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RNA modifications regulating cell fate in cancer

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The deposition of chemical modifications into RNA is a crucial regulator of temporal and spatial gene expression programs during development. Accordingly, altered RNA modification patterns are widely linked to developmental diseases. Recently, the dysregulation of RNA modification pathways also emerged as a contributor to cancer. By modulating cell survival, differentiation, migration and drug resistance, RNA modifications add another regulatory layer of complexity to most aspects of tumourigenesis.

Currently, over 170 RNA modifications are known, and most RNA species contain one or multiple distinct chemical modifications[1]. Determining the function of these modifications in RNA metabolism requires their reliable detection at single-nucleotide resolution. Only a handful of modifications can be mapped at high resolution using high-throughput (HTP) sequencing technologies[2]. These techniques have revealed that RNA modifications modulate most steps of gene expression, from DNA transcription to mRNA translation. Together, transcriptional and post-transcriptional networks are required to establish and maintain cell lineages within a tissue or organ. The deposition of RNA modifications is dynamic, and thereby allows rapid cellular responses to environmental cues[3–6]. The ability to adapt to a changing microenvironment is crucial for correct cell fate decisions during development. To ensure survival, tumour cells also dynamically adapt to often deleterious microenvironments such as that of stress or chemotherapeutic drugs. Here, we will focus on recently discovered regulatory functions of RNA modifications and discuss their emerging roles in regulating cell fate in normal tissues and cancer.

Post-transcriptional RNA modifications

Protein synthesis occurs at the ribosome and involves translation of the messenger RNA (mRNA) into amino acids via transfer RNA (tRNA). Ribosomal RNA (rRNA) is the most abundant type of RNA in a cell. Around 130 individual rRNA modifications have recently been visualized in the three-dimensional structure of the human 80S ribosome[7]. The most abundant rRNA modifications in eukaryotes are 2′-O-methylation of the ribose and the isomerisation of uridine to pseudouridine (Ψ)[8]. Most rRNA modifications occur in or close to functionally important sites and can facilitate efficient and accurate protein synthesis when they occur—for instance, at the peptidyltransferase center and the decoding site[7,8].

Tens of millions of tRNA transcripts are present in a human cell, and tRNA is the most extensively modified RNA in a cell[9]. The modifications are highly diverse, and their functions depend on the location within a tRNA and its chemical nature (Fig. 1a). The most common tRNA molecules consist of 76 nucleotides[10]. A human tRNA molecule, on average, contains 11–13 different modifications[11]. Accordingly, a large number of enzymes are involved in the site-specific deposition of the modifications (Fig. 1a). The modifications range from simple methylation or isomerisation, such as 5-methylcytosine (m^5C), N^1-methyladenosine (m^1A), pseudouridine (Ψ), 5-methyluridine (m^5U), 1-methylguanosine and 7-methylguanosine (m^1G and m^7G, respectively), and inosine (I), to complex multistep chemical modifications, such as N^6-threonylcarbamoyladenosine (t^6A) and 5-methoxycarbonylmethyl-2-thiouridine ($mcm5s^2U$)[9].

The most abundant internal modification in mRNA (and also long noncoding RNA) is N^6-methyladenosine (m^6A)[12–15]. Around 0.1–0.4% of all mRNA N^6-adenines are methylated, representing approximately 3–5 modifications per mRNA[15–17]. Other more rare modifications within eukaryotic mRNA include m^1A, N^6,2′-O-dimethyladenosine (m^6A_m), m^5C, 5-hydroxymethylcytosine (hm^5C), and Ψ (Fig. 1b)[18–25]. Some of these modifications are generated by stand-alone enzymes[26], whereas others are installed by multiprotein writer complexes comprising methyltransferases and accessory subunits (Fig. 1b)[27].

RNA modifications modulating gene expression programs

The first step of gene expression is the transcription of DNA molecules into mRNA. The deposition of m^6A into nascent pre-mRNA is carried out in the nucleus by a multicomponent writer complex[28,29] that consists of the catalytic subunit methyltransferase-like 3 (METTL3), the substrate-recognizing subunit METTL14 and many other accessory subunits (Fig. 1b)[27]. Gene-specific transcription factors and chromatin-modifying enzymes can further modulate the deposition of m^6A into nascent mRNA by repelling or recruiting the m^6A writer complex[30–33].

Two demethylases, fat mass and obesity-associated protein (FTO) and AlkB homolog 5 (ALKBH5), act as erasers of the m^6A modification (Fig. 2a)[34,35]. Several reader proteins selectively bind m^6A-containing mRNAs. Binding of YTH-domain-containing family protein YTHDC1 to m^6A regulates splicing, whereas YTHDF2 targets the transcripts for degradation[36–40]. Recruitment of YTHDF1 and YTHDF3, and YTHDC2 enhances translation (Fig. 2a)[41–43]. Binding of the insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs) to m^6A also promotes stability and translation of their targeted mRNAs (Fig. 2a)[44]. In summary, the deposition of m^6A to mRNA species regulates most aspects of RNA processing, including transcript stability, pre-mRNA splicing, polyadenylation, mRNA export and translation[27,40,45–47].

The second major step in gene expression is mRNA translation. Multiple aspects of protein synthesis are differently regulated among somatic cells and thereby contribute to cell identity and function within tissues[48]. Eukaryotic cells rely on the tight control of mRNA translation to quickly respond to a changing microenvironment, including during nutrient deprivation and stress, development and differentiation, and cancer[48–50]. All three main types of RNAs involved in translation (mRNA, tRNA and rRNA) are highly modified in mammals, and their interaction with the respective modifying enzymes often results in qualitative and quantitative changes of protein synthesis[8,9,27].

tRNA modifications modulating mRNA translation

tRNAs have multiple and versatile functions in regulating gene expression. To decode only 20 amino acids, the human genome

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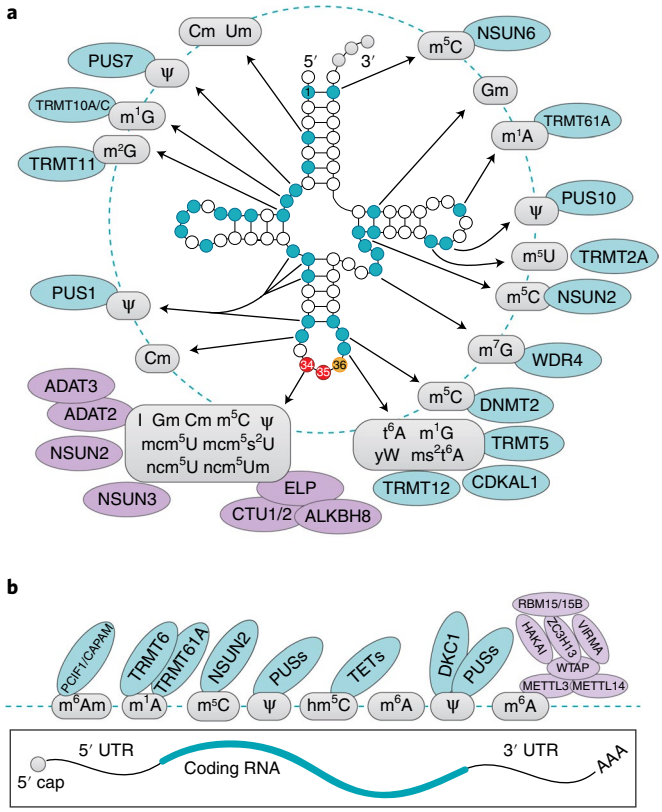

Fig. 1 | RNA modifications and their writer proteins. a, Schematic representation (modified version of refs. [75,130,131]) of a tRNA molecule and examples of RNA modifications and enzymes catalysing their deposition. Modifications at the wobble position and the corresponding catalysing enzymes are highlighted in purple. Positions 34–36 indicate anticodons. **b**, Schematic representation of the modifications internal to mRNA. Some of these modifications are enriched in the 5′ UTR, the coding sequence or the 3′ UTR of the mRNA. m^{6}A is catalysed by a multiprotein complex containing enzymes and accessory proteins (highlighted in purple). m^{1}A, N1-methyladenosine; Ψ, pseudouridine; m^{5}U, 5-methyluridine; m^{5}C, 5-methylcytosine; m^{7}G, 7-methylguanosine; ms^{2}t^{6}A, 2-methylthio-*N*^{6}-threonylcarbamoyladenosine; m^{1}G, 1-methylguanosine; yW, wybutosine; I, inosine; Gm, 2′-*O*-methylguanosine; Cm, 2′-*O*-methylcytidine; mcm^{5}U, 5-methoxycarbonylmethyluridine; mcm^{5}s^{2}U, 5-methoxycarbonylmethyl-2-thiouridine; ncm^{5}U, 5-carbamoylmethyluridine; ncm^{5}Um, 5-carbamoylmethyl-2′-*O*-methyluridine; m^{2}G, *N*^{2}-methylguanosine; Um, 2′-*O*-methyluridine; m^{6}Am, *N*^{6},2′-*O*-dimethyladenosine; hm^{5}C, 5-hydroxymethylcytidine; PUS, pseudouridylate synthase; NSUN, NOP2/Sun RNA methyltransferase family member; WDR4, WD repeat domain 4; DNMT2, DNA methyltransferase 2; TRM or TRMT, tRNA methyltransferase; CDKAL1, Cdk5 regulatory associated protein 1-like 1; ELP, Elongator protein homolog; CTU, cytosolic thiouridylase; ALKBH8, AlkB homolog 8 tRNA methyltransferase; ADAT, adenosine deaminase acting on tRNA; TET, Tet methylcytosine dioxygenase; DKC1, dyskerin pseudouridine synthase 1; RBM, RNA binding motif protein; ZC3H13, zinc finger CCCH-type containing 13; VIRMA, vir like m^{6}A methyltransferase associated; WTAP, WT1 associated protein; METTL, methyltransferase like; PCIF1/CAPAM, PDX1 C-terminal inhibiting factor 1/cap-specific adenosine methyltransferase; HAKAI, E3 ubiquitin-protein ligase Hakai.

encodes at least 610 tRNA species that are often tissue-specifically expressed[51–53]. All tRNAs carry modifications, but the extent of modification in individual tRNAs varies, and mitochondrial tRNAs are generally less modified, containing, on average, five modifications per molecule[9]. The diversity of modifications together with their highly similar L-shaped fold gives tRNAs the propensity to interact with a large number of RNAs and proteins during translation to modulate protein synthesis rates[54].

Modifications can occur along the whole L-shape fold of the tRNA, yet they are most diverse at the wobble position, where they often optimize codon usage during gene-specific translation (Fig. 1a; C34, pink)[55–57]. For example, uridines at position 34 (U34) of the wobble base of tRNA^{UUU}, tRNA^{UUC}, tRNA^{UUG} and tRNA^{UCU} can contain 5-carbamoylmethyl (ncm5) or mcm^{5}s^2 side chains. This requires the successive activities of the conserved six-subunit acetyltransferase Elongator protein (ELP) complex, the methyltransferase ALKBH8 and the thiouridylase complex CTU1–CTU2, together with the ubiquitin-related modifier (URM) pathway (Fig. 2b)[58,59]. These wobble modifications enhance base pairing and protein translation of mRNAs enriched for the corresponding codons[58–60]. Loss of the modification leads to codon-specific translation pausing of the ribosomes[61,62].

m^{5}C occurs in the anticodon loop and the variable arm of tRNAs (Fig. 1a)[63]. The methyltransferase NSUN3 is required for the formation of m^{5}C at the wobble position in mitochondrial tRNA for methionine (tRNA^{Met})[64,65]. NSUN3-dependent deposition of m^{5}C is required to initiate the subsequent biogenesis of 5-formylcytidine (f^{5}C), which is mediated by the RNA dioxygenase AlkB homolog 1 (ALKBH1)[64–67]. The formation of f^{5}C of mitochondrial tRNA^{Met} is required for the translation of AUA codons in mammalian mitochondria, and ALKBH1-knockout cells exhibited a strong reduction in mitochondrial translation and reduced respiratory complex activities[67]. Consequently, loss of these modifications due to deletion of NSUN3 also inhibits mitochondrial protein translation and impairs mitochondrial functions[64–66].

Other modifications that occur in the anticodon loop, but not at the wobble position, such as *N*^{6}-threonylcarbamoyladenosine (t^{6}A) at position 37 and m^{5}C at position 38 (Fig. 1a) increase translation elongation rates and fidelity, respectively[68,69]. Loss of DNMT2-mediated m^{5}C at C38 impairs the ability of the tRNA to discriminate Asp and Glu codons, causing codon-specific mistranslation[69]. Interestingly, C38 methylation by DNMT2 depends on deposition of queuosine (Q) at the wobble position[70]. Together, m^{5}C and Q increase translational speed of Q-decoded codons and specific near-cognate Q-decoded codons, thereby ensuring translational fidelity[70] (Fig. 2b). Depletion of queuine, the precursor for Q, which is provided through the diet and gut microbiota, results in formation of unfolded proteins, triggering the endoplasmic reticulum (ER) stress response[70].

Modifications outside the anticodon loop are often implicated in tRNA processing and cleavage. Deposition of m^{5}C and Ψ modulates the biogenesis or function of tRNA-derived small noncoding RNA fragments (tRFs)[71–73]. Loss of NSUN2-mediated m^{5}C deposition at the variable loop increases the affinity to the endonuclease angiogenin and thereby promotes cleavage of tRNAs into tRFs, eventually inhibiting global protein synthesis (Fig. 2c)[6,63]. The deposition of Ψ by PUS7 regulates the function of tRFs in targeting the translation initiation complex[72]. Only Ψ-containing tRFs efficiently decrease protein biosynthesis (Fig. 2c)[72]. RNA modifications in the anticodon loop, such as Q at the wobble position[74] and DNMT2-mediated m^{5}C at C38 (ref. [73]), also protect against ribonuclease cleavage.

In summary, in response to environmental cues, tRNA modifications can act as a rheostat of protein synthesis rates via at least two mechanisms. First, modifications outside the anticodon loop often modulate the rate of global de novo protein synthesis mostly through regulating tRNA biogenesis (Fig. 2c). Second, modifications within the anticodon loop can determine the translation speed of codon-specific genes (Fig. 2b). Because wobble base modifications usually affect gene-specific translation, they have the potential to directly modulate distinct cellular functions such as survival, growth and differentiation.

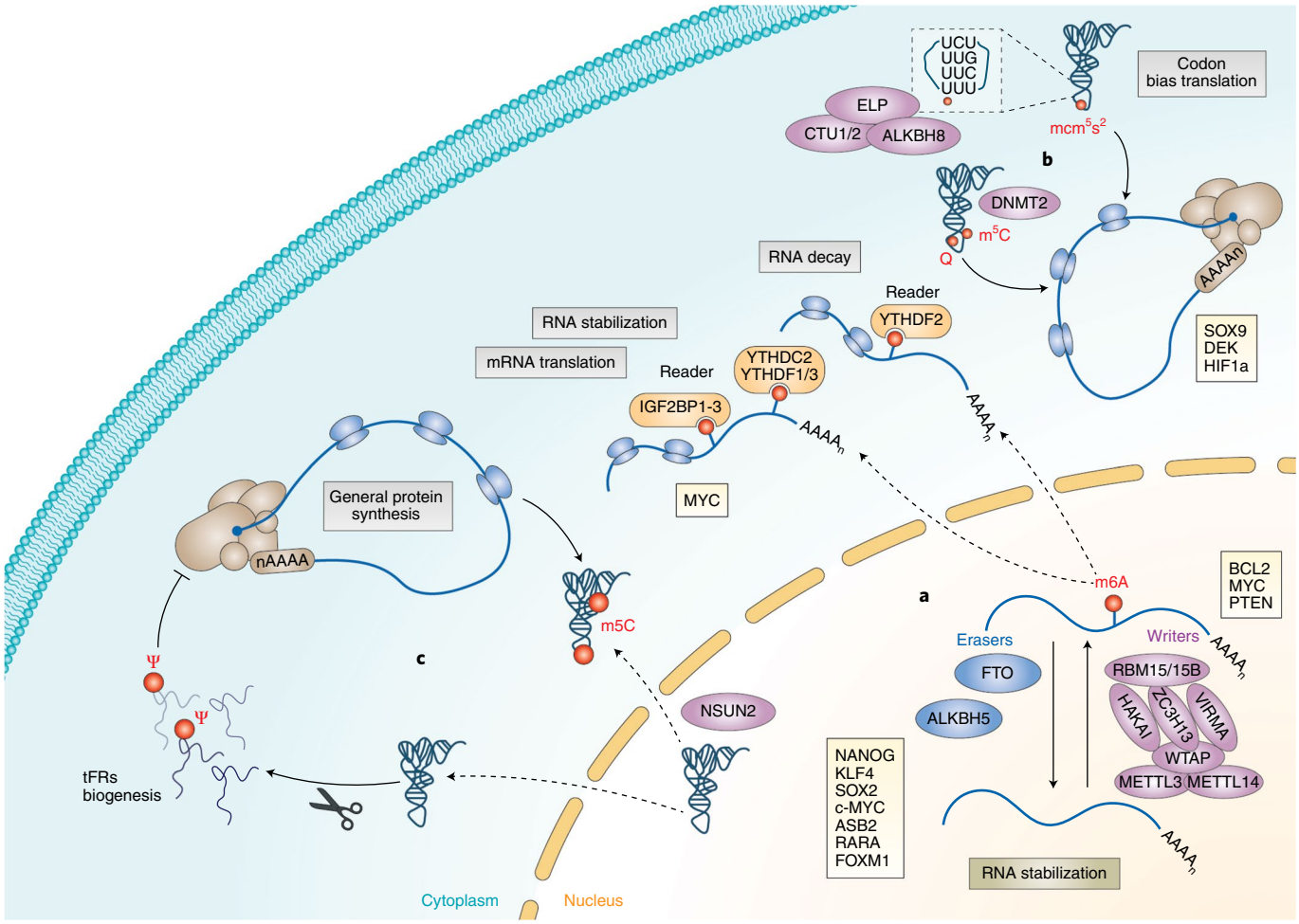

Fig. 2 | RNA modifications regulate gene expression programs. a, m^{6}A is deposited by a 'writer' multiprotein complex (i.e., METTL3, METTL14, WTAP, VIRMA, HAKAI, ZC3H13 and RBM15/15B) and removed by 'eraser' demethylases (i.e., FTO and ALKBH5). In the cytoplasm, the mRNA modifications are recognized by 'reader' proteins, resulting in stabilization or decay of the target mRNA or enhanced translation. **b**, tRNA mcm5s^{2}U modification is required for the optimal base pairing between tRNA^{UUU}, tRNA^{UUC}, tRNA^{UUG} and tRNA^{UCU} and the corresponding codons in specific mRNA targets (e.g., SOX9, DEK and HIF1α). Deposition of m^{5}C at tRNA by DNMT2 controls translational speed of Q-decoded codons. **c**, tRNAs are methylated by NSUN2 in the nucleus. The m^{5}C modification reduces the affinity to the endonuclease angiogenin in the cytoplasm. m^{5}C maintains global protein synthesis. Loss of m^{5}C facilitates the biogenesis of tRNA-derived small noncoding RNA fragments (tRFs), resulting in inhibited de novo protein synthesis. The presence of Ψ increases the efficiency of the tRFs in inhibiting protein synthesis.

The regulatory potential of RNA modifications in cancer

Because of their ability to modulate many aspects of RNA metabolism and influence protein synthesis rates, RNA modifications have emerged as important regulators in cancer[60,75,76]. Similarly to normal tissues, a tumour also contains functionally and phenotypically different cell populations. Tumour heterogeneity is the consequence of genetic changes, environmental differences and reversible alterations in cellular properties[77]. The heterogeneous cell populations are not equally tumourigenic. Some cancer cells are more differentiated with a limited tumourigenic potential. Others, potentially even rare tumour populations, exhibit stem-cell-like features that drive tumourigenesis, long-term survival and therapy resistance[78]. Though mRNA-modifying-enzymes are generally not considered to be cancer driver genes, they have been functionally linked to survival, proliferation, growth and differentiation of tumour cells[32,79–86]. Abnormal expression of tRNA modifying enzymes can also reduce the sensitivity of tumour cells towards differentiation cues or sustain the expression of specific genes required for proliferation, invasion and resistance to anticancer drugs[6,87–89].

RNA modifications regulating the fate of tumour-initiating cells. Members of the writer complex for mcm5s^{2}U tRNA modification are upregulated in melanoma as well as in colon and breast cancer[88,90,91]. ELP3, the catalytic subunit of the Elongator complex, is required for Wnt-driven intestinal tumour initiation[90]. Deletion of ELP3 in Lgr5$^+$ tumour-initiating cells (TICs) delays tumour growth, yet the number of Lgr5$^+$ cells remains unchanged[90,92]. Thus, the correct formation of mcm^{5}s^{2}U promotes the tumourigenic potential of specific cell populations (Fig. 3)[90]. A cell-type-specific function of ELP3 can be explained by the codon-specific effect of mcm^{5}s^{2}U on translation. For instance, in colon cancer cells ELP3 promotes the translation of SOX9, a downstream target of Wnt–β-catenin signalling[90,93]. In breast cancer, ELP3 enhances translation of the DEK proto-oncogene, whose mRNA is enriched for mcm^{5}s^{2}U-sensitive codons[88].

A cell-type-specific functional requirement of mcm^{5}s^{2}U is also exemplified in development. Although Elongator is required for the brain, it is dispensable for the formation of intestine and mammary glands[88,90,94–96]. Loss of ELP3 in the developing brain leads to microcephaly. Ribosome profiling in the mutant forebrain revealed

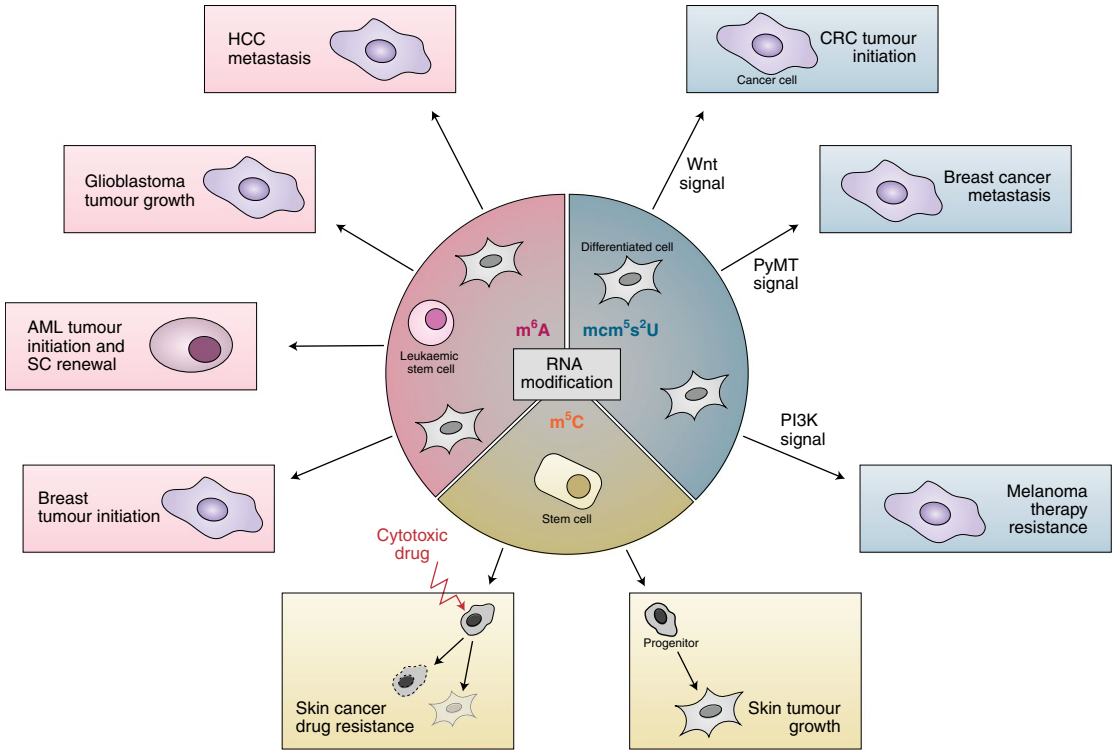

Fig. 3 | Roles of RNA modifications in cancer. RNA modifications are involved in multiple aspects of tumourigenesis. mcm^{5}s^{2}U tRNA modification is required for Wnt-driven colorectal cancer (CRC) initiation, development of lung metastasis from Polyoma Middle T (PyMT) breast tumours, and PI3K pathway-addicted resistance to therapy in melanoma. Expression of NSUN2, "writer" for m^{5}C modification, is highest in committed progenitors of skin tumours, and it is crucial for resistance to drug treatment. Lack of NSUN2 increases the number of undifferentiated stem and progenitor cells. Both positive and negative roles of m^{6}A on specific mRNAs in hepatocellular carcinoma (HCC) metastasis have been reported. Increased levels of m^{6}A suppress growth in glioblastoma tumours. m^{6}A can be advantageous or disadvantageous for the maintenance of leukaemic stem cell (SC) self-renewal and tumour initiation in AML. In breast cancer cell lines, up-regulation of m^{6}A demethylase ALKBH5 enhances tumour initiation capacity.

enhanced pausing at putative mcm^{5}s^{2}U sites. These codon-specific translation defects may cause an accumulation of unfolded or misfolded proteins and thereby explain the activation of the ER stress response, leading to activation of the unfolded protein response (UPR) pathway[94]. In contrast, melanoma cells fail to activate the UPR pathway, again indicating that mcm^{5}s^{2}U modification exerts cell-context-specific functions[87,88].

The deposition of m^{5}C to tRNA by NSUN2 is also required for normal development and is implicated in cancer[6,97–101]. Loss of the *NSUN2* gene causes growth retardation and neurodevelopmental deficits in humans and mice[63,97–99]. In cutaneous tumours, NSUN2 is absent in TICs but highly expressed in committed progenitor populations. Accordingly, deletion of NSUN2 increases the number of TICs (Fig. 3)[6]. As described for some tissue stem cells[102–105], TICs of skin tumours are also functionally maintained by low protein synthesis rates, which are at least in part maintained by tRNA fragmentation in the absence of NSUN2 (ref. [6]). Thus, similarly to the cellular response to stress or injury, in which global protein synthesis is commonly reduced[106], TICs may also require low translation rates to alleviate cellular damage and increase longevity and survival rate.

The correct deposition of m^{6}A into mRNA is essential for embryo development and cell differentiation because of its role in governing the stability of key regulatory transcripts[27]. Complete absence of m^{6}A due to deletion of METTL3 is early embryonic lethal owing to the extended transcript lifetime of key pluripotency regulators (e.g., NANOG, SOX2 and KLF4) and the resulting inability to start differentiation programs (Fig. 2a)[107,108]. Thus, the deposition of m^{6}A affects the stability of distinct groups of transcripts (for instance, pluripotency factors), allowing their synchronized regulation. This coordination of RNA metabolism then allows the cell to transit through specific cell states, such as self-renewal, proliferation or differentiation, in response to cellular signalling and environmental cues. These environmental cues may include growth factors, cytokines or external stress factors (e.g., hypoxia, oxidative stress and injury). Such a mechanism allowing fast adaptation to changing microenvironments is also required in tumours (Fig. 3).

Increased levels of m^{5}C and m^{6}A in RNA were reported in circulating tumour cells of patients with lung cancer by mass spectrometry[109]. However, several studies then showed that m^{6}A demethylation promotes cell proliferation and tumourigenesis in different types of cancer. Hypoxia-induced upregulation of ALKBH5 in breast cancer cells decreased m^{6}A levels and enhanced mammosphere formation[110]. ALKBH5 is also highly expressed in glioblastoma and sustains the proliferation of patient-derived glioblastoma cells[81]. The m^{6}A demethylase FTO is highly expressed in patients with acute myeloid leukaemia (AML)[79]. FTO enhances leukaemic oncogene-mediated cell transformation and leukaemogenesis by promoting cell proliferation and survival, and suppresses all-transretinoic acid (ATRA)-induced AML cell differentiation[79]. Knockdown of METTL3 or METTL14 also promotes tumourigenesis of primary human glioblastoma cells in vitro and in vivo, an effect that was reverted by overexpression of METTL3 or inhibition of FTO[80]. Similarly, (*R*)-2-hydroxyglutarate (R-2HG), an oncometabolite that inhibits FTO, also exerts an antileukaemic activity in vitro and in vivo[85]. Treatment with R-2HG increased m^{6}A levels, leading to

degradation of MYC and CEBPA transcripts and suppression of the relevant downstream pathways[85]. Finally, 70% of endometrial tumours exhibit m^{6}A reduction, attributed to either METTL14 mutation or METTL3 downregulation[86]. Low levels of m^{6}A mRNA modification enhance proliferation and tumourigenesis of endometrial cancer cells through activating the AKT signalling pathway[86].

Unexpectedly, the m^{6}A methyltransferase METTL3 is also more abundant in AML cells in comparison to healthy CD34-positive stem and hematopoietic progenitor cells[82] and is essential for the growth of AML cells[32,82]. Downregulation of METTL3 or METTL14 causes cell cycle arrest and differentiation of leukaemic cells through translational repression of distinct sets of transcripts, such as genes with their transcription start site occupied by the CAATT-box binding protein CEBPZ in the case of METTL3 and the MYB and MYC transcripts in the case of METTL14 (refs. [32,84]). Together, these studies indicate that elevated levels of m^{6}A are advantageous for the maintenance of an undifferentiated cell state in leukaemia. Similarly, METTL3 promotes growth, survival and invasion of human lung cancer cells[111]. Yet, in this study, METTL3 promotes translation of certain mRNAs (e.g., EGFR and TAZ) through association with ribosomes in the cytoplasm, and this function is independent of its catalytic activity and m^{6}A readers[111]. The m^{6}A reader insulin-like growth factor 2 mRNA-binding protein (IGF2BP) also promotes mRNA stability and translation of its target mRNAs such as MYC (Fig. 2a)[44].

Together, these studies reveal that aberrant methylation and demethylation of mRNA influence tumour initiation and growth. The precise underlying mechanisms that explain how both m^{6}A methylases and demethylases can promote tumourigenesis remain unclear. However, methylation and demethylation events occur on distinct and often cell-state-specific key regulatory transcripts, and the gene-region-specific m^{6}A modifications would have distinct regulatory effects on target transcripts[112]. In addition, these sets of transcripts are likely to differ in stem cells and undifferentiated or committed progenitors. Thus, depending on the cell of origin of the respective tumour and the identity of the distinct driver mutations, the degradation or stabilization of distinct sets of mRNAs may confer growth advantages. Finally, tumours are highly heterogeneous, and the distinct tumour populations may be more or less sensitive to changes in m^{6}A levels.

RNA modifications regulating tumour invasion and metastasis. Phenotypic transitions between cell states also occur in cancer and involve epithelial-to-mesenchymal transition (EMT), acquisition of cancer stem-like properties, metabolic reprogramming, the emergence of therapy resistance and programmed cell death. RNA-modifying enzymes are often required for cell survival in response to external stress stimuli (e.g., UV-radiation and oxidative stress)[113]. Tumour cells are constantly exposed to a hostile microenvironment owing to a shortage of oxygen and nutrients, and hypoxia-induced gene activity is crucial for tumour metastasis[114,115]. Although hypoxia can dynamically change tRNA modifications[116], their precise functional roles during tumour cell invasion and metastasis are unclear.

Several subunits of the mcm5s^{2}U writer complex are upregulated in breast cancer cells undergoing EMT, and ELP3 promotes translation of DEK to sustain metastasis in mouse models of invasive breast cancer (Fig. 3)[88]. Cellular migration and invasion were impaired in the absence of NSUN2 in vitro[6,117,118], and tRFs have been shown to suppress the metastatic potential of breast cancer cells[119].

The m^{6}A writer METTL3 promotes progression of liver cancer through YTHDF2-dependent degradation of suppressor of cytokine signalling 2 (SOCS2) mRNA, and knockout of METTL3 suppresses tumourigenicity and lung metastasis in vivo[83]. METTL3 promotes oncogene translation and tumourigenesis through an mRNA looping mechanism that requires the interaction with the eukaryotic translation initiation factor 3 subunit h (eIF3h)[120]. METTL3 enhances translation initiation of certain mRNAs including epidermal growth factor receptor (EGFR) and the Hippo pathway effector TAZ, and thereby promotes growth, survival and invasion of human lung cancer cells[111].

Conversely, downregulation of METTL14 enhances metastasis in hepatocellular carcinoma (HCC)[121]. Both METTL3 and METTL14 have been described in facilitating the microRNA (miRNA) processing and maturation[121,122]. METTL3 methylates primary microRNAs and marks them for recognition and processing by the microprocessor complex subunit DGCR8 (ref. [122]). Similarly, METTL14 interacts with DGCR8 to enhance the processing of miR126, a miRNA negatively associated with the invasive potential of HCC[121].

RNA modifications regulating drug resistance. Several recent studies have demonstrated a link between RNA modifications and tumour cell survival in response to chemotherapy. The coordinated depositions of m^{5}C by NSUN2 and m^{7}G by METTL1 to tRNAs were implicated in mediating sensitivity of HeLa cells towards the cytotoxic agent 5-fluorouracil (5-FU)[123,124]. 5-FU is commonly used to treat squamous cell carcinomas[125]. Removal of NSUN2 in mouse cutaneous tumours increased the number of stem and progenitor cells; however, NSUN2-lacking tumour cells were also highly sensitive towards cytotoxic drug treatment with 5-FU and cisplatin[6]. This finding highlights the importance of the dynamic deposition of m^{5}C into RNA. While stem cells and TICs lack NSUN2 to maintain a low translating stem cell state[6,126], NSUN2 upregulation, and thus methylation of the tRNA, is required to activate the appropriate survival pathways to regenerate the tumour after cytotoxic insult (Fig. 3)[6]. The high sensitivity of NSUN2-deficient tumour cells towards drug treatment is dependent on the endonuclease angiogenin and is therefore at least in part regulated via tRF formation[6].

Activation of the PI3K signalling pathway during BRAF^{V600E}-driven resistance to anti-MAPK targeted therapy in melanoma cells enhances the expression of mcm^{5}s^{2}U writer enzymes[87]. The resulting tRNA wobble modification mcm5s^{2}U sustains resistance in melanoma through rewiring protein synthesis towards a translational bias for mcm^{5}s^{2}U-dependent codons, which are found, for instance, in the HIF1α mRNA. The enhanced synthesis of the HIF1α protein thereby promotes glycolysis and maintains the metabolic requirements for the melanoma cells[87]. The resistant cells are resensitized to drug treatment through depletion of the mcm^{5}s^{2}U writers (Fig. 3)[87]. Together, these recent studies highlight the importance of RNA modification pathways in most aspects of tumourigenesis.

Concluding remarks

RNA modifications are key players in regulating cell fate decision during development. More recently, RNA modifications have also emerged as important regulators of cancer. Similarly to stem cells in most adult tissues, TICs also maintain the tumour in the long term. An important feature of TICs is to efficiently adapt self-renewal, proliferation and survival pathways to external cues. A dependency on RNA modifications to switch cell fates, for example, from a proliferating tumour cell to a quiescent TIC in response to chemotherapy, may represent a window of opportunity to specifically target tumour-initiating or resistant cell populations.

Cancer cells rapidly adapt to extreme environmental conditions by changes in specific metabolic pathways and through translational control, mediating an adaptive response to oncogenic stress conditions[50,127]. RNA modifications have emerged as one mechanistic link between metabolism and enhanced codon-dependent translation of HIF1α, for instance, to promote glycolytic metabolism[87]. Similarly, RNA modifications promote gene-specific translation of one or several groups of tumour driver and suppressor genes. Thus, the modulation or inhibition of RNA modification pathways offers therapeutic strategies to target specific tumour populations, such as slow cycling TICs or resistant tumour cells.

Depending on tumour heterogeneity, distinct RNA modification patterns may be used to identify TICs or to distinguish resistant cells from drug-responsive tumour populations. However, whether this could be exploited as a biomarker is difficult to predict for several reasons. First, the tumour population of interest might be marked by the absence, rather than the presence, of distinct modifications. Second, methods suitable for easy, sensitive and reliable high-throughput detection of RNA modifications are currently not available. Third, aberrant expression of an RNA modifier is often required for the misexpression of cell-type-specific gene clusters. Thus, putative biomarkers may only be suitable for distinct subtypes of tumours.

Though aberrant expression of RNA modifying enzymes has now been described for most aspects of tumourigenesis, the precise contributions of the enzymes and respective modification to tumour initiation, growth, metastasis and resistance need to be further investigated. Currently, it also remains unclear how specific modifications influence different tumour cell populations and how precisely they regulate survival, longevity and resistance of cancer cells. In addition, the dynamic expression patterns of writer, reader and eraser proteins complicate the identification of the precise functional consequences of aberrant deposition of modifications on RNA metabolism and tumour cell fate decisions. Furthermore, with the exception of some tRNA modifications[70,128,129], it is currently largely unclear how different modifications influence each other and affect the binding to RNA-binding proteins. The development of tools for the identification and quantification of RNA modifications will be essential to further unearth their roles in the different steps of cancer development.

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Competing interests

M.F. consults for STORM Therapeutics.

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