In response to environmental changes, signaling pathways rewire gene expression programs through transcription factors. Epigenetic modification of the transcribed RNA can be another layer of gene expression regulation. N6-adenosine methylation (m6A) is one of the most common modifications on mRNA. It is a reversible chemical mark catalyzed by the enzymes that deposit and remove methyl groups, m6A recruits effector proteins that determine the fate of mRNAs through changes in splicing, cellular localization, stability, and translation efficiency. Emerging evidence shows that key signal transduction pathways including TGFβ (transforming growth factor-β), ERK (extracellular signal-regulated kinase), and mTORC1 (mechanistic target of rapamycin complex 1) regulate downstream gene expression through m6A processing. Conversely, m6A can modulate the activity of signal transduction networks via m6A modification of signaling pathway genes or by acting as a ligand for receptors. In this review, we discuss the current understanding of the crosstalk between m6A and signaling pathways and its implication for biological systems.

Keywords: ERK, mTOR, N6-methyladenosine, RNA modification, signaling, TGFβ

INTRODUCTION

N6-methyladenosine (m6A) is a methylation modification of adenosine on RNA. m6A is evolutionarily conserved, ranging from yeasts, plants, insects to mammals (Meyer and Jaffrey, 2017; Yue et al., 2019). In mammalian cells, m6A is detected on 0.1%-1% of adenosines in mRNA with an average of 2-3 sites per transcript (Perry et al., 1975). Transcriptome-wide sequencing revealed that m6A occurs in the consensus motif DRA*CH (D = A, G, or U; R = A or G; A* = m6A-modified A; H = A, C, or U) (Dominissini et al., 2012; Meyer et al., 2012). Considering that DRACH appears once every ~57 nucleotides in mRNA, many transcripts have the potential to be modified with m6A. Nevertheless, only 20%-30% of coding genes are methylated in cells (Dominissini et al., 2012; Meyer et al., 2012), indicating a specific site selection mechanism of m6A modification.

Indeed, cells tightly regulate m6A modification using specialized enzymes, m6A writers and erasers. The m6A-modified RNAs then recruit m6A-binding proteins (readers) that guide these RNAs for RNA biogenesis processes such as pre-mRNA splicing, nuclear export, stabilization, degradation, and translation. Aberrant m6A modifications by overactivation or suppression of these enzymes lead to human diseases such as cancer, diabetes, and neurological disorders. There are comprehensive review papers about the molecular functions of m6A enzymes (Meyer and Jaffrey, 2017; Wiener and Schwartz, 2021; Zaccara et al., 2019) and their pathophysiological functions (Barbieri and Kouzarides, 2020; He and He, 2021; Huang et al., 2020; Kasowitz et al., 2018). In this review, we will focus on how the signal transduction pathways, which play key roles in diverse physiological and pathological conditions, coordinate cellular processes through m6A. Given that m6A also controls signaling pathways through RNA modification or acting as a ligand, understanding the crosstalk be-
tween signal transduction networks and m6A RNA processing will provide us insights into the complex biological systems.

**THE m6A PROCESSING PROTEINS: WRITER, ERASER, AND READER**

The m6A methyltransferase (writer) consists of the enzymatic m6A-methyltransferase like (METTL) complex (MAC) and the scaffolding MAC-associated complex (MACOM) (Fig. 1). METTL3 is a catalytic core of MAC, which methylates target mRNAs on the adenosine of DRACH sequence (Bokar et al., 1994; Dominissini et al., 2012; Meyer et al., 2012). METTL14 acts as a scaffolding protein of MAC by recognizing the substrate RNA and interacting with Wilms’ tumor 1-associated protein (WTAP) of MACOM (Bujnicki et al., 2002; Liu et al., 2014; Ping et al., 2014). MACOM consists of several adaptor proteins including WTAP, VIRMA (vir-like m6A methyltransferase associated), RBM15 (RNA-binding motif protein 15), HAKAI, and ZC3H13 (zinc finger CCCH domain-containing protein 13) (Knuckles et al., 2018; Patil et al., 2016; Růžička et al., 2017; Śledź and Jinek, 2016; Wang et al., 2021; Yue et al., 2018). The MACOM complex does not have catalytic activity, but it is necessary for efficient m6A methylation by recruiting RNA substrates and stabilizing the MAC-MACOM complex in the nucleus and nuclear speckles.

Similar to DNA and histone methylations, m6A RNA methylation is a reversible process regulated by the demethylase enzymes (erasers): Fat mass and obesity-associated protein (FTO) and AlkB homolog 5 (ALKBH5) (Jia et al., 2011; Zheng et al., 2013) (Fig. 1). They belong to AlkB homolog iron(II) and αKG-dependent dioxygenases, which include nine proteins with different substrate preferences toward single-stranded (ss) or double-stranded (ds) DNA and RNA substrates (Guengerich, 2015). Interestingly, although FTO and ALKBH5 exhibit comparable catalytic activities for m6A demethylation on ssRNA, the reaction steps are quite different. While ALKBH5 directly converts m6A to adenosine, FTO produces two intermediates N6-hydroxymethyladenosine (hm6A) and N6-formyladenosine (fm6A) during the demethylation process (Chen et al., 2014; Fu et al., 2013). This could be one of the reasons why FTO can demethylate another m6A-related modification, N6,2’-O-dimethyladenosine (m6Am) (Maurer et al., 2017; Zhang et al., 2019). In contrast to the tissue-specific function of ALKBH5 in testes (Zheng et al., 2013), FTO is expressed in most tissues and involved in various human diseases including diabetes, obesity, and several cancers (Hirayama et al., 2020; Losman et al., 2020; Song et al., 2019; Zhao et al., 2014). Therefore, the substrate pools and specificity of FTO may dynamically change depending on the cellular context. Studies illuminating the predominant substrate of FTO in specific tissues and pathophysiological conditions will be needed.

The m6A binding proteins that determine the fates of m6A-methylated mRNA are classified as readers, which include several proteins such as YT521-B homology (YTH) and insulin-like growth factor-2 mRNA-binding protein (IGF2BP) family proteins (Fig. 1). As a common function, YTHDF1/2/3 promote the degradation of m6A-containing mRNAs (Lee et al., 2020; Patil et al., 2018; Wang et al., 2014; Zaccara and Jaffrey, 2020). YTHDF1 and YTHDF3 facilitate protein translation of m6A-methylated mRNAs by promoting ribosome assembly (Li et al., 2017; Shi et al., 2017; Wang et al., 2015). YTHDF2 undergoes liquid-liquid phase separation with mRNAs containing multiple m6A residues (Ries et al., 2019; Wang et al., 2014). Similar to YTHDF1 and YTHDF3, YTHDC2 induces degradation of m6A-modified mRNAs while enhancing their translation efficiency (Hsu et al., 2017; Mao et al., 2019; Tanabe et al., 2016; Wojtas et al., 2017; Zhou et al., 2021). The last YTH family protein, YTHDC1, facilitates pre-mRNA splicing and nuclear export of m6A-modified mRNAs (Kasowitz et al., 2018; Roundtree et al., 2017a; 2017b; Xiao et al., 2016; Xu et al., 2014). In contrast to the YTH family proteins, IGF2BP family proteins increase both the stability and translation efficiency of m6A-modified mRNAs, maximizing the expression of m6A-modified genes (Huang et al., 2018).

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**Fig. 1. Key players of the m6A RNA modification process.** The deposition of m6A on mRNA is mediated by the writer complex which consists of m6A-METTL complex (MAC) and scaffolding MAC-associated complex (MACOM). MAC includes METTL3, a catalytic core protein, and METTL14, a scaffolding protein, which methylates adenosine in the consensus motif (DRACH, D = A, G, or U; R = A or G; A = m6A-modified A; H = A, C, or U). MACOM consists of adaptor proteins including Wilms’ tumor 1-associated protein (WTAP), VIRMA (vir-like m6A methyltransferase associated), RBM15 (RNA-binding motif protein 15), HAKAI, and ZC3H13 (zinc finger CCCH domain-containing protein 13). FTO and ALKBH5 demethylate m6A (erasers). The m6A binding proteins (readers) include YT521-B homology (YTH) and insulin-like growth factor-2 mRNA-binding protein (IGF2BP) family proteins, which determine the fate of m6A-methylated mRNA such as splicing, nuclear export, stability, and translation. The chemical structure of m6A is shown in the circle.
TRANSFORMING GROWTH FACTOR-β (TGFβ) SIGNALING REWIRES GENE EXPRESSION PROGRAM THROUGH m6A MODIFICATION

TGFβ signaling pathway plays an essential role in cell fate decisions, including pluripotency maintenance, differentiation, senescence, apoptosis, and tumorigenesis (Derynck and Zhang, 2003). TGFβ family proteins are ligands for TGFβ receptors (TGFβR), which includes TGFβ, nodal, activin, bone morphogenetic protein (BMP), and growth differentiation factor (GDF) (Derynck and Zhang, 2003; Zhang et al., 2017). The activated TGFβR phosphorylates downstream signaling proteins, the receptor-regulated SMADs (R-SMADs). Each TGFβ family ligand activates distinct R-SMADs. For example, BMP and GDF promote the phosphorylation of SMAD1, SMAD5, and SMAD8 (Hata and Chen, 2016). On the other hand, TGFβ, nodal, and activin promote SMAD2 and SMAD3 phosphorylation (Hata and Chen, 2016). The phosphorylated R-SMADs form a heterodimer such as SMAD2-SMAD3 (SMAD2/3), which subsequently binds with a common binding partner SMAD4 (co-SMAD). The SMAD complex then moves into the nucleus and associates with transcription factors and chromatin remodeling proteins to induce transcription of target genes (Derynck and Zhang, 2003; Hata and Chen, 2016) (Fig. 2).

In addition to their well-established role in transcription, a novel function of TGFβ-SMAD signaling has been revealed in m6A modification (Fig. 2). TGFβ ligands activin and nodal maintain embryonic stem cell (ESC) stemness through SMAD2/3-dependent pluripotency gene expression (James et al., 2005). On the other hand, considering the rapid differentiation of ESCs upon TGFβ withdrawal, ESCs would have also developed some mechanisms for such a rapid, efficient transition. From interactome analysis of SMAD2/3, Bertero et al. (2018) found that the phosphorylated SMAD2/3 interacts with the m6A writer complex, METTL3, METTL14, and WTAP. m6A methylation of SMAD2/3 target genes, Nanog, Nodal, and Lefty1, leads to degradation of these transcripts, thereby inducing ESC differentiation. In line with their findings, Mettl3 knockout induces prolonged Nanog expression and impaired differentiation in ESCs (Batista et al., 2014; Geula et al., 2015).

Interestingly, SMAD2/3 do not directly control the activity of m6A writer complex (Bertero et al., 2018). Instead, SMAD2/3 induce m6A methylation of its target genes by recruiting the m6A writer complex to the active transcription sites (Fig. 2). In another study, Huang et al. (2019) showed that trimethylation of histone H3 at Lys36 (H3K36me3) recruits m6A writer complex to the active transcription elongation sites. It may seem odd that transcription factors and elongation markers recruit m6A enzymes to label newly transcribed mRNAs with m6A for degradation. However, this priming system would be most efficient when a timely cell fate transition is required. For example, in early development of zebrafish embryos, maternal mRNAs are marked with m6A and degraded by the YTHDF2 reader protein during the maternal-to-zygotic transition (Zhao et al., 2017).

TGFβ signaling also triggers m6A modification of target genes for epithelial-mesenchymal transition (EMT) of cancer cells (Fig. 2). TGFβ treatment increases m6A levels in diverse cancer cells, including cervical, liver, breast, and lung cancers. Particularly, TGFβ induces methylation of SNAIL mRNA, an important transcription factor in EMT. The methylated SNAIL mRNA binds to an m6A reader protein, YTHDF1, which then induces translation of SNAIL by recruiting eEF-2 (eukaryotic elongation factor-2). Interestingly, METTL3 depletion stabilized SNAIL mRNA, implying that m6A modification promotes its degradation while inducing translation (Lin et al., 2019). Further investigation is needed to examine whether these opposite effects of m6A on SNAIL mRNA fates are mediated solely by YTHDF1 or through other m6A reader proteins that are activated by TGFβ signaling.

REGULATION OF m6A PROCESSING ENZYMES BY EXTRACELLULAR SIGNAL-REGULATED KINASE (ERK)

ERK is a member of the mitogen-activated protein kinases (MAPKs) family (Roberts and Der, 2007). MAPK pathway is a phosphorylation cascade composed of GTPase-activated kinase (MAPKKK) that phosphorylates and activates an intermediate kinase (MAPKK), which in turn phosphorylates and activates the effector kinase (MAPK) (Lavoie et al., 2020). In the ERK MAPK pathway, the epidermal growth factor (EGF) binds to the receptor tyrosine kinase, EGF receptor (EGFR), to activate RAS GTPase (Boriack-Sjodin et al., 1998). Then the

Fig. 2. TGFβ controls gene expression through m6A modification. Upon TGFβ stimulation, SMAD2/3 interact with METTL3, METTL14, and WTAP, to induce m6A methylation and degradation of pluripotency genes for differentiation of embryonic stem cells. On the other hand, in cancer cells, TGFβ induces SNAIL mRNA methylation during EMT. The methylated SNAIL mRNA binds with YTHDF1, which induces SNAIL translation through interaction with a translation elongation factor eEF-2 (eukaryotic elongation factor-2).
GTP-loaded RAS promotes the kinase activity of RAF (MAPKK), which is followed by MEK (MAPK) activation (Lavoie and Therrien, 2015). Finally, MEK activates ERK (MAPK) that controls a wide range of cellular processes by phosphorylating downstream target proteins (Wee and Wang, 2017). Because of its key role in regulating cell proliferation, survival, and differentiation, ERK signaling is one of the frequently activated signaling pathways in human cancers (Davies et al., 2002; Li et al., 2018).

The activity of RNA processing enzymes such as RNA polymerase, splicing enzymes, and translation factors are often regulated by phosphorylation (Nosella and Forman-Kay, 2021; Thapar, 2015). In a recent paper, Sun et al. (2020) provided direct evidence for the phosphorylation-dependent regulation of m^6A processing enzymes. To find a new regulator of m^6A modification, the authors performed a CRISPR knockout screen using a GFP reporter system that contains m^6A modification site on a circular RNA sequence. Once the GFP RNA is assembled by back splicing of the circular RNA (Yang et al., 2017), m^6A methylation on that RNA drives translation and expression of GFP. From the screen, several genes in the MAPK signaling pathway were identified (Sun et al., 2020). Mechanistically, it turns out that the effector protein of MAPK signaling pathway, ERK, phosphorylates m^6A writer proteins METTL3 and WTAP. The ERK-dependent phosphorylation of METTL3 and WTAP strengthened their interaction; however, ERK did not influence the binding between METTL3 and METTL14 (Sun et al., 2020). The association between METTL3 and METTL14 is strong and not affected by other phosphorylation events either, including a serine residue of METTL14 that forms a salt bridge with METTL3 (Schöller et al., 2018; Wang et al., 2016).

Even though the binding of METTL3-METTL14 was not regulated by ERK-dependent phosphorylation, ERK controlled the activity of MAC complex through METTL3 stabilization (Sun et al., 2020) (Fig. 3). While the non-phosphorylated METTL3 is degraded by ubiquitination, the phosphorylated METTL3 recruits ubiquitin-specific protease 5 (USP5) which removes ubiquitin from METTL3. From the m^6A-GFP reporter CRISPR screen, Sun et al. (2020) also found several E3 ubiquitin ligases that decrease m^6A levels. Knockdown of ubiquitin ligase candidates SPOP, ANAPC1, or TRIM28 restored METTL3 expression. Depletion of SPOP or ANAPC1 decreased K11 and K48 ubiquitination of METTL3, the ubiquitination sites targeted by USP5 (Sun et al., 2020). In contrast to the negative effect of TRIM28 on m^6A modification on the m^6A-GFP reporter, TRIM28 did not affect global m^6A levels (Yue et al., 2018). Considering that TRIM28 was identified as an interacting protein of the m^6A writer complex, TRIM28 may regulate m^6A modification of specific target genes by localizing the writer complex to the target transcripts like MACOM complex proteins.

In addition to m^6A writers, ERK-dependent regulation of the m^6A reader protein is reported (Fig. 3). Fang et al. (2021) found that ERK phosphorylation status correlates with YT-HDF2 expression level in the glioblastoma tissues. Upon EGF stimulation or EGFR overexpression, ERK phosphorylates YT-HDF2 to induce stabilization of YT-HDF2. The stabilized YT-HDF2 degrades m^6A-modified liver X receptor alpha (LXRA) and human immunodeficiency virus type 1 enhancer binding protein 2 (HIVEP2) genes, which elevates cholesterol uptake and proliferation of glioblastoma cells (Fang et al., 2021).

Under stress conditions, such as heat shock, m^6A modification of mRNA is globally increased (Meyer et al., 2015; Ries et al., 2019; Zhou et al., 2015). Yu et al. (2021) found that m^6A rapidly accumulates upon reactive oxygen species (ROS) stress, within five minutes. Such rapid response indicates that ROS may directly influence the activity of m^6A enzymes. Surprisingly, ERK signaling plays a crucial role in this stress response by promoting sumoylation of m^6A eraser, ALKBH5 (Fig. 3). In hematopoietic stem and progenitor cells, ROS-induced ERK and another MAPK, JNK (c-Jun N-terminal kinase), phosphorylate ALKBH5. ALKBH5 phosphorylation promotes its interaction with UBC9, a SUMO E2 conjugating enzyme, and disassociates ALKBH5 from the deSUMOylase SENP1. Consequently, the increased sumoylation on ALKBH5 prevents its binding with the substrate mRNAs and leads to the elevation of m^6A levels on mRNAs (Yu et al., 2021). Among the m^6A readers, the mRNA stabilizing reader, IGF2BP (Huang et al., 2018), plays a major role in ROS-ERK-ALKBH5-dependent gene expression regulation. In response to ROS, IGF2BP1/2/3 stabilize mRNA of FA core complex association protein 20 (FAAP20) and alpha-thalassemia/mental retardation X-linked (ATRX), which are critical enzymes for DNA repair under oxidative stress (Yu et al., 2021). These seminal studies show that ERK signaling pathway insistently controls m^6A modification process through the regulation of writers, readers, and erasers, to rewire gene expression programs in diverse physiological and pathological conditions.

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**Fig. 3. Dynamic regulation of m^6A enzymes by ERK.** METTL3 phosphorylation by ERK inhibits its degradation by recruiting ubiquitin-specific protease 5 (USP5). ERK-mediated METTL3 phosphorylation also enhances the interaction between METTL3 and WTAP (bottom panel). ERK stabilizes YT-HDF2 through phosphorylation (right panel). ALKBH5 phosphorylation by ERK sustains its sumoylation and induces disassociation of ALKBH5 from m^6A-modified mRNA (left panel).
ACTIVATION OF m6A WRITER COMPLEX BY MECHANISTIC TARGET OF RAPAMYCIN COMPLEX 1 (mTORC1) SIGNALING

mTORC1 is a serine/threonine kinase that promotes anabolic process including synthesis of proteins, nucleic acids, and lipids (Saxton and Sabatini, 2017; Shimobayashi and Hall, 2014). In response to extracellular stimuli such as growth factors and nutrients, PI3K-Akt initiates the signaling cascade that activates mTORC1. Akt inhibits tuberous sclerosis complex (TSC) 1/2, a GTPase activating protein that inhibits mTORC1-activating small GTPase Rheb (Saxton and Sabatini, 2017; Shimobayashi and Hall, 2014). To turn on the gene expression program, mTORC1 activates RNA processes from transcription, splicing, to translation through its downstream proteins including ribosomal protein S6 kinase (S6K), SRPK (serine/arginine-rich protein specific kinase), elf4B (eukaryotic translation initiation factor 4B), and 4E-BP (elf4E-binding protein) (Lee et al., 2017; Ma and Blenis, 2009).

mTORC1’s role in promoting m6A RNA modification has also been elucidated. Cho et al. (2021) and Villa et al. (2021) found that mTORC1 increases expression of the MACOM component, WTAP (Fig. 4). mTORC1/S6K enhances WTAP translation through elf4A/4B-dependent unwinding of secondary structure in WTAP’s 5’-untranslated region. The elevation of WTAP expression enhances m6A methyltransferase activity (Cho et al., 2021; Villa et al., 2021). In another paper, Tang et al. (2021) found that mTORC1-mediated activation of the chaperonin protein, chaperonin containing tailless complex polypeptide 1 (CCT) complex, stabilizes the MAC component proteins, METTL3 and METTL14 (Fig. 4). In addition to regulating enzyme expression, mTORC1 promotes m6A modification by increasing S-adenosylmethionine (SAM) level (Villa et al., 2021) (Fig. 4). SAM is a methyl donor for m6A, whose increase can stimulate the activity of methyltransferase enzymes (Bokar et al., 1997; Kim and Lee, 2021; Tuck, 1992). mTORC1 induces SAM synthesis through cMyc-dependent expression of MAT2A (methionine adenosyl transferase 2A) (Villa et al., 2021). SAM has been shown to induce condensation of METTL3 through liquid-liquid phase separation and promotes the association of MAC with WTAP at nuclear speckles (Han et al., 2022). Therefore, induction of SAM levels could be another way of increasing the activity of m6A methyltransferase complex by mTORC1. Further studies are necessary to elucidate how mTORC1-dependent expression and localization changes of MAC (METTL3 and METTL14) and MACOM (WTAP) proteins coordinate m6A processing in physiological and disease conditions.

Surprisingly, mTORC1-induced m6A modification induced degradation of mRNAs, which seems to be the opposite of canonical mTORC1 function in promoting macromolecule synthesis. However, those mRNAs methylated by mTORC1 include cell growth-suppressing genes such as cMyc suppressor and autophagy machinery (Cho et al., 2021; Tang et al., 2021). For example, mTORC1 induces methylation of MAX dimerization protein 2 (MXD2), which is followed by YTHDF2/3-mediated degradation of MXD2 mRNA (Cho et al., 2021). MXD2 is a cMyc inhibitor that competes with cMyc for binding with a transcription activator MAX (Mathsyaraja et al., 2019; Schreiber-Agus et al., 1995). The decreased MXD2 expression results in cMyc activation, which induces the proliferation of cancer cells derived from diverse tissues including kidney, breast, lung, and colon (Cho et al., 2021). These findings demonstrate a complex interplay of mTORC1, cMyc, and m6A signals in tumorigenesis.

REGULATION OF CELLULAR SIGNALING BY m6A

In the signal transduction cascade, feedback inhibition of upstream signaling by the downstream components plays crucial role in preventing overactivation of signal transduction cascade (Mendoza et al., 2011). Such negative feedback loops are often hijacked by cancers to promote cell proliferation and survival. From a genome-wide sequencing study of endometrial cancers, Liu et al. (2018) found that METTL14 is frequently mutated in cancers with a predominant mutation of arginine 298 to proline. The arginine 298 locates in the RNA binding domain of METTL14 and mutation of this residue decreases m6A methylation activity of the MAC complex (Śledź and Jinek, 2016; Wang et al., 2016). In the endometrial cancers that do not contain METTL14 loss-of-function mutations, the expression of METTL3 is decreased, indicating that decreasing the activity of MAC either by decreasing METTL3 expression or through METTL14 mutation promotes repression of m6A RNA modification in breast cancers with METTL14 loss-of-function mutations (Cho et al., 2021).

Fig. 4. mTORC1 activates the activity of m6A writer complex.

mTORC1 activates the m6A writer complex in three ways. mTORC1-mediated activation of chaperonin protein, chaperonin containing tailless complex polypeptide 1 (CCT) complex, stabilize METTL3 and METTL14 (middle panel). mTORC1 induces WTAP expression through eukaryotic initiation factor 4A (elf4A)/4B-dependent translation (right panel). mTORC1 also stimulates S-adenosylmethionine (SAM) synthesis through cMyc-mediated upregulation of MAT2A (methionine adenosyl transferase 2A) (left panel).
endometrial cancers (Liu et al., 2018). Transcriptome-wide m^6^A sequencing of endometrial tumors revealed that m^6^A modification is decreased in the group of genes that regulate Akt activity. Interestingly, the decreased m^6^A modification of Akt phosphorylation-inducing genes, such as mTOR, proline rich protein 5 (PRR5), and PRR5-like (PRR5L), led to stabilization of those transcripts. In contrast, the decreased m^6^A modification of Akt phosphatase, PH domain leucine rich repeat protein phosphatase 2 (PHLPP2), reduced translation of PHLPP2. Together, these changes increase Akt phosphorylation and thus activate Akt downstream signaling for proliferation of endometrial cancer cells (Liu et al., 2018) (Fig. 5A). In another study, using a reverse phase protein microarray assay (RPMA), Vu et al. (2017) found that the activity of Akt signaling pathway components is increased by METTL3 knockdown in leukemia cells. Specifically, the decreased m^6^A modification of phosphatase and tensin homolog (PTEN) mRNA, a negative regulator of Akt, decrease PTEN translation; and, the decreased PTEN expression lead to activation of Akt signaling in METTL3 knockdown cells (Vu et al., 2017) (Fig. 5A).

In addition to the gene expression regulation, an unexpected function of m^6^A has been uncovered as an extracellular signaling molecule. Considering the existence of G-protein coupled receptors (GPCR) that are activated by nucleotide ligands such as adenosine receptor (AR) (Borea et al., 2015), Ogawa et al. (2021) performed a screen to identify a new nucleotide ligand for ARs. From the screen, several adenosine derivatives including 1-methyladenosine (m^1^A), m^6^A, and m^6^Am activated adenosine A3 receptor (A3R), with m^6^A being the most potent activator. In fact, m^6^A activated A3R approximately 10-fold higher than adenosine, with EC_{50} (half maximal effective concentration) of 10 nM in contrast to that of adenosine being 100 nM. The ligand binding domain of A3R has hydrophobic amino acids including valine, leucine, and isoleucine, which could form van der Waals interactions with the methyl group on m^6^A. In contrast, adenosine would be less stable in the ligand binding pocket due to the lack of those intermolecular interactions. Indeed, when the ligand binding pocket of other ARs was mutated to contain those hydrophobic amino acids, they were also activated by m^6^A (Ogawa et al., 2021).

When m^6^A was treated onto A3R-expressing cells, the AR downstream signals such as ERK and intracellular calcium transport were induced, which was abolished by AR antagonist. Upon cytotoxic stresses such as ROS that activate AR signaling, m^6^A was produced in cells by lysosomal degradation of mRNA and rRNA to initiate the m^6^A-AR-ERK signaling (Ogawa et al., 2021). Although Ogawa et al. (2021) tested the activity of only single nucleoside m^6^A molecules, it is possible that m^6^A-containing oligonucleotides can also act as a ligand for GPCRs. The RNase T2 ribonucleases in the lysosome generate both mono- and oligo-nucleotides (Fujisawa et al., 2017) which can be secreted outside of the cells. It will be exciting to dissect the potential roles of m^6^A-containing nucleic acids as receptor-binding signaling molecules in various developmental and disease processes.

**CONCLUDING REMARKS**

The regulation of m^6^A modification by multiple signaling pathways demonstrates cells’ abilities to dynamically determine their fate by rewiring gene expression via post-transcriptional gene modifications beyond the gene transcription level. Although research has begun to identify the effects of individual signaling pathways on m^6^A processing, there remains open questions regarding the potential for cross-talk between interwoven signaling pathways. For example, phosphorylation of METTL3 by ERK signaling induces m^6^A methylation of pluripotency genes such as Nanog, Klf2, Sox2, and Lefty1, which results in degradation of these transcripts and mouse ESC differentiation (Sun et al., 2020). In another study, Bertero et al. (2018) observed that upon TGFβ stimulation, the transcription factors SMAD2/3 bind with METTL3 to promote m^6^A modification and degradation of pluripotency genes including NANOG and LEFTY1. Considering that ERK-mediated METTL3 phosphorylation strengthens its interaction with WTAPE and USP5 (Sun et al., 2020), the phosphorylated METTL3 could also interact with other proteins. Future work will be needed to elucidate whether METTL3 phosphorylation induces its interaction with SMAD2/3 and triggers m^6^A modification of SMAD2/3 target genes, which could be the nexus between TGFβ and ERK signals for stem cell differentiation. Building a comprehensive signaling map for the m^6^A-dependent gene expression program will provide
ACKNOWLEDGMENTS
We thank members of the Lee laboratory for helpful discussions, especially Laurence Seabrook, Yujin Chun, and Sunhee Jung, for their feedback on the manuscript. We apologize to authors whose work was not cited due to space limitations. This work was supported by the Department of Defense grant TS200022.

AUTHOR CONTRIBUTIONS
K.-H.J., C.R.H., and G.L. researched data for the review. C.R.H. made substantial contributions to the m6A processing enzyme section and figures. K.-H.J. made substantial contributions to the rest of the review. K.-H.J., C.R.H., and G.L. wrote, reviewed, and edited the manuscript.

CONFLICT OF INTEREST
The authors have no potential conflicts of interest to disclose.

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