Remodeling of the m6A RNA landscape in the conversion of acute lymphoblastic leukemia cells to macrophages

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TO THE EDITOR:
Leukemia cells show an altered transcriptome and proteome that can be associated to many genetic and epigenetic defects. Adding complexity to the biology of transformed cells and its RNA and protein landscape, it has recently been shown that cancer cells also exhibit a distorted pattern of chemical modifications of the RNA molecule [1, 2], the so called epitranscriptome. More than 150 differentially modified nucleotides have been reported in various RNA species, affecting transcript structure, stability, splicing, nuclear export, targeting or translational efficiency [1, 2]. The most abundant internal modification of messenger RNA (mRNA) [3] is the methylation of adenosine (A) in the form of m6A, affecting numerous features of RNA activity and metabolism [4]. The identification of an m6A eraser, FTO [5], represented the first proof of a reversible mRNA modification and has further stimulated epitranscriptome research in cellular differentiation and carcinogenesis. Since then, the molecular pathways of m6A have been carefully dissected revealing that the mark is established by an RNA methyltransferase writer complex with a catalytic subunit, METTL3 and several assistant proteins (METTL14, WTAP, RBM15, KIAA1429, and ZC3H13) [4]. In addition to FTO, m6A can also be reversed by ALKBH5 [4]. The m6A mark is “read” by m6A-binding proteins, such as members of the YTH family (YTHDF1-3 and YTHDC1-2), IGF2BP1-3 proteins, and heterogeneous nuclear ribonucleoproteins (hnRNPs) [4].

In hematopoiesis, the generation of all the different types of mature blood cells from hematopoietic stem cells (HSCs) requires a tight control of RNA activity and disrupted patterns of m6A RNA modification and alterations in its associated proteins impair physiological hematopoiesis and are also observed in hematological malignancies [6–8]. In this regard, m6A marking was shown to be important for the resolution of the naive state of embryonic stem cells to primed cells, the control of cell fate decisions in early hematopoiesis and the maintenance of hematopoietic stem cell identity and symmetric commitment [8, 9]. Less is known about the role of m6A RNA decoration in transition of differentiated stages of more mature hematopoietic cells in physiological and pathological microenvironmental conditions. Examples of hematopoietic cell lineage conversion and plasticity include B-cell lymphomas that transdifferentiate to histiocytic/dendritic cell sarcoma and B-cell acute lymphoblastic leukemia (ALL) patients that escape both antibody treatments and chimeric antigen receptor (CAR) T-cell therapy against CD19 by converting to AML. We have recently identified that myeloid-lineage transdifferentiation is associated with a reconfiguration of the DNA methylation landscape [10]. We now investigated whether such transdifferentiation also involves a switch in the m6A RNA epitranscriptome.

Our experimental model involves transdifferentiation of pre-B cells into macrophages. Following early work showing that murine B-cell precursors can be induced by C/EBPa to convert into functional macrophages, we used murine and human cellular models of pre-B cells containing C/EBPa fused with the estrogen receptor hormone-binding domain (C/EBPaER) that transdifferentiate to macrophages upon 17β-estradiol exposure [11, 12]. Primary human BCR-ABL1(+)- ALL cells can also be reprogrammed into macrophage-like cells by C/EBPa expression [13]. To identify possible remodeling of the m6A RNA landscape upon cell conversion, we have herein studied the human precursor B-ALL cell line RCH-A CV transfected with the transgene C/EBPaER, designated below as BlaER1, at the start (0 h) and end (168 h) of transdifferentiation timepoints upon 17β-estradiol exposure, using m6A-seq raw data have been deposited in the Sequence Read Archive (SRA) BioProject (accession number PRJNA734010).

m6A profiling of efficiently transdifferentiated cells, which was validated by the shift in the corresponding CD19 and CD11B markers (Fig. 1A) and additional B-cell and macrophage markers (Fig. S1), revealed that whereas 406 m6A peaks (corresponding to 326 transcripts) were stable between the 0 h and 168 h time...
cellulation. As to the precise location within the mRNA molecule of these m6A peaks, 62.5% (3796) were localized in gene-body related sequences, corresponding to 36.6% (1922) and 30.9% (1874) intron and exon RNA sequences, respectively; whereas 19% (1153) were in 5′-UTR, 3′-UTR and 5′-Untranslated Region; 3′-UTR, 3′-Untranslated Region. One key issue related to the impact of m6A marks in mRNA function relates to the location of the modification [1, 2]. The first and best-recognized activity of m6A is to induce mRNA instability [15], particularly when deposited at 3′-UTR m6A sites on gene 5′-UTR m6A sites on gene. To study the effect of identified differential 3′-UTR m6A sites on gene expression in our model, we took advantage of the available microarray expression data of the start (0 h) and end (168 h) cell conversion timepoints [12]. We did not observe any overall association between the presence of m6A peaks and expression levels (Fisher's exact test, 2-Tail, P = 0.78), even when stratified for losses and gains of m6A vs upregulation or downregulation of the corresponding transcripts (Fisher's exact test, 2-Tail, P = 0.29) (Fig. S4). We observed, in agreement with the previously published literature [1, 2, 4], that an increase in 3′-UTR m6A sites was associated with transcript downregulation, taking into consideration the 3′UTR m6A sites that exhibited a corresponding transcript in the expression microarray (Fisher's exact test, 2-Tail, P = 0.017). Most importantly, and in agreement with the GO results (Fig. 1C and Fig. S3), we found an enrichment in genes related to ribosomal translation, splicing, and processing [44], and best-recognized activity of m6A is to induce mRNA translation. To study the effect of identified differential 3′-UTR m6A sites on gene expression in our model, we took advantage of the available microarray expression data of the start (0 h) and end (168 h) cell conversion timepoints [12]. We did not observe any overall association between the presence of m6A peaks and expression levels (Fisher's exact test, 2-Tail, P = 0.78), even when stratified for losses and gains of m6A vs upregulation or downregulation of the corresponding transcripts (Fisher's exact test, 2-Tail, P = 0.29) (Fig. S4). We observed, in agreement with the previously published literature [1, 2, 4], that an increase in 3′-UTR m6A sites was associated with transcript downregulation, taking into consideration the 3′UTR m6A sites that exhibited a corresponding transcript in the expression microarray (Fisher's exact test, 2-Tail, P = 0.017). Most importantly, and in agreement with the GO results (Fig. 1C and Fig. S3), we found an enrichment in genes related to ribosomal translation, splicing, and processing [44], and best-recognized activity of m6A is to induce mRNA translation. To study the effect of identified differential 3′-UTR m6A sites on gene expression in our model, we took advantage of the available microarray expression data of the start (0 h) and end (168 h) cell conversion timepoints [12]. We did not observe any overall association between the presence of m6A peaks and expression levels (Fisher's exact test, 2-Tail, P = 0.78), even when stratified for losses and gains of m6A vs upregulation or downregulation of the corresponding transcripts (Fisher's exact test, 2-Tail, P = 0.29) (Fig. S4). We observed, in agreement with the previously published literature [1, 2, 4], that an increase in 3′-UTR m6A sites was associated with transcript downregulation, taking into consideration the 3′UTR m6A sites that exhibited a corresponding transcript in the expression microarray (Fisher's exact test, 2-Tail, P = 0.017). Most importantly, and in agreement with the GO results (Fig. 1C and Fig. S3), we found an enrichment in genes related to ribosomal translation, splicing, and processing [44], and best-recognized activity of m6A is to induce mRNA translation.
Having shown the above associations that support a functional role for m6A in pre-B cell to macrophage transdifferentiation, particularly targeting 3'UTRs of ribosome-associated transcripts, we experimentally validated this model by knocking down the main m6A RNA methyltransferase, METTL3 [4]. Efficient shRNA-mediated downregulation of METTL3 in the BLaER1 pre-B cells using three different target sequences (Fig. 2B and Fig. S7) lead to a significant decrease in transdifferentiation rate as measured by the sorted out population of successfully transdifferentiated cells, particularly by decorating the 3'UTRs of genes related to the ribosome translational machinery. These findings highlight the role of this epitranscriptomic signal, and the proteins controlling and mediating its deposition and downstream effects, in hematological transdifferentiation pathways.

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(RPS25, RPL23A, RPS3, RPS21, RPS27, RPS14, and RPL3) and we confirmed their significant downregulation at the end of the cell conversion (Fig. 2A). Importantly, using the Actinomycin D assay to determine the mRNA stability of our top candidate RPS25, which is downregulated at the RNA (Fig. 2A) and protein (Fig. S5) levels in our model, we observed that transdifferentiation induced a reduction of transcript stability (Fig. S5). This results is in agreement with the proposed role of a gain of m6A mark in the 3'-UTR [1, 2, 4]. Interestingly, cells in a middle time point of the conversion process (72 h) show intermediate values for all the above-described parameters (B-cell vs macrophage markers, m6A-seq, and ribosomal protein expression patterns) (Fig. S6), supporting that the described transdifferentiation model reflects a gradual change from one cell type to the other.

Overall, these results indicate a relevant activity of m6A RNA marks in the succesfull generation of a macrophage from pre-B cells, particularly by decorating the 3'-UTRs of genes related to the ribosome translational machinery. These findings highlight the role of this epitranscriptomic signal, and the proteins controlling and mediating its deposition and downstream effects, in hematological transdifferentiation pathways.
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Author Contributions

AB-C and ME conceived and designed the study; AB-C, VO-B, and LMV performed molecular analyses and studied cellular models; AB-C, DP, and CGP analyzed multimomics data; NAW and BA performed the m^6A quantification; NK, CA, SMM, and GR performed the RNA high-throughput sequencing using the m^6A antibody. ME wrote the manuscript with contributions and approval from all authors.

Competing Interests

ME is a consultant of Ferrer International and Quimatryx. NAW and BA are employees of STORM Therapeutics Ltd. The remaining authors declare that they have no conflict of interest.

Additional Information

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