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RAPID COMMUNICATION

Altered expression of m^{1}A regulatory genes is associated with oncogenic pathways, overall survival, and infiltration of immune cells in diverse human cancers

Of over 170 types of RNA modifications discovered, N^6-methyladenosine (m^{6}A) is the most actively studied. m^{6}A confers oncogenic potential, maintains cancer stem cells, and is associated with cancer-related outcomes.[1] The role of RNA modifications including m^{1}A is poorly understood. m^{1}A is abundant in transfer and ribosomal RNAs but has also been found on messenger RNAs.[1–4] m^{1}A stabilizes these RNA species to regulate essential biological processes including translation and ribosome biogenesis, which are frequently dysregulated in cancers.[2–4] However, the role of aberrant m^{1}A RNA methylation in cancer development and its utility in predicting clinical outcomes is currently unclear. Understanding the function of m^{1}A RNA methylation in diverse cancers requires the characterization of genetic alterations and aberrant expression of m^{1}A regulatory genes. These genes include m^{1}A writers (*TRM61A*, *TRIM61B*, *TRIMT61B*, *TRMT10C*), erasers (*ALKBH1*, *ALKBH3*) and readers (*YTHDF1*, *YTHDF2*, *YTHDF3* and *YTHDC1*).[2–4] Here, we present the frequency of molecular abnormalities affecting m^{1}A regulatory genes and their association with cancer-related pathways, prognosis, and tumor immune cell infiltration across 33 types of human cancers.

We first determined the frequency of mutations in m^{1}A writers, readers, and erasers across 33 cancer types available via the Cancer Genome Atlas (TCGA). Although the average mutation frequency was low (0.11%–1.23%) (Table S1), one-fifth of cancer types have concomitantly high mutation frequency in 8/10 (>80%) m^{1}A regulatory genes (Fig. 1A and Table S1). These cancer types were predominantly those bearing a higher mutation burden including endometrial carcinoma (UCEC), melanoma (SKCM), lung squamous cell carcinoma (LUSC), colon, gastric and rectal adenocarcinomas (COAD, STAD and READ) (Fig. 1A; Fig. S1A). Some cancers harbored uniquely higher mutation frequencies for individual m^{1}A regulatory genes, such as *TRMT61B* in mesothelioma (MESO), *YTHDC1* in kidney cancer (KICH), and *YTHDF2* in diffuse large B-cell lymphoma (DLBC) (Fig. 1A).

We next determined the copy number alteration (CNA) in m^{1}A regulatory genes across the 33 cancer types. Our analysis revealed an increased prevalence of copy number gain over the loss (Fig. 1B) and this observation held true with stringent analysis that include "amplification" and "deep deletion" only (Fig. S1B). Among the m^{1}A writer genes, *TRM61B*, *TRMT10C*, and *TRMT6* were more often amplified in cancers (Fig. S2 and Table S2). *TRMT61A* showed cancer-specific patterns of genetic alteration, where ovarian cancer (OV), LUSC, and adenoid cystic carcinoma (ACC) harbored a higher frequency of amplification while deletion was prevalent in DLBC and bladder carcinoma (BLCA) (Fig. S2 and Table S2). Among readers, amplification of *YTHDF1* and *YTHDF3* were more frequent in individual cancers (up to 15%) compared to *YTHDF2* and *YTHDC1* (<4%) (Fig. 1B; Fig. S2 and Table S2). Genes encoding m^{1}A erasers were less frequently altered (<2% in individual cancers) (Fig. S2 and Table S2).

We further explored the changes in gene expression of m^{1}A regulatory genes in cancers and matched normal tissues. Consistent with the prevalence of m^{1}A writer amplification (Fig. S2 and Table S2), we also observed a general increase in their expression across most cancer types (Fig.1 C; Fig. S3). Intriguingly, kidney cancers (KIRP, KIRC, and KICH) showed downregulation of *TRMT6* expression, indicating some uniqueness of *TRMT6* expression in cancers

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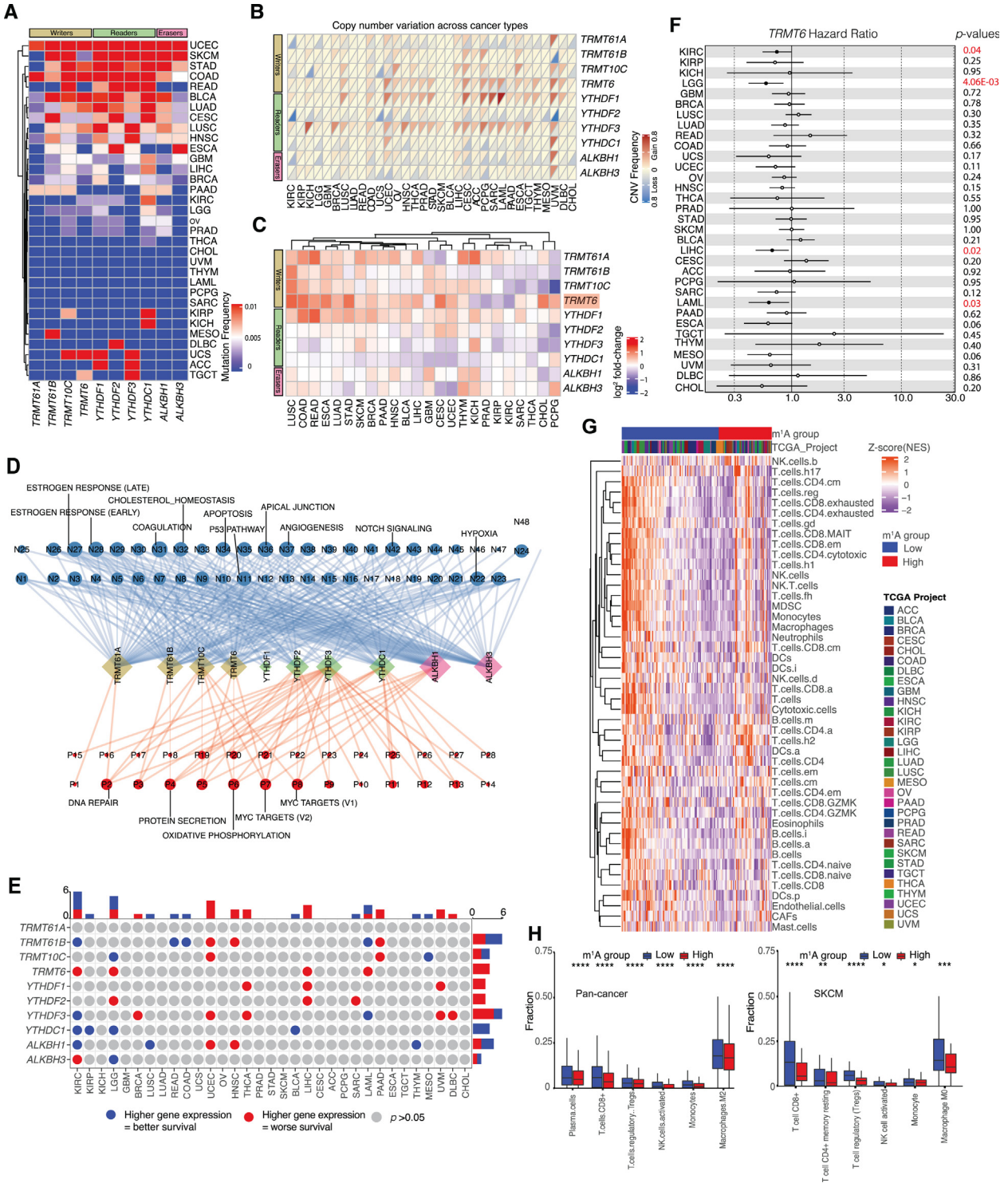

Figure 1 Alterations of m^{1}A regulatory genes and associations with tumorigenic pathways, overall survival, and immune cell infiltration in 33 human cancers. **(A)** The mutation frequency distribution of m^{1}A regulatory genes across 33 cancer types. Gradients from dark blue to bright red indicate the lowest to the highest frequency of mutations. **(B)** The CNV alteration frequency of m^{1}A regulatory genes across 33 cancer types. The upper and lower part of each grid shows the frequency of copy number gain and loss respectively. **(C)** The gene expression alteration of m^{1}A regulatory genes in 24 cancer types, whereby matched tumor and normal samples were available. The heatmap represents $\log_2$ fold-change (tumor/normal). Red and blue show up-regulated and down-regulated genes respectively. **(D)** Network diagram demonstrating the association between the expression of m^{1}A regulatory genes and cancer hallmark-related pathways. Red nodes show positively-correlated pathways, and blue nodes show negatively-correlated pathways. The size of each node corresponds to the number of linked genes within each pathway. **(E)** Summary of the association between expression of m^{1}A regulatory genes and patient survival across 33 cancer types. Red represents higher gene expression

that originate from kidney cells (Fig. 1C; Fig. S3). Glioblastoma (GBM), thymoma (THYM), KICH and pheochromocytoma and paraganglioma (PCPG) exhibited increased expression of m^{1}A erasers, often with concomitant downregulation of one or more writers (Fig. 1C). Overall, our results indicate a diverse pattern of m^{1}A regulator dysregulation that may underpin the development and maintenance of different cancer types.

To explore molecular mechanisms by which altered expression of m^{1}A regulators may contribute to cancer pathogenesis, we correlated the expression of individual m^{1}A regulatory genes with the activity of cancer-related pathways. In general, the expression of m^{1}A regulatory genes was negatively correlated with major cancer pathways except for DNA repair, protein secretion, oxidative phosphorylation, and MYC signaling pathways (Fig. 1D and Table S3). m^{1}A regulatory genes may therefore be involved in the activation of specific cancer-related pathways.

We next examined the clinical relevance of m^{1}A regulators by performing overall survival (OS) analysis based on the expression of m^{1}A regulatory genes. Overall, we found that the expression of one or more m^{1}A regulatory genes was associated with patient survival in 19 cancers (Fig. 1E). Cancers with the highest number of m^{1}A regulatory genes that were significantly associated with OS include KIRC ($n = 6$), low-grade glioma (LGG, $n = 5$), UCEC ($n = 4$), hepatocellular carcinoma (LIHC, $n = 3$), and acute myeloid leukemia (LAML, $n = 3$).

Although *TRIM61A* expression was not associated with OS in any cancer type, nine other genes showed significant association with OS in at least two cancer types (Fig. 1E). *YTHDC1* displayed a feature of tumor suppressor genes whereby higher expression of *YTHDC1* was significantly associated with better survival in four cancer types, including KIRC (hazard ratio, HR = 1.52, $P = 0.0069$), KIRP (HR = 1.86, $P = 0.048$), LGG (HR = 1.71, $P = 0.0031$), and BLCA (HR = 1.5, $P = 0.0073$) (Fig. S4). Conversely, three m^{1}A regulatory genes, *TRMT6*, *YTHDF1*, and *YTHDF2*, displayed oncogenic characteristics reflected in poorer OS. Higher expression of *TRMT6* was significantly associated with worse OS in four cancer types, including KIRC (HR = 0.73, $P = 0.043$), LGG (HR = 0.58, $P = 0.0037$), LIHC (HR = 0.66, $P = 0.02$), and LAML (HR = 0.62, $P = 0.026$) (Fig. 1F; Fig. S5A). Poorer OS in LIHC was also significantly associated with higher expression of genes encoding m^{1}A readers, YTHDF1 and YTHDF2 (Fig. S5B), indicating the potential contribution of multiple overexpressed m^{1}A regulators to adverse prognosis in this type of cancer.

Notably, higher expression of *TRM61B* and *TRMT10C* were significantly associated with both better and worse survival in different cancers (Fig. S6A, B). Higher expression of *TRMT61B* and *TRMT10C* showed a significant association with worse survival in UCEC (Fig. S6C). Higher expression of *TRMT61B* and *TRMT10C* was significantly associated with better survival in KIRC and LGG respectively (Fig. S6D). Thus, aberrant expression of m^{1}A regulatory genes predicts OS in a cancer-type-specific manner.

We then determined the infiltration of immune cells based on the overall high or low expression of m^{1}A regulatory genes and observed higher enrichment of immune cell infiltration in the m^{1}A-low group (Fig. 1G). A deconvolution method (CIBERSORT) was used to characterize the immune cell composition between the m^{1}A-low and -high groups from their gene expression profiles. Plasma cells, T cells CD8, T cells regulatory (Tregs), activated NK cells, monocytes and macrophages showed significantly higher infiltration in the m^{1}A-low group of a pan-cancer cohort (Fig. 1H). Of clinical relevance, we confirmed the same trend in the patients with SKCM, a cancer subtype that is amenable to immunotherapy (Fig. 1H). The m^{1}A-low group also exhibited significantly higher ESTIMATE, immune and stromal scores compared to the m^{1}A-high group (Fig. S7). These results indicate that m^{1}A-low tumors tend to be associated with increased immune cell infiltration and represent "hot tumors" that may be more responsive to immunotherapy.[5]

Overall, we provide a global landscape of the genetic alterations and expression changes of m^{1}A regulatory genes across diverse cancer types. We reveal the strong association between the dysregulation of m^{1}A regulatory genes, the activation of cancer pathways, and overall survival in a tumor-type-specific manner. Low m^{1}A gene expression was also associated with increased immune cell infiltration and may be clinically useful to infer responsiveness to immunotherapy. This work will serve as a critical resource to understand the role of aberrant RNA methylation in cancer and guide the development of novel therapeutic interventions.

associated with worse survival; blue represents higher gene expression associated with better survival, and grey means not significant (log-rank P values > 0.05). **(F)** The distribution of *TRMT6* hazard ratio (HR) across 33 cancer types. Solid circle and P values in red indicate that expression of *TRMT6* is significantly associated with patient survival (HR < 1 indicates worse survival; >1 indicates better survival). **(G)** Heatmap showing the normalized enrichment scores of 46 immune cell infiltrates in the TCGA pan-cancer cohort of 9776 patients using the single-sample Gene Set Enrichment Analysis (ssGSEA) scores. Rows represent tumor-infiltrating cells, and columns represent samples (blue and red indicate the m^{1}A-low and -high groups respectively). "Red color cluster" represents "hot" tumors with more immune cell infiltration, and "blue color cluster" represents "cold" tumors with less immune cell infiltration. **(H)** Box plots showing the immune cell infiltration composition of tumor microenvironment in the m^{1}A-low and -high groups across 33 cancer types (left) and melanoma (SKCM) only (right). The Wilcoxon Rank Sum test was used to compare the two subgroups. Blue and red indicate the m^{1}A-low and -high groups respectively. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Author contributions

JJ-LW conceived the study and interpreted the data. RS performed data analysis. JJ-LW and RS wrote the manuscript.

Conflict of interests

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.11.001.

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