REVIEW ARTICLE

Cancer Science WILEY

Significant epitranscriptomes in heterogeneous cancer

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Abstract

Precision medicine places significant emphasis on techniques for the identification of DNA mutations and gene expression by deep sequencing of gene panels to obtain medical data. However, other diverse information that is not easily readable using bioinformatics, including RNA modifications, has emerged as a novel diagnostic and innovative therapy owing to its multifunctional aspects. It is suggested that this breakthrough innovation might open new avenues for the elucidation of uncharacterized cancer cellular functions to develop more precise medical applications. The functional characteristics and regulatory mechanisms of RNA modifications, ie, the epitranscriptome (ETR), which reflects RNA metabolism, remains unclear, mainly due to detection methods being limited. Recent studies have revealed that N6-methyl adenosine, the most common modification in mRNA in eukaryotes, is affected in various types of cancer and, in some cases, cancer stem cells, but also affects cellular responses to viral infections. The ETR can control cancer cell fate through mRNA splicing, stability, nuclear export, and translation. Here we report on the recent progress of ETR detection methods, and biological findings regarding the significance of ETR in cancer precision medicine.

KEYWORDS
diagnosis, modification, MODOMICS, RNA, therapy

1 | INTRODUCTION

Numerous previous studies have extensively investigated cellular biology in cancer, in terms of gene controls, which are the result of genetic information on DNA, acquired modifications in histones, dynamic regulations by proteins, and the fate determining flow of metabolites. These findings have led to the acceptance of conceptual conclusions as to whether cancer is fundamentally a genetic disease, although various factors beyond simple alterations in DNA sequences and expression orchestrate the biologically malignant phenotypes of tumors.[1]

It was reported that, although cellular reprogramming by induced pluripotent stem cell technology[2] is fundamentally epigenetic

manipulation mainly using transcription factors, subsequent studies have indicated that epigenetic regulation associated with induced pluripotent stem cell derivation could drive the development of particular types of cancer in mouse experiments, suggesting the significance of epigenetics in cancer.[3,4] In addition, in the process of cancer metastasis, the biological mechanism of epithelial-mesenchymal transition (EMT) is controlled by epigenetic regulations rather than genetic events, suggesting a role of the transcriptional and epigenetic landscapes, and the gene regulatory network in controlling EMT states.[5] In addition, it has been shown that focus on phenotypic drug development might not target the appropriate molecular variant in rare driver mutations, as variants of the same protein can express more than one phenotype.[6] This indicates that a multifaceted

FIGURE 1 Biochemistry of the epitranscriptome (ETR) in the central dogma. The modification of DNA has been examined extensively in the term of epigenetics. According to the genetic codes on DNA, ETR denotes the modification of RNA, which is involved in the splicing and translation on ribosomes, leading to the production of proteins. The acquired histone modification is involved in the regulation of transcription.[87] SAM, S-adenosylmethionine
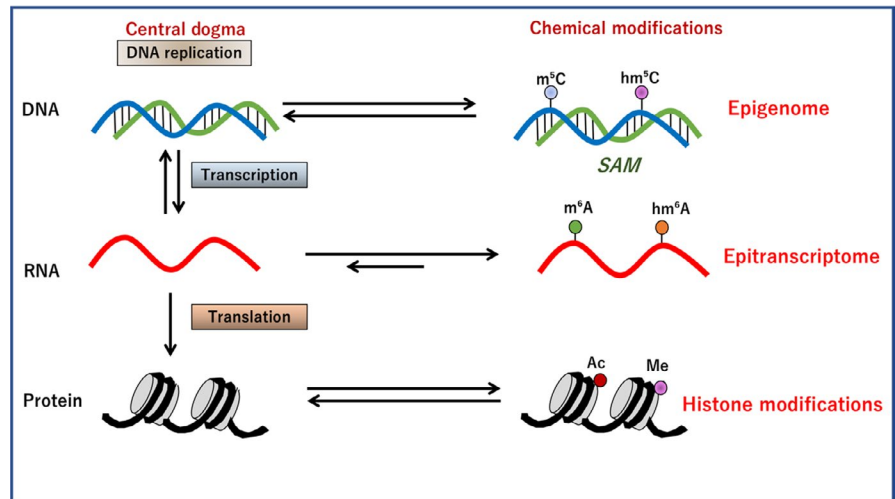
understanding beyond genetics is required in order to ensure further precision in cancer research and medicine.

Recently, other diverse information, including RNA modifications, has emerged in cell physiology,[7] technology,[8] and structure chemistry.[9] Although RNA modifications are not readily readable using bioinformatics, their significance has been emphasized for the diagnosis and therapy of diseases (Figure 1). Whether RNA modifications are associated with tumor heterogeneity remains to be fully elucidated.

This systemic review considers the recently emerged modifications of ribonucleotides and epitranscriptomes (ETRs) in cancer, and discussed the significance of tumor heterogeneity. A published database of references (https://www.ncbi.nlm.nih.gov/pubmed/) between February 1959 and 2019 was reviewed using the following search terms: "RNA methylation", 21 521 papers; "epitranscriptome",

164 papers; and "RNA MODOMICS", 12 papers. Information of ETRs in cancer was then updated.

2 | HISTORICAL REVIEW OF THE DETECTION OF RNA MODOMICS

Since the 1960s, the important role of post-transcriptional modification of RNAs in the synthesis of eukaryotic RNA has be traced; it was reported that one of the most notable features of rRNA synthesis is the specific methylation that occurs on the 45S precursor RNA molecule in a study of HeLa cells.[10] In 1974, methylated nucleotides were identified in a study of Novikoff hepatoma cells by methyl labeling of RNA with L-[methyl-^{3}H] methionine, which was detected by liquid chromatography.[11] This study found that, although rRNA
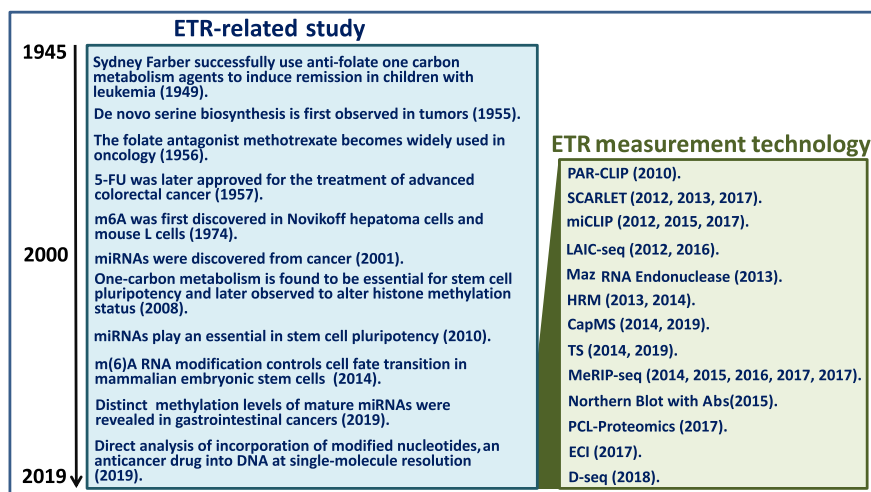

FIGURE 2 Historical view of the study of the epitranscriptome (ETR). Although ETR-related studies began in the 1950s, ETR measurement technologies were in practice in the 21st century. 5-FU, 5-fluorouracil; CapMS, captured mass spectrometry; D-seq, direct m^{6}A sequencing; ECI, electrochemical immunosensor with silver SiO_2; HRM, high-resolution melting; LAIC-seq, m^{6}A levels and isoform-characterization sequencing; MeRIP-seq, methylated RNA immunoprecipitation sequencing; miCLIP, m^{6}A individual nucleotide resolution cross-linking and immunoprecipitation; miRNA, microRNA; PCL-Proteomics, photo-crosslinkers and quantitative proteomics; SCARLET, site-specific cleavage and radioactive-labeling followed by ligation-assisted extraction and thin-layer chromatography; TS, tunnel current sequencing

and tRNA possess complex patterns in base-methylation, the distribution in mRNA was relatively simple, consisting predominantly of N6-methyladenosine.[12]

Although previous studies have revealed the diversity of chemical modifications of RNA, two-thirds of all known RNA modifications were reported before 1980, and the observed variations have remained relatively unchanged over several decades.[13] A historical review of the techniques to detect RNA modifications, namely RNA MODOMICS, is summarized in Figure 2.

3 | DATABASE FOCUSING ON RNA MODOMICS

In 1994, the RNA Modification Database was introduced as a focal source for information of naturally occurring RNA modifications.[13] The database incorporated a searchable interface for detailed data of 109 currently known RNA modifications.[11]

In 1999, the RNA Modification Database provided a comprehensive list of 95 different natural modifications of RNA, including information on chemical structure, phylogenetic distribution, structure characterization, and chemical synthesis.[14]

In 2008, MODOMICS (http://modomics.genesilico.pl) was made available as a database of 119 different modifications of tRNAs, rRNAs, snRNAs, and small nucleolar RNA, regarding comprehensive information of the chemical structures of modified ribonucleosides, their synthetic pathways, the location of modified residues in RNA sequences, and RNA modifying enzymes.[15] The modifications in RNAs were shown using a liquid chromatography-mass spectrometry (LC-MS) technique for tRNAs,[16] by ^{15}N or ^{13}C stable isotope labeling and LC-MS/MS in *Escherichia coli* and yeast,[17] and by LC-MS and LC-MS/MS analysis in archaeal tRNA(Ile).[18]

The biochemical and physiological roles of modifications have been found in the decoding process of tRNAs, as examined and reviewed by Gu et al,[19] and the methylation process of rRNA was investigated.[20-23]

4 | CONSENSUS SEQUENCE OF RNA MODOMICS

In molecular biology, the complementarity of DNA or RNA sequences defines the association between 2 structures, each following a lock-and-key association. Complementarity includes the principles of DNA replication, RNA transcription, and translation of peptides, in addition to various functions modulating the central dogma, such as RNA interference[24] and competing endogenous RNAs[25] and genome editing.[26]

It has been shown that the structural modifications of nucleotides, mainly as ribose analogs, are aimed at the application of siRNAs to limit their sensitivity to ribonucleases, poor cellular uptake, and rapid size-mediated renal clearance. The correction of thermal asymmetry is achieved by the incorporation of nucleotide substitutions, or blocking sense strand incorporation in the RNA-induced silencing complex. Locked nucleic acids also increase the sensitivity and specificity of short RNA and DNA targets.[27] These technologies are used for the validation of potent drug targets for anticancer therapy and the silencing of thermodynamically unfavorable targets, ie, mutations of oncogenes and tumor suppressor genes and chimeric fusion genes.[27] The modification of bases in DNA or RNA allows the 4 ribonucleotide residues to gain diverse functions similar to those of the side chains of amino acid residues.

Most mammalian m^{6}A sites are found within the consensus sequence, typically "GGACU"; more precisely, Rm^{6}ACH (R denotes G or A; H includes A, C, or U), or Pu(G>A)m^{6}AC(A/C/U) (where Pu represents purine).[28] Previous studies have revealed that the enriched binding motif, GGACC, is observed in methyltransferase-like protein 14 (METTL14), an RNA-binding scaffold protein that recognizes the substrate in a component of m^{6}A methyltransferase complex, whereas the motif NHGGAC is found in METTL3, a catalytic core of m^{6}A methyltransferase, which formed a complex with METTL14 (N indicates G, C, A, or U).[28] The overlay of METTL3 and METTL14 is GUCGGAC.[29] Wilms tumor 1-associating protein (WTAP) can interact with the complex of METTL3 and METTL14 to affect methylation of the GACU site.[28]

5 | WRITING, ERASING, AND READING IN RNA MODOMICS

Among the various conserved internal chemical modifications of RNAs, the N6-methyladenosine (m^{6}A) is the most prevalent modification, which is found in almost all eukaryotic RNAs,[30] and the importance of these modifications has been reported.[31] Generally, the m^{6}A modification is post-transcriptionally installed by a multi-component N6-adenosine methyltransferase (MT) complex.[28] It was reported that the ~200-kDa (MT) complex, which was isolated from mammalian cell nuclear extract, revealed only a 70-kDa protein, which is involved in the inhibition of methyltransferase activity and was termed MT-A70 or METTL3.[32] A recent study showed that human METTL3 and METTL14 form a stable heterodimer core complex that functions in the deposition of cellular m^{6}A on mammalian nuclear RNAs and catalyzes m^{6}A RNA methylation.[28] As a mammalian splicing factor, WTAP can interact with this complex and affect this methylation.[28] Therefore, the core complex of METTL3-14 with WTAP exerts a function of methylation at N6-adenosines, as writers for ETR codes[33] (Figure 3).

By contrast, functionally significant demethylases that reverse this methylation, such as fat mass and obesity-associated protein (FTO)[34] and α-ketoglutarate-dependent dioxygenase (ALKB) homolog 5 (ALKBH5)[29] have been identified. A recent study also revealed distributions of m^{6}A in the mammalian transcriptomes[35] and indicated diverse regulatory functions of this dynamic modification.[36] Therefore, these factors function as erasers to ETR codes[33] (Figure 3).
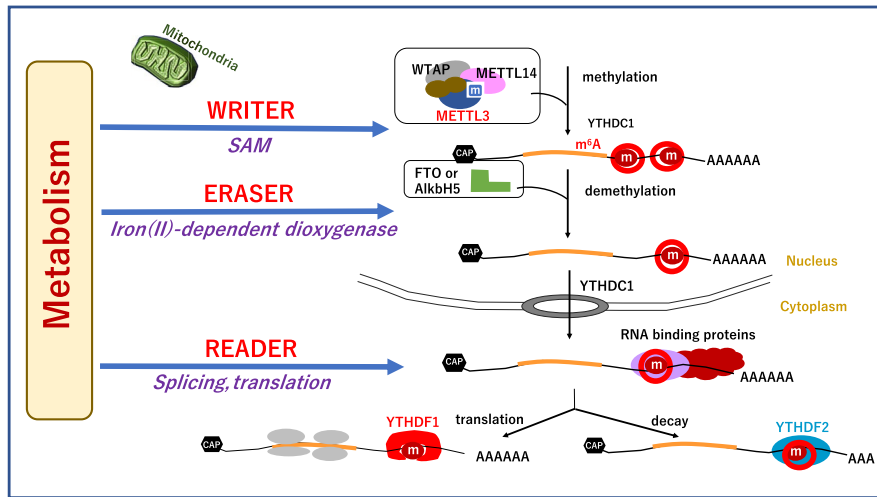
FIGURE 3 Writers, erasers, and readers of the epitranscriptome. In response to the extracellular stimuli and cellular metabolism, the methyltransferase-like protein 3 (METTL3)/MELLT14/Wilms tumor 1-associating protein (WTAP) writer complex is involved in the methylation of RNAs at the specific residue. Fat mass and obesity-associated protein (FTO) or α-ketoglutarate-dependent dioxygenase homolog 5 (ALKBH5) is involved in demethylation, recognized by reader groups, including YT521-B homology N6-methyladenosine RNA binding protein 1 9YTHDF1), YTHDF2, and heterogeneous nuclear ribonucleoproteins (HNRNPA2).[88] CAP, RNA 5'-CAP; m, methylated base; SAM, S-adenosylmethionine

Readers, including heterogeneous nuclear ribonucleoproteins and YT521-B homology (YTH) N6-methyladenosine RNA binding protein 1 (YTHDF1) have been reported. A recent study revealed that oncogene *c-myc* promotes the expression of ERT m^6A reader YTHDF1 in colorectal cancer.[37] Therefore, reader, writer, and eraser proteins for m^6A were discovered.[33] Furthermore, it is reported that YTHDF2, another YTH domain family member, is involved in the localization of bound mRNA from the translatable pool to mRNA decay sites, such as processing bodies, through a carboxy-terminal domain of YTHDF2 selectively binding to m^6A-containing mRNA.[38] Taken together, studies have indicated that the dynamic m^6A modification is recognized by different binding proteins to affect the translation status and lifetime of RNA[33,37,39] (Figure 3).

6 | RNA MODOMICS IN EMBRYONAL STEM CELLS

The study of murine embryonal stem cells (ESCs) has identified METTL3 as a regulator for terminating murine naïve pluripotency.[40] The preimplantation epiblasts and naïve ESCs with METTL3 knockout were depleted of the m^6A modification and were viable[40]; however, the adequate termination of their naïve pluripotency failed and they underwent aberrant and restricted lineage priming at the postimplantation stage, which led to early embryonic lethality.[40] This suggests that m^6A serves a role in determining the fate of murine ESCs, which functionally influence naïve and primed pluripotency in an opposing manner. m^6A predominantly and directly reduces the stability of mRNA.[40] It is suggested that METTL3 is involved in the balance between pluripotency and lineage-committed differentiation and that the

depletion of METTL3 results in the stabilization of target genes; ie, METTL3-deficient naïve pluripotent cells upregulate the already high naïve pluripotent genes to create a "hyper"-naïve pluripotent state, and METTL3 depletion occurring in primed cells could induce the determination of lineage commitment and tip the balance toward differentiation.[41]

In addition to METTL3, a recent study indicated a synergistic role of METTL14. The knockdown of METTL3 and METTL14 in mouse ESCs resulted in a lack of m^6A modification and loss of self-renewal capability.[42] The study indicated that m^6A modification was inversely correlated with mRNA stability and gene expression, and that the human antigen R and microRNA pathways were linked to the effects. This supports the hypothesis that m^6A modification is required to maintain mouse ESCs in a naïve state and might be relevant to thousands of mRNAs, long non-coding RNAs (lncRNAs) and microRNAs in various cell types.[42] In addition, a recent study indicated that the depletion of mouse and human METTL3 resulted in the impairment of ECSs exiting from a naïve state to a state of differentiation, suggesting a role of the m^6A modification in marking transcriptome flexibility in cells.[43]

7 | RNA MODOMICS IN NON-CODING RNAS AND VIRAL INFECTION

m^6A is the most prevalent and reversible modification in mRNAs and non-coding RNAs (ncRNAs), including lncRNAs.[28] The cellular ETR finely sculpts a dynamic proteome in response to diverse cues during cellular processes, including differentiation, immune tolerance, and neuronal signaling.[44] The ETR of cancer emerges and chemical modifications of RNA serve a central role in the control of mRNA and

ncRNA activity, which could assist in the development of anticancer drugs.[45]

The involvement of viral epitranscriptomics has been reported in viral RNAs that replicate in the nuclei of host cells, as shown in the study of the Rous sarcoma virus in the 1970s,[46] Flaviviridae virus,[47] Zika virus,[48] Kaposi sarcoma-associated herpesvirus,[38] HIV,[49] influenza A virus,[50] and tobacco mosaic virus.[51]

8 | RNA MODOMICS IN DIFFERENT TYPES OF CANCER

8.1 | Glioma

A study on glioma indicated that the knockdown of METTL3 or METTL14 resulted in the changes in mRNA m^6A enrichment and alterations in the mRNA expression of target genes, including ADAM19, which serves a critical role in glioblastoma stem cells.[52] In addition, the inhibition of FTO, an m^6A demethylase, reportedly suppressed tumor progression and prolonged the lifespan of glioblastoma stem cell-grafted mice in vivo.[52] This study indicated that m^6A is critical for self-renewal and tumorigenesis in glioblastoma stem cells, presumably through the regulation of target RNAs, and that m^6A modification is a promising therapeutic target for glioblastoma.[52]

8.2 | Leukemia

It has been shown that FTO is expressed at high levels in the leukemic cells of patients with acute myeloid leukemia (AML) with t(11q23)/myeloid/lymphoid or mixed-lineage leukemia rearrangements, t(15;17)/promyelocytic leukemia - retinoic acid receptor alpha (RARA), Fms-related tyrosine kinase 3 - internal tandem duplication, and/or nucleophosmin 1 mutations, suggesting that FTO serves a critical oncogenic role in AML.[53] This study suggested that FTO decreases the expression of targets, including ankyrin repeat and SOCS box protein 2 and RARA, through removal of the m^6A modification in these mRNAs, and thus inhibits all-trans-retinoic acid-induced AML cell differentiation, which leads to leukemic transformation, providing profound insight into the disease mechanism and for therapeutic drug development.[53] Additionally, METTL3 plays a critical role in cell growth of myeloid leukemia, in which METTL3 controls the function of target genes.[54] METTL3 is involved in remodeling chromatin, which is necessary for the maintenance of myeloid leukaemia.[54]

8.3 | Lung cancer

A study on METTL3-mediated translation indicated that METTL3 enhances mRNA translation through an interaction with the translation initiation machinery, which promotes the translation of certain mRNAs, including epidermal growth factor receptor and the Hippo pathway effector transcriptional coactivator with PDZ-binding motif (TAZ) in human cancer cells.[55] This study indicated that METTL3 promotes the growth, survival, and invasion of human lung cancer cells,[55] suggesting that METTL3 could be a possible target of therapy.

8.4 | Breast cancer

A study on human breast cancer cells indicated that the exposure to hypoxia resulted in the stimulated expression of AlkB homolog 5 (AlkBH5), an m^6A demethylase, which decreased the methylation level of NANOG mRNA, increased NANOG levels, and increased breast cancer stem cells.[56] AlkB homolog 5 was involved in the demethylation of m^6A in sequence AAACU in the 3′-UTR of NANOG mRNA.[56] Exposure to hypoxia stimulated expression in a hypoxia-inducible factor (HIF)-1α- and HIF-2α-dependent manner.[56] In addition, hypoxia induced an zinc finger protein 217 (ZNF217)-dependent inhibition of the m^6A modification of mRNAs encoding NANOG and Kruppel-like factor 4 in breast cancer.[57] Zinc finger protein 217 or AlkBH5 are involved in the control of pluripotency factor expression in breast cancer in hypoxia.[57]

8.5 | Colorectal cancer

A recent study indicated that m^6A modification serves a role in the malignant behaviors of colorectal cancer.[37] Epigenetic data obtained by ChIP indicated that the oncogene *c-Myc* is involved in the transcription of m^6A reader YTHDF1 in colorectal cancer.[37] Immunohistochemical analysis of YTHDF1 showed that its expression was associated with various malignant tumor behaviors, and the expression of YTHDF1 was identified as an independent prognostic factor in patients.[37]

8.6 | Pancreatic cancer

A previous hospital-based case-control study of genetic variations indicated an association between variations in the *FTO* gene and pancreatic cancer risk in Japan.[58] The significant association of the *FTO* rs9939609 variant polymorphism was shown in endometrial cancer and pancreatic cancer, particularly in Asian populations, and could be a potential biomarker in the early diagnosis of these types of cancer.[59] An association between the *FTO* rs9939609 variant and malignant pleural mesothelioma risk was also reported.[60]

Another study on pancreatic cancer found that METTL3-depleted cells showed higher sensitivity to anticancer reagents, including gemcitabine, 5-fluorouracil, cisplatin, and irradiation, suggesting that METTL3 is a potent target for enhancing therapeutic efficacy in patients with pancreatic cancer.[61]

8.7 | Measurement and detection technology of ETR

Several technologies have been reported for the measurement and detection of ETRs. For measurement purposes, several methods have been reported, including the dot blot,[53] northern blot with Ab,[62] Maz RNA endonuclease (MazF is an *E. coli* toxin),[63] high-resolution
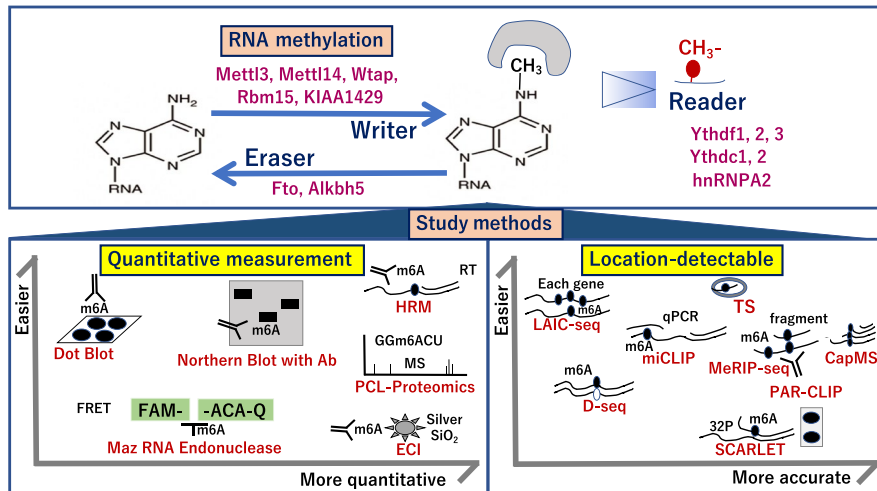
FIGURE 4 Study methods of RNA MODOMICS. For measurement purposes, several methods have been reported, including dot blot, northern blot with Ab, Maz RNA endonuclease, high-resolution melting (HRM), photo-crosslinkers and quantitative proteomics (PCL-Proteomics), and electrochemical immunosensor (ECI) with silver SiO_2. For location detection, several methods have been reported, including m^6A levels and isoform-characterization sequencing (LAIC-seq), direct m^6A sequencing (D-seq), m^6A individual nucleotide resolution cross-linking and immunoprecipitation (miCLIP), methylated RNA immunoprecipitation sequencing (MeRIP-seq), photo activatable ribonucleoside-enhanced cross-linking and immunoprecipitation (PAR-CLIP), site-specific cleavage and radioactive-labeling followed by ligation-assisted extraction and thin-layer chromatography (SCARLET), tunnel current sequencing (TS), and captured mass spectrometry (CapMS). The figure indicates each technology according to ease of quantitative assessment (left) and accuracy (right). qPCR, quantitative PCR
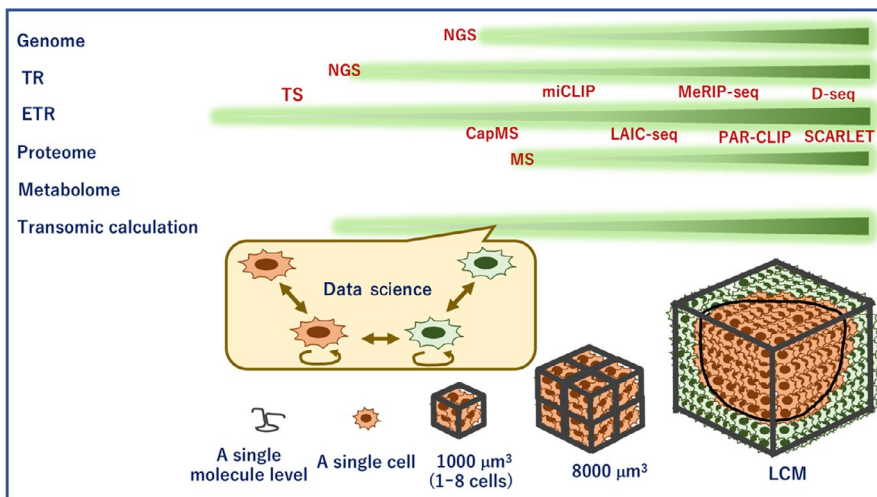

FIGURE 5 Tissue study by epitranscriptome (ETR) and other technologies. Information on genome, transcriptome (TR), ETR, proteome, and metabolome levels is assessed by various techniques, as indicated. The data obtained through each technique are analyzed by transomic approaches. Although bulk tissues are examined by laser-capture microdissection (LCM), high-speed next-generation sequencing (NGS) can evaluate data of a single cell, and tunnel current sequencing and captured mass spectrometry (MS) can get obtain data for each molecule at the subcellular level of ETR. CapMS, captured mass spectrometry; D-seq, direct m^6A sequencing; LAIC-seq, m^6A levels and isoform-characterization sequencing; MeRIP-seq, methylated RNA immunoprecipitation sequencing; miCLIP, m^6A individual nucleotide resolution cross-linking and immunoprecipitation; PAR-CLIP, photo activatable ribonucleoside-enhanced cross-linking and immunoprecipitation; SCARLET, site-specific cleavage and radioactive-labeling followed by ligation-assisted extraction and thin-layer chromatography; TS, tunnel current sequencing

melting (HRM),[64] RNA photo-crosslinkers and quantitative proteomics (PCL-Proteomics),[65] and electrochemical immunosensor (ECI) with silver SiO_2.[66]

Several methods have been reported for location detection purposes, including the detection of m^6A levels and

isoform-characterization sequencing (LAIC-seq),[67] direct m^6A sequencing (D-seq),[68] m^6A individual-nucleotide-resolution cross-linking and immunoprecipitation (miCLIP),[69] methylated RNA immunoprecipitation sequencing (MeRIP-seq),[35] photo activatable ribonucleoside-enhanced cross-linking and immunoprecipitation
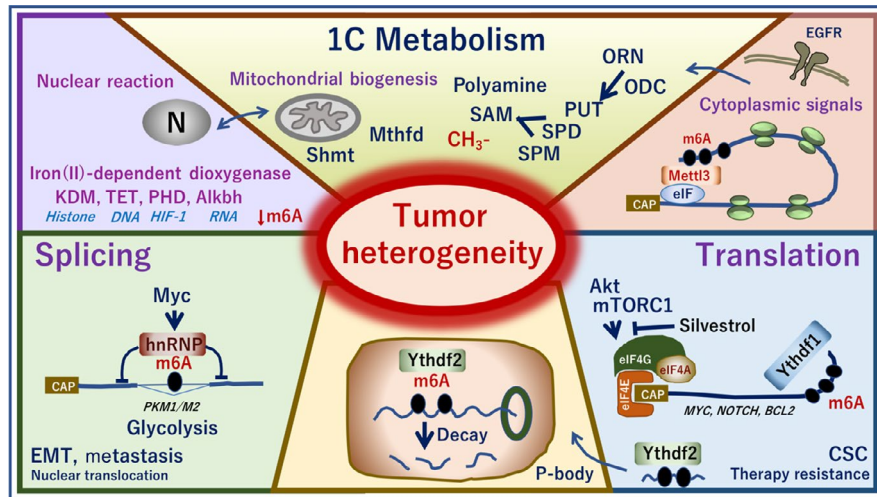
FIGURE 6 RNA MODOMICS and heterogeneity of tumors. In response to the upstream signals, including epidermal growth factors, one-carbon metabolism serves a critical role in mitochondrial biogenesis through methylenetetrahydrofolate dehydrogenase (Mthfd) and serine hydroxymethyltransferase 2 (Shmt). Polyamine flows including metabolites, ornithine, spermidine, and spermine regulate the methylation reaction. The mitochondrial metabolites control α-ketoglutarate/2-oxoglutarate dioxygenase (Alkbh) and iron (II)-dependent dioxygenase, histone lysine demethylase (KDM), and ten-eleven translocation methylcytosine dioxygenase 1 (TET), which catalyzes the conversion of the modified DNA base 5-methylcytosine to 5-hydroxymethylcytosine, prolyl hydroxylase domain enzymes, an enzyme involved in the hypoxia-inducible factor (HIF) signaling pathways. ALKBH5 demethylates specifically by oxidative demethylation m6A RNA, the most prevalent internal modification of mRNA in higher eukaryotes. m6A is involved in the RNA splicing by heterogeneous nuclear ribonucleoproteins (hnRNP) that are known as splicing factors. YT521-B homology 6-methyladenosine RNA binding protein 1 (YTHDF1) recognizes m6A and binds the mRNA's 3′UTR. YHTDF1 recruits the eIF4 family and induces protein translation, which is regulated by the Akt-mTORC1 pathway. m6A markings are also involved in the P-body decay by YTHDF2. These mechanisms are altered in tumors to various levels and contribute to the heterogeneity of tumors. ALKBH5, α-ketoglutarate-dependent dioxygenase ALKB homolog 5; CAP, RNA 5'-CAP; CSC, cancer stem cell; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; ODC, ornithine decarboxylase; ORN, ornithine; PHD, plant homeodomain; PKM, pyruvate kinase; PUT, putrescine; SAM, S-adenosylmethionine; SPD, spermidine; SPM, spermine

(PAR-CLIP),[70] site-specific cleavage and radioactive-labeling followed by ligation-assisted extraction and thin-layer chromatography (SCARLET),[71] tunnel current sequencing (TS),[72] and captured mass spectrometry (CapMS) (M. Konno and H. Ishii, unpubl. data, 2019) (Figures 4 and 5).

Eventually TS can be applied to DNA,[73] methylcytosines in DNA,[74] RNA,[75] post-translational signaling,[76] and DNA-incorporated anticancer reagent trifluorothymidine (trifluridine)[77] (Figure 5).

8.8 | Tumor heterogeneity affected by ETRs

Epitranscriptome modifications regulate all stages of the RNA life cycle, from transcription to decay.[78] As recent studies have indicated a mechanism in which RNA methylation is involved, information regarding aberrant modification is suggested to be involved in cancer initiation and progression, and serve as a biomarker for early stage diagnosis in several types of cancer.[78] In response to extracellular stimuli, signals including epidermal growth factor elicit METTL3 function in collaboration with TAZ.[55] Serine metabolism in one-carbon metabolism supports the methionine cycle and DNA and RNA methylation in cancer cells.[79] In terms of one-carbon metabolism, a previous study indicated that polyamine flow and S-adenosylmethionine metabolism exert a critical role in the maintenance of therapy-resistant cancer stem cells.[80] One-carbon metabolism is

involved in the function of signaling organelle mitochondria through mitochondrial products, including α-ketoglutarate/2-oxoglutarate, and succinate and fumarate interfering mechanisms to 2-oxoglutarate and iron (II)-dependent dioxygenases, including demethylases of DNA and RNA[81] (Figure 6). Computational analysis has indicated the imbalanced mechanism of isocitrate dehydrogenase in colorectal cancer.[82]

As oncogene *c-Myc* is involved in the transcriptional control of ETR reader YTHDF1,[37] in addition to METTL3,[61] a recent study indicated that METTL3 promotes ovarian cancer growth and invasion through EMT.[83] Furthermore, the RNA editing process, which irreversibly recodes primary RNA sequences by enzymes apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like and adenosine deaminase, RNA-specific, are reported to be involved in dynamically altering RNA molecules by writers and demethylases in response to environmental conditions.[84] Furthermore, it has been proposed that malignant RNA processing plays a role in the generation and maintenance of cancer stem cells through mechanisms of RNA methylation, RNA editing, and RNA splicing, the functional consequences of which contribute to aberrant regulation in human malignancies.[85] Therefore, it is expected that ETR affects the heterogeneous phenotypes of tumor biology through the mechanisms of RNA splicing, nuclear-cytoplasmic transport, translation, and regulation of gene expression by RNA interference,[84] although
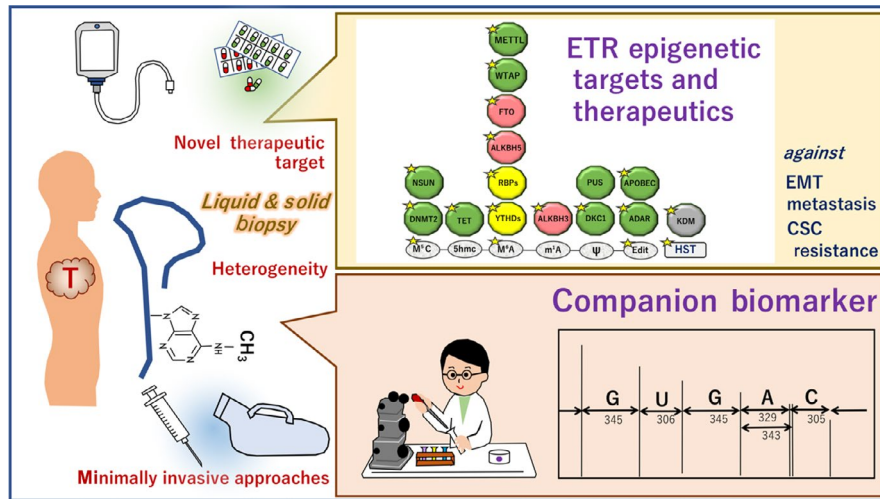
FIGURE 7 Nucleotide precision medicine by focusing on the epitranscriptome (ETR). By liquid and solid biopsy, precise information including ETR can be obtained. The ETR can be useful for companion biomarker and novel drug discovery. Writers of RNA modification (green), readers (orange), and erasers (red)[55]; yellow stars show targets that are reportedly involved in tumorigenesis.[55] ADAR, adenosine deaminase RNA; ALKBH, α-ketoglutarate-dependent dioxygenase homolog; APOBEC, apolipoprotein B mRNA editing enzyme catalytic; CSC, cancer stem cell; DKC1, dyskerin pseudouridine synthase 1; DNMT2, DNA methyltransferase 2; EMT, epithelial-mesenchymal transition; FTO, fat mass and obesity-associated protein; HST, histones as possible therapeutics; KDM, histone demethylase; METTL3, methyltransferase-like protein; NSUN, NOP2/Sun RNA methyltransferase; PUS, pseudouridine synthase; RBP, RNA binding protein; TET, tet methylcytosine dioxygenase; WTAP, Wilms tumor 1-associating protein; YTHD, YTH N6-methyladenosine RNA binding protein

genetic codes serve a role in defining a fundamental phenotype. Of note, ETR information is difficult to read through standard deep sequencing, and further development of measuring technologies is required.[86]

Whether ETR information can contribute to profiling responsiveness to anticancer reagents, and the subclass of cancer diseases, remains a challenge. To overcome this, a perceptive view for the multiomic profiling of tumor tissues, through a combination of deep sequencing data and ETR sequencing, is required to improve precision medicine for intractable cancer (Figure 7).

9 | CONCLUSION

The ETR is involved in the initiation and progression of tumors through a mechanism involving RNA splicing, nuclear-cytoplasmic transport, translation, and the regulation of gene expression by RNA interference and editing. The present review will facilitate further development of novel technology to profile tissue RNA MODOMICS for precision medicine.

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