Unlocking miRNA Regulation: Potential and Pitfalls of Single-Cell miRNA-mRNA Co-Sequencing

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Résumé

Introduction

microRNAs (miRNAs) are small non-coding RNA that play pivotal roles in the post-transcriptional regulation of gene expression, influencing various physiological and pathological processes.

In recent years, advancements in single-cell experimental techniques have revolutionized our understanding of cellular heterogeneity. Single-cell experiments enable to capture the dynamic of individual cells within a complex microenvironment and could improve the understanding of the role of microRNA in various biological processes. However, single-cell miRnome datasets are still relatively novel and scarce.

Wang *et al.* (Nat. Comm., 2019) has conducted microRNA and mRNA co-sequencing for 19 K562 single-cells as a proof of concept. The article presents a short statistical analysis of correlations between microRNAs and their predicted mRNA targets.

Materials and methods

We performed Gene Set Enrichment Analysis (GSEA) to assess whether the expression levels of microRNAs are statistically anti-correlated with those of their predicted targets. We varied various parameters to assess their individual contributions.

Results

We showed that only a small proportion of microRNAs significantly anti-correlate with their targets, even when focusing on highly expressed microRNAs with many predicted targets.

After correcting for bias in the null hypothesis estimation, we demonstrate a trade-off between analyzing a small number of targets with high confidence versus including more targets. This trade-off applies to both target conservation through evolution and predicted target efficacy scores. While conserved targets or high-scoring targets show extreme enrichment in anti-correlation, the significance of this enrichment is lower compared to that observed when including a larger number of targets.

We hypothesize, through a comprehensive analysis, that only the most expressed microRNA,

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miR-92a-3p, is responsible for the anti-correlation with its predicted targets in the investigated biological situation, underscoring the necessity of systemic analysis.

We confirmed our results with an independent dataset of miRNA and mRNA co-sequencing of 32 human primary cells from Xiao *et al.* (Genome Biology, 2018). In this other dataset, the highly abundant let-7-5p family, whose members are anti-correlated with their targets, appears to drive all significant microRNA-mRNA anti-correlations.

Discussion

We performed an analysis of two independent public datasets to thoroughly comprehend their potential and limitations. This type of dataset is exceptionally valuable for gaining a deeper knowledge of how microRNAs impact the expression of their mRNA targets. However, analysis must be conducted using systematic approaches and with utmost care.

Reference

Velut, L., Fancello, L., Cherradi, N., & Guyon, L. (2025). **Single-cell microRNA-mRNA co-sequencing techniques convey large potential for understanding microRNA regulations but require careful and systemic approaches**. *Nature Communications*, *16*(1), 5255.

Mots-Clés: microRNA, sequencing, single, cell