A Meta-analysis: Evaluating the Effect of METTL3/METTL14 on m6A Level Based on Knockdown Samples

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2021, Hangzhou, China. ACM, New York, NY, USA, 4 pages. https://doi.org/10.1145/3469678.3469715

1 INTRODUCTION

As is the case with DNA and proteins, RNA undertakes chemical modifications which can affect its function, metabolism, and distribution [1]. To date, more than 150 kinds of modifications have been discovered in RNA molecules with which ribosomal and transfer RNA transcripts harbor the majority of the diverse chemical modifications [1, 2]. N6-methyladenosine (m6A) modification is the most prevalent internal modification in eukaryotic mRNAs, which was initially discovered in the 1970s [2]. M6A methylation is a reversible process regulated by the activities of methyltransferase (“writer”) and demethylase (“eraser”); it is catalyzed by “writers”, which is usually a 1MDa complex (MTase) composed of multiple catalytic subunits, namely Methyltransferase-like 3 (METTL3) and Methyltransferase-like 14 (METTL14), a splicing factor (WTAP) and other unnamed subunits [3]. Methyl groups can be temporarily removed from the mRNA transcripts by RNA demethylases (“eraser”). ALKBH5 (alkB homolog5) and FTO (Fat mass and Obesity-associated protein) are two typical m6A eraser proteins [3].

METTL3 and METTL4 play significant roles in various biological processes. It is reported that METTL3 could promote oncogene expression, regulate the development of cancer cell by facilitating translation in it [4-7]. Moreover, the role of METTL3 in controlling myeloid differentiation of leukemia cells as well as normal hematopoietic [8], cardiac homeostasis and hypertrophy [9], gene expressions, and sustenance of oncogenic signaling [10] has been uncovered. The various biological function of METTL14 has been identified as well. Research confirmed that METTL14 could prevent bladder tumorigenesis and self-renewal of bladder TIC [11]. METTL3 and METTL4 can also cooperatively perform specific functions. For instance, the RNA methyltransferase complex of METTL3, METTL4, and WTAP can regulate mitotic clonal expansions in adipogenesis [12] and METTL3/METTL4 mediated mRNA methylation can modulates murine spermatogenesis [13].

Various literatures are available focusing on the consequent effect of knocking down the methyltransferase. Researches shows that the knockdown of METTL3 or METTL14 would upregulates
the expression of certain transcripts such as the pluripotency factor Nanog [14]. Also, depletion of these two protein negatively affect the embryonic stem cell differentiation [15]. Knocking down of METTL3 or METTL14 genes in T cells would lead to slower mRNA decay and suppressed T-cell homeostatic proliferation and differentiation [16]. The critical role of methyltransferase on cancer development was explored as well. A number of literatures reported that the knockdown or depletion of METTL3 or METTL14 results in decreased m6A modifications, which means the methylation level would possibly degrade[13]. Some independent also confirmed that the mRNA methylation would be influenced by the knockdown of METTL3 and/or METTL14. However, no literature has been published to systematically study the relationship between the knockdown of methyltransferase and methylation level based on published literatures or data. Therefore, we designed this project to evaluate the quality and strength of the current evidence regarding the effects of the knockdown of METTL3/METTL14 on mRNA m6A methylation levels under different cell line.

2 MATERIAL AND METHODS
2.1 Data Sets and Preprocessing
Data from the 17 datasets were collated and grouped based on their cell line and the gene knockdown (METTL3 or METTL14 gene). There are respectively 8 and 6 samples for METTL3 and METTL14 genes and each sample was further divided into control group and knockdown group. Information about datasets can be accessed from: https://github.com/yuxuanwu17/meta_analysis/tree/master/citation. We also did a PRISMA flowchart to present the process of data selection. The PRISMA flowchart can be accessed from: https://github.com/yuxuanwu17/meta_prisma

2.2 Data Extraction and Measures
Leading authors, publication citation, GSE number, cell line, and treatment (control or knockdown of METTL3/METTL14 gene) were collected from the collected dataset. The quality and reliability of data were measured based on the journal where they published, profiling approaches used, and the appropriateness of the experiments.

Experiment groups were prepared via the RNA methylation differential analysis with MeRIP-seq data [17] and exome Peak R/Bioconductor package [18]. By calculating the difference between methylation level under different treatments, samples were categorized as “experimentally positive” or “experimentally negative”. For a given sample, if its experimental group has a higher methylation level than its control group, then it would be considered as “experimentally positive” and vice versa.

2.3 Statistical Analysis
The RNA methylation level in the experimental group and control group were compared and assessed. We used a R package “metafor” to perform meta-analysis. Additionally, we calculated the risk ratios (RRs) and used random-effects model to calculate 95% CIs.

The data within the studies were pooled and evaluated by statistical heterogeneity I 2, which is the percentage of the total variation across studies that is due to heterogeneity rather than by chance. An I 2 of 25%, 50%, and 75% were considered as low, moderate, and high, respectively. A Forest plot was made to compare and visualize the relationship between each sample, demonstrating the 95% CIs and effect sizes.

When an inconsistency was discovered between the RR for the same outcome, subgroup analysis was applied to determine the data which contribute significantly to the high heterogeneity. The potential influence of each sample was determined, ordered, and visualized by the R package introduced by Baujat [19]. Samples were eliminated by order and the heterogeneity of the left samples was analyzed until the heterogeneity was reduced to an acceptable level. Furthermore, the remaining sample was further divided into ten pieces and each piece was considered as housekeeping genes. Then data were recombined and conduct the subgroup analysis to figure out the lowest heterogeneity combination. Funnel plots were produced to visualize the potential publication bias since researchers tend to publish strong effect sizes study. Additionally, both the rank correlation test and Egger’s regression test was used as an objective measurement of potential bias.

3 RESULT AND DISCUSSION
3.1 The Basic Model
In the basic model, eight studies of methyltransferase knockdown based on different cell lines are included. As shown in the forest plot reported in Figure 1, by knocking down the METTL3 genes, there is a high possibility (Log RR = 6.93, 95% CI = 3.63-13.25, p-value < 0.01) that the m6A methylation level increases from the level where the METTL3 genes exist. The positive association between knockdown of METTL3 and m6A methylation level is proved significant with high confidence by examining that, across all studies included, the Log Risk Ratios (RR) are far from 1 with narrow 95% confidence intervals (CI). As for the basic model of METTL14, the m6A methylation level increases from the level where the METTL14 genes exist with a high possibility (Log RR = 11.53, 95% CI = 2.96-44.95, p-value < 0.01). The significant positive association between knockdown of METTL14 and m6A methylation level is larger than that in the case of METTL3, while the 95% CI is wider in the sense of less certainty. Data from the 17 datasets were collated and grouped based on their cell line and the gene knockdown (METTL3 or METTL14 gene). There are respectively 8 and 6 samples for METTL3 and METTL14 genes and each sample was further divided into control group and knockdown group.

However, strong heterogeneity or inconsistency of METTL3 (Q(df = 7) = 3461.82, I2 = 99.9%, tau2 = 0.8716) and METTL14 (Q(df = 5) = 3526.23, I2=100%, tau2 = 2.8867) samples are found among the studies, and through the visual inspection, all the CIs are indeed poorly overlapped. The inconsistent effect across the studies reflects the variability from the systematic review, and is usually caused by the difference in samples or methodology. In this case, as those studies were conducted under different cell lines, it is not surprising that analysis suffers from huge heterogeneity. The significant heterogeneity reported by the Random Effect model indicates that the discrepancy of cellular environment and activities should be considered as the source of between-study variability in meta-analysis.
3.2 Quality Assessment

Investigating heterogeneity is a critical feature of a meta-analysis of studies. To control over heterogeneity, we considered both exclusion rules and moderator analysis. Specifically, we first examined the Baujat plot and excluded the identified studies contributing to heterogeneity, and then we explored the source of heterogeneity by performing subgroup analysis and meta-regression relating design features to an outcome.

The RE model can be used to illustrate differences in the study samples. q value is obtained from homogeneous distribution and is widely used in heterogeneity detection. The I² index represents the total variability of a set of effects, and the log risk ratio is a measure of the effect size.

We used the Baujat diagrams to show the studies’ contribution to heterogeneity. The number in the Figure 2 represented the studies mentioned in the data section. As shown in Figure 2, the Baujat diagrams generated by the METTL3 study dataset determine the impact on the overall results of each study and the Pearson product differences. Because the sixth study was located in the upper right quadrant, it showed an important contribution of heterogeneity to overall results. By contrast, other studies are closer to its origins. So we kept all the research, except for one in the upper right. Similarly, we exclude the fourth study because it is at the far right of the Baujat plot of METTL14.

Furthermore, since the inconsistency (I²) dropped but is still high enough, based on Random Effect model, we introduced design features and conducted subgroup analysis. Including the aforementioned cell lines as a source of variability, we also specified the gene type as one characteristic to serve as a design feature for subgroup analysis. We kept the numbers of characteristics to a minimum to avoid the over-complexity. Instead of analyzing two design features separately, we employed meta-regression
We successfully verified the positive relationship between the knockdown of methyltransferase and methylation levels in different cell lines. Knocking down of Mettl3 or Mettl14 tends to increase the methylation level within cells and the positive relationship is common over different cell lines and gene types. However, the degree of methylation level change responding to the knockdown of methyltransferase gene varied in different cell line and gene types. To our knowledge, this is the first meta-analysis that investigate the association between methyltransferase and methylation levels, which provides an insight to their relationship.

However, there are a few limitations to this project. This meta-analysis is not generally comprehensive due to the lack of experiments or studies studying the relationship between methyltransferase and methylation levels so far. Therefore, the number of works or literature included is limited. Fortunately, the area of m6A methylation is increasingly explored and become popular within bioinformatics communities. Our meta-analysis model could be extended and improved by incorporating newly published studies and data. In addition, publication bias is observable from the funnel plot. More effort will be paid when review, select literature and collect data for our future meta-analysis study.

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