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CONCISE REVIEW

STEM CELLS®

The epitranscriptome landscape of small noncoding RNAs in stem cells

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Abstract

Stem cells (SCs) are unique cells that have an inherent ability to self-renew or differentiate. Both fate decisions are strongly regulated at the molecular level via intricate signaling pathways. The regulation of signaling networks promoting self-renewal or differentiation was thought to be largely governed by the action of transcription factors. However, small noncoding RNAs (ncRNAs), such as vault RNAs, and their post-transcriptional modifications (the epitranscriptome) have emerged as additional regulatory layers with essential roles in SC fate decisions. RNA post-transcriptional modifications often modulate RNA stability, splicing, processing, recognition, and translation. Furthermore, modifications on small ncRNAs allow for dual regulation of RNA activity, at both the level of biogenesis and RNA-mediated actions. RNA post-transcriptional modifications act through structural alterations and specialized RNA-binding proteins (RBPs) called writers, readers, and erasers. It is through SC-context RBPs that the epitranscriptome coordinates specific functional roles. Small ncRNA post-transcriptional modifications are today exploited by different mechanisms to facilitate SC translational studies. One mechanism readily being studied is identifying how SC-specific RBPs of small ncRNAs regulate fate decisions. Another common practice of using the epitranscriptome for regenerative applications is using naturally occurring post-transcriptional modifications on synthetic RNA to generate induced pluripotent SCs. Here, we review exciting insights into how small ncRNA post-transcriptional modifications control SC fate decisions in development and disease. We hope, by illustrating how essential the epitranscriptome and their associated proteome are in SCs, they would be considered as novel tools to propagate SCs for regenerative medicine.

KEYWORDS

adult stem cells, cancer stem cells, epigenetics, epitranscriptome, microRNAs, noncoding RNAs

1 | INTRODUCTION

The unique properties of stem cells (SCs), such as self-renewal, differentiation, migration, and homing, make them well suited for regenerative medicine.[1] Stem cell therapies have shown considerable promise in the treatment of diverse medical conditions, including regrowth of cartilage for osteoarthritis, pancreatic β cell regeneration for diabetes, and neural stem cell transplant for spinal cord injuries.[2-4] While many stem cell-based regenerative therapies are moving toward clinical practice, there are still challenges to be addressed before they can be effectively and safely applied *en masse*.[5] Current challenges for stem cell therapies include acquisition of stem cells with sufficient plasticity, precise (yet comprehensive) cell reprogramming, storing and maintaining populations with the required stemness, pretreatment, therapeutic delivery mechanisms, and control over cell growth and migration (tumorigenesis) post-treatment.[6] Considerable efforts are ongoing to comprehend fundamental mechanisms that govern stem cell biology in order to address the aforementioned hurdles. Specifically, the emergence of systems-level (OMICs) approaches has been essential in uncovering gene expression profiles and epitranscriptome signatures in stem cells.[7-9] This work has revealed the emerging importance of an expanding set of small noncoding RNAs (ncRNAs) in regulating cell biology. Moreover, these RNAs have been found to be extensively decorated with chemical modifications that comprise a complex regulatory layer on their functionality.[10-15] As a fundamental governor of cell behavior, elucidating the epitranscriptome (the cell-wide collection of post-transcriptional chemical modifications in the RNA pool) would represent a significant step forward in addressing challenges faced by stem cell therapies.

Transcriptome and proteome-wide studies have shown that, while the majority of genomic loci are actively transcribed, only a small fraction (~1.5%-2%) is faithfully translated into protein.[16,17] The revelation that small ncRNAs, and their modifications, should have such importance in regulating stem cell biology is, therefore, not altogether surprising. This untranslated fraction, the ncRNA, carry out a broad range of key roles and are categorized into, *housekeeping ncRNAs*, which include transfer RNAs (tRNAs) and ribosomal RNAs, and *regulatory ncRNAs*, long and small, ncRNAs, the latter of which tend to exhibit more dynamic expression patterns across temporal and spatial cellular profiles. Small ncRNAs, in this review, include all untranslated transcripts with a length of <200 nt.[18] These ncRNAs include a surprisingly diverse range of species with distinct processing pathways, secondary structures, localizations, and molecular functions.[10,15,19] RNA post-transcriptional modifications are analogous to the well-developed model of epigenetic alterations observed in DNA. In contrast to DNA, there is an ever-growing array of chemical modifications that RNA can undergo (with 163, at the time of writing).[20] Recently, the development of next-generation sequencing (NGS) techniques, tailored to RNA modification detection, has revealed part of the epitranscriptome landscape of small ncRNAs. In doing so, both housekeeping ncRNAs and regulatory ncRNAs, such as micro RNAs (miRNAs), small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), PIWI-interacting RNAs (piRNAs), and vault-RNAs (VT-RNAs), have been found heavily modified as shown in Figure 1.[11,12,19,20]

Significance statement

The epitranscriptome has recently emerged as a major regulator of stem cell biology. It comprises the epigenetic signatures decorating both coding and noncoding RNAs (ncRNAs). The latter is further divided into large and small RNAs. Distinct small ncRNAs, such as transfer RNAs and small vault RNAs, depend on RNA modifications to govern skin stem cell differentiation. Molecularly, the epitranscriptome can control the translation of global programs or specific mRNAs. Post-transcriptional modifications coordinate stem cell decisions by orchestrating RNA processing, RNA structure, and reader-eraser protein binding. The epitranscriptome field still remains in its infancy with many RNA modifications roles unknown. This study foresees the epitranscriptome to play key roles in regenerative medicine and RNA-based therapeutics in the near future.

RNA modifications involve post-transcriptional base additions and alterations of nitrogenous bases, ribose moieties, or terminal phosphates (Figure 2). RNA modifications can span the entire RNA transcript with positional precision being modulated by structural or sequence elements.[21-24] RNA-binding proteins (RBPs) involved in RNA modifications are generally referred to as *writers*, *erasers*, and *readers*.[12,25,26] Writers are enzymes that directly deposit modifications on RNA. Whereas erasers are enzymes that mediate modification removal from targeted RNAs. Readers, on the other hand, are RBPs that recognize and bind to RNA-modifications, to elicit a biological response. Accordingly, in some described cases, it is through coordinated actions of writers, readers, and erasers that RNA modifications influence molecular and cellular decisions.

For several RNA modifications, however, the corresponding writers, readers, and erasers remain unknown making basic research on the epitranscriptome essential. The functional roles of small ncRNA modifications involve DNA replication (3′ modifications in Y-RNAs), RNA processing (m5C in VT-RNAs), transcription (3′ 2′-O-methylation [Nm] in piRNAs), RNA binding interactions (m6A in snoRNAs), splicing and RNA processing (m6A in snRNA), and translation (m5C in tRNAs). In stem cells, small ncRNA modifications are increasingly being recognized for their roles in governing self-renewal, pluripotency, lineage commitment, and differentiation. The regulation of miRNA biogenesis comprises several good examples of how the epitranscriptomic landmarks control gene expression.[27,28] miRNAs participate in a variety of signaling pathways, including those that govern cell cycle, self-renewal, proliferation, apoptosis, commitment, and differentiation and are therefore central players in coordinating stem cell behaviors.[29-31]

In this report, we review findings from recent studies connecting the epitranscriptome landscape of small ncRNAs with stem cell governance in development, homeostasis, and disease. Descriptions are

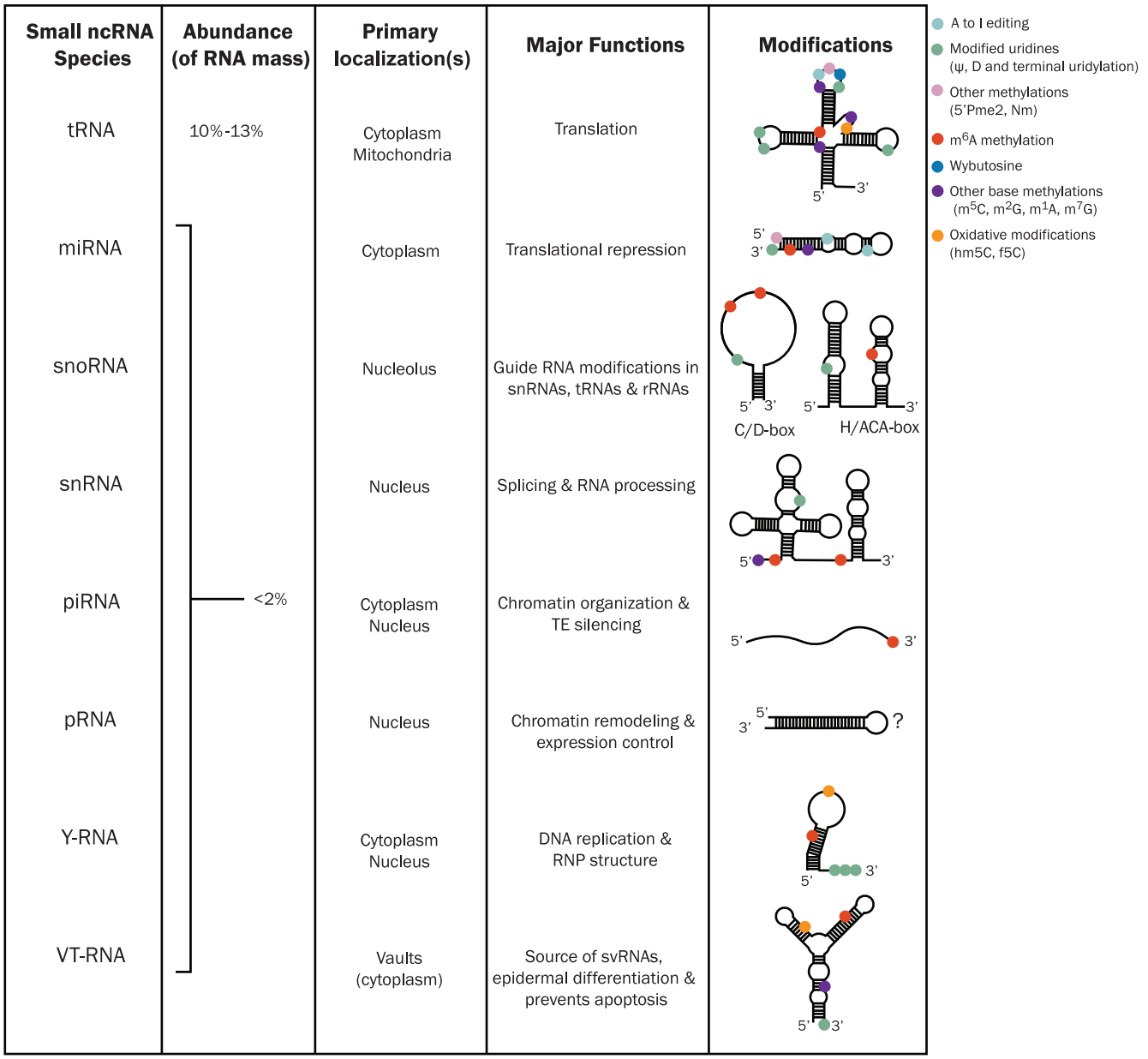

FIGURE 1 Novel features of the small ncRNA transcriptomes. Here, we show major small ncRNA species alongside their typical abundance in cells, primary localization, major functions, and known epitranscriptome modifications. The positions of major modifications are denoted (colored circles) on representative secondary structures (colored by modification type, according to the side legend). miRNA, micro RNA; ncRNA, noncoding RNA; piRNA, PIWI-interacting RNA; pRNA, promoter-associated RNA; snRNA, small nuclear RNA; snoRNA, small nucleolar RNA; tRNA, transfer RNA; VT-RNA, vault RNA. A to I editing indicates adenosine to inosine editing. ψ, pseudouridylation; D, dihydrouridine; terminal uridylation, addition of one or more uridyl nucleotides at the RNA terminal; 5′Pme2, 5′ terminal phosphate methylation, Nm: 2′-O ribose methylation; m6A, 6-methyladenosine; m5C, 5-methylcytosine; m2G, 2-methylguanidine; m1A, 1-methyladenosine; m7G, 7-methylguanidine; hm5C, 5-hydroxymethylcytosine and 5-formylcytosine

given how the epitranscriptomic signatures influence small ncRNA transcripts, their interactions with key readers, writers and erasers, and, ultimately, their roles in actively regulating stem cells, from which an emerging view of epitranscriptome-RBP landscape is presented. Novel insights into pathways governing stem cells and unresolved topics are highlighted in an effort to be exploited for stem cell research and regenerative applications.

2 | THE EPITRANSCRIPTOME IN STEM CELLS

2.1 | Embryonic stem cells

Pluripotency is a plastic state of ESCs that requires multilayer regulation checkpoints. Besides master regulator genes, such as octamer-

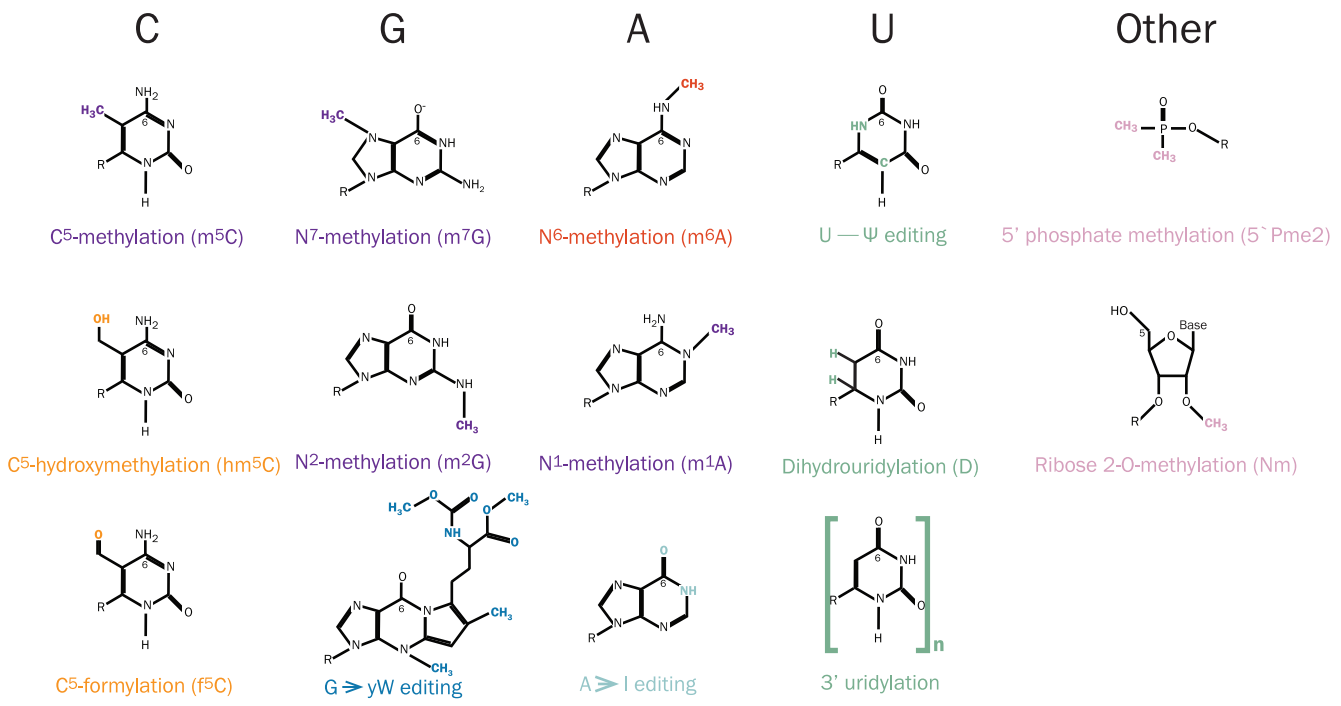

FIGURE 2 Major RNA post-transcriptional modifications of eukaryotic small ncRNAs. The structures of RNA bases, 5′ phosphate, and ribose moieties with major chemical modifications are highlighted. Where relevant, these color conventions are continued throughout subsequent figures. ncRNA, noncoding RNA

binding transcription factor4 (OCT4), the epitranscriptome also plays crucial roles in maintaining the plasticity required for ESCs to self-renew or differentiate into all three germ layers (ectoderm, mesoderm, and endoderm). Indeed, in mouse ESCs, both self-renewal and neuronal differentiation require 7-methylguanosine (m7G) methylated tRNAs.[23] Only 22 species of tRNAs are m7G modified at the anticodon loop in cultured mouse ESCs. Knockdown of methyltransferase-like1 (METTL1), which is part of m7G writer complex along with tRNA 7-guanine methyltransferase (WDR4), increases ribosomal occupancy at codons corresponding to m7G modified tRNAs.[23] Molecularly, lack of m7G disrupts the translation of genes controlling cell cycle and brain development.[23] In contrast, human ESCs negatively control their proteome by highly expressing pseudouridine (Ψ) writer PUS7, which also modifies tRNAs.[32] PUS7-modified tRNAs are further processed into smaller tRNA fragments (tRFs). Small tRFs often target the translational machinery at the initiation complex to reduce protein translation.[32] Pseudouridylation in ESCs is essential for proper germ layer organization, as well as hemopoietic lineage commitment, by serving as a balance between growth and differentiation.[32]

The production of tRFs into 5′ and 3′ fragments is further controlled by distinct 5-methylcytosine (m5C) modifications deposited by DNA (cytosine-5-)-methyltransferase2 (DNMT2) and NOP2/Sun RNA Methyltransferase2 (NSUN2) shown in Figure 3.[33] Both enzymes mainly modify tRNAs at the anticodon loop with only NSUN2 having a wider range of targets including mRNAs. During stress conditions, m5C-modified tRNAs inhibit tRF production by repelling angiogenin (ANG), a tRNA endonuclease, binding thereby allowing specific protein translation response programs. In vivo, NSUN2 is detected in the inner cell mass of mouse embryos with no evident roles in germ-layer commitment. Additionally, in vitro, NSUN2 has not yet been found crucial for ESCs, although DNMT2 seems to regulate embryonic differentiation into cardiac linages via perturbing the transcriptional activity of the noncoding RNA 7SK.[34] Interestingly, m5C in mouse ESCs cluster near translation starting sites and within coding regions of both total and nuclear mRNAs.[35] Although the exact function of m5C in ESCs remains unknown, we speculate it coordinates between stress responses and specific translational programs.

Pluripotency exit in ESCs is controlled by fine-tuning Let-7 miRNA processing and oligo-uridylation mediated degradation. During ECS self-renewal, Dicer-dependent processing of Let-7 precursor (pre) miRNAs into mature miRNAs is blocked by LIN28 RNA-binding proteins.[36] LIN28 interact with Let-7 pre-miRNAs and recruit terminal uridyl-transferases such as Terminal Uridylyl Transferase (TUT)4/7 to facilitate terminal uridylation.[21,37] Polyuridylated let-7 pre-miRNAs are recognized and degraded by exonuclease DIS3-Like 3′-5′ exoribonuclease2 (DIS3L2).[36] During pluripotency exit, LIN28 proteins are actively repressed allowing Let-7 pre-miRNA maturation. Molecularly, mature Let-7 miRNAs silence mRNAs promoting pluripotency such as EGR1 and LIN-41/TRIM71, thereby initiating major differentiation decisions.[36,38] Pluripotency exit in ESCs is also regulated by 5′ phosphate-methylation (5′Pme). 5′Pme modifications have been found in miR-145 and miR-21 which, in ESCs, promote differentiation toward vascular cells and inhibit self-renewal, respectively. Both miR-145 and miR-21 regulate each other and act upon the K-Ras, SOX2, Nanog, OCT4, and TGF-β pathways, wherein they coordinate differentiation decisions as illustrated in Figure 4B.[39-41]

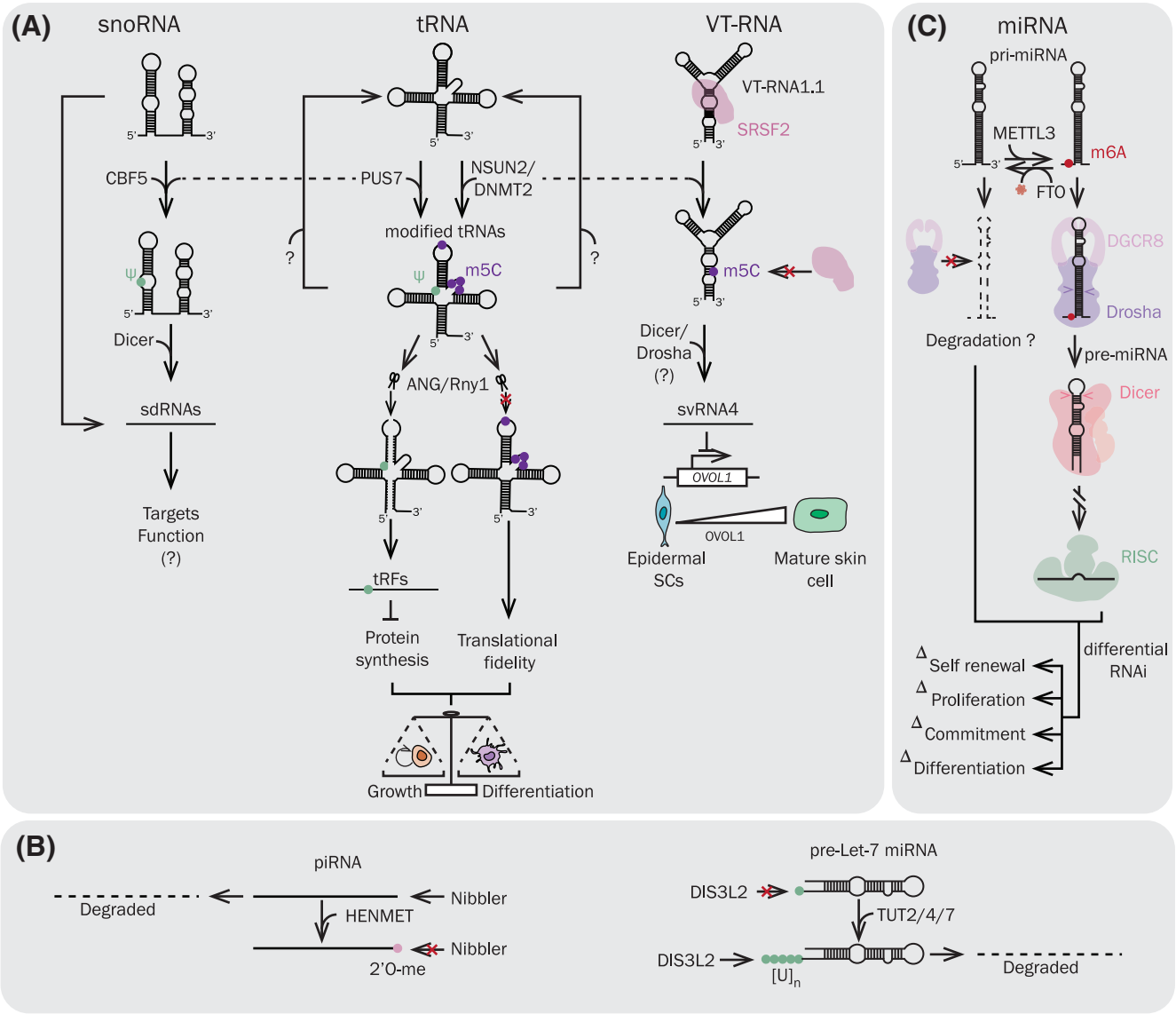

FIGURE 3 How the epitranscriptome of small ncRNAs controls RNAi and translation. A, The occurrence and position of RNA modifications placed by writers such as the pseudouridine synthases CBF5 and PUS7 or methyltransferases NSUN2 and DNMT2 in several small ncRNAs (such as snoRNA, tRNA, and VT-RNA) dictate endonuclease (eg, angiogenin [ANG], Ro-associated 1 [Rny1], Dicer, or Drosha) processing. In this way, small ncRNA modifications modulate the production of regulatory RNA fragments (eg, snoRNA-derived sdRNAs, tRNA-derived tRFs and VT-RNA-derived svRNAs), which coordinate stem cell decisions by silencing mRNAs or altering the translational machinery (as observed for certain tRFs).[106] B, In piRNAs, 3′ terminal ribose methylation by HEN1 orthologues (HENMET in humans) protect against 3′-5′ endonuclease activity (Nibbler in *Drosophila*, possibly PARN in humans[107]). Conversely, terminal uridylation of Let-7 miRNAs by TUTases are carefully coordinated as oligouridylation labels pre-Let-7 miRNAs for degradation by the endonuclease DIS3L2. C, The METTL3-mediated deposition of m6A at the 5′ end of pri-miRNAs promotes engagement with Drosha, thereby facilitating pre-miRNA synthesis. Conversely, the m6A eraser, FTO (a dioxygenase) removes m6A from pri-miRNAs, thereby reducing Drosha-DGCR8 recognition. Such differential processing alters cell's miRNA pool hence adjusting RNAi and, ultimately, stem cell decisions through suppression of transcripts involved in self-renewal, proliferation, commitment, and differentiation. The delta symbol (Δ) has been used to represent "a difference in." Annotations for proteins and modifications are colored according to their associated graphic. ? indicates currently unknowns. iPSC, induced pluripotent stem cell; NSC, neuronal stem cell; ncRNA, noncoding RNA; piRNA, PIWI-interacting RNAs; RNAi, RNA interference; SC, stem cell; snoRNA, small nucleolar RNAs; tRNA, transfer RNA; VT-RNA, vault RNA

Both miR-145 and miR-21 are thought to be targets of the 5′Pme writer BCDIN3 domain containing RNA methyltransferase (BCDIN3D), which negatively regulate the activity of miRNAs by preventing their Dicer-dependent maturation.[42]

Normal ESC differentiation is also determined by 6-methyladenosine (m6A) modifications.[43,44] m6A marks are deposited by a large writer complex composed of METTL3, METTL14, and Wilm's tumor 1 associated protein (WTAP).[45] METTL3 depletion in human and mouse ESCs

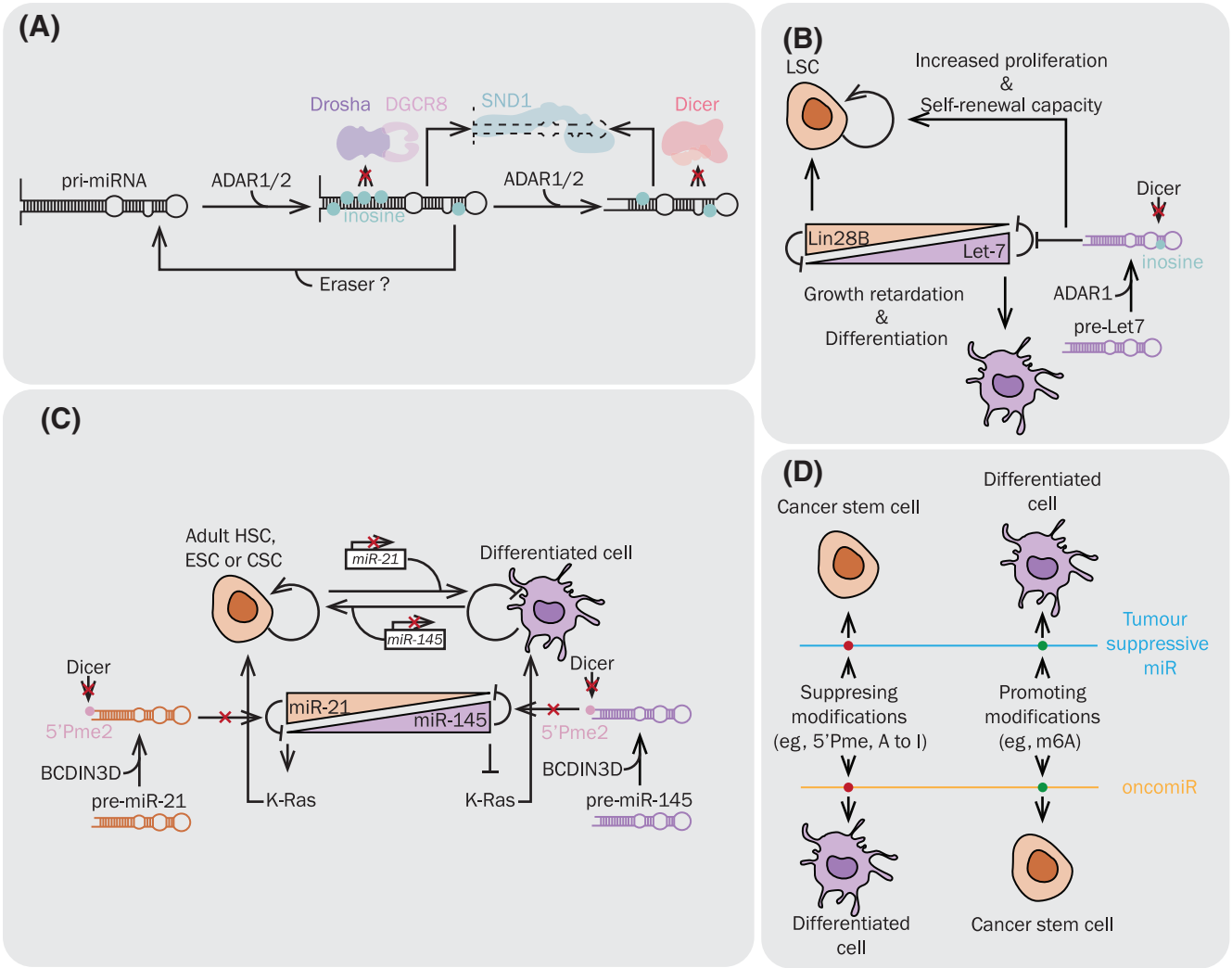

FIGURE 4 The epitranscriptome signatures of stem cell miRNAs in homeostasis and disease. A, Modifications edited by ADAR1 or ADAR2 (adenosine deaminases, which convert adenosine to inosine) on pri-miRNAs and pre-miRNAs provide additional layers of control over miRNA biogenesis. A to I editing of pri-miRNA and pre-miRNAs flag them for degradation by endonuclease SND1, as has been observed for Let-7 miRNAs. B, The activity of Let-7 miRNAs is suppressed by Lin28B (a Let-7 miRNA-binding protein). In normal HSC development, Let-7 miRNA expression is elevated in line with Lin28B reduction. However, in some cancer low levels of Let-7 caused by ADAR-editing coincides with high levels of Lin28B (a dysregulation seen in LSCs), which promotes the continuance (or establishment) of proliferation and self-renewal. C, miR-21 and miR-145 comprise an antagonistic regulatory axis (wherein the expression of each miR ultimately results in the suppression of the other). As certain stem cells (eg, HSCs and ESCs) undergo differentiation, miR-21 levels are decrease in favor of increasing the expression of miR-145 and, in doing so, lose their self-renewal capacity (indicated by circular arrows). Both miR-21 and miR-145 coordinate self-renewal decisions by modulating K-Ras activities (K-Ras activation is promoted by miR-21, whereas inhibited by miR-145). The BCDIN3D-dependent installation of 5′ phosphate methyl groups on pre-miR-21 and pre-miR-145 prevents their Dicer-dependent maturation. By preventing miR-21 or miR-145 maturation, BCDIN3D also blocks subsequent suppression of their antagonistic counterparts (which for miR-21, is miR-145 and vice versa), hence forming an epitranscriptome regulatory circuit governing differentiation, the dysregulation of which appears to be linked to the regression of normal cells into CSC like cells with self-renewal capacity. D, Concept of contextual effects of miRNA modifications in tumorigenesis. Here, we demonstrate how identical modifications in different miRNAs (marked as red circles for modifications suppressing miRNA activities [eg, tagging the miRNA for degradation] or green circles for modifications enhancing miRNA activities [eg, RNA-stabilizing modifications]) can give rise to distinct fate decisions (ie, whether it is an oncomiR or tumor suppressive miR). Arrows marked with a Red "X" indicate a lost interaction as a result of the epitranscriptome. Annotations for proteins and modifications are colored according to their associated graphic. CSC, cancer stem cell; HSC, hematopoietic stem cell; LSC, leukemic stem cell; miRNAs, micro RNAs

perturbs differentiation by stabilizing pluripotent maintaining genes such as Nanog.[43] Although m6A's role in ESC pluripotency is linked to mRNA metabolism, recent findings identifying METTL16 as a novel m6A writer of small ncRNAs could connect m6A modified ncRNAs with ESC differentiation. Indeed, both ncRNA targets of METTL16 7SK and 7SL levels increase during ESC differentiation, thus suggesting a functional role of m6A modified ncRNAs in pluripotency.[46] METTL16 contains a unique N-terminal domain that enables methylation within predicted

small ncRNA loops such as Y-RNAs and VT-RNAs.[43,47] More research is needed to uncover the functional role of METTL16 in ESCs.

2.2 | Germline stem cells

Germline stem cells (GSCs) are unipotent cells that only give rise to haploid gametes. RBPs, which are expressed in GSCs, with various functions including small-RNA-mediated transposable element (TE) repression, ensures their restricted plasticity. PIWI (P-element-induced Wimpy testis), Tudor, and Argonaute proteins are all involved in the PIWI-interacting (pi) RNA pathway suppressing TEs in GSCs.[48,49] piRNAs are small interfering ncRNAs thought to be expressed shortly after fertilization where, for certain cells, their role has been supplanted by tRFs.[50,51] piRNAs are involved in numerous SC behaviors, including GSCs asymmetrical division, germ layer organization, survival, and differentiation.[52,53] Approximately 20% of piRNAs in mammalian cells map to TEs or repeat regions.[54] The majority of piRNAs, therefore, work on heterosilencing RNA targets, similar to miRNAs. Yet, while piRNAs inhibit RNA by PIWI proteins, miRNAs inhibit RNA by recruiting Argonaute proteins. Furthermore, classic miRNAs depend on Dicer for biogenesis; however, piRNAs do not depend on Dicer.[55] Most piRNAs are stabilized by 3′ end Nm modifications deposited by (HEN1) orthologs, HEN-methyltransferase 1 (HENMT1) in humans.[56,57] Nm-modified piRNAs resist degradation by inhibiting exonucleases such as Nibbler as we summarized in Figure 3B.[58] Currently what remains is to underpin the biological roles of Nm and its writer HEN1 in GSC behavior. In contrast to HEN1, m5C writer NSUN2 has been functionally linked to normal GSC maturation.[10] In NSUN2 knockout mice, the meiotic progression of spermatocyte into the pachytene stage is absent.[10] Precisely how NSUN2 is modulating GCS differentiation is currently unknown. However, we suspect a disrupted balance between m5C controlled tRFs and piRNAs to play a vital role, as both work to maintain genomic stability by silencing TEs.[50,51,59]

2.3 | Neuroepithelial stem cells

Neuroepithelial stem cells (NESCs) are multipotent cells that contribute to the central nervous system development by giving rise to neurons, astrocytes, and oligodendrocytes. m5C-modified small ncRNAs such as tRNAs have been directly associated with NESC locomotion. In humans and mice, NSUN2 loss-of-function mutations cause brains to develop smaller during embryogenesis.[13] Human isolated neuroepithelial progenitors express NSUN2; however, its expression mitigates with differentiation.[13] In *NSUN2* knockout mice, neural intermediate progenitors exhibit lower migration rates resulting in smaller cerebral cortexes.[13] It is therefore thought that NSUN2 is required for NESC migration toward differentiation initiation cues. Molecularly, the cellular accumulation of 5′ tRFs in the absence of NSUN2 interferes with global translation programs causing NESCs to lack responsiveness to growth factors such as FGF-2.[13,60]

2.4 | Skin stem cells

Skin is a versatile tissue maintained by numerous SCs some of which are unipotent such as epidermal SCs while others like bulge SCs are multipotent. In NSUN2 knockout mice, bulge SCs residing in the hair follicle exhibit delayed differentiation when stimulated during hair cycle initiation.[14] Normally NSUN2 expression peaks in bulge SCs as they enter anagen, the growth phase of hair cycle. When NSUN2 is ablated in the skin, quiescent bulge SCs still accumulate at the onset of anagen causing an overall delay in differentiation.[14] Similarly, isolated bulge SCs and epidermal SCs from NSUN2 knockout mice display differentiation difficulties in vitro.[14] The accumulation of 5′ tRFs disrupts migratory responses in epidermal cells, which is thought to be the underlining mechanism limiting differentiation.[14,61] In humans, NSUN2 also targets VTRNA1.1 with a single m5C at C69.[11,62] In vitro human epidermal progenitors inhibit differentiation by promoting VTRNA1.1 methylation-dependent processing into RNA-induced silencing complex (RISC)-bound small vault RNAs (svRNAs).[63,64] Methylated VTRNA1.1 prevents the binding of serine/arginine rich splicing factor2 (SRSF2) to its putative binding site, which spans the methylated C69. Without the protection conferred by SRSF2 binding, VTRNA1.1 is preferentially cleaved into svRNA4, which in turn inhibits epidermal differentiation.[64] Exactly how svRNA4 is blocking skin differentiation is not clear; however, svRNA4 predicted targets, such as Ovo Like Transcriptional Repressor1 (OVOL1), could be its mode of action.[63] This might indeed be the case as low levels of OVOL1 is required to stop epidermal differentiation.[63,64] Currently, m5C is the only RNA post-transcriptional modification linked to skin SC functions. Further studies are needed to determine whether other epitranscriptome signature plays any role in skin SCs.

2.5 | Hemopoietic stem cells

Hematopoiesis is a complex process driven by a monarchy model of hemopoietic stem cells (HSCs) differentiating into all myeloid and lymphoid lineages. Any perturbations in HSCs would eventually have consequences on hematopoiesis and blood. On the epitranscriptome level, mice lacking DNMT2 exhibit immure hematopoietic systems due to low HSC numbers.[65] Functionally, DNMT2 methylate tRNA Asp^{GTC}, Gly^{GCC}, and Val^{AAC} at C38 thereby increasing global translation fidelity.[65] HSCs lacking DNMT2 accumulate tRFs, which cause ribosomes to fail in discriminating between Asp and Glu codons during protein synthesis. Codon infidelity in HSCs lead to ubiquitination mediated degradation of mistranslated or truncated proteins thereby affecting self-renewal and differentiation.[65] Interestingly, mice lacking the m6A writer METTL3 also exhibit low HSCs with restricted myeloid and erythroid linage differentiation.[66,67] In zebra fish, METTL3 depletion similarly results in a reduction of hematopoietic stem and progenitor cells (HSPCs) with >300 modified ncRNAs.[68] In contrast, METTL3 overexpression in human HSPCs prevents myeloid lineage differentiation while promoting cellular growth.[69] The epitranscriptome landscape of small ncRNAs in HSPCs appears to have received less attention

compared to coding RNAs. Small ncRNA epitranscriptome-focused studies are needed to shed light on the importance of RNA post-transcriptional modifications in HSPCs regulation.

2.6 | Cancer stem cells

The emergence of cancer, in its myriad forms, is a notoriously complex development. The link between the epitranscriptome and cancer adds a new regulatory layer to an already challenging process.[70] Most often, the onset of cancer appears linked to dysregulation in gene profiles. There, the epitranscriptome of small ncRNAs is being directly implicated in tumorigenesis by regulating the transcriptome of cancer stem cells (CSCs). miRNA post-transcriptional modifications are among the most well-studied small ncRNAs in CSCs. For example, deamination of adenine (A) to inosine (I) editing of pre-miRNAs is directly involved in CSC regulation.[71] Both A to I editing proteins, Adenosine Deaminase RNA Specific 1 and 2 (ADAR1 and ADAR2), stimulate CSCs by modifying Let-7 pre-miRNAs and blocking their Drosha-independent maturation as detailed in Figure 4A.[72,73] Loss of Let-7 mature miRNAs enhances tumorigenicity by increasing CSCs self-renewal and drug resistance.[74,75] Activated ADAR pathways are reported in CSCs of leukemia, hepatocarcinomas, oral carcinomas, breast cancer, colon cancer, lung cancer, ovarian cancer, and pancreatic cancer.[73,76-78] Since ADAR Let-7 miRNAs editing is a common mechanism governing CSCs, it is a promising candidate in cancer targeted therapy (Figure 4B). Molecularly, A to I editing prevents miRNA maturation by impairing DGCR8 loading, Drosha's cofactor, and Dicer cleavage.[76,79] It is thought that inosine sites within miRNAs disrupt endonuclease recognition sites by altering RNA secondary structures. ADAR1 mediated A to I editing also works through another mechanism whereby flagging miRNAs for degradation by staphylococcal nuclease domain-containing1 (SND1).[72]

Another epitranscriptome signature linked to CSC self-renewal is mono-methylated 5′ terminal mono-phosphate (O-methylated 5′Pme) and its writer Bicoid interacting three domain containing RNA methyltransferase (BCDIN3D). BCDIN3D is highly expressed in cancer CSCs and a well-known hallmark for tumor invasiveness.[80] BCDIN3D deposits 5′Pme on oncogenic miR-21 and tumor suppressor miR-23b and miR-145.[42] 5′Pme modified pre-miRNAs are not processed into mature miRNAs due to Dicer recognition difficulties. It is therefore no surprise that miR-21 and miR-145 are dysregulated in colon, among other, types of cancers.[39,40,81-84] Molecularly, miR-21 and miR-145 coordinate CSC decisions by targeting TGF-β, OCT4, SOX2, and K-RAS mRNAs. Accordingly, miR-21 promotes SC self-renewal while miR-145 and miR-23b promote differentiation (Figure 4C).[82,85,86] It is thought that BCDIN3D expands CSC compartments by having a greater affinity to miR-145 than miR-21.[42] This could explain the association between elevated BCDIN3D expression and poor prognosis in cancer patients.[80] Fully understanding the small ncRNAs epitranscriptome landscape of ADAR and BCDIN3D in CSC should provide novel targets for targeted cancer therapies.

m6A is also another common signature of small ncRNAs in cancer. Among the known pri-miRNAs decorated with m6A, the following have been linked to CSCs: miR-17, miR-93, miR-106b, and miR-222.[22,87] Using m6A tailored NGS, clusters of m6A are often found near 5′ region of pri-miRNAs. These m6A modifications facilitate pre-miRNA processing by enhancing DROSHA-recognition.[87] Additionally, m6A reader HNRNPA2B1 further increases the affinity of pri-miRNAs to DROSHA leading to more pre-miRNA copies.[22] m6A-regulated miR-17 is part of the oncomiR-1 cluster (miR-17-92) that controls pancreatic CSCs and numerous other CSCs.[88,89] Similarly, NSC proliferation and fate decisions are regulated by miR-17-92 clusters during development.[90] Mature miR-93 supports colon and breast cancer metastasis by modulating CSC proliferation and differentiation.[91,92] In contrast, miR-106b and miR-222 promote metastasis by inducing CSC like characteristics in cancer cells.[93-95] In the case of m6A, the relative activities of its erasers Fat Mass and Obesity Associated Dioxygenase (FTO) and AlkB homolog5 (ALKBH5) are critical in orchestrating how m6A control transcripts.[96,97] As a clearer view of normal (and aberrant) miRNA expression profiles is developed, these small ncRNAs are being recognized for their potential to serve as biomarkers with diagnostic potential.[98] Realizing these potential benefits for translation to clinical applications is an ongoing effort. However, it is clear that the biological consequences of small ncRNA biogenesis depend on modifications promoting processing (eg, m6A) or inhibiting processing (eg, 5′Pme and inosines) illustrated in Figure 4D. Enhancing the mechanistic view of the small ncRNA epitranscriptome in relation to oncogenesis will no doubt help to identify the relevant oncogenic processes in clinical cases, leading to more personalized approaches in their treatment.

2.7 | Epitranscriptome-associated RBPs

RNA post-transcriptional modifications in principle influence RNA by altering structure and/or RBP interactions. Historically, epitranscriptome associated RBPs have been classified into writers, erasers, and readers. Although this characterization of RBPs has been convenient for initial epitranscriptomic studies, recent body of evidence is illustrating a more complex picture. Specifically, the discovery of RBPs in which their binding is mitigated by the presence of modifications. For instance, m5C inhibits angiogenin from binding to tRNAs and SRSF2 from binding to VT-RNAs.[60,64] In fact, Sajini et al recently discovered many small ncRNA-BPs where their binding was limited by m5C modifications.[64] Similarly, the authors found classic readers that preferentially interacted with m5C modified RNAs. In order to differentiate between RBPs attracted or repelled by modifications, we coin the term “Repellers” here to describe RBPs that loss binding efficiency to RNA in the presence of distinct post-transcriptional modifications.

In addition, several writers were recently found to process modified RNA bases rather than deposit de novo modifications. This is particularly true for writers involved in oxidizing RNA modifications such as ALKBH1 and 2′-O-specific methyltransferase (FTSJ1),[99] a member of the TRM7 family. In the case of ALKBH1, NSUN3 modified tRNAs

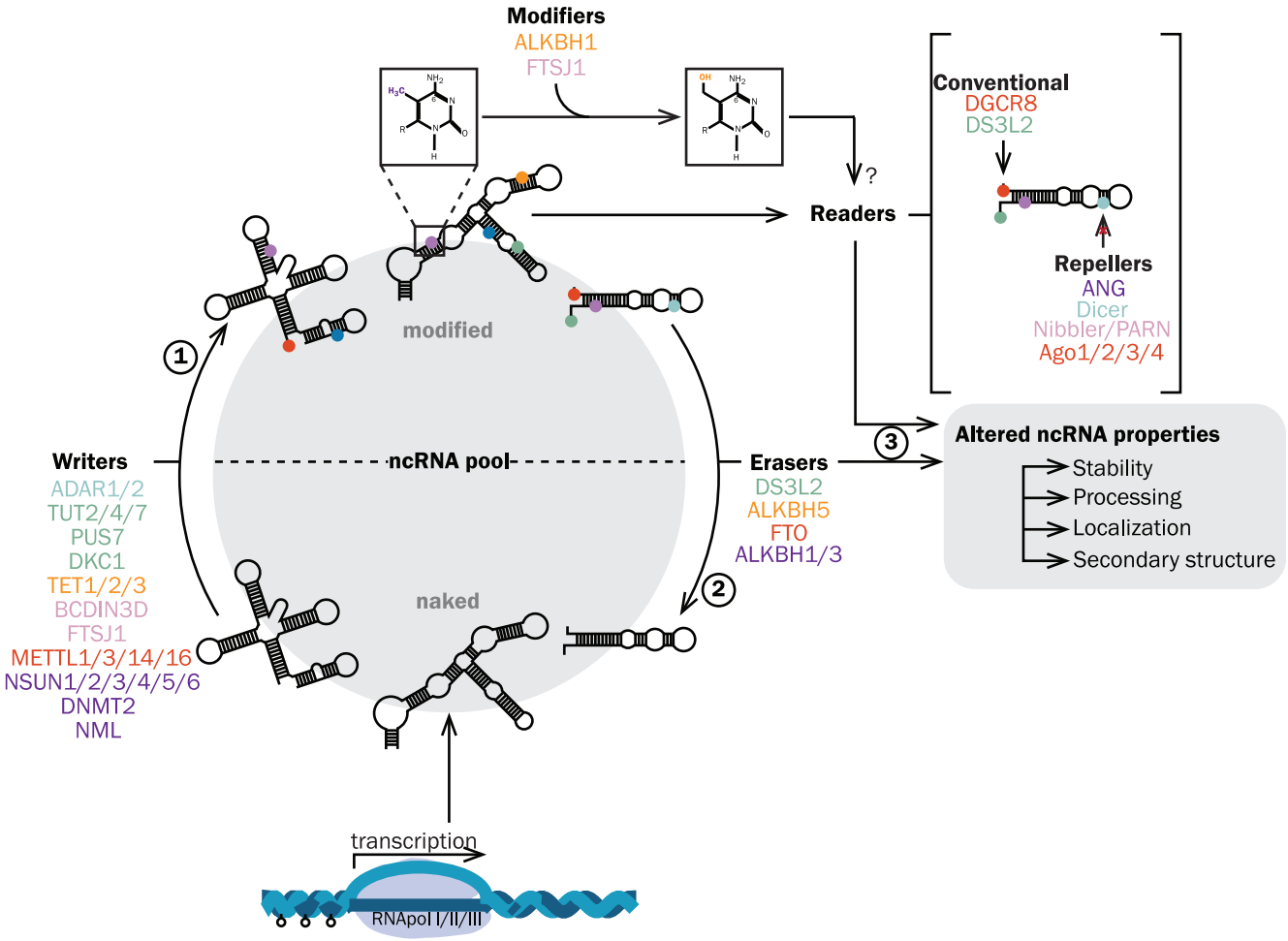

FIGURE 5 An emerging view of the epitranscriptome by specialized RBPs. (1) Writers modify naked RNA. Modifiers process post-transcriptional modifications into novel modifications. Readers recognize and bind to modified RNA. Repellers lose binding to modified RNA. Erasers remove RNA modifications (2). The interactions of RBPs with modifications determine RNA stability, processing, localization, and translation (3) enabling additional stem cell regulatory layers. Examples for each class of epitranscriptome-associated RBPs are given and color-coded according to their respective modifications in previous figures. RBP, RNA-binding proteins

are oxidized by ALKBH1 into 5-hydroxymethylcytosine (hm5C) and, subsequently, into 5-formylcytosine (f5C) (Figure 5) . The verdict is still out whether such forms of oxidative modifications are simply intermediary byproducts, akin to those of the multistep erasing events observed during FTO mediated m6A demethylation,[100] or functionally important. FTSJ1 on the other hand, both deposits Nm on naked RNA bases and converts modified bases into Nm. FTSJ1 recognizes hydroxy-methylated bases, such as hm5C (in such cases converts them into 5-hydroxymethyl-2-O-methylcytosine [hm5Cm]).[99] We have summarized the epitranscriptome associated RBPs using our proposed nomenclature in Table S1.

3 | DISCUSSION

The fine-tuning of mechanisms governing stem cell behavior is required for successful and safe tissue homeostasis. Understanding such mechanisms will aid in deciphering the imbalances in homeostasis that results in CSCs development or in using stem cells for regenerative medicine. Recently, the epitranscriptome has emerged as a major regulator of stem cell biology. Both coding and noncoding RNAs are decorated by modifications that are essential for SC decisions. In this review, we focused on the landscape of small ncRNA modifications and how they modulate SCs in health and disease. Currently, the epitranscriptome remains at its infancy with many modifications and their associated RBPs unknown. The number of RNA modifications continues to expand as new sequencing techniques are devised to probe these modifications at the transcriptome-wide level with single nucleotide resolution. Here, we offer further insights on where the field is heading and predict that small ncRNA modifications will play a key part in future SC therapies.

The potential of the epitranscriptome to improve regenerative medicine is currently being investigated. Indeed, some epitranscriptome-based mechanisms have already reached clinical applications while others are underway. Here, we summarize the current epitranscriptome-based approaches that are used or could been used to modulate SCs for

regenerative medicine. First, incorporating natural occurring post-transcriptional modifications within synthetic mRNA to enhance local translation and reduces innate immunogenicity. Due to mammalian cells having foreign RNA sensing receptors such as pattern recognition receptors, Toll-like receptors, and nucleotide-binding oligomerization domain-like receptors (NLRs), synthetic mRNA must evade cellular sensing to avoid immunoreactions. Numerous epitranscriptome signatures are able to mitigate synthetic mRNA immunogenicity and simultaneously improve translational rates. Among the most commonly used RNA modifications are m6A, m1A, m5C, hm5C, Ψ, and 5-methoxyuracil. Highly modified synthetic mRNAs are today used to generate induced pluripotent SCs (iPSCs) or transdifferentiate somatic cells for regenerative applications.[101,102] Synthetic mRNAs are very attractive molecules to generate iPSCs due to their activities being potent, transient, and nonintegrative.

Second, modulating writers, readers, erasers or repellers that coordinate epitranscriptome pathways regulating SC homeostasis. This approach has recently been explored in HSCs, which by far are the most successful model for SCs therapies. HSCs isolated from YTH domain-containing family protein2 (YTHDF2) conditional knockout mice were found to exhibit significant self-renewal capabilities without perturbing differentiation.[103] Similar phenotypes were also observed in YTHDF2 knockdown human umbilical HSCs.[103] Interestingly, YTHDF2$^{-/-}$ HSCs maintained better self-renewal qualities during secondary serial dilation transplants and did not give rise to any blood malignancies.[103] The longer-term effect of YTHDF2 ablation in HSCs is not known but, at least, the short-term consequences on potency and safety seem negligible.[103] More studies are needed to investigate the longer-term efficacy and safety of YTHDF2 loss in HSCs. We postulate that if safe, small molecules could be used to inhibit m6A reader YTHDF2 activity during HSCs collection and transplantation to increase transplant efficiency. It is worth noting that m6A modulates different fate decisions by coordinating cell context reader and eraser interactions. For instance, YTHDF2 cross-talks with FTO to degrade cyclin A2 (CCNA2) and cyclin-dependent kinase2 (CDK2) mRNAs in order to inhibit adipocyte differentiation.[103,104] Therefore, it is of equal importance when dissecting the epitranscriptome roles in SC decisions to probe for spatial and temporal writer, reader, repellers, and eraser interactions.

Finally, chemically engineering small ncRNAs with natural occurring modifications to improve their stability, specificity, affinity, and delivery into cells. Antisense oligonucleotide (ASO) drugs and small interfering RNA (siRNA)-based drugs are small ncRNAs that act on precursor mRNAs to alter splicing or mature mRNAs to induce decay. Both ASO and siRNA drugs relay on chemical modifications to properly function within bodily fluids. For instance, the licensed antisense drug Kynamro for familial hypercholesterolemia uses a Nm modification to enhance mRNA-affinity and RNA stability. Similarly, siRNA drugs, such as Arbutus (which is currently undergoing human trials for Hepatitis-B), are terminally modified with Nm to improve RISC binding and stability. We are not aware of any approved ASO or siRNA drugs in regenerative medicine; however, with the growing importance of small ncRNAs in SC biology, we foresee their rapid movement in this field to support regenerative medicine.[105]

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

J.M.W.R.M.: manuscript writing, generated all figures, revised the manuscript; A.H.: figure design, financial support, revised the manuscript; A.S.: conception and design, financial support, manuscript writing, figure design, revised the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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SUPPORTING INFORMATION

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