REVIEW

m6A readers, writers, erasers, and the m6A epitranscriptome in breast cancer

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Abstract

Epitranscriptomic modification of RNA regulates human development, health, and disease. The true diversity of the transcriptome in breast cancer including chemical modification of transcribed RNA (epitranscriptomics) is not well understood due to limitations of technology and bioinformatic analysis. N-6-methyladenosine (m6A) is the most abundant epitranscriptomic modification of mRNA and regulates splicing, stability, translation, and intracellular localization of transcripts depending on m6A association with reader RNA-binding proteins. m6A methylation is catalyzed by the METTL3 complex and removed by specific m6A demethylase ALKBH5, with the role of FTO as an ‘eraser’ uncertain. In this review, we provide an overview of epitranscriptomics related to mRNA and focus on m6A and its detection. We summarize current knowledge on altered levels of writers, readers, and erasers of m6A and their roles in breast cancer and their association with prognosis. We summarize studies identifying m6A peaks and sites in genes in breast cancer cells.

Key Words
- epitranscriptome
- m6A
- mRNA
- breast cancer

Introduction

Breast cancer (BC) is the most commonly diagnosed cancer worldwide, although countries with the highest human development index, related to life expectancy, education, and wealth, have the highest incidence of BC (Wilkinson & Gathani 2022). Breast tumors are characterized by cellular heterogeneity, abnormal gene expression, and chaotic signaling associated with genetic, epigenetic, and epitranscriptomic changes (Lüönd et al. 2021, Yang et al. 2021). BC is divided into four major clinical subtypes based on varying expression of hormone receptors (HR), estrogen receptor α (ERα, referred to as ER+) and progesterone receptor (PR) and human EGF receptor (HER2 ERBB2−) (Dunnwald et al. 2007, Dai et al. 2016), or the lack of these receptors in triple-negative BC (TNBC). These molecular markers provide treatment options. Due to early diagnosis and improved treatment options, the death rate from BC has decreased significantly during the last 20 years (Siegel et al. 2021, 2022, Sung et al. 2021). Based on individual risk assessment, patients with HR+/HER2− primary tumors receive 5–10 years of single agent endocrine therapies (ET): an aromatase inhibitor (AI, e.g. letrozole) for postmenopausal women or tamoxifen (TAM) and ovarian suppression for premenopausal women (Burstein et al. 2010, 2014, 2016). For metastatic ER+ BC, patients are treated with combined ET and CDK4/CDK6 inhibitors, PI3K inhibitors, PARP inhibitors, and or anti-PD-L1 immunotherapy, depending on molecular profiling (reviewed in Loibl et al. 2021). Although the PAM50 risk of recurrence score (PAM50-ROS) is an example of ‘precision medicine’ and ‘patient-customized therapy’
with a predictive recurrence value in ET-treated ERα+ BC patients (Giorgi Rossi et al. 2021), >43,700 women in the US will die of metastatic BC in 2022 (Siegel et al. 2022).

If a breast tumor expresses little (<1%) or no ER/PR but has a high expression of HER2, the patient is treated with the humanized monoclonal antibodies trastuzumab and pertuzumab directed against the extracellular domain of HER2 (ERB2) (von Minckwitz et al. 2017) and trastuzumab deruxtecan (T-DXd), an antibody-drug conjugate with a topoisomerase I inhibitor payload for metastatic of unresectable disease (Bardia et al. 2022). Patients with TNBC receive chemotherapy +/- radiation therapy as first-line therapy (Bianchini et al. 2016). New FDA-approved therapeutic options used in combination with chemotherapy for patients with local recurrent unresectable or metastatic TNBC include PD-L1 inhibitors (atezolizumab and pembrolizumab (Torres & Emens 2022)), PARP inhibitors (olaparib or talazoparib, depending on germline BRCA gene mutations) (Cardoso et al. 2020), and sacituzumab govitecan (SG) that is antibody conjugate with anti-trophoblast cell surface antigen-2 (Trop-2), targeting Trop-2-expressing cancer cells, linked with cytotoxic moiety SN-38 (govitecan) with topoisomerase I inhibitor activity (Michaleas et al. 2022). A recent pilot clinical trial using personalized immunotherapy, infiltrating lymphocytes(TILs), showed that 6 of 42 patients with metastatic BC developed objective cancer regression (Zacharakis et al. 2022).

For ~25–40% of BC patients with ER+ breast tumors, ET resistance develops and overall survival (OS) for patients with metastatic disease is only ~44.6 mos (Polley et al. 2021). Significant progress has been made in understanding the molecular mechanisms for ET resistance (reviewed in Clarke et al. 2015) and includes mutations in ESR1 and ESR1 fusion transcripts in ~30% of metastatic tumors from patients on aromatase inhibitor ET therapy (reviewed in Toy et al. 2017, Piscuoglio et al. 2018). Increased progression-free survival (PFS) has been realized with the addition of cyclin-dependent kinase 4 and 6 (CDK4 and 6) inhibitors to ET for women with advanced BC (Cardoso et al. 2020, Sledge et al. 2020). However, metastatic spread occurs early in tumor progression and likely precedes the detection of the primary tumor (Sanger et al. 2011, Celii-Terrassa & Kang 2016). Thus, identifying features in the primary tumor that reflect its metastatic potential and blocking progression is key to preventing recurrent disease. Whether epitranscriptomic RNA modifications in breast tumors may hold clues to metastatic potential and the mechanism(s) and targets that these modifications regulate are currently unknown. Analysis of BC data in ONCOMINE revealed changes in the transcript levels of writers, readers, and erasers of the most common RNA modification, N-6-methyladenosine (m6A) with two readers, RBM15B and ZC3H13, positively associated with ER and PR expressions and with longer relapse-free survival (RFS) (Zhang et al. 2020). However, there are conflicting data with respect to associations of these and other m6A writers, readers, and erasers in BC (Liu et al. 2022a). There are few studies on how the position of m6A affects transcript splicing, stability, or translation in BC cells or tumors, indicating the need for closer examination of the importance of the regulation of the epitranscriptome in subtype-specific BC and metastatic disease.

An overview of epitranscriptomics

Over 160 chemical modifications of transcribed RNA have been identified (Mathlin et al. 2020). The proteins that add the chemical modification recognize the specific RNA base modification and remove it. The modification is termed as ‘writers, readers, and erasers’. The term ‘epitranscriptomics’ refers to these chemical modifications of transcribed RNA and includes modifications of adenosine (A), guanosine (G), cytidine (C), and uridine (U) (reviewed in McCown et al. 2020). The modomics database of RNA modification pathways summarizes these modifications (Boccaletto et al. 2022). These modifications affect the structure of RNA and its interactions with proteins, including readers (Jones et al. 2022, Kierzek et al. 2022, Szabat et al. 2022). tRNAs are the most extensively modified cellular RNAs with over 100 chemical modifications (Boccaletto & Bagiński 2021) and each tRNA contains 11–13 modifications (Delaunay & Frye 2019). The tRNA chemical modifications within the D-, T-, and anticodon- loops regulate tRNA stability, folding, and interaction with the elongator complex for translational fidelity (Pereira et al. 2018). About 130 modifications have been identified in mRNA, including isomerization of uridine to pseudouridine (Ψ) and 2′-O-methylation of the ribose (Am) (Delaunay & Frye 2019). Mitochondrial-encoded rRNAs are also highly modified (Laptev et al. 2020). There are 13 known internal mRNA modifications located in the 5′ and 3′ UTRs as well as exons and introns (Anreiter et al. 2021). m6A is the most common dynamic modification of the transcriptome and regulates the functioning and processing of miRNAs, long ncRNAs (lncRNAs), and primary miRNA (pri-miRNAs) to precursor (pre)-miRNAs (Zaccara et al. 2019). In mammalian mRNA, ~0.1%–0.4% of RNA are exposed to m6A modification, with an average of 3–5 m6A sites per transcript (Roundtree et al. 2017a). m6A is also enriched...
in circRNAs, lncRNAs, and U6 snRNA (Zaccara et al. 2019). m6A is enriched in the 3'UTR and within exons, highlighting its role in regulating mRNA processing, alternative splicing, stability, and translation (Roundtree et al. 2017a, Kretschmer et al. 2018) and plays an important role in various cancers (reviewed in Niu et al. 2022).

m6A is dynamic and is added by a METTL3 'writer' complex at DRACH (RRACH) (D or R = A/G/U, R = A/G, H = A/C/U) consensus motifs and removed by 'erasers' FTO and ALKBH5 (Knuckles & Bühler 2018) (Fig. 1). FTO and ALKBH5 are α-ketoglutarate-dependent dioxygenases sensitive to D-2-hydroxyglutarate-mediated inhibition connecting glucose metabolism to m6A (Su et al. 2018). Demethylases FTO and ALKBH5 are the 'erasers' of m6A methylation (Zhao et al. 2020), although since FTO is nuclear, it may only demethylate m6Am in the 5' N-terminal cap of mRNA (Mauer et al. 2019). The role of m6A depends on the proteins that recognize and 'read' this marker, including YTHDC1-3, YTHDF1-3, and HNRNPA2B1 (Licht & Jantsch 2016, Lewis et al. 2017). An additional number of RNA-binding proteins and transcription factors, including glucocorticoid receptor (GR), also bind m6A (An et al. 2020).

The mechanisms of regulation and roles of epitranscriptomic mRNA modifications other than m6A are largely unknown (Zhao et al. 2020). Common epitranscriptomic marks in mRNA are involved in translation efficiency, i.e. m6A, m6Am, m1A, m5C, Ψ, and hm5C, RNA structure and stability, export, and degradation (Kumar & Mohapatra 2021) (Table 1). The biological roles of the epitranscriptome in human biology and disease and the writers, readers, and erasers of these modifications are of great research interest. New strategies are being developed to address the technical challenges in identifying and understanding the biological importance of the epitranscriptome.

**METTL3 methyltransferase complex**

METTL3 is the ‘writer’ of the m6A modification in mRNA. The METTL3 complex (875 kDa) includes the METTL3–METTL14 heterodimeric complex and auxiliary proteins that enhance the m6A methyltransferase activity: WTAP (Wilms tumor suppressor-1-associated protein),
### Table 1  Selected mRNA epitranscriptomics (Wiener & Schwartz 2021).

<table>
<thead>
<tr>
<th>Modification</th>
<th>Role in mRNA</th>
<th>Writer</th>
<th>Readers</th>
<th>Erasers</th>
<th>Role in breast cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ψ (pseudouridine)</td>
<td>Enriched in alternatively spliced regions (Martinez et al. 2022); enhanced mRNA translation (Hoernes et al. 2019); role in mRNA not well-understood (Borchardt et al. 2020)</td>
<td>PUS1, PUS3, PUS7, PUS7L, PUS10, DKC1, TRUB1 (Pereira et al. 2018, Anreiter et al. 2021, Chen et al. 2021a, b)</td>
<td>Unknown for mRNA (Levi &amp; Arava 2021)</td>
<td>Unknown for mRNA (Cerneckis et al. 2022)</td>
<td>Role in mRNA modification is unknown (Xue et al. 2022)</td>
</tr>
<tr>
<td>m5C (5-methylcytosine)</td>
<td>mRNA stability, translation, and export (Yang et al. 2017, Chen et al. 2021a, b); enriched in CG-rich regions in the 3'UTRs of genes and immediately downstream of translation initiation sites and has conserved, tissue-specific and dynamic features across mammalian transcriptomes (Yang et al. 2017)</td>
<td>NSUN2 (Yang et al. 2017)</td>
<td>ALYREF/THOC4, the mammalian mRNA export adaptor (Yang et al. 2017)</td>
<td>TET2 (Chen et al. 2021b)</td>
<td>m5C writer NSUN2 was upregulated in breast cancer and promoted proliferation, migration, invasion, and tumorigenicity (reviewed in Li et al. 2022c)</td>
</tr>
<tr>
<td>m1A (1-methyladenosine)</td>
<td>Affects mRNA structure and interaction with RNA-binding proteins (Dominissini et al. 2016)</td>
<td>TRMT6, TRMT61A, TRMT61B (mt) (Li et al. 2016b, Pereira et al. 2018)</td>
<td>YTHDF1–3 (Anreiter et al. 2021)</td>
<td>ALKBH1 (Liu et al. 2016), ALKBH3 (Chen et al. 2019c)</td>
<td>Demethylation of m1A promotes breast cancer invasion (reviewed in Kumari et al. 2021)</td>
</tr>
</tbody>
</table>
metTl3 methyltransferase complex proteins and their role in breast cancer.

<table>
<thead>
<tr>
<th>Protein (other names)</th>
<th>Function</th>
<th>Breast cancer activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>METTL3</td>
<td>m6A methyltransferase</td>
<td>Oncogene (Cai et al. 2018, Wang et al. 2020a, Pan et al. 2021)</td>
</tr>
<tr>
<td>METTL14</td>
<td>Heterodimer partner for METTL3 m6A methyltransferase</td>
<td>Tumor suppressor in TNBC (Shi et al. 2020)</td>
</tr>
<tr>
<td>WTAP</td>
<td>Stabilizes the METTL3-METTL14 heterodimer to nuclear speckles and interacts with splicing factors (Horiuchi et al. 2021)</td>
<td>Oncogenic (Sun et al. 2020)</td>
</tr>
<tr>
<td>CBLL1 (Hakai)</td>
<td>E3 ubiquitin ligase, but its enzymatic activity is dispensable for its function in stabilizing the METTL3 complex by interacting with WTAP and VIRMA (Zhang et al. 2022)</td>
<td>Oncogene, but inhibits metastasis (Wang et al. 2022)</td>
</tr>
<tr>
<td>VIRMA (KIAA1429)</td>
<td>Cofactor that guides the METTL3 complex to bind RNA</td>
<td>Tumor suppressor and low expression associated with TAM resistance (Zheng et al. 2021); higher expression associated with higher RFS (Zheng et al. 2021b)</td>
</tr>
<tr>
<td>ZC3H13 (KIAA0853)</td>
<td>Component of the WTAP complex</td>
<td>Oncogene (Qian et al. 2019); upregulated in breast tumors (Fang et al. 2021)</td>
</tr>
<tr>
<td>RBM15/15B</td>
<td>RNA-binding proteins that interact with METTL14</td>
<td>Oncogene, positively associated with ER/PR expression (Zhang et al. 2020); high expression correlated with reduced OS (Mu et al. 2021, Tai et al. 2022)</td>
</tr>
</tbody>
</table>

CBLL1 (Cbl proto-oncogene-like 1, Hakai), VIRMA (Vir-like m6A methyltransferase associated), RBM15 and RBM15B (RNA-binding motif protein 15), and ZC3H13 (zinc finger CCCH-type containing 13) (reviewed in Zaccara et al. 2019, Covelo-Molares et al. 2021) (Table 2). Crystal structure studies revealed that METTL3 and METTL14 interact over a large surface area via their methyltransferase domains (MTD) to bind RNA (Zaccara et al. 2019). METTL3 catalyzes the m6A methylation by taking the methyl from the cofactor S-adenosylmethionine (SAM) and releasing S-adenosylhomocysteine (SAH), while METTL14 is catalytically inactive (Wang et al. 2016, Zhou & Pan 2016). METTL14 stabilizes METTL3 and contributes to RRACH (R=A/G, H=A/U/C) binding site. METTL3/METTL14 was also shown to preferentially bind RNA G-quadruplex motifs (G4) that are enriched in human gene promoters, suggesting that G4s are involved in transcription regulation by the MTC (Yoshida et al. 2022). The MTC is localized to gene promoters to deposit m6A mRNA modification (Han et al. 2022). CBLL1 (Hakai) is an E3 ubiquitin ligase that interacts with WTAP and is required to writer complex interactions (Zaccara et al. 2019). ZC3H13 also interacts with WTAP and RBM15/15B and is required for m6A methylation (Zaccara et al. 2019). BioID, a proximity-dependent labeling approach in which a bait protein is fused to an E. coli biotin ligase (BirA*), is used to label proteins within a 10 nm radius (Liu et al. 2018) and when coupled to liquid chromatography-tandem mass spectrometry (LC-MS/MS), it was used to identify METTL3-interacting proteins in HEK-293T cells (Covelo-Molares et al. 2021). In addition to the proteins listed in Table 2, a number of other proteins involved in transcription, for example, STAT5B, ZEB2; chromatin remodeling, i.e. BCL7C; DNA replication, i.e. CHFR and MCMBP; and protein modification, for example, SUGT1, DNAJA2, FKBP4/5, were identified (Covelo-Molares et al. 2021). These results have not been validated or extended in other cell lines and in BC cells.

A novel circRNA from the METTL3 gene, circMETTL3 (623 nt originating from exon-2 and exon-3), was identified as highly expressed in breast tumors (Li et al. 2021b). Experiments in ZR-75-1 and SUM1315 BC cells...
demonstrated that siRNA-targeting METTL3 inhibited cell proliferation, migration, invasion, and xenograft tumor growth in vivo (Li et al. 2021b). circMETTL3 was reported to sponge miR-31-5p, thus increasing its target CDK1 which drives BC progression (Li et al. 2021b).

**MTC transcripts and proteins in breast cancer**

Examination of BC data in The Cancer Genome Atlas (TCGA) showed decreased transcript levels of METTL3, METTL14, and WTAP (1.575-, 1.200-, and 1.883-fold) in breast tumors compared to normal controls (Wu et al. 2019). Similar findings for METTL3, METTL14, and WTAP using the ONCOMINE BC data were reported; additionally, ZC3H13 transcript was also decreased, but RBM15B and VIRMA transcripts were increased (Zhang et al. 2020). qRT-PCR examination of 36 breast tumor samples (no histopathological data were included) identified significant reductions in the expression of METTL3, METTL14, and WTAP (Wu et al. 2019). These breast tumors had reduced global m6A levels in total RNA (primarily rRNA and tRNA with ~5% mRNA), although the inter-sample variability was high, with some tumor samples showing elevations in gene transcript and in m6A (Wu et al. 2019).

Other studies reported increased METTL3 in BC cells and tumors. Increased METTL3 mRNA expression in breast tumors correlated with increased expression of late endosomal/lysosomal adaptor, MAPK, and MTOR activator 5 (LAMTOR5, HBXIP) in BC cell lines and patient breast tumor arrays (TMA) (Cai et al. 2018). This study demonstrated that HBXIP increased let-7g which directly targeted and reduced METTL3 in MCF-7 luminal A (ER+/PR+, HER2-) BC cells and that METTL3-induced m6A methylation of HBXIP increased HBXIP protein expression (Cai et al. 2018). METTL3 transcript levels were higher in GEPIA database, breast tumors, and BC cell lines (including MCF-7 and MDA-MB-231 TNBC cells) compared to MCF-10A (immortalized ‘normal’ breast epithelial) cells (Wang et al. 2020a). Knockdown of METTL3 by shRNA (~60% decrease in protein) in MCF-7 and MDA-MB-231 cells inhibited cell proliferation and reduced BCL2, an apoptosis regulator (Wang et al. 2020a). High METTL3 transcript was associated with lower RFS in the Kaplan–Meier plotter (Gyorffy et al. 2010) used in the study (Wang et al. 2020a). Higher METTL3 protein correlated with higher levels of m6A (dot blot) in BC cell lines (MCF-7, T47D (luminal A), MDA-MB-231, SKBR3 (ER−/PR−/HER2(ERBB2)+, and BT474 (ER+/PR+/HER2+)) and knockdown of METTL3 (shRNA) increased sensitivity to growth inhibition by chemotherapeutics in vitro: ADR, 5-FU, cisplatin, paclitaxel, and carboplatin (Li et al. 2022b). METTL3 promoted dsDNA break repair and homologous recombination repair (HHR) after DNA damage induced by etoposide (ETO) and adriamycin in MCF-7 and MDA-MB-231 cell lines (Li et al. 2022b). The mechanism involved METTL3 m6A methylation of EGF which increased EGF translation via interaction with m6A reader YTHDC1. In turn, EGF increased the expression of RAD51, a DNA repair enzyme, RAD51 recombinase (Li et al. 2022b). Inhibiting DNA repair is an important target in BC to enhance the efficacy of chemotherapy, for example, the use of PARP1 inhibitors (McCann et al. 2019). METTL3 expression was upregulated in BC stem cells (BCSC) and increased the expression of AF4/FMR2 family member 4 (AFF4) which binds the MYC and SOX2 gene promoters to regulate ‘stemness’ (Gao et al. 2020).

METTL14 mRNA and protein expression was increased in breast tumors and some BC cell lines (Sun et al. 2020). LINC00942 (LINC942) upregulated in BC was shown to promote METTL14-mediated m6A methylation by interacting with METTL14 and upregulating the transcription of specific target genes, CXXCR4 and CYP1B1, that play roles in EMT and BC tumorigenesis (Sun et al. 2020). Upregulation of onecogenic IncRNA UCA1 in T47D BC cells inhibited METTL14 transcription by recruiting DNMT1 and DNMT3A/B to increase METTL14 promoter DNA methylation. Consequently, the decrease in METTL14 reduced m6A in pri-miR-375 which increased pre-miR-375 and mature miR-375 and reduced SOX12 translation, resulting in increased proliferation and invasion and reduced apoptosis (Zhao et al. 2022).

Aurora kinase A (AURKA) positively regulated METTL14 protein expression in BC cells in a protein kinase-independent manner by inhibiting its ubiquitination-mediated degradation to increase m6A content in the DROSHA mRNA transcript resulting in increased DROSHA mRNA stability and binding of IGF2BP2 in MDA-MB-231 BCSC (Peng et al. 2021).

Nuclear WTAP protein expression was higher in breast tumor specimen (n = 347) compared to noncancerous breast tissue (n = 23) with higher staining associated with tumor size >2 cm and higher tumor grade (II or III) and in ER tumors compared to TNBC which had the highest WTAP staining (Wang et al. 2022). We reported that WTAP transcript abundance was higher in LCC9 endocrine-resistant BC cells compared to MCF-7 parental cells (Klinge et al. 2019). Whether ER inhibits WTAP expression in a ligand-dependent manner is unknown. HER2 status had no correlation with WTAP staining (Wang et al. 2022). Interestingly, higher WTAP staining...
was observed in LN metastatic-negative compared to LN metastatic-positive breast tumors. The authors suggested this result to indicate a role for WTAP as a suppressor of metastasis; however, no metastatic tumors from distant site were examined (Wang et al. 2022). There was no association between WTAP protein expression and BC patient survival (Wang et al. 2022).

Hypoxia increases the expression of METTL14 in MDA-MB-231 cells (Panneerdoss et al. 2018). Knockdown of METTL14 inhibited the growth, migration, and invasion of MDA-MB-231, MDA-MB-468, and BT-549 TNBC cells in vitro and MDA-MB-231 tumor xenographs in vitro by ~30% (Panneerdoss et al. 2018). The authors found that METTL14 and m6A eraser ALKBH5 constitute a positive feedback loop with the RNA-binding protein HuR (ELAV1) to regulate the stability of target genes, i.e. cyclin D1, SMAD3, cyclin E1, TGFβ1, VEGFA, HMG2A, and PDGF, in MDA-MB-231 cells (Panneerdoss et al. 2018).

METTL3 methylation of m6A on pri-miRNAs (Alarcón et al. 2015b) and RNA-dependent interaction of the m6A reader HNRNPA2B1 with DGCR8, a component of the DROSHA complex, stimulate processing of selected pri-miRNA-m6A to precursor miRNA (pre-miRNA) (Alarcón et al. 2015a). METTL3 protein expression was increased in adriamycin-resistant MCF-7 BC cells (MCF-7/ADR) and enhanced pri-miR-221-3p m6A methylation and increased mature miR-221-3p expression which decreased homeodomain-interacting protein kinase 2 (HIPK2) protein expression in MCF-7/ADR cells (Pan et al. 2021).

VIRMA (KIAA1429) is the largest (202 kDa) component of the METTL3 complex and acts as a scaffold and bridges the METTL3/METTL14/WTAP complex with RNA and is upregulated in BC at the transcript and protein levels (Qian et al. 2019). Knockdown of VIRMA (KIAA1429) in MCF-7 and MDA-MB-231 BC cells inhibited proliferation, migration, invasion, and colony formation (Qian et al. 2019). VIRMA was shown to have the most copy number variations (CNV) among m6A readers, writers, and erasers (Zhao et al. 2021). Further studies are needed to validate this report.

RNA-binding proteins as m6A readers

While studies have identified altered m6A in transcripts in human disease, their potential mechanism of action and involvement in human pathologies is not well understood. A focus of m6A research has been characterizing the protein complexes responsible for identifying the reversible and dynamic m6A mark in mRNA and investigating the downstream effects resulting from m6A modification (reviewed in Zaccara et al. 2019, Zaccara & Jaffrey 2020). At the time of this review, 20 RNA-binding proteins (RBPs) have been identified to bind to m6A-methylated regions of the mRNA transcript and regulate transcript outcome for protein expression (Wang et al. 2014, Wang et al. 2015, Xiao et al. 2016, Du et al. 2016). m6A readers with known relevance to BC, their cellular location, and molecular functions are summarized in Table 3. These m6A readers have been shown to play a role in embryonic stem cell fate (Batista et al. 2014, Lasman et al. 2020) energy homeostasis (Edupuganti et al. 2017), human circadian rhythm (Robinson et al. 2019), and various cancers (Jing et al. 2021, von Hagen et al. 2021). Evidence suggesting that the dysregulation of m6A readers plays a pivotal role in BC progression will be described below.

YTH domain m6A-binding proteins

The earliest identified m6A readers contain a broadly conserved YTS21-B homology (YTH) domain with tryptophan residues forming a hydrophobic pocket that specifically recognizes and encapsulates m6A-containing RNA (Luo & Tong 2014). Five YTH domain-containing proteins have been identified in the mammalian genome; YTHDC1, YTHDC2 – and three YTHDF paralogs – YTHDF1, YTHDF2, and YHDF3 (reviewed in Zaccara & Jaffrey 2020). In 2012, the binding affinity of YTHDC1, YTHDF2, and YHDF3 to m6A was validated using affinity chromatography followed by mass spectrometry (MS) (Dominiessini et al. 2012). The direct binding of these reader proteins as well as YTHDC2 has been validated using individual nucleotide-resolution UV crosslinking and immunoprecipitation (iCLIP) (Patil et al. 2016). YTHDF family members are primarily cytoplasmic and contain the YTH domain located at the C-terminus as well as a larger domain enriched with Q, N, and P residues (Meyer & Jaffrey 2017). Studies suggest that m6A-containing mRNAs have a reduced half-life compared to mRNAs without m6A (Batista et al. 2014, Geula et al. 2015); however, each YTHDF paralog has different functions related to both mRNA stability and translation efficiency. Binding of YTHDF1 to m6A in the 3’ UTR recruits eukaryotic translation initiation factor 3 (eIF3) to facilitate 5’ cap and polyA tail addition, as well as the forming of the cyclic structure to promote translation (Wei et al. 2022). In contrast, binding of YTHDF2 to m6A sites in the 3’UTR leads to shortening of the polyA tail length and localizes m6A-modified mRNA transcripts to processing bodies in the cytoplasm, promoting mRNA

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Table 3 RNA-binding proteins identified as m6A readers. Each m6A reader's location, function, and role in breast cancer are listed.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Location</th>
<th>Cellular function</th>
<th>Additional roles in breast cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>YTHDF1</td>
<td>Cytoplasm</td>
<td>RNA translation efficiency (Wang et al. 2015)</td>
<td>YTHDF1 mediated translation of Sprd2, a gene that encodes for SPRD2, a negative regulator of ERK signaling, in murine bone marrow-derived macrophages (BMDMs) (Yin et al. 2021). Elongation and translation of KRT7, which is higher in BT-549 cells, are mediated by the YTHDF1/eEF-1 complex (Chen et al. 2021a). YTHDF2 is upregulated in TCGA-BRCA analysis of TNBC tumor tissue (Wang et al. 2020b).</td>
</tr>
<tr>
<td>YTHDF3</td>
<td>Cytoplasm</td>
<td>RNA degradation or translation efficiency (Li et al. 2017, Shi et al. 2017)</td>
<td>YTHDC1 is downregulated in TCGA-BRCA analysis of TNBC tumor tissue (Wang et al. 2020b).</td>
</tr>
<tr>
<td>YTHDC2</td>
<td>Cytoplasm</td>
<td>RNA translation efficiency (Hsu et al. 2017, Mao et al. 2019)</td>
<td></td>
</tr>
<tr>
<td>IGF2BP2</td>
<td>Cytoplasm</td>
<td>RNA stability (Simon et al. 2014, Dai et al. 2015, Pu et al. 2020)</td>
<td>Overexpression of IGF2BP2 (p62/IMP2) in MDA-MB-231 cells increased MYC expression and cell migration, while reducing cell adhesion (Li et al. 2015).</td>
</tr>
<tr>
<td>IGF2BP3</td>
<td>Cytoplasm</td>
<td>RNA stability (Yang et al. 2020)</td>
<td>IGF2BP3 interacts with circFOXK2 and miR-370 to promote cell migration and invasion in BT-549 cells (Zhang et al. 2021b). IGF2BP3 depletion repressed TRIM25 expression and reduced MCF-7 and MDA-MB-231 proliferation (Wang et al. 2019).</td>
</tr>
<tr>
<td>HNRNPA1</td>
<td>Nucleus</td>
<td>Alternative splicing (Loh et al. 2015)</td>
<td>HNRNPA1 is higher in breast, colorectal, and prostate cancer cells and is associated with increased expression of type I and type II methyltransferases (PRMTs) (Li et al. 2021a). Alternative splicing of CD44 was mediated by HNRNPA1 in MDA-MB-231 cells and was associated with metastatic progression (Loh et al. 2015).</td>
</tr>
<tr>
<td>HNRNPA2B1</td>
<td>Nucleus</td>
<td>Alternative splicing and miRNA processing (Alarcón et al. 2015a)</td>
<td>Based in TCGA breast cancer datasets, HNRNPA2B1 is increased with elevated histological grade and higher in MDA-MB-468 TNBC cells compared to MCF-7 cells (An et al. 2021). Plays a role in endocrine therapy resistance in an MCF-7 cell model (Klinge et al. 2019; Petri et al. 2021).</td>
</tr>
<tr>
<td>HNRNPC</td>
<td>Nucleus</td>
<td>m6A switch recognition, alternative splicing (Liu et al. 2015)</td>
<td>HNRNPC is upregulated in TCGA-BRCA analysis of TNBC tumor tissue (Wang et al. 2020b).</td>
</tr>
<tr>
<td>FMR1</td>
<td>Nucleus/cytoplasm</td>
<td>RNA trafficking, translation regulation (Darnell et al. 2011)</td>
<td>Genotype analysis of BRCA patients identified an association between FMR1 expression and the BRCA1/2 mutation (Tea et al. 2013).</td>
</tr>
<tr>
<td>LRPPRC</td>
<td>Nucleus/mitochondria</td>
<td>mRNA maturation (Cui et al. 2019)</td>
<td>LRPPRC suppression by miR-1 in stem cells isolated from MCF-7 cells induced mitochondrial damage (Zhang et al. 2019).</td>
</tr>
</tbody>
</table>

(Continued)
YTHDC1 is predominantly nuclear (Patil et al. 2016). An investigation of YTHDC1 nuclear-interacting proteins in HeLa cells revealed the enrichment of splicing enhancer-binding SR proteins, SRSF1 (ASF/ SF2), SRSF3 (SRp20), SRSF7 (9G8), SRSF9 (SRp30c), and SRSF10 (SRp38), identifying a role for YTHDC1 in alternative splicing (Xiao et al. 2016). The function of cytoplasmic YTHDC2 is less defined, but it includes a DEAD-box RNA helicase domain that may be targeted to specific mRNAs by m6A (Patil et al. 2016). A direct role for YTHDC2 in translation efficiency was discovered using luciferase-based tethered reporter assays in HeLa cells (Hsu et al. 2017).

**IGF2BP family m6A-binding proteins**

Insulin-like growth factor-2 (IGF2) mRNA-binding proteins (IGF2BPs) constitute a conserved family of single-stranded RBPs that specifically recognize m6A-RNA and influence transcript outcomes (Bell et al. 2013, Du et al. 2021, Korn et al. 2021). The role of IGF2BPs in regulating transcript outcomes has been studied since 2002, well before IGF2PBs were discovered to be m6A readers, and several synonyms for IGF2BP exist in the literature, including, but not limited to, IMP, CRD-BP, VICKZ, and ZBP (Kobel et al. 2012, Samuels et al. 2020, Zhou et al. 2022). These three IGF2BP proteins have a 56% aa sequence identity with two RNA-recognition motifs (RRMs) at their N-termini and hnRNP-K homology (KH) domains at their C-termini (Bell et al. 2013). This sequence homology suggests that the IGF2BP proteins share biochemical functions. IGF2BPs form ribonucleoprotein (RNP) granules in the cytoplasm and encapsulate their target mRNAs in protein–RNA complexes (mRNPs), preventing premature decay (Lemm & Ross 2002, Vikesaa et al. 2006, Kobel et al. 2007).

**IGF2BP family members in breast cancer**

IGF2BPs are involved in breast cancer proliferation, migration, and invasion since 2002, well before IGF2PBs were discovered to be m6A readers. A study in human astrocytes exploring the role of YTHDF2 in mRNA degradation showed that YTHDF3 influences proliferation and migration of glioma cells by suppressing UBX domain protein 1 (UBXN1), thus inducing NF-kB activation (Chai et al. 2021). The role of YTHDF3 is less clear, although it appears to share many targets with YTHDF1 and YTHDF2, suggesting dynamic and cooperative activity between the YTHDF proteins regulating translation and decay of common mRNAs (Shi et al. 2017).

Table 3 Continued.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Location</th>
<th>Cellular function</th>
<th>Additional roles in breast cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRRC2A</td>
<td>Nucleus/cytoplasm</td>
<td>RNA stabilization (Chen et al. 2019a)</td>
<td>PRRC2A is upregulated in breast cancer compared to normal samples in TCGA breast cancer data (Yang et al. 2021). A study in human astrocytes exploring the role of YTHDF2 in mRNA degradation showed that YTHDF3 influences proliferation and migration of glioma cells by suppressing UBXN1, inducing NF-kB activation (Chai et al. 2021). The role of YTHDF3 is less clear, although it appears to share many targets with YTHDF1 and YTHDF2, suggesting dynamic and cooperative activity between the YTHDF proteins regulating translation and decay of common mRNAs (Shi et al. 2017). YTHDC1 is predominantly nuclear (Patil et al. 2016). An investigation of YTHDC1 nuclear-interacting proteins in HeLa cells revealed the enrichment of splicing enhancer-binding SR proteins, SRSF1 (ASF/SF2), SRSF3 (SRp20), SRSF7 (9G8), SRSF9 (SRp30c), and SRSF10 (SRp38), identifying a role for YTHDC1 in alternative splicing (Xiao et al. 2016). The function of cytoplasmic YTHDC2 is less defined, but it includes a DEAD-box RNA helicase domain that may be targeted to specific mRNAs by m6A (Patil et al. 2016). A direct role for YTHDC2 in translation efficiency was discovered using luciferase-based tethered reporter assays in HeLa cells (Hsu et al. 2017).</td>
</tr>
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</table>
In human colon cancer cells, IGF2BP3 stabilized and prevented degradation of CCND1 and VEGFA, increasing proliferation and angiogenesis (Yang et al. 2020). Collectively, these studies established the role of IGF2BPs in mRNA stability and show that IGFBP1/2/3 bind to transcripts harboring the m6A mark and preventing mRNA degradation in human disease, including BC, as will be described below.

HNRNP m6A-binding proteins

The role of heterogeneous ribonucleoproteins (HNRNPs) in alternative splicing, mRNA packaging, and transport contributing to proteomic diversity in eukaryotes is well studied (Geuens et al. 2016, Kedzierska & Piekielko-Witkowska 2017). HNRNPs were first identified and characterized in HeLa cells as A, B, and C groups comprising HNRNP particles that form on all nascent transcripts (Beyer et al. 1977) and act as key proteins in mRNA biogenesis (Osheim et al. 1985, Choi et al. 1986). The major classes of HNRNPs each contain conserved domains that facilitate RNA-binding: the RRM, the quasi-RRM, an arginine-glycine-glycine (RGG) box, and a KH domain (Swanson & Dreyfuss 1988, Gorlach et al. 1992, Siomi & Dreyfuss 1995). HNRNPs A/B are divided into four subgroups: HNRNP A1, A2/B1, A3, and A0, though the more highly expressed A1 and A2/B1 are the most studied for the role they play in mRNA translation (Park et al. 2015), splicing (Mayeda & Krainer 1992), and trafficking (Shan et al. 2000). Only in the past 10 years, HNRNP proteins have been associated with m6A-RNA mechanisms. In 2012, Tavazoie and others investigated how HNRNPs stabilize mammalian mRNA (Goodarzi et al. 2012), and in 2015, they established a clear connection between HNRNPA2/B1 and m6A-RNA by showing that HNRNPA2/B1 recognizes m6A in mRNA in MDA-MB-231 cells to mediate alternative splicing (Alarcón et al. 2015a).

HNRNPA2/B1 was reported to bind to m6A on primary microRNA (pri-miRNAs) transcripts in MDA-MB-231 cells and interact with the DROSHA complex to induce pri-miR to premature (pre)-miR processing (Alarcón et al. 2015b). We reported that HNRNPA2B1 was increased in endocrine-resistant ER+ BC cells, played a role in sensitivity to tamoxifen and fulvestrant, and regulated miRNA expression (Klinge et al. 2019, Petri et al. 2021); this will be described in more detail below. HNRNPC contains only one RBD and forms self-oligomers for RNA binding (Swanson et al. 1987). In 2015, HNRNPC was reported to bind to a secondary RNA structure that occurs when m6A remodels RNA to form truncated hairpins. This m6A-remodeled structure, also known as an m6A-switch, creates accessible U-tracts which significantly improves HNRNPC binding affinity and facilitates RNA splicing (Liu et al. 2015c). HNRNPC is another HNRNP that takes advantage of the m6A-switch mechanism by preferentially binding to target transcripts remodeled by m6A. Depletion of HNRNPC in HEK-293T cells altered the expression and splicing of hundreds of transcripts altered by METTL3 or METTL14 knockout (Liu et al. 2017a). Together, these reports validate the connection between m6A-remodeling and HNRNP m6A-readers in regulating mRNA metabolism.

Other m6A-binding proteins

Additional RBPs have been identified and characterized as m6A readers. High-efficiency diazirine-based RNA photo-crosslinking of m6A-mRNA in HeLa cells combined with LC-MS/MS proteomics identified novel m6A-RNA–protein interactions, including the leucine-rich pentatricopeptide repeat containing LRPPRC and fragile X-messenger ribonucleoprotein 1 (FMR1) proteins as m6A-binders (Arguello et al. 2017). Gene set enrichment analysis from GEO datasets of lung adenocarcinoma patients found that downregulation of LRPPRC was correlated with immune response activation, suggesting that LRPPRC may regulate the tumor environment (Li et al. 2021c). FMR1 encodes FMRP, which contains an RGG domain that recognizes the m6A-modified RNA (Myrick et al. 2015). FMRP associates with CDS of transcripts, stalling ribosomes and regulating translation at the elongation stage (Darnell et al. 2011). Another RBP that recognizes m6A-RNA is staphylococcal nuclease-like (SN-like) domain-containing protein 1 (SN1), a member of the Tudor family of proteins that binds to methylated DNA. SN1 was first identified in Schizosaccharomyces pombe but is conserved from yeast to humans (Chen et al. 2011). Although the exact mechanism of the m6A/SN1 axis is not understood, a pan-cancer analysis of TGCA datasets shows a correlation between increased SN1 expression and poor OS and RFS in many cancers, including BC (Cui et al. 2020). Other RBPs with roles in translation have been identified as possible m6A readers. For example, eukaryotic initiation factor 3 (eIF3), a component of the 43S preinitiation complex, interacts with YTHDF1 or YTHDF3 at m6A-modified RNA in the 5’-end of transcripts (Tarun et al. 1997, Shi et al. 2017). However, binding of eIF3 to m6A in the 5’UTR of transcripts does...
not require YTHDF1, suggesting that eIF3 directly binds m6A (Meyer et al. 2015). The identification of RBPs that recognize m6A and their roles in transcriptional-translational regulation requires further investigation.

**m6A readers in breast cancer**

Dysregulation of m6A modification and the expression of m6A readers are associated with cancer progression (Bai et al. 2019, Liu et al. 2020b). Analysis of data from TCGA for YTHDF1 and YTHDF3 showed increased transcript expression of these readers in breast tumors compared to normal tissue that was correlated with reduced OS of BC patients (Anita et al. 2020). In a separate study, gene set enrichment analysis of microarray expression datasets from TCGA database showed distinct enrichment of the WNT signaling pathway in patient samples with high YTHDF1 expression and knockdown of YTHDF1 in MCF-7 cells reduced the expression of WNT pathway proteins (Hu et al. 2021). YTHDF1 and YTHDF3 were associated with lower OS and higher expression rates of genes related to c-Myc signaling and T cell differentiation in TCGA BC data (Li et al. 2022a). Despite evidence that YTH domain proteins play an important role in mRNA translation and stability in cancer, few studies have evaluated their specific role(s) in BC (Table 3). The REPIc (RNA Epitranscriptome Collection) database only lists one m6A-Seq/MetaRIP-seq data set from BC cells (Liu et al. 2020a). YTHDF1, with METTL14, promoted double-stranded break (DSB) DNA damage repair and YTHDF1 knockdown increased sensitivity to adriamycin, cisplatin, and olaparib in MDA-MB-231 cells (Sun et al. 2022). FOXM1, which promotes BC metastasis, is a YTHDF1 target and binding of YTHDF1 to FOXM1 mRNA for accelerated translation of FOXM1 in MCF-7 cells (Chen et al. 2022). Binding of YTHDF1 to PKM2 mRNA stabilized the transcript to increase translation efficiency, which increased glycolytic activity in MBA-MD-231 and MCF-7 cells (Yao et al. 2022).

Depletion of YTHDF2 increased apoptosis in MDA-MB-231 by increasing mitochondrial stress, proteotoxicity, endoplasmic reticulum stress (ERS), and the unfolded protein response (UPR) (Einstein et al. 2021b). Interestingly, eCLIP for YTHDF identified unique YTHDF target genes in MDA-MB-231, MCF-7, and SKBR3 cells that overlapped with m6A sites identified by m6A-RIP-seq (Einstein et al. 2021b). shYTHDF2 upregulated transcript levels of genes promoting cell migration, wound healing, EMT, and metastasis in MDA-MB-231 cells (Einstein et al. 2021b). In summary, YTHDF2 was demonstrated to bind m6A peak-containing transcripts for degradation in MDA-MB-231 cells, thus ‘safeguarding’ the cells from ERS and proteotoxicity (Einstein et al. 2021b). By binding m6A, YTHDF2 stabilized ATF3 transcripts, thus increasing ATF3 protein, a transcription factor that was identified as a potential driver of TAM resistance in TAM-resistant MCF-7 cells (Liu et al. 2021). A study evaluating TNBC tissue samples identified an association between YTHDF3 expression, lymph node metastasis, and higher histological grade (Lin et al. 2022). YTHDF3 was reported to increase the binding of eIF3 to ST6GALNAC5, GJA1, EGFR, and VEGFA mRNAs in MDA-MB-231 cells, suggesting YTHDF3-stimulated translation of key genes that drive BC brain metastasis (Chang et al. 2020). YTHDC1 overexpression in MDA-MB-231 cells promoted DNA replication and DNA damage repair and induced resistance to the adriamycin (Zhou et al. 2021). Taken together, these studies suggest that the YTH domain family of m6A readers plays a dynamic role in altering the expression of genes in pathways associated with BC progression and metastasis.

MYC is an important oncogenic transcription factor in TNBC (Fallah et al. 2017) and MYC mRNA has been reported as a downstream target of IGF2BP proteins (Huang et al. 2018) as well as YTHDF1 (Zheng et al. 2022). Binding of IGF2BPs by FGFI3-ASI, a long ncRNA (lncRNA) decreases glycolysis and stemness in MDA-MB-468 cells and prevents MYC mRNA stabilization by IGF2BPs (Ma et al. 2019). IGF2BPs also interact with circCD44 to stabilize MYC mRNA in BT549 cells (Li et al. 2021d). Further evidence for IGF2BP regulation of MYC mRNA in BC is listed in Table 3.

IGF2BP1 bound directly to m6A sites on MIR210HG, an oncogenic lncRNA that promotes proliferation and EMT in MCF-7 cells (Shi et al. 2022b). Similar associations between IGF2BP1 and other lncRNAs with roles in breast tumor progression have been established, for example, upregulation of LINC00483, an oncogenic lncRNA associated with advanced tumor stage (Qiao et al. 2021). IGF2BP2 stabilized DROSHA mRNA for translation in MDA-MB-231 cells and DROSHA induced the expression of genes promoting sphere formation and cell stemness, i.e. STC1, GRB10, and SLCO4A1 (Peng et al. 2021). METTL3 and IGF2BP3-mediated m6A modification increased PD-L1, a cell surface immune checkpoint inhibitor, in MDA-MB-231 and HCC38 cells (Wan et al. 2022). These studies characterizing IGF2BPs and their interaction with m6A in target transcripts suggest lncRNA-epitranscriptomic regulation of BC progression and drug resistance.

The role of HNRNPs in BC progression and drug-resistance is being actively investigated (Klinge et al. 2019, An et al. 2021, Li et al. 2021a, Petri et al. 2021). There are
16 HNRNP family members named alphabetically A–U classified by their RNA-binding domains and have diverse structural features and functions (reviewed in Geuens et al. 2016). HNRNPA2B1 expression is higher in breast tumors compared to normal breast tissue (Liu et al. 2019, Ma et al. 2020) and regulates stability, alternative splicing, DNA repair, and telomere maintenance (Liu & Shi 2021). HNRNPA2B1 plays a role in the processing of selected pri-miRNAs to pre-miRNAs in HEK-293T cells (Alarcón et al. 2015b). HNRNPA2B1 stimulated miRNA processing in MDA-MB-231 cells by recognizing m6A on pri-miRNAs and interacting with the DROSHA complex protein DGCR8, to increase pri-miRNA processing to pre-miRNAs (Alarcón et al. 2015b). However, some studies suggest that HRNPA2B1 may also interact with negative regulators of miRNA biogenesis and suppress miRNA processing (Suster & Feng 2021). Analysis of the miRome in MCF-7 cells with overexpressed HNRNPA2B1 was found to upregulate and downregulate hundreds of miRNAs and identified dysregulated pathways associated with BC progression, metastatic spread, and TAM resistance (Klinge et al. 2019). Knockdown of HNRNPA2B1 inhibited proliferation in MCF-7 and MDA-MB-231 BC cells by downregulating signal transducer and activator of transcription 3 (STAT3) and extra-cellular-signal-regulated kinase 1/2 (ERK1/2) (Hu et al. 2017). In addition, overexpression of HNRNPA2B1 increased endocrine resistance, cell motility, and stem cell properties in MCF-7 cells, whereas knockdown of HNRNPA2B1 restored TAM and fulvestrant sensitivity to endocrine-resistant LCC9 and LY2 BC cells (Petri et al. 2021). Investigating other HNRNPs has revealed novel m6A regulatory mechanisms in BC. For example, HNRNPC expression promoted cell proliferation by inducing MAPK signaling in MDA-MB-231 and MCF-7 cells (Lv et al. 2021). HNRNPK promotes invasion and metastasis in MDA-MB-231 and MDA-MB-468 cells by interacting with PROX1 and stimulating the WNT pathway (Zhu et al. 2022).

Emerging roles have been identified for other m6A-associated RBPs in BC progression. FMR1 and LRPPC are overexpressed in BC tissue data from TCGA compared to normal tissue (Zhang et al. 2020). Additionally, GTEx data revealed a higher expression of LRPPC in breast tissue (Zhang et al. 2020). SND1 is upregulated in breast primary invasive ductal carcinomas and induced RhoA degradation, thus disrupting F-actin cytoskeletal organization, by promoting expression of Smurf1, an E3 ubiquitin ligase (Yu et al. 2015). The role of elf3 subunits in BC tumorigenesis has been well-documented (Fan & Guo 2015, Zheng et al. 2016, Han et al. 2020, Morris et al. 2021) and there is clear evidence for an m6A–eIF3 interaction (Meyer et al. 2015). Further studies investigating RBPs involved in BC progression, drug resistance, and metastasis are necessary to define their role in m6A-mediated RNA metabolism in BC.

**m6A erasers in breast cancer**

ALKBH5 and FTO are members of a conserved family of AlkB non-heme Fe(II)/2-oxoglutarate (2OG)-dependent dioxygenases with nine ALKB homologues with diverse substrates and specificities (reviewed in Rajecka et al. 2019). Knockout phenotypes for ALKBH5 and FTO are divergent: infertility and impaired spermatogenesis vs growth retardation, lean phenotype, and impaired adipogenesis, respectively (reviewed in Rajecka et al. 2019) ALKBH5 and FTO were described as the two mammalian non-heme Fe2+ and α-ketoglutarate (αKG)-dependent dioxygenases that act as m6A mRNA demethylases that catalyze the oxidation of m6A in a two-step reaction that requires O2 and generates CO2, succinate, and Fe(III) and ultimately yields adenosine and formaldehyde (reviewed in Meyer & Jaffrey 2014, Purslow et al. 2021).

**ALKBH5**

There is evidence that ALKBH5 is the specific m6A demethylase, whereas FTO is the m6Am demethylase (Mauer et al. 2017, Mauer & Jaffrey 2018, Mauer et al. 2019). Hypoxia (1% O2) increased ALKBH1 expression in MCF-7 luminal A and in MDA-MB-231, SUM159, and MDA-MB-435 TNBC cell lines in a HIF-1α- and HIF-2α-dependent manner and reduced m6A methylation in total RNA (Zhang et al. 2016). Further experiments in MCF-7 and MDA-MB-231 cells revealed that ALKBH5 demethylated m6A in NANOG mRNA which increased total NANOG mRNA transcript levels and enriched BCSC populations in these cell lines (Zhang et al. 2016). NANOG is a pluripotency factor that promotes BC tumorigenesis and metastasis (Lu et al. 2014). Knockdown of ALKBH5 in MDA-MB-231 cells reduced mammary tumor growth in vivo (Zhang et al. 2016). Immortalization of primary human mammary epithelial cells (HMECs) by stable expression of hTERT, P5330D, Cyclin D1, CDK4R24C, or C-MYC58A increased FTO and ALKBH5 while decreasing METTL3 protein expression (Fry et al. 2018). siALKBH5 increased overall m6A levels and led to reduced long-term viability, migration, and invasion of MDA-MB-231 cancer cells (Panneerdoss et al. 2018). Doxorubicin treatment increased m6A levels in breast tumor patient samples, but overexpression of PRMT5 (protein arginine...
methyltransferase S) expression in MDA-MB-231 BC cells decreased m6A levels and decreased m6A methylation of BRCA1 to increase BRCA1 stability by increasing ALKBH5 nuclear translocation through an interaction with ALKBH7 (Wu et al. 2022). The drug tadalafl was recently demonstrated to inhibit PRMT5 methyltransferase activity and reduce ALKBH5 nuclear localization (Wu et al. 2022). Tadalafl increased total RNA m6A methylation and specific m6A methylation on the 3′-UTR of BRCA1 and decreased BRCA1 mRNA stability and protein expression, enhancing the doxorubicin-induced MDA-MB-231 cell apoptosis (Wu et al. 2022). These findings suggest that blocking nuclear localization of ALKBH5 may enhance chemotherapeutic responses in BC.

FTO

FTO was originally identified in genome-wide association studies as having a modest, but significant, contribution to childhood and adult obesity (Blakemore & Froguel 2008). FTO was reported to share motifs with Fe(II)- and 2-oxoglutarate-dependent oxygenases and showed high mRNA expression in the brain, notably in the hypothalamic nuclei and in the arcuate nucleus that regulates satiety and feeding behaviors (Church et al. 2009). FTO was identified as an m6A demethylase in vitro and in HeLa and HEK-293T cell studies (Jia et al. 2011). Case–control analysis of 354 breast tumors and 364 controls revealed that 3 single nucleotide polymorphisms (SNPs) in intron 1 of FTO were associated with BC risk (Kaklamani et al. 2011). Another study found an association with an intron 1 SNP in FTO (rs9939609) with BC risk in a large study (62,328 BC cases and 83,817 controls) of women of European ancestry (Zhao et al. 2016). In women of African ancestry, FTO SNP rs17817449 was associated with both overall BC and ER+ disease (Feng et al. 2017). Reduced FTO transcript expression was associated with TAM resistance and early distant relapse time in an analysis of two datasets (GSE26971, GSE58644) (Zhou et al. 2018).

FTO localizes to nuclear speckles in HeLa cells (Jia et al. 2011) and MCF-7 cells and was shown to affect RNA modifications including the 3-methyluridine/uridine and pseudouridine/uridine ratios in total brain RNA (Berulava et al. 2013). Transgenic mice with a CRISPR/Cas9-engineered mutation modeling the human FTO-R316Q mutation (mouse FTO-R313A) resulting in a catalytically inactive FTO protein showed reduced bone mineral density and content, reduced body length and mass, and reduced viability (Sachse et al. 2018). Bovine milk consumption was suggested to be an epigenetic regulator of FTO transcription via milk exosome-containing bovine miR-29s that downregulate human cell DNA methyltransferases (DNMTs), thus reducing CpG island DNA methylation in the FTO gene and increasing FTO mRNA and protein (Melnik 2015). Persistent consumption of cow’s milk is associated with hepatocellular cancer, luminal A BC, prostate cancer, and Parkinson’s disease by milk exosomal regulation of mTORC1 signaling (Melnik 2021).

Conclusions as to whether FTO is increased or decreased in breast tumors compared to normal breast depend on the type of study (transcripts vs protein) and publication (Table 4 and below). FTO transcript expression was higher in MCF-7 compared to MDA-MB-231 and HCC1937 TNBC cell lines (Liu et al. 2017b). In silico examination of FTO transcript expression (GSE9014) found FTO upregulated in breast tumors and protein was higher in IHC staining of three breast tumors vs normal breast, correspondingly, an m6A dot blot showed lower m6A levels in breast tumors compared to normal breast (Niu et al. 2019). Likewise, examination of Oncomine data identified increased FTO and ALKBH5 in breast tumors (Wu et al. 2019). However, neither FTO nor ALKBH5 transcript expression was associated with OS in the TCGA BC dataset (Chang et al. 2020). Analysis of BC and normal breast tissues in the RNA-sequencing expression data in GEPIA found lower FTO but no difference in ALKBH5 in breast tumors (Shi et al. 2020). However, the distance-metastasis-free survival (DMFS) of the FTO high-expression TNBC patients was shorter than that of the FTO low-expression group, indicating that FTO is a risk factor for DMFS in TNBC.

High FTO transcript abundance was associated with ER+/PR−/HER2+ and higher grade (II and III) breast tumors and with lower OS (Niu et al. 2019, Xu et al. 2020). SKBR3 and MDA-MB-453 ER+/PR−/HER2+ BC cells had higher FTO than MCF-10A, MCF-7, T47D, BT474, MDA-MB-231, or BT549 BC cells (Xu et al. 2020). Lentiviral-mediated stable knockdown of FTO in MCF-7 and MDA-MB-231 cells inhibited cell proliferation and colony formation and reduced cell apoptosis (Niu et al. 2019). Likewise, shFTO inhibited 4T1 mouse mammary tumor growth in vivo (Niu et al. 2019). RNA-seq in shFTO-MDA-MB-231 cells identified Bnip3, an apoptosis inhibitor, as upregulated by FTO knockdown in an m6A-modification-dependent manner (Niu et al. 2019). Thus, the authors concluded that increased FTO reduced m6A in the Bnip3 mRNA resulting in lower Bnip3 protein and increased BC cell proliferation (Niu et al. 2019). Similarly, knockdown of FTO by siRNA in SKBR3 and MDA-MB-453 cells inhibited cell migration and invasion, although not cell proliferation. Overexpression of FTO in SKBR3 and MDA-MB-453 cells stimulated
Table 4  m6A erasers ALKBH5 and FTO in breast cancer.

<table>
<thead>
<tr>
<th>Protein (other names)</th>
<th>Function</th>
<th>Breast cancer</th>
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| ALKBH5 (AlkB homolog 5, RNA demethylase) | Dioxygenase that specifically demethylates m6A (Zheng et al. 2013). ALKBH5 is mainly nuclear and is specifically the m6A demethylase (Mauer et al. 2017). | • IHC staining showed increased ALKBH5 in breast cancer tissues compared to normal breast tissue (Liu et al. 2019).  
• ER+ or PR+ status was associated with high mRNA levels of ALKBH5 (Wu et al. 2019).  
• Transcript expression was downregulated in breast cancer compared to adjacent normal breast (Zhang et al. 2020).  
• No difference in transcript expression between breast tumors and normal breast tissue (Shi et al. 2020, Zhao et al. 2021).  
• Copy number loss in breast tumors (Yang et al. 2021). No difference in copy number variations or somatic mutations of ALKBH5 in breast cancer (Zhao et al. 2021).  
• IHC showed FTO staining was both nuclear and cytoplasmic and higher in breast cancer than adjacent normal tissue and the percent of FTO-positive expression was higher in TNBC and HER2+ than ER+/PR+ breast cancer (Tan et al. 2015).  
• Another study found reduced FTO IHC staining in breast carcinoma tissues compared to normal breast tissue (Liu et al. 2019).  
• Lower transcript expression in breast cancer tissues compared to normal tissue (Zhang et al. 2020, Zhao et al. 2021). |
| FTO (fat mass- and obesity-associated gene (FTO) alpha-ketoglutarate-dependent dioxygenase) | RNA demethylase mediates oxidative demethylation of different RNA species, such as mRNAs, tRNAs, and snRNAs, and acts as a regulator of fat mass, adipogenesis, and energy homeostasis (Jia et al. 2011). FTO targets m6Am (Mauer et al. 2017, Mauer & Jaffrey 2018, Mauer et al. 2019). FTO also demethylates 3-meT in ssDNA and 3-meU in ssRNA (reviewed in Chen & Du 2019). FTO demethylates m6A, m6Am, and m1A in the nucleus and cytoplasm (Wei et al. 2018). |  |

cell migration and invasion and decreased total m6A abundance (Xu et al. 2020). RNA-seq in FTO-knockdown SKBR3 cells identified miR-181b-3p as upregulated and ARSLB (ADP ribosylation factor-like GTPase SB) as a direct target of miR-181b-3p, although a direct role for FTO removal of m6A in downregulating miR-181b-3p, thus increasing ARSLB in BC was not proven (Xu et al. 2020).  
FTO was reduced, while METTL3 and m6A levels were increased in ‘Lung metastasis breast cancer cells’ MDA-MB-231MF3 and BT-549MF3 which are TNBC cell lines established by three rounds of tail vein injection and ‘lung metastasized cell’ re-injection (Chen et al. 2021a). However, chemical inhibitors of FTO (CS1 and CS2 at 0.1 and 1 µM) inhibited leukemia stem/initiating cell self-renewal and MDA-MB-231 cell proliferation in vitro and as tumor xenografts in vivo (Su et al. 2020). While this finding suggests that inhibition of FTO may inhibit BC metastasis, the study of the lung metastatic MDA-MB-231 cells suggests caution. More research is needed on mechanism and role of FTO in metastatic disease.

m6A in mRNA and its detection
m6A modification of mRNA regulates RNA splicing, stability, translation, and intracellular localization and plays a role in many human diseases including cancers (breast, lung, hepatocellular, renal, prostate, gastric, bladder, and colorectal) and other diseases, for example, osteoporosis and type II diabetes (reviewed in Destefanis et al. 2021, Lan et al. 2021). The number of m6A in transcripts is variable, some mRNAs can contain 20 m6A sites, generally found in genes that regulate development and cell fate (Zaccara et al. 2019).

The most common methods to detect m6A in mRNA were recently reviewed (Zaccara et al. 2019, Moshtitch-Moshkovitz et al. 2022). These include m6A-RNA immunoprecipitation (RIP)-seq (called meRIP-seq, m6A-RNA-seq, or m6A-seq), in which an m6A antibody is used to identify bound transcripts by Illumina Seq; m6A- cross-linking and immunoprecipitation (CLIP)-seq which employs UV cross-linking to identify m6A.
sites at higher specificity compared to m6A-seq; miCLIP (modified CLIP) that relies on inducing specific mutations (C to T conversions) during RT (Roberts et al. 2021), LC-MS (Thüring et al. 2016) and deamination adjacent to RNA modification targets (DART-seq) that uses a fusion protein consisting of the m6A-binding YTH domain tethered to the cytidine deaminase APOBEC1 to direct C-to-U editing at cytidine residues that invariably follow m6A sites (Meyer 2019, Tegowski et al. 2022). Direct RNA sequencing by Oxford nanopore-based single-molecule sequencing, which maintains and directly detects nucleic acid modifications as a single strand of RNA, passes through a pore (Kono & Arakawa 2019, Lorenz et al. 2020, Parker et al. 2020, Leger et al. 2021), without the need for added reagents or processing, i.e. antibodies specific for m6A for RNA immunoprecipitation (RIP)-seq or cDNA generation which introduce biases and only localizes the m6A mark within ~100 nt, rather than the precise position of the m6A (Pratanwanich et al. 2021). A computational method (xPore) was demonstrated to identify m6A positions at single-base resolution, within the DRACH/RRACH motif (Jenjaroenpun et al. 2020), from nanopore reads (Pratanwanich et al. 2021). The nascent RNA m6A methylome was also identified by methylation inscribed nascent transcripts sequencing (MINT-Seq) to capture nascent RNAs RNA metabolic labeling used in TT-Seq combined with m6A-RIP-seq (Lee et al. 2021). An m6A-Atlas of the m6A epitranscriptome includes m6A sites in human, mouse, rat, and other species, as well as 46 human cell lines (Tang et al. 2021). Using the m6A-Atlas, m6A, m1A, m5C, m6Am, m7G, and Ψ modifications were identified in 2294 gene transcripts involved in pharmacokinetic and pharmacodynamics, for example, drug-metabolizing enzymes and transporters, receptors, and targets (Liu et al. 2022b). Based on their analysis, the authors concluded that m6A is a biomarker for anti-tumor drugs in TNBC (Liu et al. 2022b).

**Roles for m6A mRNA modification in cellular functions**

As indicated above, the presence and position of m6A in mRNA transcripts regulate stability, splicing, and intracellular location by the association of reader proteins with the m6A mark. Recently, YTHDC1 and unknown m6A readers recognized m6A and recruited KDM3B and other demethylases of H3K9me2, to the chromatin regions around the transcription start site of CYP2B6 in Huh-7 and HepaRG hepatoma cells, resulting in increased transcription (Isono et al. 2022). This suggests that protein interactions between chromatin modifiers and m6A readers are potential mechanism for transcript in initiation as well as transcript stability and splicing. m6A is involved in the maintenance of the ‘stemness properties’ of cancer stem cells and the interaction between CSC and the tumor immune microenvironment (TIME) (reviewed in Zhang et al. 2021d). m6A modification of MYC mRNA regulates transcript stability by binding YTHDF1 to increase translation (Zheng et al. 2022). Interestingly, m6A modification on mRNA accumulates within 2 min at sites of DNA damage in U2OS cells generated by global UVC irradiation, allowing recruitment of DNA polymerase kappa (Pol κ) (Xiang et al. 2017). Tonicity-responsive enhancer-binding protein (TonEBP) was demonstrated to recruit METTL3 to R-loops, three-stranded DNA–RNA hybrids with ssDNA, to methylate m6A and stimulate homologous recombination (HR) repair in YTHDC1-dependent manner at DNA double-stranded breaks (Zhang et al. 2020). There are few studies of how transcript-specific m6A affects gene expression or pathways in BC. A recent study reported increased expression of ALKBH5 in HER2+ SKBR3 and BT474 BC cell lines that are trastuzumab- and lapatinib-resistant and in breast tumors from patients who responded poorly to these treatments (Liu et al. 2022c). m6A-RIP seq identified m6A peaks in the SKBR3 and BT474 and their HER2 therapy-resistant derivatives and correlated increased ALKBH5 with increased m6A demethylation and expression of EGFR, FOXO1, and GLUT4 in the resistant cells. RIP-qPCR identified YTHDF2–m6A interaction stabilized the GLUT4 mRNA transcript. m6A demethylation of GLUT4 by the increase in ALKBH5 in the resistant cell lines resulted in increased glycolysis and ritonavir, an FDA-approved HIV protease inhibitor that also inhibits GLUT4, restored sensitivity to trastuzumab in the resistant cells (Liu et al. 2022c).

**Identification of m6A sites in genes in breast cancer cells**

There are relatively few studies identifying m6A sites in genes in BC cell lines and few cell lines have been studied (Table 5). Most studies have used some form of m6A-RIP-seq to identify m6A peaks and a few have knocked down METTL14 or METTL3 as a control to verify m6A identification. In MDA-MB-231 TNBC cells, 15,981 and 17,312 m6A peaks from 6796 and 7194 m6A-containing transcripts in control and METTL14-silenced MDA-MB-231 cells, respectively (Panneerdoss et al. 2018). METTL14 target genes including TGFβ1, SMAD3, cyclin E1,
<table>
<thead>
<tr>
<th>Method of m6A-RIP-seq</th>
<th>Breast cancer cell line(s)</th>
<th>Other treatments</th>
<th>Comments, reference, and data availability</th>
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<tbody>
<tr>
<td>Magna MeRIP™ m6A kit (Millipore) Illunina ONT Nanopore direct RNA-seq with xPore computational analysis m6A-enhanced cross-linking and immunoprecipitation (m6A-eCLIP)</td>
<td>MDA-MB-231</td>
<td>sIMETTL14</td>
<td>GEO database GSE81164 (Panneerdoss et al. 2018) The data are available through the ENA (PRJEB40872) (Pratanwanich et al. 2021) Observed divergent numbers of m6A peaks in the three replicates: GEO database GSE147440 (Roberts et al. 2021) m6A-seq analysis identified 47,539 and 38,383 m6A peaks from 6284 to 5373 m6A-modified transcripts in BT-549 and BT-549®®®® cells, respectively (Chen et al. 2021a); KRT7 and KRT7-AS showed increased expression and m6A deposition and increased transcript stability by binding IGF2BP1/HuR and increased interaction of YTHDF1/EIF-1 with KRT7 mRNA to increase elongation in BT-549®® cells (Chen et al. 2021a) Unique and common m6A peaks were identified when comparing MDA-MB-231 vs MCF-7 or SKBR3 cells and between MCF-7 and SKBR3 cells (Einstein et al. 2021a); GEO: GSE137258 No information about data availability (Wan et al. 2022)</td>
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<tr>
<td>Magna MeRIP™ m6A kit (Millipore) Illunina HiSeq 2000 system</td>
<td>MCF-7 with two replicates</td>
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<td>MDA-MB-231</td>
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<td>MCF-7 and MDA-MB-231</td>
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<td>Performed three biological replicates/cell line</td>
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<td>BT-549 and BT-549® cells</td>
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<td>MDA-MB-231</td>
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<td>BT-549® cells</td>
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<td></td>
<td>Myc-Induced human mammary epithelial cells (HMECs); MDA-MB-231, MDA-MB-231-LM2, MCF-7, SKBR3 cells in biological duplicates</td>
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<td></td>
<td>+/- shMETTL3 or IGF2BP2</td>
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<tr>
<td>M6A-RIP-seq (Mouse mAb anti-m6A (Synaptic Systems; 202 011)-Illumina HiSeq4000</td>
<td>MYC-induced human mammary epithelial cells (HMECs); MDA-MB-231, MDA-MB-231-LM2, MCF-7, SKBR3 cells in biological duplicates</td>
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<td>Magna MeRIP™ m6A kit (Millipore) Illunina HiSeq X Ten platform</td>
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<td>MDA-MB-231</td>
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Cyclin D1, MMP9, VEGFA, and HMGA2 were found to be hyper m6A methylated and showed decreased expression in METTL14 KDa cells, suggesting that increased m6A methylation may increase the stability of these transcripts in MDA-MB-231 cells (Panneerdoss et al. 2018). Another study in MDA-MB-231 cells knocked down either METTL3 or the reader IGF2BP2 to identify specific m6A peaks by m6A-RIP-seq and reported that most m6A peaks were identified in the CDS (Wan et al. 2022). Epitranscriptomic microarray and meRIP-seq identified 30 sequences from 24 genes that harbored both hypo-methylation and downregulated m6A peaks after shMETTL3 in MDA-MB-231 cells including PD-L1 (CD274). IGF2BP2 reduced the CD274 transcript and PD-L1 protein, suggesting that m6A deposition and binding by IGF2BP2 enhance transcript stability, and m6A-RIP-qPCR identified binding of IGF2BP3 to CD274 mRNA in an m6A-dependent manner (Wan et al. 2022). In MCF-7 cells, knockdown of METTL3 and METTL14 increased ERα and YTHDC1 protein levels (Lee et al. 2021), which can have secondary effects on measured outcomes. m6A-enhanced cross-linking and immunoprecipitation (m6A-eCLIP) was developed to specifically identify m6A sites in MCF-7 and MDA-MB-231 cells, although the authors neither describe the m6A-containing genes nor compare the two cell lines (Roberts et al. 2021). Secondary lung metastatic MDA-MB-231-LM2 tumor cells showed more m6A peaks (14,873 peaks) and m6A-peak modified genes (4512 genes) that were unique from the parental MDA-MB-231 cell line; however, MYC-induction of HMECs did not alter m6A peaks (Einstein et al. 2021a). Unique and common m6A peaks were identified when comparing MDA-MB-231 vs MCF-7 or SKBR3 cells and between MCF-7 and SKBR3 cells; however, the genes containing different numbers of m6A peaks in the different cell lines were not discussed (Einstein et al. 2021a). Direct RNA seq using nanopore and xPore identified m6A sites in transcripts from HEK-293T cells and compared those in transcripts from cancer cell lines: MCF-7, K562 (leukemia), HCT116 (colon cancer), A549 (non-small cell lung cancer), and HepG2 (hepatocellular) (Pratanwanich et al. 2021). The number of m6A sites was lower in MCF-7 cells compared to the other cell lines; however, because the paper was focused on the computational analysis, the authors neither discuss which transcripts showed reduced m6A nor interpret their data relative to breast tumor samples.

Using MINT-seq, -three to five times more m6A peaks were identified in the nascent transcripts of MCF-7 cells.
Compared to MeRIP-seq (Lee et al. 2021). The authors reported pervasive m6A signals on nascent RNAs in MCF-7 cells, including 2207 enhancer RNAs (eRNAs) and 1201 upstream antisense RNA (uarRNAs) (eRNAs and uarRNAs are ncRNAs whose expression is correlated with the activity of functional enhancers by modifying chromatin accessibility at promoters, stabilizing enhancer-promoter interactions, transcription factor-DNA interactions, and RNA Pol II stability (reviewed in Kim et al. 2015, Li et al. 2016a, Cardiello et al. 2020). Approximately 18.8% of putative active eRNAs contained one or more m6A peaks (Lee et al. 2021). The m6A-marked transcripts had higher abundance, suggesting the m6A increased eRNA stability. Estradiol (E2, 100 nM for 24 h) did not significantly alter m6A peak levels in the 226 eRNAs induced by E2 in MCF-7 cells (Lee et al. 2021). The authors concluded that m6A modification is ‘largely hard wired to nascent sequences based on sequences, for example, the RRACH motif’ (Lee et al. 2021), as originally proposed (Darnell et al. 2018). The RRACH motif f (R = A, G; H = A, C, U) (Jenjaroenpun et al. 2020) is recognized by the METTL3 complex (Licht & Jantsch 2016, Knuckles & Bühler 2018).

Conclusions and future directions

Abundant data support the role of epitranscriptomic modification of mRNA and the readers, writers, and erasers of m6A in various diseases and cancers, including BC. The number of publications on m6A in BC is doubling each year (Fig. 2) with 64 papers in 2022 at the time of submission of this review. Whether m6A is ‘hard wired’ by the RRACH motif (Darnell et al. 2018) or modified depending on regulation of the METTL3 complex, other RNA-binding proteins, RNA structure, intranuclear localization, for example, condensates, and the epigenome remain to be examined in a cell- and tissue-type manner in BC. How the ‘expososome’, including the microbiome, environmental chemical exposures, and endocrine- and metabolism-disrupting chemicals (Koul et al. 2020, Koval et al. 2022), affect these events to regulate the epitranscriptome in BC is unknown. The advent of direct RNA sequencing and single-cell RNA sequencing promises to yield new understanding of epitranscriptomics not only of m6A but less abundant modifications in BC progression, endocrine therapy resistance, and metastasis. For example, identifying specific sites of m6A that impact on transcript stability and reader binding will be important in determining the role of m6A on endocrine-resistance in BC as seen for HER2 resistance and GLUT4 expression (Liu et al. 2022c).

Future studies to determine the mechanism by which gene- and position-specific m6A sites affect transcript stability and protein abundance, may employ inducible Crispr/dCas13b-METTL3 and Crispr/dCas13b-ALKBH5 programmed to respectively methylate and demethylate specific m6A sites on a transcript (Li et al. 2020, Zhang et al. 2021c, Shi et al. 2022a). The regulation and selectivity, role of specific cell types within a breast tumor and the tumor microenvironment, nuclear-mitochondrial or retrograde signaling of epitranscriptomic regulation of mRNA transcript processing and pathway regulation remains to be elucidated.

Declaration of interest

The authors declare no conflict of interest.

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Author contribution statement

BJP wrote the Readers section and edited the manuscript. CMK wrote all other sections, edited and revised the manuscript.

References


Arguello AE, Deliberto AN & Kleiner RE 2017 RNA chemical proteomics reveals the N(6)-methyladenosine (m6A)-regulated protein-RNA interactome. Journal of the American Chemical Society 139 17249–17252. (https://doi.org/10.1021/jacs.7b09213)


Ling Z, Chen L & Zhao J 2020 m6A-dependent up-regulation of DRC1 by METTL3 and ELAVL1 promotes growth, migration, and colony formation in osteosarcoma. Bioscience Reports 40 BSR20200282. (https://doi.org/10.1042/BSR20200282)


Roberts JT, Pormann AM & Johnson AM 2021 Identification of m6A residues at single-nucleotide resolution using eCLIP and an accessible custom analysis pipeline. RNA 27 527–541. (https://doi.org/10.1261/rna.078543.120)


Roundtree IA, Evans ME, Pan T & He C 2017a Dynamic RNA modifications and DR2. Cell 168 87–100. (https://doi.org/10.1016/j.cell.2017.05.045)

Research and Treatment 191 291–302. (https://doi.org/10.1007/s10549-021-06423-0)


Yao X, Li W, Li L, Li M, Zhao Y, Fang ZX, Zeng X & Luo Z 2022 YTHDF1 upregulation mediates hypoxia-dependent breast cancer growth and metastasis through regulating PKM2 to affect glycolysis. Cell Death and Disease 13 258. (https://doi.org/10.1038/s41419-022-04711-1)


Yoshida A, Oyoshi T, Suda A, Futaki S & Imanishi M 2022 Recognition of G-quadruplex RNA by a crucial RNA methyltransferase component,