudouridine, but we also have recognized the involvement in tumors of some of the genes involved in these epitranscriptomic pathways, such as the m6A RNA methyltransferase WTAP cooperates with WT1 protein to regulate proliferation (61, 62), suggesting that this tumor type (which is also characterized by the aberrant imprinting of ncRNAs) could be particularly associated with a disrupted pattern of RNA chemical modifications. Table 2 summarizes the most relevant RNA modifiers involved in cancer overall.
paired tumoral samples, how these RNA modifications could shift in carcinogenesis from early benign lesions to metastases, and how heterogeneous these marks are within a given tumor. In a few words, future work must address which of these events are critical in tumorigenesis, and it must follow the genetic and DNA epigenetic studies that have tackled these key issues of cancer biology in the last 25 years.

Many advances in science depend on the existence of the proper methodology to assess the desired parameters. For ncRNA modifications, the development of the above-mentioned epitranscriptomic techniques that mix bisulfite modification-based protocols or immunoprecipitation against the different marks with deep sequencing has been a major achievement. However, some of these approaches do not provide single-nucleotide resolution, or they often depend on the batch specificity of the antibodies employed for the analysis. At the same time, most of these methods are unable to discriminate among the simultaneous presence of different RNA modifications in the same molecule, thus losing part of the richness and complexity of the epitranscriptome. For example, the same IncRNA can undergo m^5C, m^5A, and pseudouridylation, but do all these modifications take place at the same time? Do they cooperate or antagonize each other? New tools to address these questions are needed. Among these, it is worth highlighting the advances in combination mass spectrometry (86), isoform-characterization sequencing (87), and the use of single-molecule real-time sequencing (88), a third-generation sequencing technique.

Interestingly, part of the information necessary to advance the field may already exist but may be overlooked. Careful data mining of the many RNA-sequenced and fully sequenced genomes and epigenomes publicly available can provide further clues about possible aberrant RNA modification patterns and their possible causes and consequences. For example, a simple review of the data available at The Cancer Genome Atlas has shown that underexpression of RNA demethylases and overexpression of mRNA methyltransferases occur in human cancers and the use of the cBioPortal suggests the presence of additional mutations in some of these enzymes (89). This list that could grow with the use of other platforms such as the Catalogue of Somatic Mutations in Cancer genome browser, which has been recently updated with new genomic and epigenomic data from 1,001 cell lines (90). Interestingly, for some of these cell lines, such as HeLa or MDA-MB-231, epitranscriptomic data are also available, and different data-sets can be cross-examined. Unfortunately, many of these databases were produced from poly(A) fractions, and thus chemical modifications of many ncRNAs cannot be analyzed.

New experimental data, however, will be also fundamental in moving forward. Among the more than 100 differently modified RNA nucleotides (1), it is not well known how many are present in human cells. For other RNA modifications that have been identified, the information is scarce, but exciting. Interesting cases include the O-methylation of the 5’ monophosphate of pre-miRNAs mediated by BCDIN3D that negatively regulates miRNA maturation (91); the 5-methoxycarbonylmethyluridine (mcm^5U) modification of tRNA regulated by the cancer-related enzymes ALKBH8 and hTRM9L (92); the existence of further modifications of an already-modified nucleotide, such as the methylation of pseudouridine in RNA by EMG1 (93); and the presence of intermediate RNA demethylation nucleotides such as formylcytosine, carboxylcytosine, N^6-hydroxymethyladenosine, and N^6-formyladenosine. It is also relevant to further define the spectrum of modified RNAs: Beyond tRNAs, rRNAs, and mRNAs, how many and which miRNAs and IncRNAs are modified? This is a key issue because there is an increasing number of IncRNAs, such as lincRNAs (94), transcribed-ultraconserved regions (95, 96), and pseudogenes (97, 98), with key roles in tumorigenesis that could be regulated by RNA modifications, changing their biogenesis, stability, and intracellular localization, or shifting their interacting RNA targets. Genetic and functional studies, using CRISPR and similar tools, will also be necessary to identify the missing machineries involved in RNA modification, such as the enzymes responsible for circRNA formation, the readers for m^5C, or the methyltransferases for m^5A. In addition, in vivo studies crossing the available genetically modified mice for the writers, readers, and erasers of RNA modifications with well-established knockout and knock-in mouse models of recognized tumor-suppressor genes and oncogenes will provide another read-out of the contribution of this epigenetic layer to cellular transformation. These experiments are absolutely necessary to distinguish those RNA modifiers that could truly be drivers in carcinogenesis from those events that could be just passengers for tumorigenesis.

Finally, a better definition of the RNA modifications present in human cells, the existence of differences in comparison with cancer tissues, and the characterization of the proteins responsible for the epitranscriptomic patterns will facilitate the translational use of RNA epigenetics. We can imagine these different scenarios as diagnostic and predictive biomarkers or even as potential targets for innovative therapeutic strategies. In the two first cases, the use of RNA changes in miRNAs and circRNAs, very stable RNA molecules, for the detection of cancer cells in serum or biological fluids as well as the determination of the epitranscriptome in RNA molecules involved in DNA repair and, thus, in the assessment of chemotherapy sensitivity, is a possibility worth exploring. For treatment, we can start designing synthetic-lethal strategies for the recognized defects in the RNA modification machinery, or approaches aimed at inhibiting or activating specific RNA-modifying enzymes. Related to this last point, the elucidation of the crystal structures for some of the proteins involved in RNA modifications, such as m^5A modifiers and readers (99–101), represents an important step forward. Interestingly, the drug 5-azaacetyldine, used in leukemia treatments (102), inhibits both DNA and m^5C RNA methyltransferases by competing with cytosine, leading to RNA hypomethylation (103, 104); thus, it is worth exploring how much of the growth-inhibitory effects of DNA-demethylating agents relate to a change in m^5C RNA levels. On the other hand, we can improve the effects of anticancer RNA medicines by adding appropriate chemical modifications to the RNA drug backbone. Exciting times are coming; stay tuned.

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