Mini-Review

DOI: 10.5582/irdr.2023.01073

Single-cell metabolomics in rare disease: From technology to disease

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SUMMARY With the development of clinical experience and technology, rare diseases (RDs) are gradually coming into the limelight. As they often lead to poor prognosis, it is urgent to promote the accuracy and rapidity of diagnosis and promote the development of therapeutic drugs. In recent years, with the rapid improvement of single-cell sequencing technology, the advantages of multi-omics combined application in diseases have been continuously explored. Single-cell metabolomics represents a powerful tool for advancing our understanding of rare diseases, particularly metabolic RDs, and transforming clinical practice. By unraveling the intricacies of cellular metabolism at a single-cell resolution, this innovative approach holds the potential to revolutionize diagnosis, treatment, and management strategies, ultimately improving outcomes for RDs patients. Continued research and technological advancements in single-cell metabolomics are essential for realizing its full potential in the field of RDs diagnosis and therapeutics. It is expected that single-cell metabolomics can be better applied to RDs research in the future, for the benefit of patients and society.

Keywords rare diseases, single cell omics, inborn errors of metabolism, single-cell metabolomics

1. Introduction

Rare diseases (RDs) are characterized by their low prevalence in the general population (*1*). Currently, around 6,000-8,000 RDs have been identified, but there are still many undiagnosed and unknown diseases (*2*). This poses a growing public health concern, particularly because the majority (50-75%) of RDs primarily affect children, and a significant proportion (approximately 80%) have a genetic basis (*3-5*).

The prevalence of RDs can vary significantly depending on the region and specific disease type (*6*). The European Union Regulation on orphan medicinal products defines RDs as diseases that affect fewer than 1 in 2,000 individuals in Europe. Similarly, the American Orphan Drug Act defines RDs as diseases that affect fewer than 200,000 patients in the United States. In China, it is estimated that there are approximately 20 million RD patients (*6*). It is important to note that the exact morbidity for most RDs is not currently available, highlighting the need for further research and awareness in this field. Metabolic related diseases comprise a significant portion of rare diseases, representing the majority (*7*). These diseases encompass inborn metabolic abnormalities as well as other rare metabolic conditions with low incidence in the general population (*8*). Inborn errors of metabolism (IEMs) are a subset of rare metabolic diseases that result from defects in enzymes, co-factors, or transport proteins due to mutations affecting crucial metabolic enzymes (*8*). Currently, there are more than 1450 known types of IEMs (*9*). It shows low prevalence but high death rate in IEMs patients. A study estimated the global birth prevalence of all-cause IEM, which is 50.9 per 100,000 live births, resulting in almost 0.4% of child deaths worldwide in 2018 (*10*).

Over the years, there have been significant advancements in basic research, clinical case registration, and the development of orphan drugs for rare diseases (*11,12*). However, it is widely recognized that patients with rare diseases face significant challenges in accessing a definitive diagnosis and effective treatment, particularly in many regions of the world (*13*). The widespread use of next-generation sequencing technology has had a transformative impact on diagnostic accuracy

and cost-effectiveness, surpassing older technologies (*14,15*). Exome sequencing (ES) has played a crucial role in identifying previously unknown diseases as rare diseases (*16,17*). As integrated technologies, such as genomic, transcriptomic, metabolomic, proteomic, and methyl profiling analyses, are increasingly considered for clinical use, functional studies should be conducted to facilitate efficient diagnosis and treatment of rare diseases (*18-21*).

Metabolomics is a field of study that focuses on the analysis of metabolites, including amino acids, sugars, and lipids (*22*). These metabolites have been shown to play vital roles in cellular signaling and various biological processes (*23*). Different from proteomics and genomics, metabolomics provides insights into real-time biochemical activity (*22*). By analyzing metabolomics datasets, researchers can uncover relationships between cellular activities, metabolic processes, and biological mechanisms in both health and disease (*24*). There are three commonly used categories of analytical workflows in cell metabolism analysis: testing the general inputs and outputs of metabolism, characterizing metabolic enzymes through enzyme activity assays, and utilizing steady-state metabolomics analysis through mass spectrometry (MS) technology (*25*). Single-cell technology enables qualitative and detailed analysis of the extensive molecular information carried by a large number of biomolecules at the single-cell level (*26*). Single-cell metabolomics can identify phenotypic heterogeneity between individual cells and discover seemingly similar cell subpopulations to decipher disease specificity, explore stage differences in disease progression, and provide evidence for disease treatment.

Capillary electrophoresis electrospray ionization (CE-ESI) is one of the new techniques for single-cell metabolite analysis (*27*). This technique allows for in situ micro-sampling of live single cells, eliminating the need for cell dissection and separation. CE-ESI bridges the technical gap between comprehensive non-targeted metabolomics and live single-cell analysis (*28*). Another technique, probe-based electrospray ionization (ESI), has been specifically designed for in situ single-cell metabolite analysis (*28*). The development of above techniques offers dual benefits: reduced detection limitations resulting by low sample dilution and the ability to recognize unknown molecules. In the field of single-cell proteomics by mass spectrometry (SCoPE-MS), single cells are amplified to generate sufficient signals for peptide sequencing using tandem mass tags (TMT) (*29*). Looking ahead, we can expect more advanced labeling workflows to be developed for exploring single-cell metabolomics.

2. Single-cell metabolomics in IEM

IEM can affect various organs throughout the body, and the clinical manifestations vary among patients,

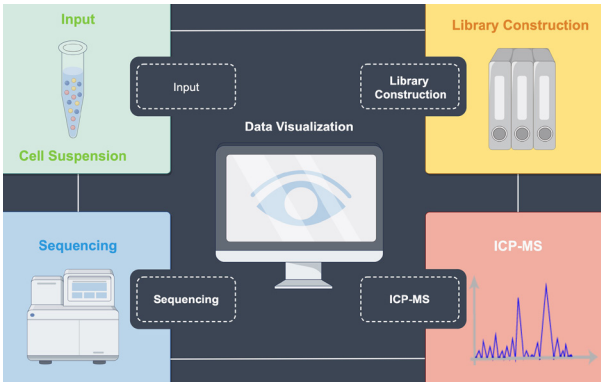

Figure 1. The work flow of single cell sequencing technology combined with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) technology.

often lacking specificity (*10*). This makes it challenging to determine when to perform IEM testing and which specific laboratory tests to conduct due to the absence of characteristic signs and symptoms. However, the development of tandem mass spectrometry (MS) has significantly improved the detection capability for a wider range of diseases from a single blood spot (*30*). Previously, MS was used to test blood and urine samples, relying on the detection of metabolites that are indicative of specific diseases. If the results of metabolite testing suggest a potential disorder, Sanger or next-generation sequencing can be employed to confirm the diagnosis (*31*).

Metabolomics testing is crucial because it is often quicker than other methods, providing valuable information about disease severity and helping to elucidate the significance of mutations found in transcriptome sequencing (*32*). In recent years, numerous methods of analysis have been improved to enable single-cell level analysis, basing on inductively coupled plasma mass spectrometry (ICP-MS) (*33*). The short dwell time of cells in single-cell ICP-MS, which lasts only milliseconds, allows for precise measurement of single-cell metals (*34,35*) (Figure 1). Single-cell ICP-MS has become increasingly important in studying the metal-related properties of cells and its role in investigating cell metal-drug penetration in drug research (*36*).

In addition to single-cell ICP-MS, another valuable concept in sample introduction is the plotting of single cells on a flat surface, which facilitates qualitative and quantitative analysis at the single-cell and sub-cellular levels (*37*). With advancements in high sensitivity and spatial-temporal resolution imaging, researchers now have the ability to conduct detailed qualitative and quantitative analyses at these specific levels (*37,38*). Combination of high-resolution imaging of Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) and single-cell technology prompted new generations of agents based on metals in precision medicine (*39,40*).

Leigh syndrome (LS) is a rare and devastating

early mitochondrial disease which primarily infringes infants and young children. It is considered one of the most severe mitochondrial diseases in children, which belong to the largest class of IEM (*41*). The prevalence of LS is estimated to be around 1 in 36,000 newborns (*42*). Unfortunately, the limited number of patients and the lack of validated disease models have hindered the exploration of potential mechanisms of LS neuronal pathology (*43*). The scarcity of treatments and medications for LS results in a high mortality rate, with many patients not surviving past three years of age (*44*). In an effort to shed light on LS pathology and explore potential therapeutic strategies, a study utilized patient-derived induced pluripotent stem cells and CRISPR/Cas9 engineering to develop workable human LS model. By integrating single-cell multi-omics analysis, the study uncovered abnormal metabolic states in neurons derived from mutant nerve cultures and brain organoids. It was discovered that metabolic defects caused by mutations in the SURF1 gene, a key gene in LS, disrupt the ability of differentiated cells to maintain proliferative and glycolytic states, leading to impaired neuronal morphogenesis and maturation. Single-cell metabolomics data played a crucial role in revealing the importance of metabolic programming in LS (*45*). As technology continues to advance and its application expands, single-cell metabolomics holds promise for exploring various diseases (Figure 2).

3. Single-cell metabolomics in other metabolism-related RDs

Inflammatory breast cancer (IBC) is a rare and aggressive form of breast cancer, which accounts for 1% to 5% of breast cancer cases and is associated with a higher death rate of 8% to 10% (*46*). A study investigating metabolic heterogeneity in IBC found elevated levels of N-acetylaspartic acid, a major metabolite (*47*). This analysis was conducted using clinical IBC samples and IBC cell line models, and it incorporated single-cell metabolomics techniques. The findings of this study revealed the crucial role of JAK2/STAT3 signaling in IBC resistance and identified potential biomarkers and therapeutic targets for IBC (*48*). These results demonstrate the potential of single-cell metabolomics in unraveling the metabolic characteristics and underlying mechanisms of IBC, providing valuable insights for the development of personalized treatment strategies and improving patient outcomes.

4. The future feasible application of new single-cell metabolomics technologies in RDs

In a recent study, a novel microfluidic device using surfacing enhanced Raman spectroscopy (SERS) was reported. This device enables the dynamic screening of single circulating tumor cells (CTCs), thereby providing valuable insights into the differential expression of multiple protein biomarkers in response to therapy (*51*). This automated detection technology holds immense clinical significance in the diagnosis and therapeutic efficacy monitoring of RDs. By enabling the detection and analysis of single CTCs, it offers a powerful tool for understanding disease heterogeneity from the single-cell perspective. This advancement has the potential to significantly improve our understanding of RDs and aid in the development of personalized treatment strategies. The integration of microfluidics and SERS in this device provides a practical and efficient method for screening and analyzing single cells, paving the way for future advancements in the field of single-cell analysis, especially in RDs researches.

5. Conclusion

Metabolomics plays a crucial role in systems biology and the study of various diseases. It combines emerging analytical tools with bioinformatics methods to study the role, abundance, content, downstream pathways and distribution of metabolic molecules in organisms. This

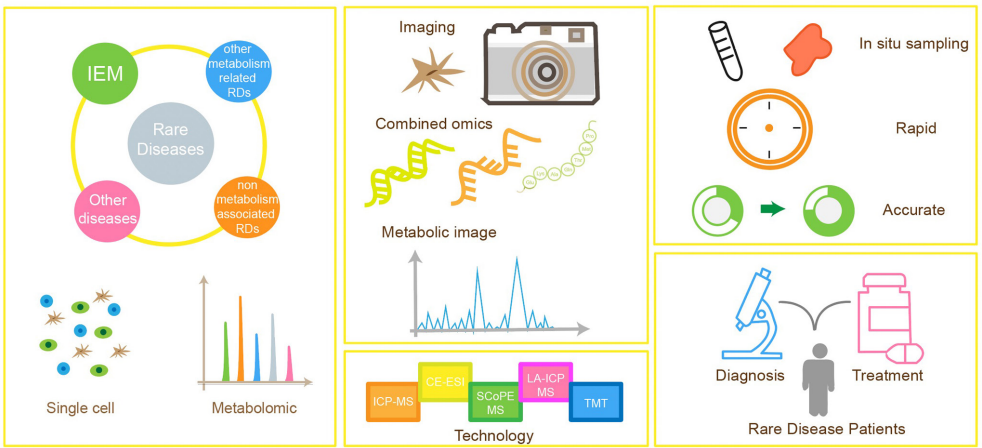

Figure 2: The use of single-cell metabolomics in rare diseases.

approach has proven to be valuable in understanding the mechanism of action and facilitating the diagnosis, including rare diseases (RDs). Single-cell metabolomics is an emerging field that focuses on analyzing the metabolome of individual cells to gain greater insights into cellular heterogeneity and disease processes in RDs. It has the potential to significantly enhance the ability to study the single-cell metabolome of RDs.

Integrating cellular omics, such as transcriptomics, peptidomics, and proteomics, is an important current goal in single-cell metabolomics research. By utilizing both single-cell transcriptome and metabolome measurements, researchers can assess the fit of disease models and identify models that closely resemble the patient's condition. This integration provides valuable insights into the details of gene transcription, translation, protein modifications, and metabolite interactions, enabling a better understanding of cell phenotype and fate. Overall, single-cell metabolomics is a rapidly evolving field which holds great promise for mastering RD-related knowledge, improving diagnostics and treatment strategies in the future.

Funding: This work was supported by two projects of the National Natural Science Foundation of China (grant no. 82374243 to L Wang, grant no. 82304906 to LL Li).

Conflict of Interest: There is no conflict of interest to disclose.

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Received August 28, 2023; Revised May 12, 2024; Accepted May 22, 2024.

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Released online in J-STAGE as advance publication May 24, 2024.