RNA modifications regulating cell fate in cancer

Sylvain Delaunay [1] and Michaela Frye [1,2]*

[1] University of Cambridge, Department of Genetics, Downing Street, Cambridge CB2 3EH, UK, [2] German Cancer Center (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

*Correspondence to: mf364@cam.ac.uk; m.frye@dkfz-heidelberg.de

Abstract

The deposition of chemical modifications into RNA is a crucial regulator of temporal and spatial accurate 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 resistance, RNA modifications add a novel regulatory layer of complexity to most aspects of tumourigenesis.

Post-transcriptional RNA modifications

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]. However, these newly developed technics have revealed that RNA modifications modulate most steps of gene expression from RNA transcription to protein translation. 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.

Protein synthesis occurs at the ribosome and involves the translation of the messenger RNA (mRNA) into amino-acids via transfer RNAs (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 ribosome [3]. The most abundant rRNA modifications in eukaryotes are 2′-O-methylation of the ribose and the isomerisation of uridine to pseudouridine (Ψ) [4]. 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 [3,4].

Tens of millions of tRNA transcripts occur in a human cell, and tRNA is the most modified RNA in a cell [5]. The modifications are highly diverse, and their functions depend on the location within a tRNA and its chemical nature (***Figure 1a***). The most common tRNA molecules consist of 76 nucleotides [6]. A human tRNA contains between 11 to 13 different modifications [7]. Accordingly, a large number of enzymes are involved in the site-specific

deposition of the modifications (***Figure 1a***). The modifications range from simple methylation or isomerization events, such as m^5C, m^1A, Ψ, 5-methyluridine (m^5U), 1- and 1/7-methylguanosine (m^1G, m^7G), and inosine, to complex multistep chemical modifications, such as N6-threonylcarbamoyladenosine (t^6A) and 5-methoxycarbonylmethyl-2-thiouridine (mcm^5s^2U) [5].

The most abundant internal modification in mRNA (and also long non-coding RNA) is N6-methyladenosine (m^6A) [8-11]. Around 0.1 to 0.4% of all mRNA adenines are methylated, representing approximately 3-5 modifications per mRNA [11-13]. Other rarer modifications within eukaryotic mRNA include N1-methyladenosine (m^1A), N6-2'O-dimethyladenosine (m^6A_m), 5-methycytosine (m^5C), 5-hydroxymethylcytosine (hm^5C), and pseudouridine (Ψ) (***Figure 1b***) [14-21]. Some of these modifications are generated by stand-alone enzymes [22], others are installed by multi-protein writer complexes and accessory subunits (***Figure 1b***) [23].

RNA modifications modulate 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 methyltransferase complex [24,25]. The multi-protein writer complex installing m^6A consists of the Methyltransferase Like catalytic subunits (METTL3, METTL14), and many other accessory subunits [23]. Gene-specific transcription factors and chromatin modifying enzymes can further modulate the deposition of m^6A into nascent RNA by repelling or recruiting the m^6A writer complex [26-28].

Two demethylases, Fat Mass and Obesity-associated protein (FTO) and AlkB Homolog 5 (ALKBH5) act as erasers of the m^6A modification (***Figure 2a***) [29,30]. Several reader proteins

selectively bind m^{6}A containing mRNAs. For instance, binding of YTH N6-Methyladenosine RNA Binding Protein 2 (YTHDF2) targets the transcripts for degradation [31-34]. Recruitment of YTHDF1/3 enhances translation (***Figure 2a***) [35,36]. The deposition of m^{6}A and other additional mRNA modifications contribute to most aspects of RNA metabolism such as transcript stability, pre-mRNA splicing, polyadenylation, mRNA export, and translation [23,37,38].

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 [39]. Eukaryotic cells rely on the tight control of mRNA translation to quickly respond to a changing micro-environment, including nutrient deprivation and stress, development and differentiation, and cancer [39-41]. 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 [4,5,23].

tRNA modifications modulating mRNA translation

Transfer RNAs have multiple and versatile functions in regulating gene expression. To decode only 20 amino acids, the human genome encodes at least 610 tRNAs that are often tissue-specifically expressed [42-44]. All tRNAs carry modifications, but the extent of modifications in individual tRNAs varies and mitochondrial tRNAs are generally less modified, containing on average of five modifications per molecule [5]. 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 [45].

RNA modifications can occur along the whole L-shape of the tRNA, yet they are the most diverse at the wobble position, where they often optimize codon usage during gene-specific translation (***Figure 1a;*** *C34 pink*) [46-48]. For example, uridines in position 34 of the wobble base of $tRNA^{UUU}$, $tRNA^{UUC}$, $tRNA^{UUG}$ and $tRNA^{UCU}$ can contain a 5-carbamoylmethyl (ncm^5) or 5-methoxycarbonylmethyl-2-thiouridine (mcm^5s^2) side-chains. This requires the successive activities of the conserved acetyltransferase six-subunit Elongator complex, the methyltransferase ALKBH8, and the thiouridylase CTU1/CTU2, together with the URM pathway (Ubiquitin-Related Modifier pathway) (***Figure 2b***) [49,50]. The wobble modification enhances base-pairing and protein translation of mRNAs enriched for the corresponding codons [49-51]. Loss of the modification leads to codon-specific translation pausing of the ribosomes [52,53].

Cytosine-5 methylation (m^5C) occurs in the anti-codon loop and the variable arm of tRNAs (***Figure 1a***) [54]. The methyltransferase NSUN3 is required for the formation of m^5C at the wobble position in mitochondrial tRNA for start methionine ($tRNA^{Met}$) [55,56]. NSUN3-dependent deposition of m^5C is required to initiate the subsequent biogenesis of 5-formylcytidine (f^5C), which is mediated by the RNA dioxygenase AlkB Homolog 1 (ALKBH1) [55-58]. Consequently, loss of these modifications due to deletion of NSUN3 inhibits mitochondrial protein translation and impairs mitochondrial functions. Other modifications that occur in the anticodon loop, but not at the wobble position, such as t^6A at position 37 and m^5C at position 38 modulate translation elongation rates and fidelity respectively [59,60].

Modifications outside the anticodon loop are often implicated in tRNA processing and cleavage. Deposition of m^5C and Ψ modulates the biogenesis of tRNA-derived small non-coding RNA fragments (tRFs) [61-63]. Loss of NSUN2-mediated methylation at the variable loop increases the affinity to the endonuclease angiogenin, and thereby promotes cleavage of tRNAs into tRFs, which then inhibit global protein synthesis (***Figure 2c***) [54,64]. The deposition of Ψ by PUS7 also influences the biogenesis of tRFs; yet interestingly, loss of PUS7 leads to increased protein biosynthesis [62]. Deposition of Queuosine (Q) at the wobble anticodon position of tRNAs protects against ribonuclease cleavage [65], and Q-tRNA levels promote DNMT2-mediated methylation [66]. Together, m^5C and Q control translational speed of Q-decoded codons as well as at near-cognate codons [66]. Loss of DNMT2-mediated methylation at the anti-codon loop (C38) causes tRNA-specific fragmentation and codon-specific mistranslation [60]. Depletion of queuine, the precursor for Q, which is provided through the diet and gut microbiota, results in unfolded proteins triggering the endoplasmic reticulum stress response [66].

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 *de novo* protein synthesis. Second, modifications within the anticodon loop can determine the translation speed of codon-specific genes. Because wobble base modifications usually affect gene-specific translation, they have the potential to directly modulate distinct cellular functions such as survival, growth or differentiation.

The regulatory potential of RNA modifications in cancer

Due to their ability to modulate many aspects of RNA metabolism and influence protein synthesis rates, several RNA modifications emerged as important regulators in cancer [51,67,68].

Similar to normal tissues, also a tumour contains functionally and phenotypically different cell populations. Tumour heterogeneity is the consequence of genetic change, environmental differences, and reversible changes in cellular properties [69]. The heterogenous cell populations are not equally tumourigenic. Some cancer cells are more differentiated with a limited tumorigenic potential. Others, potentially even rare tumour populations, exhibit stem cell-like features that drive tumourigenesis, long-term survival, and therapy resistance [70]. While RNA modifying-enzymes are generally not considered to be cancer driver genes, they have been functionally linked to sustain cell survival, proliferation, growth or differentiation of tumour-initiating cells. Abnormal expression of RNA modifying enzymes can reduce the tumour cell's sensitivity towards differentiation cues (m^6A, m^5C) or sustain the expression of specific genes required for proliferation, invasion and resistance to anti-cancer drugs (mcm^5s^2U, m^6A, m^5C).

RNA modifications regulating the fate of tumour-initiating cells

Members of the mcm^5s^2U writer complex are upregulated in melanoma as well as colon and breast cancer [71-73]. ELP3, the catalytic subunit of the Elongator complex, is required for Wnt-driven intestinal tumour initiation [72]. Deletion of ELP3 in $Lgr5^+$ tumour initiating cells delays tumor growth, yet the number of $Lgr5^+$ cells remains unchanged [72,74]. Thus, the correct formation of mcm^5s^2U promotes the tumourigenic potential of specific cell populations [72]. A cell type-specific function of ELP3 can be explained by the codon-specific effect of mcm^5s^2U on translation. For instance, in colon cancer cells, ELP3 promotes translation SOX9, a down-stream target of Wnt/β-catenin signaling [72,75]. In breast cancer, ELP3 enhances translation of the DEK proto-oncogene, whose mRNA is enriched for mcm^5s^2U sensitive codons [71].

A cell type-specific functional requirement of mcm^5s^2U is also exemplified in development. While Elongator is required for the brain, it is dispensable for the formation of intestine and mammary glands [71,72,76-78]. Loss of ELP3 in the developing brain leads to microcephaly. Ribosome profiling in the mutant forebrain revealed enhanced pausing at putative mcm^5s^2U sites. These codon-specific translation defects may cause an accumulation of unfolded or misfolded proteins and thereby explain the activation of the endoplasmic reticulum (ER) stress response, leading to the activation of the Unfolded Protein Response (UPR) pathway [76]. In contrast, melanoma and breast cancer cells fail to activate the UPR pathway, again indicating that mcm^5s^2U modification exerts cell context-specific functions [71,79].

The deposition of m^5C by NSUN2 is also required for normal development and implicated in cancer [64,80-84]. Loss of the *NSUN2* gene causes growth retardation and neuro-developmental deficits in human and mice [54,80-82]. In cutaneous tumours, NSUN2 is absent in tumour-initiating cells but highly expressed in committed progenitor populations. Accordingly, deletion of NSUN2 increases the number of tumour-initiating cells (***Figure 3***) [64]. As described for some tissue stem cells, also tumour-initiating cells of skin tumours are functionally maintained by low protein synthesis rates, which is at least in part maintained by tRFs in the absence of NSUN2 [64,85-87]. Thus, similar to the cellular response to stress or injury, in which global protein synthesis is commonly reduced [88], tumour-initiating cells may also require low translation rates to alleviate cellular damage and increase longevity and survival rate.

The correct deposition of m^6A into mRNA is essential for embryo development and cell differentiation due to its role in governing the stability of key regulatory transcripts [23]. Complete absence of m^6A due to deletion of METTL3 is early embryonic lethal due to the

195 extended transcript lifetime of key pluripotency regulators (e.g. *Nanog*, *Sox2*, and *Klf4)* and
196 the inability to start differentiation programs (***Figure 2a***) [89,90]. Thus, the deposition of m^6A
197 affects the stability of distinct groups of transcripts, for instance pluripotency factors,
198 allowing their synchronized regulation. This coordination of RNA metabolism then allows
199 the cell to transit through specific cell states, such as self-renewal, proliferation or
200 differentiation, in response to cellular signaling and environmental cues. These
201 environmental cues may include growth factors, cytokines, or external stress factors (e.g.
202 hypoxia, oxidative stress, or injury). Such a mechanism allowing the fast adaptation to
203 changing micro-environments is also required in tumours (***Figure 3***).
204
205 Increased levels of m^5C and m^6A in RNA was first reported in circulating tumour cells of
206 lung cancer patients by mass spectrometry [91]. However, several studies then showed that m^6A
207 de-methylation promotes proliferation and tumourigenesis in different types of cancer.
208 Hypoxia-induced up-regulation of ALKBH5 in breast cancer cells decreased m^6A and
209 enhances mammosphere formation [92]. ALKBH5 is also highly expressed in glioblastoma and
210 sustains the proliferation of patient-derived glioblastoma cells [93].
211
212 The m^6A de-methylase FTO is highly expressed in patients with acute myeloid leukemia
213 (AML) [94]. FTO enhances leukemic oncogene-mediated cell transformation and
214 leukemogenesis by promoting cell proliferation and survival and inhibiting all-trans-retinoic
215 acid (ATRA)-induced AML cell differentiation [94]. Knockdown of METTL3 or METTL14
216 also promotes tumourigenesis of primary human glioblastoma cells *in vitro* and *in vivo,* an
217 effect that was reverted by overexpression of METTL3 or inhibition of FTO [95]. Similarly, R-
218 2-hydroxyglutarate (R-2HG), an oncometabolite that inhibits FTO, also exerts an anti-
219 leukemic activity *in vitro* and *in vivo* [96]. Treatment with R-2HG increased m^6A leading to

degradation of *Myc*/*Cebpa* transcripts and suppression of the relevant down-stream pathways [96]. Finally, 70% of endometrial tumours exhibit m^6A reduction, either attributed to METTL14 mutation or METTL3 downregulation [97]. Low levels of m^6A enhances proliferation and tumorigenesis of endometrial cancer cells, through AKT signaling pathway [97].

Unexpectedly, the m^6A methyltransferase METTL3 is also more abundant in AML cells when compared to healthy CD34-positive stem and progenitor hematopoietic cells [98], and is essential for the growth of acute myeloid leukaemia cells [28,98]. Downregulation of METTL3 or METTL14 causes cell cycle arrest and differentiation of leukaemic cells through transcriptional repression of distinct sets of transcripts, such as genes containing a CAATT-box binding protein at the transcription start site in the absence of METTL3 and *Myb* and *Myc* in the absence of METTL14 [28,99]. Together, these studies indicate that elevated levels of m^6A is advantageous for the maintenance of an undifferentiated cell state in leukemia. Similarly, METTL3 promotes growth, survival, and invasion of human lung cancer cells [100]. Yet in this study, METTL3 promoted translation of certain mRNAs (e.g. *Egfr* and *Taz*) through association with ribosomes in the cytoplasm, this function was independent of its catalytic activity and m^6A readers [100]. The m^6A reader Insulin-like growth factor 2 mRNA-binding proteins (IGF2BP) also promotes mRNA stability and translation of its target mRNAs, for example *Myc* (***Figure 2a***)[101].

Together, these studies reveal that aberrant methylation and de-methylation of mRNA influences tumour initiation and growth. The precise underlying mechanisms how both m^6A methylases and de-methylases can promote tumourigenesis remain unclear. However, methylation and de-methylation events occur on distinct and often cell-state specific key regulatory transcripts at gene-specific regions [102]. 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^6A levels.

RNA modifications regulating tumour invasion and metastasis

Phenotypic transitions between cell states also occurs in cancer and include epithelial-to-mesenchymal transition (EMT), 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) [103]. Tumour cells are constantly exposed to a hostile microenvironment, due to shortage of oxygen and nutrients; and hypoxia-induced gene activity is crucial for tumour metastasis [104,105]. Although hypoxia can dynamically change tRNA modifications [106], their precise functional roles during tumour cell invasion and metastasis is unclear.

Several mcm^5s^2U writers are upregulated in cells undergoing EMT, and ELP3 promotes translation of LEF1 to sustain metastasis in invasive breast cancer mouse models [71]. Cellular migration and invasion is impaired in the absence of NSUN2 *in vitro* [64,107,108] and tRNA-derived cleavage products have been shown to modulate the metastatic potential of breast cancer cells [109].

The m^6A writer 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 (***Figure 3***) [100]. METTL3

has been also described to promote liver cancer progression through an YTHDF2-dependent mechanism and knockout of METTL3 suppressed tumorigenicity and lung metastasis *in vivo* [110].

Conversely, down□regulation of METTL14 enhances metastasis in hepatocellular carcinoma (HCC) [111]. Both METTL3 and METTL14 have been described to modulate the microRNA (miRNA)-guided RNA silencing pathway [111,112]. METTL3 methylates pri-miRNA and marks them for recognition and processing by the microprocessor complex subunit DCR8 [112]. Similarly, METTL14 interacts with DGCR8 to enhance miR126 processing, a miRNA associated with invasive potential of HCC cell lines [111].

RNA modifications regulating drug resistance

Several recent studies demonstrated a link between RNA modifications and tumour cell survival in response to chemotherapeutic drug treatments. The coordinated modification of tRNAs by NSUN2 and METTL1, that mediates m^7G methylation in tRNAs, was first implicated in mediating sensitivity of Hela cells towards the cytotoxic agent 5-Fluorouracil (5-FU) [64,113,114]. 5-FU is commonly used to treat squamous cell carcinomas [115]. Removal of NSUN2 in mouse cutaneous tumours increases the number of undifferentiated stem and progenitor cells; however, NSUN2-lacking tumour cells are also highly sensitive towards cytotoxic drug treatment with 5-FU and cisplatin [64]. This finding highlights the importance of the dynamic deposition of m^5C into RNA. While stem and tumour-initiating cells lack NSUN2 to maintain a low translating stem cell state [64,116], NSUN2 up-regulation, and thus methylation of the RNA, is required to activate the appropriate survival pathways to regenerate the tumour after cytotoxic insult (***Figure 3***) [64]. The high sensitivity of the tumour

cells towards drug treatment is angiogenin-depended and is therefore at least in part regulated via tRF formation [64].

Activation of the PI3K signaling pathway in melanoma cells enhances the expression of mcm^5s^2U writers [79]. The tRNA wobble modification mcm^5s^2U is also required for specific codon decoding during translation and sustains resistance in melanoma [79]. Rewiring of protein synthesis during $BRAF^{V600E}$-driven resistance to targeted therapy induces a translational bias for mcm^5s^2U-dependent codons, which are for instance found in the *Hif1a* mRNA. The enhanced synthesis of the HIF1α protein thereby promotes glycolysis and maintains the metabolic requirements for the melanoma cells [79]. The resistant cells are re-sensitized to drug treatment through depletion of the mcm^5s^2U writers (***Figure 3***) [79]. Together, these recent studies highlight the importance of RNA modification pathways in most aspects of tumourigenesis.

Summary and future prospective

RNA modifications are key players in regulating cell fate decision during development. More recently, RNA modifications also emerged as an important regulator of cancer. Similar to stem cells in most adult tissues, also tumour-initiating cells maintain the tumour in the long term. An important feature of tumour-initiating cells 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 cells to a quiescent tumour-initiating cell in response to chemotherapeutic drug treatment, may represent a window of opportunity to specifically target tumour-initating 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 [41,117]. RNA modifications emerged as one mechanistic link between metabolism and enhanced codon-dependent translation of HIF1α for instance to promote glycolytic metabolism [79]. 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 offer novel therapeutic strategies to target specific tumour populations, such as slow cycling tumour-initiating populations or resistant tumour cells.

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

While 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 needs 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. In

addition, the dynamic expression patterns of writer, reader and eraser proteins complicates 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, it is currently largely unclear how different modifications influence each other and affect the binding to RNA-binding proteins. The development of new tools for the identification and quantification of RNA modification will be essential to further unearth their roles in the different steps of cancer development.

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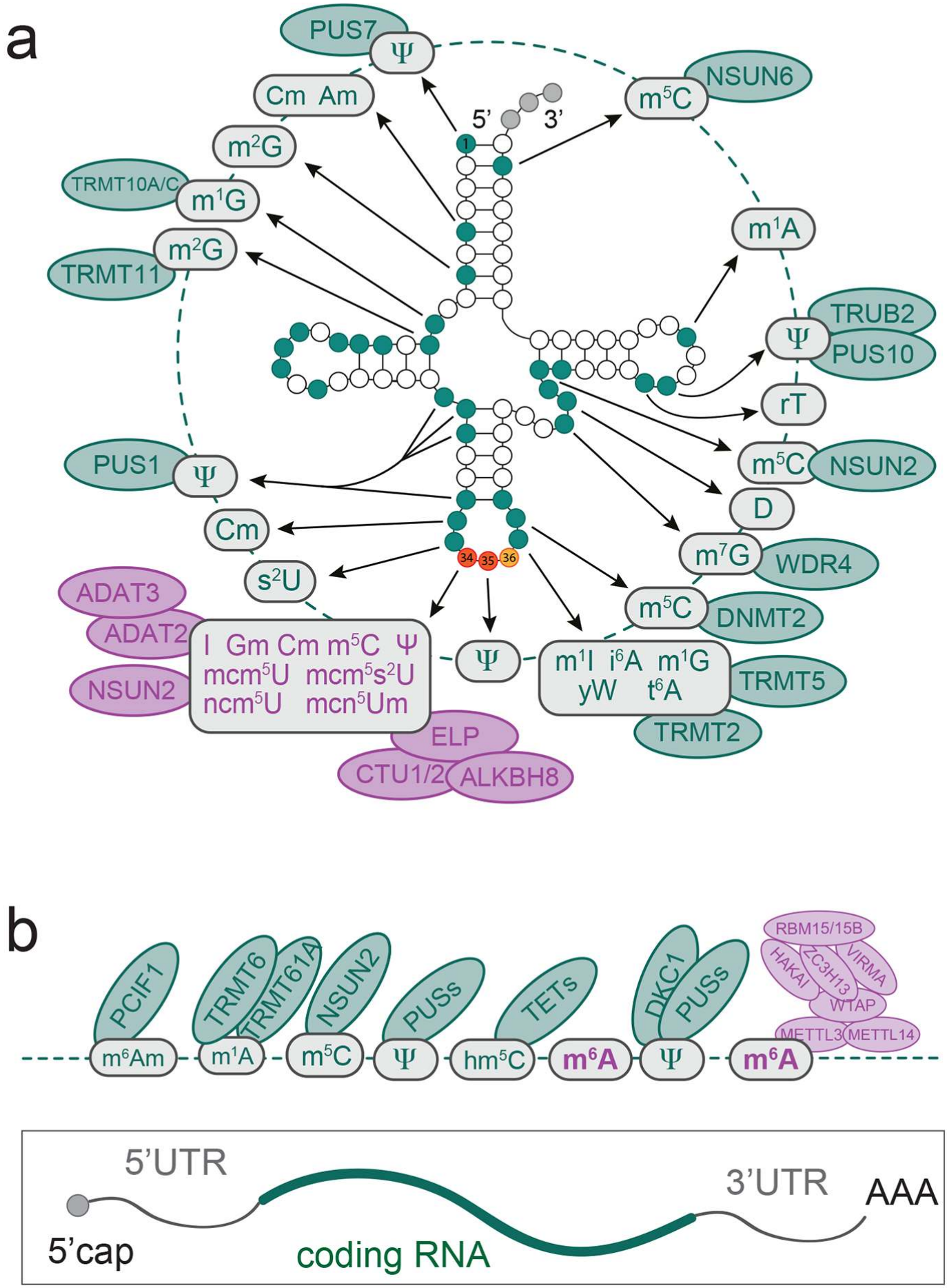
Figure Legends

Figure 1: RNA modifications and their writer proteins. a, Schematic representation of a tRNA molecule and examples of RNA modifications and their modifying enzymes. Modifications at the wobble position are highlighted in purple. **b**, Schematic representation of the modifications internal to messenger RNA. Some of these modifications are enriched in the 5'UTR, the coding sequence or the 3'UTR of the mRNA. m^6A is catalyzed by a complex of multi-proteins containing enzymes and accessory proteins. Abbreviations: m^1A: N1-methyladenosine; Ψ: pseudouridine; rT: ribothimidine; m^5C: 5-methylcytosine; D: dihydrouridine; m^7G, 7-methylguanosine; m^1I: 1-methylinosine; i^6A: N6-isopentenyladenosine; m^1G: 1-methylguanosine; yW: wybutosine; t^6A: N6-threonylcarbamoyladenosine; I: inosine; Gm: 2'-O-methylguanosine; Cm: 2'-O-methylcytidine; mcm^5U, 5-methoxycarbonylmethyluridine; mcm^5s^2U, 5-methoxycarbonylmethyl-2-thiouridine; ncm^5U: 5-carbamoylmethyluridine; ncm^5Um: 5-carbamoylmethyl-2'-O-methyluridine; s^2U: 2-thiouridine; Am: 2'-O-methyladenosine; m^2G: N2-methylguanosine; m^6Am: N6,2'-O-dimethyladenosine; hm^5C: 5-hydroxymethylcytidine. PUS: Pseudouridylate Synthase; TRUB2: TruB Pseudouridine Synthase Family Member 2; NSUN2, NOP2/Sun RNA methyltransferase family member 2; WDR4, WD repeat domain 4; DNMT2, DNA methyltransferase 2; TRM or TRMT, tRNA methyltransferase; ELP, Elongator protein homolog; CTU: Cytosolic Thiouridylase; ALKBH: AlkB Homolog 8, TRNA Methyltransferase; ADAT3, adenosine deaminase acting on tRNA 3; 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^6A Methyltransferase Associated; WTAP: WT1 Associated Protein; METTL: Methyltransferase Like.

Figure 2: RNA modifications regulate gene expression programs. a, m^6A is deposited by a '*writer*' multi-protein complex (i.e. METTL3, METTL14) and removed by '*eraser*' demethylases (i.e. FTO, ALKBH5), which induce stabilization or decay of the target mRNA. In the cytoplasm, the mRNA modifications are recognized by '*reader*' proteins. **b**, The deposition of mcm^5s^2U modification is required for the optimal base pairing between $tRNA^{UUU}$, $tRNA^{UUC}$, $tRNA^{UUG}$ and $tRNA^{UCU}$ and the corresponding codons enriched in specific mRNA targets (i.e. *Sox9*, *Dek*, *Lef1*). **c**, tRNAs are methylated by NSUN2 in the nucleoli. The m^5C modification reduces the affinity to the endonuclease angiogenin in the cytoplasm. m^5C maintains global protein synthesis. Loss of m^5C alters the biogenesis of tRNA-derived small non-coding RNA fragments (tRFs), which inhibit *de novo* protein synthesis.

Figure 3: Roles of RNA modifications in cancer. RNA modifications are involved in multiple aspects of tumourigenesis. mcm^5s^2U is required for Wnt-driven colorectal cancer (CRC) initiation, development of lung metastasis from PyMT breast tumours, and PI3K pathway-addicted resistance to therapy in melanoma. m^5C levels are high 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. Elevated levels of m^6A on specific mRNA inhibit metastasis in hepatocellular carcinoma (HCC) and growth in glioblastoma tumours. m^6A is also advantageous for the maintenance of a cell-undifferentiated state in leukemia and promotes tumour initiation. In breast cancer cell lines, up-regulation of ALKBH5 enhances tumour initiation capacity.

Figure 1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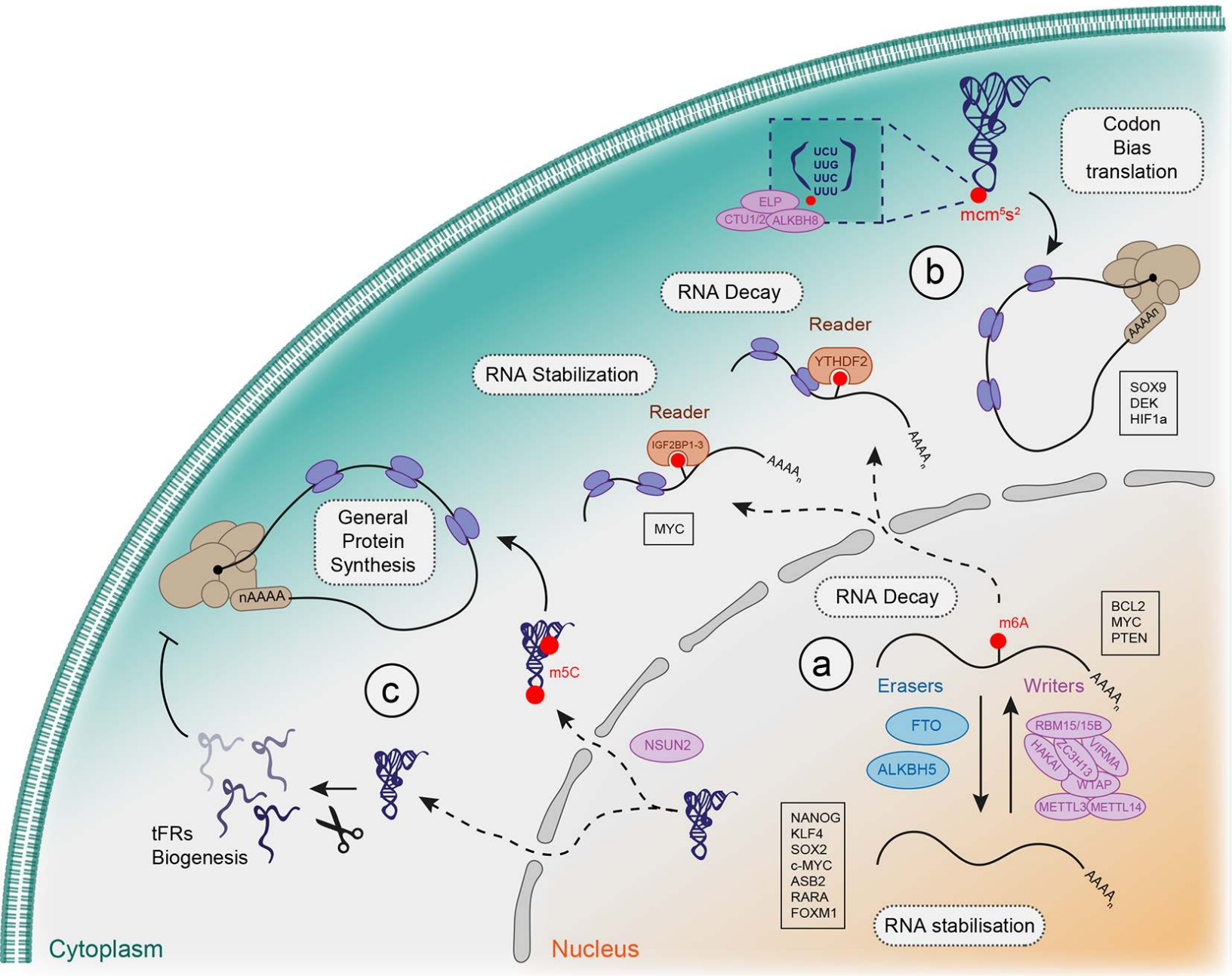
5'cap
coding RNA

Figure 2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AAAAn
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General Protein Synthesis
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NSUN2
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RNA Decay
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BCL2
MYC
PTEN
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AAAAn
FTO
ALKBH5
RBM15/15B
ZC3H13
VIRMA
HAKAI
WTAP
METTL3
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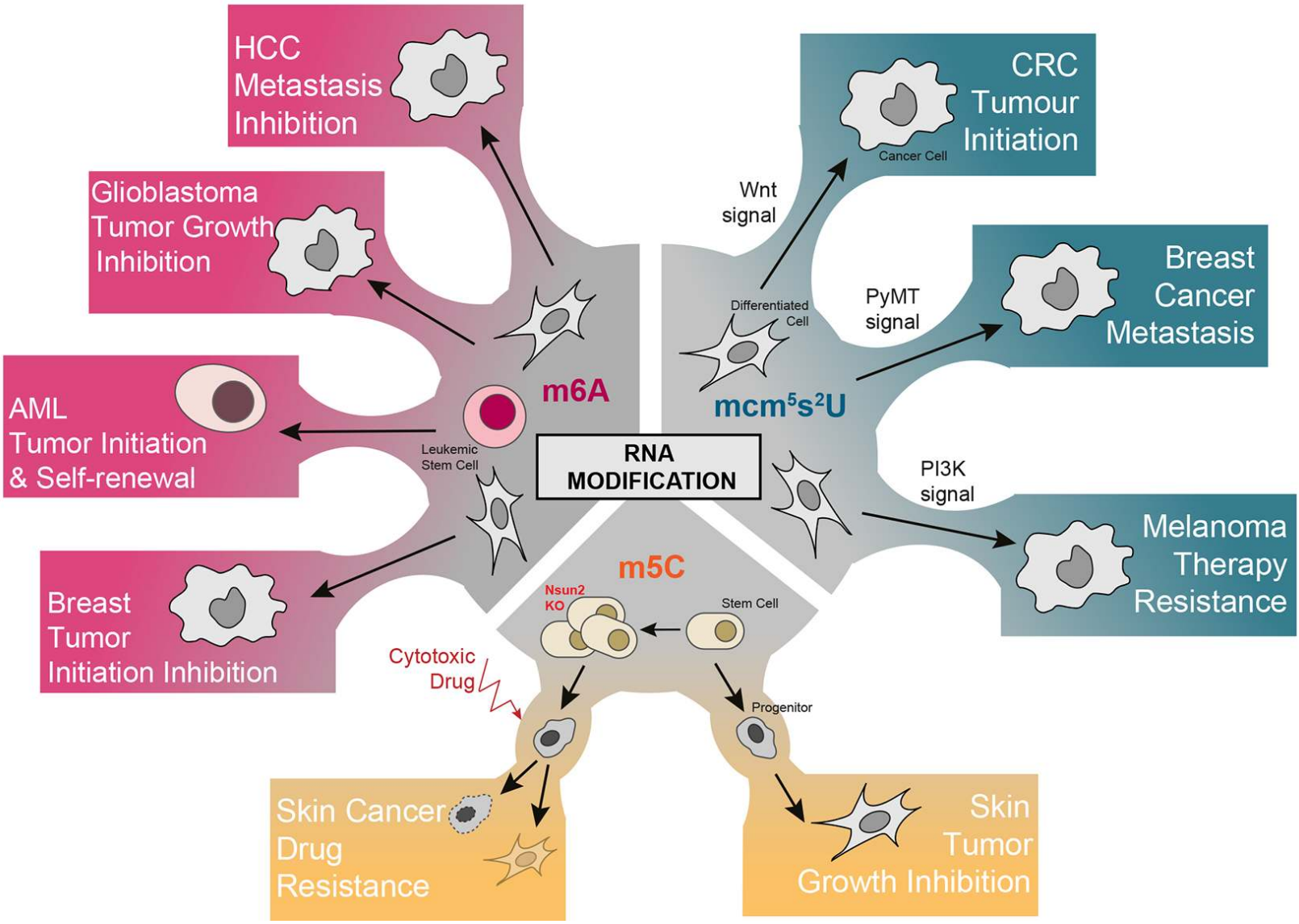
Cytoplasm
Nucleus

Figure 3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Leukemic
Stem Cell
RNA
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Therapy
Resistance
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