Emerging Transcriptomic Approaches to Decipher Mycobacterial Complexities

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Abstract

The word "transcriptome" generally refers to overall RNA species transcribed from DNA (or RNA itself) or transcripts that are present in a cell. The transcriptome includes messenger RNA (mRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), and even noncoding RNAs, such as miRNA, having a regulatory function. TB pathogenesis is highly dynamic and is determined by the interaction between the host defense strategy and the tactics employed by *Mycobacterium* species to survive inside the host. Recently, several reports suggested that differential expression of host miRNAs serves as hallmark of disease progression at both cellular and organism level in various diseases, including tuberculosis. Next-generation sequencing (NGS) is a revolutionized massive parallel sequencing technique that uses RNA-seq, which is a recent NGS-based technology for transcriptome profiling which is most often used to analyze differential gene expression and differential splicing of mRNA. RNA-seq is more sensitive than other technologies such as microarray as it directly determines the cDNA and thus provides a great insight about physiological state of cell, healthy or diseased. In this chapter, we present a detailed overview of RNA-seq technology and its data analysis, applications, and advances made so far. With the advancement in NGS, RNA-seq has also developed. Single RNA-seq is the further refinement of RNA-seq analysis in a single cell, which allows studying the complex biological processes especially useful in cancer studies. Until recently, the technique was limited to mRNA expression of either pathogen or host cell. However, in the recent years, more advanced RNA-seq technology enables "dual

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RNA-seq" analysis for better understanding of host-pathogen interaction. Dual RNA-seq does not require pre-designed species-specific probes and thus provides a better understanding of the interaction, virulence factor, or immune response mechanism. With increasing sensitivity of dual RNA-seq, it is now being applied to understand the host-pathogen interaction in tuberculosis, which is the key study in development of therapeutic strategies to control "*Mycobacterium tuberculosis*." With more refinement at lower cost, RNA-seq is expected to replace the microarray technique in the near future.

Keywords

RNA · miRNA · NGS · M.tb · Diagnostics

7.1 Introduction

The word "transcriptome" generally refers to overall RNA species transcribed from DNA (or RNA itself) or transcripts that are present in a cell. The transcriptome includes messenger RNA (mRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), and even noncoding RNAs, such as miRNA, having a regulatory function. Recently, there emerged a high-throughput sequencing technology which involves analysis of RNA at single nucleotide level, called RNA sequencing (RNA-seq). RNA-seq is an NGS-based sequencing technology with many applications, such as mRNA expression quantification, differential splicing of mRNA, fusion genes detection, and RNA editing. RNA-seq is a powerful tool that directly determines the cDNA and hence provides a great insight of genomic functions and physiological state of cell. RNA-seq has advantage over qPCR and microarrays as it provides complete transcriptome of the organism including coding and noncoding genes, intergenic regions, and small RNAs [1]. *Mycobacterium tuberculosis* (*M. tb*) is the causative agent of tuberculosis and is the leading cause of death globally [2]. It is estimated that around one-fourth of the world population is infected with TB; however, only 5%–10% of these cases advance to active TB in their lifetime [2]. In this chapter, we will discuss emerging transcriptomic approaches to decipher complexities related to *Mycobacterium tuberculosis.*

7.2 MicroRNAs: Biogenesis and Mechanism of Action

MicroRNAs (miRNAs) are a class of highly conserved, small, noncoding single-strand RNA molecules that are approximately 18–25 nucleotides in length. They play an important role in the posttranslational expression of genes and are involved in cell development, differentiation, proliferation, apoptosis, etc. [3]. miRNAs are divided into two classes, the intragenic and intergenic miRNAs, based on their genomic loci. Intergenic miRNAs are located between two genes and can be monocistronic (with their own promoters) or polycistronic, wherein various

miRNAs are transcribed together, while the intragenic miRNAs are known to be present in the introns or exons of genes and are co-regulated with their host genes by Pol II. miRNA biogenesis pathway begins with the transcription of miRNA by RNA Pol II to form hairpin-like structure called the pri-miRNA. These are then cleaved by a nuclease called Drosha, resulting in the formation of pre-miRNA. The pre-miRNA is transported to the cytoplasm by exportin-5 through a Ran-GTP-dependent mechanism and is further excised by Dicer to form the mature miRNA duplex. A single strand of this miRNA duplex then binds to the other accessory proteins such as Ago-2 and GW182 and is incorporated in the RNA-induced silencing complex (RISC) for posttranscriptional regulation of mRNAs [4]. The microRNAs usually bind to a specific region, about 5–8 nucleotides long, in the 3′-UTR region of the messenger RNA, resulting in the degradation of the target mRNA or inhibition of translation.

7.2.1 miRNAs in Tuberculosis: New approaches for Diagnosis and Host-Directed Therapy

TB pathogenesis is highly dynamic and is determined by the interaction between the host defense strategy and the tactics employed by *Mycobacterium* species to survive inside the host. Currently, TB diagnostic methods primarily focus on diagnosing active tuberculosis, thus demanding more definitive biomarkers that differentiate latent and active TB for a more specific and efficient diagnosis. Owing to the limitations in traditional methods for diagnosis of TB, researchers have emphasized the need for development of diagnostic tools based on host biomarkers that can be used for assessment of disease status and monitoring of treatment outcomes. In pursuit of the same, several classes of molecules are currently being scrutinized as prospective biomarkers, miRNAs being one of these biomarkers.

7.2.2 Role of Host miRNAs in Tuberculosis

Recently, several reports suggested that differential expression of host miRNAs serves as hallmark of disease progression at both cellular and organism level in various diseases, including tuberculosis. Interestingly, the pathogen has evolved various mechanisms to survive inside the macrophages and establish dormancy/latency. Notably, miRNAs have been emerging as critical regulators of immune response in *M. tb* infection. Here, we mention some of the important host-bacterial interactions that are modulated by miRNAs in tuberculosis.

7.2.2.1 Host miRNAs Regulate Autophagy and Phagolysosome Maturation in Tuberculosis

Autophagy is a well-known cell-autonomous defense mechanism conducted against many intracellular microorganisms. To ensure their own survival, pathogenic bacteria modulate many host cell processes including autophagy. Accumulating

evidences suggest that host miRNAs regulate several autophagy-related genes (ATGs), thus favoring mycobacterial survival. Wang et al. [5] showed that the expression of host miR-155 was enhanced after mycobacterial infection using in vitro and in vivo models. This enhanced expression accelerated the formation of phagolysosome and helps in eradicating the infection. Kim et al. [6] showed that miR-125a inhibits autophagy and antimicrobial effects against *Mycobacterium tuberculosis* by targeting the UV radiation resistance-associated gene (UVRAG). miR-20a inhibits autophagy by targeting ATG7 and ATG16L1, hence favoring mycobacterial survival inside the host macrophages. miR-125a-3p, miR-33, miR-144-3p, miR-23a-5p, and miR-142-3p are potential inhibitors of autophagy in *Mycobacterium tuberculosis* infection. Notably miR-155, miR-17-5p, and miR-26a target Ras homolog enriched in the brain (Rheb), Mcl-1, STAT3, and KLF4, respectively, play a role by inhibiting the infection.

One of the well-studied mechanisms of immune escape by *M. tb* is mediated by arresting phagosome-lysosome maturation. Prevention of acidification of phagosome with lysosomal enzymes, such as cathepsin proteases, plays a crucial role in persistence of *M. tb* inside host macrophages. miR-106-5p is known to target cathepsin S and inhibits lysosomal enzymatic activity. Another microRNA, miR-142-3p, inhibits internalization by phagosomes by targeting N-WASP and PKC-alpha genes [7]. Polarization of macrophages as M2 renders macrophages as anti-inflammatory and poorly microbicidal. A transcription factor, KL4, drives M2 polarization of macrophages and is negatively regulated by miR-26a. Notably, in patients with TB, miR-26a is found to be downregulated [8].

7.2.2.2 Host miRNAs Regulate Innate Immunity in Tuberculosis

Innate immune cell activation is regulated by miR-155, miR-146a, miR-21, and miR-9. miR-125a-3p can inhibit antimicrobial responses and host defense against *M. tb* infection by targeting the gene encoding autophagy UV radiation resistance-associated protein [6]. miR-125b inhibits the production of TNF-α in alveolar macrophages during *M. tb* infection. TNF-α is a well-known pro-inflammatory cytokine for the *M. tb* clearance. Targeting miR-125a and miR-125b will be helpful in increasing the antibacterial responses and also helps in clearance of pathogen by increasing the TNF-α levels during *M. tb* infection. miR-146a and miR-155 are the most well-studied miRNAs in TB, which significantly influence the host-pathogen interactions [9].

miR-146a expression, driven by the transcription factor NF-kB, also represses the mycobacteria-associated inflammatory response and helps in bacterial proliferation in RAW264.7 macrophages via IRAK-1/TRAF-6 pathway. miR-146a enhances mycobacterial survival in RAW264.7 macrophages via suppression of nitric oxide (NO) production [10]. miR-155 is induced upon infection of murine macrophages with mycobacteria and positively regulates the TLR signaling [5]. It is known to play a protective role against mycobacterial infections during the earlier stage of infection. On the other hand, it augments the survival of macrophages, thereby providing a niche for the mycobacterial replication. miR-155 levels in serum negatively regulate the activity of the NK cells. Increased levels of miR-155 lead to decreased

NO synthesis and enhance the mycobacterial load [11]. Targeting the miR-146a and miR-155 will be helpful in mycobacterial killing by increased NO production and increased NK cell activity. MyD88 signaling facilitates bacterial containment and is crucial for raising innate and adaptive immune response against *M. tb*. miR30a targets 3′-UTR of MyD88, thus inhibiting MyD88/TLR signaling in macrophages and promoting bacterial growth inside host cells. miR-27a targets 3′-UTR of TICAMI, a TLR3 adaptor, to inhibit TLR3 pathway [12]. miR-146a, an anti-inflammatory, regulates NF-κB signaling and favors *M. tb* growth. miR-223 is significantly increased in macrophages infected with *M. tb* and negatively regulates NF-κB signaling and pro-inflammatory cytokines [12].

7.2.2.3 Host miRNA Regulates Apoptosis in Tuberculosis

Apoptosis is an important mechanism to inhibit/kill intracellular mycobacteria. Host miRNAs that negatively regulate apoptosis are upregulated during *M. tb* infection. FOXO1, a positive regulator of apoptosis, is targeted by miR-582-5p in patients with active TB. Increased levels of miR-223 are associated with decreased apoptosis in *M. tb*-infected macrophages. Fas, a central component in apoptosis pathway, is targeted by let-7b-5p miRNA, thus inhibiting apoptosis in THP1 macrophages [12].

7.2.3 Host miRNAs as Diagnostic Biomarkers in Tuberculosis

An ideal biomarker for TB should have high sensitivity and specificity and the ability to be detected based on minimally invasive procedures [13]. A reliable biomarker for TB must be able to differentiate between healthy people and those with active TB and LTBI, as well as account for differences in expression because of variation in stages of infection and human-induced biases such as differences in age, sex, and comorbidities in patients [14]. Hence, exploring circulating host miRNA profiles has been considered a viable step in the direction of finding a potential biomarker for TB (Fig. 7.1).

7.2.3.1 Exosomal miRNAs as Biomarkers in Tuberculosis

Pulmonary TB accounts for around 80% of all TB forms, but tuberculosis meningitis (TBM) accounts for approximately 50% of TB-related mortality. There is a pressing need to have new and definitive biomarkers to improve TB diagnosis, especially for lethal forms such as TBM. Recently, Hu et al. showed that exosomal miRNAs could serve as reliable biomarkers for PTB and TBM combining microarray and electronic health record (EHR) data approaches [15]. They identified six differentially expressed human miRNAs in PTB and TBM patients compared to healthy controls. Integration of microarray and EHR data provided a better superior model in the differential diagnosis of PTB and TBM.

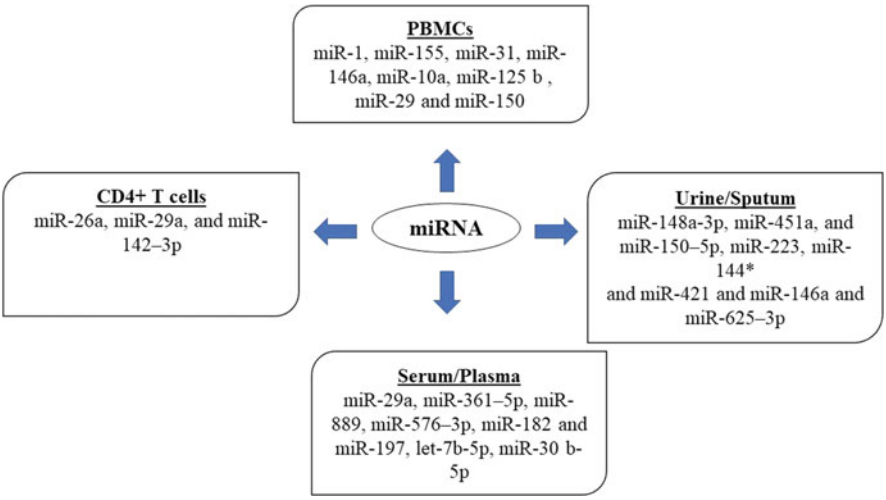

Fig. 7.1 A schematic illustration showing a list of miRNAs as biomarkers in tuberculosis for different biological specimen/samples

7.2.4 Role of miRNAs in Extrapulmonary TB

Extrapulmonary TB (EPTB) accounts for around 3% of TB cases. There is an urgent need to develop accurate and rapid diagnostic approaches to cure EPTB. Bone TB/skeletal TB is one form of EPTB, and spinal TB is one of the commonest bone TB found around the world. Yang et al. showed a negative correlation between host miRNA-155 and matrix metalloproteinase-13 (MMP-13) expression in patients with spinal TB-induced intervertebral disc, suggesting new approaches to treat and diagnose spinal TB [16].

TBM is one of the severe forms of TB of the central nervous system. Due to lack of accurate and efficient diagnostic methods, it is difficult to discriminate TBM from other similar diseases such as viral meningitis (VM). Interestingly, Pan et al. showed that four miRNAs (miR-126-3p, miR-130a-3p, miR-151a-3p, and miR-199a-5p) clearly show differential expression between TBM and VM patients, thus improving our understanding of pathogenesis between TBM and VM [17].

7.2.5 Host miRNA Profile in Response to TB Treatment

miRNA profiling post TB treatment helps in identifying drug-resistant mutants. In TB-susceptible patients (those who respond to anti-TB therapy), miR-320a is increased compared to drug-resistant TB patients. Notably, Wang et al. identified 37 upregulated and 63 downregulated miRNAs in humans cured of TB as compared to untreated TB patients [18]. Further, expression of miRNAs involved in Th1 response was modulated post TB treatment including miR-155 and miR-326.

miR-16 was expressed at lowest levels in MDR patients compared to all controls and healthy individuals.

7.2.6 Limitations with miRNA Profiling for Diagnostics in Tuberculosis

A plethora of studies have been conducted in order to explore the differential expression of miRNAs in different stages of TB infection. These studies show a diverse profile of upregulated and downregulated miRNAs between patients with LTBI and active TB infection. The inconsistencies in the results from the various studies are majorly due to the biases introduced as a result of methodological differences, the choice of sample, data analysis techniques, cut off criterion, etc. (i.e., human-induced biases) or because of the varying stages of TB infection in the samples taken [19]. Application of miRNAs as biomarkers in TB infection studies is often complicated by the ambiguity in the different stages of infection and the lack of differentiation between recent and long-standing latent TB infection and newly acquired or treated infections, which makes it difficult to compare the samples among the groups [19]. Moreover, the analysis of the same between the two sexes is done in an identical manner even though there is little to no similarity in the miRNA expression profiles of the two [20].

Previous studies have also shown various challenges associated with the delivery of miRNAs to their respective targets. These include a low half-life of miRNA in biological fluids; easy degradation and eradication of miRNA particles in vivo because of nuclease activity, phagocytosis, or renal clearance owing to their low molecular weight; inadequate penetration of the miRNA into tissues due to impediment caused by various physicochemical barriers; and intracellular disposition of miRNA for aggregating within endosomes of naked miRNA leading to inefficient gene-silencing or inducing immuno- or neurotoxicity [21, 22]. Recent studies have concluded that miRNAs are not as specific in their targeting action as they were previously assumed to be and can bind to complementary sequences as well as other targets having sequence similarity, which may lead to deleterious effects, ones that may even completely negate the therapeutic effects of miRNA [23].

Other potential drawbacks of miRNA studies in tuberculosis infection may arise depending on the techniques used in their profiling. NGS analysis or microarray techniques are most commonly used for searching possible miRNA biomarkers due to their advantages in allowing the identification and analysis of multiple targets [23]. However, both techniques have their own set of disadvantages as well, which may be extended to miRNA studies.

7.3 RNA-seq

At present, many technological platforms are available for sequencing, namely, Illumina, Roche 454, ABI SOLiD, Ion Torrent, and PacBio [24]. Illumina is the most commonly used platform because of its high sequence yield, but Ion Torrent and PacBio are gaining more popularity over Illumina platform due to their low cost and longer reads generation. Millions of reads are generated after RNA sequencing; hence, in-depth analysis is required. There is no single set of pipeline for the analysis as it depends on the availability of reference genome or transcriptome and also on the objective of the researcher [25]. Figure 7.2 summarizes an overview of the conventional RNA-seq data analysis workflow comprising both experimental design and computational analysis [25, 26]. Experimental workflow includes RNA extraction, reverse transcription polymerase chain reaction (RT-PCR), and cDNA library construction followed by sequencing and imaging. Computational analysis includes different software for each step varying from quality control to functional profiling. Programs are selected based on the aim of the work. Apart from command line tools, many other web-based, user-friendly pipelines such as Galaxy (galaxyproject.org), iDEP (ge-lab.org/idep), and UTAP (openpbs.org) are also available for RNA-seq dataset analysis, making it convenient for biologist not having bioinformatics expertise.

7.3.1 Differential Gene Expression Analysis

Differentially expressed gene (DEG) analysis is a process for identification of genes and comparison of their expression levels under different biological conditions, tissues, or stages of development.

7.3.2 Computational Analysis on RNA-seq

Recent studies have suggested that noncoding transcripts are not just noise or junks, they are biologically important; hence, they are now being implicated in human pathology [27]. The advancement of RNA-seq in recent years has facilitated the expression profiling of both long and short noncoding transcripts. Apart from expression profiling, quantification of exogenous RNA content, transcriptional elongation, and DNA variance can also be estimated using different RNA-seq approaches. ENCODE software tool helps in the sequencing data of long noncoding RNA through RNA-seq [28]. PathSeq tool is highly sensitive and specific and helps in discrimination of human from nonhuman sequences by use of data of transcriptome and whole-genome sequencing data [29]. Detection of virus can also be done through VirusSeq, ViralFusionSeq, and VirusFinder [28]. Software such as asSeq and AlleleSeq help in differentiation of expression of alleles by transcriptome and whole-genome sequencing data [28]. There are RNA-editing databases, such as DARNED, REDidb, dbRES, and RADAR, which are useful for removing

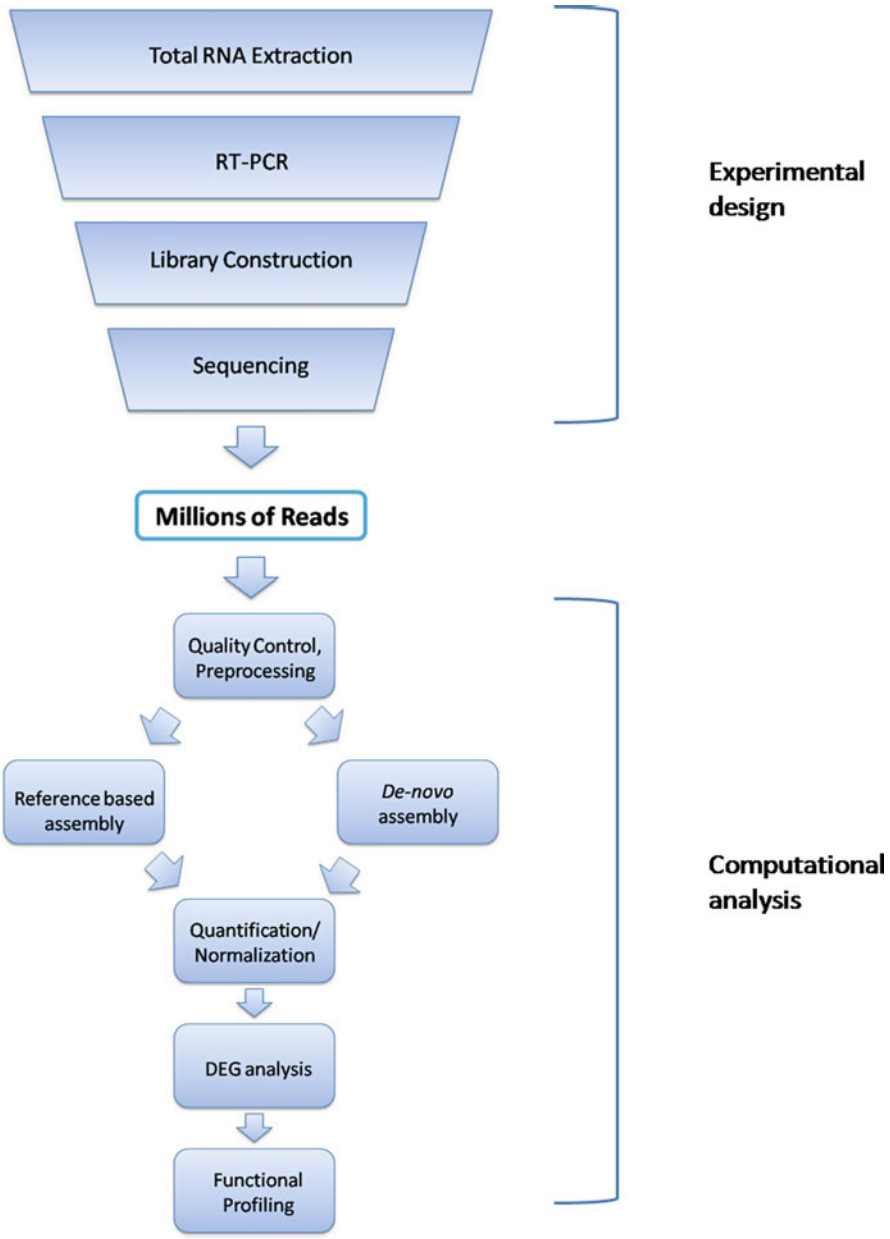

Fig. 7.2 Overview of RNA-seq data analysis workflow

RNA-editing sites [28]. Tools such as single-nucleotide variation quality, RNAmapper, RSMC, and SNPiR are useful for detection of DNA variations [28].

7.3.3 Advancements in RNA-seq

Although RNA-seq is a high-throughput technology, it has certain limitations. So some refinements have taken place in RNA-seq technology to overcome those limitations, some of which are as follows:

1. It is unable to determine polarity of RNA transcript, which is important for correct annotation of novel genes as it gives information related to the function of a gene, both at the level of RNA and the protein. To overcome this, single-strand RNA-seq technique was developed. Deoxy-UTP is incorporated in the second strand of cDNA. After this step, there is destruction of the uridine-containing strand which helps in the identification of the orientation of the transcript as shown in Fig. 7.3a [30].
2. RNA is extracted from the large population of cells in the sample. As a result, important differences between cells may be ignored, so a protocol of single-cell RNA-seq was developed as shown in Fig. 7.3b [31].
3. When host-pathogen interaction takes place, changes in the gene expression occur in both host and pathogen. So an advancement in RNA-seq has taken place which helps in studying the expression of the genes simultaneously in both host and pathogen known as dual RNA-seq, as shown in Fig. 7.3c [32].

7.4 Use of RNA-seq in Deciphering Complexities in *M. tb*

M. tb is able to disseminate successfully because of immune evasion depicted by it after infection in host, survival, and persistence. Successful pathogenesis and transmission of *M. tb* are mainly due to drug resistance, drug tolerance, and reactivation from dormancy. Recently developed technique of RNA-seq has provided in-depth information related to important attributes of *M. tb* such as dormancy, survival, infection, and immune evasion.

7.4.1 Dormancy

Fatty acid metabolism, rather than carbohydrate utilization, plays an important role in latency and dormancy. Fatty acid metabolism has been recently studied in depth through global transcriptome of *M. tb* by the use of strand-specific RNA-seq. *M. tb* bacilli were grown in media containing even-length long-chain fatty acids (LC-FAs) as the sole source of carbon and compared with those grown with dextrose supplementation [33]. The study revealed that there were a shift toward the glyoxylate cycle, increase in expression of several genes of reductive stress, and regulation like

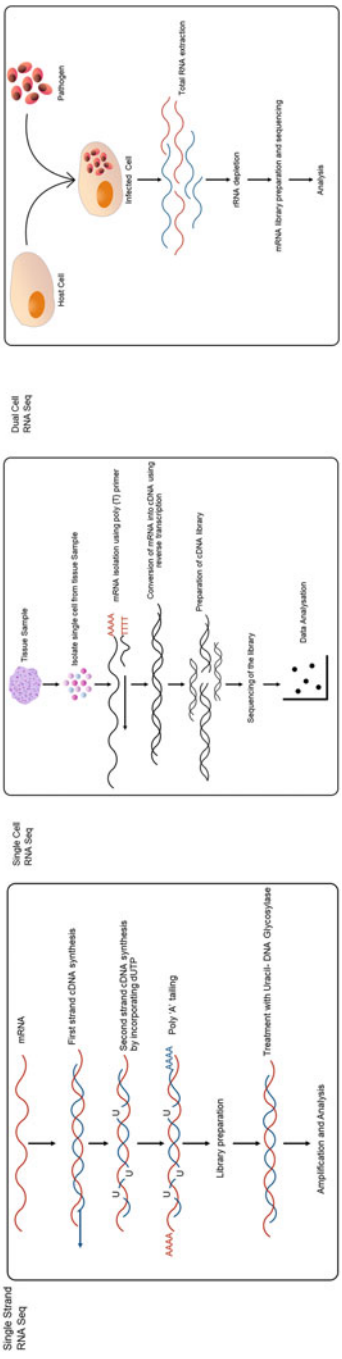

Fig. 7.3 Advancements in RNA seq. (**a**) Single-strand RNA seq. (**b**) Single-cell RNA seq. (**c**) Dual RNA-seq

whiB3, dosR, and Rv0081. There was also an upregulation of tRNA, which gave an attribute of drug tolerance by dormant bacterium. Lipid signature genes were also identified [33]. Another group did a transcriptome profiling using a mixture of cholesterol and palmitic, stearic, and oleic acids under aerobic and hypoxic conditions over three metabolic stages: exponential stage, stationary stage, and non-replicating persistence stage 1 of hypoxia [34]. They used cholesterol and long-chain fatty acids as a carbon source under aerobic and hypoxic conditions because sputum from TB patients showed the presence of lipid-rich environment where mycobacteria are residing and it mainly comprises cholesterol and palmitic, stearic, and oleic acids. The results revealed the differential expression of a core of 368 genes out of which 183 were downregulated and 185 were upregulated. This mainly helped in the induction of a machinery that leads not only to tolerance of drug but also in the maintenance of iron and production of sulfide, which is essential for enzymes and coenzymes necessary for redox balance and production of acetyl-CoA and methylmalonyl-CoA for de novo lipid biosynthesis [34]. Thus, these RNA-seq-based investigations have provided some clues related to target pathways in *M. tb* metabolism and hence paved the way to find alternate targets for therapy.

7.4.2 Survival

There are many hypothetical genes in *M. tb* which play an important role in survival of *M. tb* inside the host cell and a couple of genes from the host, playing roles in favor of the pathogen. As *M. tb* is an intracellular pathogen, it is able to face stress inside the macrophage and granuloma. It is thus necessary to study the response of *M. tb* to similar stresses which will help in identification of virulence factors and pathways necessary for the survival of *M. tb*. *M. tb* were exposed to stress such as oxidative, nitrosative, and alkylation stresses or mitomycin C-induced double-strand break in DNA. Nearly 700 DE genes were seen under nitrosative stress [35]. Mitomycin C stress mainly affects replication, repair, and recombination. Genes of the T7 secretion system (T7SS) and proline-glutamic acid/proline-proline-glutamic acid (PE/PPE) family responsible for the virulence of bacteria and survival were differentially expressed [35]. RNA-seq was also performed under various conditions of incubation, such as non-inhibitory to cidal condition based on the replication of mycobacteria or their killing profile [35]. There was an absence of replication of mycobacteria under inhibitory condition, and it lead to the expression of a unique transcriptome involving modulation of genes in response to stress, reprogramming in metabolic pathways, oxidative stress, response to dormancy, respiration, and virulence [35]. RNA-seq also gave transcription pattern which involved remodeling of cell wall, leading to increase in infection, resistance to antibiotics, and immune evasion.

7.4.3 Infection

Studies related to host-pathogen interactions in TB are important for the development of new strategies to control *M. tb*. In one of these studies, transcriptome analyses were carried out in macrophages which were infected with H37Rv (Rv), a virulent strain of *M. tb*, or H37Ra (Ra), an attenuated strain. Seven hundred fifty differentially expressed genes (DEGs) were identified and analyzed, of which *solute carrier family 7 member 2* (*SLC7A2*) was more suppressed in Rv-infected macrophages as compared to Ra-infected macrophages [36]. SLC7A2 transporter is required by M1 macrophages to produce NO. M1 macrophages help in removal of bacteria because they produce pro-inflammatory cytokines and NO. Thus, intracellular survival of *M. tb* is also regulated by induction of SLC7A2.

Different cell lineages of the host showed differentially expressed genes as well as change in the expression of *M. tb* genes in pathogenesis through dual RNA-seq. Three thousand four hundred fifty-three genes were upregulated and 3119 downregulated in alveolar macrophages (AMs) compared with uninfected macrophages. The NRF2 pathways were upregulated in AMs infected with *M. tb*, while expression of pro-inflammatory cytokines and molecules for prevention of inflammation-associated intracellular damage was lower than infected interstitial macrophages (IMs). These results revealed that the infected AMs were more permissive for bacterial growth. In IMs, 3614 genes were upregulated, and 3298 genes were downregulated [32]. Transcriptional profiling of uninfected and infected IM populations depicted that pro-inflammatory pathways such as activation of NF-kB and immune response through Th1 pathway got induced. Genes responsible for adhesion and chemotaxis were upregulated in IM-infected macrophages which recruited the other host cells to granuloma from peripheral blood. There was an induction of type II toxin-antitoxin-chaperon (higB1-higA1-secB) under the conditions of stress and hence played role in bacterial persistence [32].

Acquisition of nutrient in the presence of environment of the host is critical for the intracellular pathogens to survive. For the quantification of interactions in metabolic pathways between the host and *M. tb*, dual RNA-seq was performed. This resulted in identification of alterations in metabolic pathways which were specific to infection. The in silico data suggested that *M. tb* was able to consume 33 different nutrients during initial phase of macrophage infection, which is utilized by the tubercle bacilli for the generation of energy for its intracellular growth [37].

PathSeq tool has helped in the discovery of a novel transcriptional machinery for remodeling of cell wall of mycobacteria when *M. tb* infects alveolar macrophage. It has been shown that MadR modulates two mycolic acid desaturases *desA1/A2* for promotion of remodeling of cell wall when *M. tb* infects macrophages and helps in mycolate biosynthesis upon entering dormancy [38]. Thus, RNA-seq has provided an insight that disrupting MadR is lethal and can be an antitubercular target for initial and later stages of infection.

7.5 Application of RNA-seq in Developing Better Diagnosis and Treatment of Tuberculosis

It is important to understand response against tuberculosis (TB) infection for diagnosis of people having an active or a latent TB infection (LTBI). It is also important to identify LTBI individuals who are at greater risk of active TB development. The RNA-seq analysis was done on samples from Galicia (Spain) and Mozambique where more TB is prevalent [39]. The results show high expression of genes that code for immunoglobulin chains. There was an increase in the expression of immunoglobulin receptors such as FCGR1A and FCGR1B. Genes responsible for T cell regulation such as programmed cell death 1 ligand 1 (CD274) and 2 (PDCD1LG2) were also upregulated in samples obtained from active TB patients. Authors have identified two profiles within LTBI contacts. Transcriptional profiles of the 77.8% subgroup were similar to that of uninfected contacts, whereas the second subgroup (22.2%) showed transcription profiles similar to confirmed TB patients. Thus, these kinds of studies can be useful to study LTBI contacts at risk, progressing to active TB without any clinical follow-ups. Single-cell RNA-seq datasets obtained from peripheral blood monocytes of healthy control, LTBI and active tuberculosis revealed natural killer (NK) cell subset ($CD3^{-}CD7^{+}GZMB^{+}$) got depleted in patients suffering from TB. Hence, it can serve as a biomarker for diagnosis of TB which will help in distinguishing LTBI from healthy control [40].

Antibiotics have a property to either stimulate or repress gene expression in bacteria. Through transcriptional profiling mode of action of various drugs, their efficacy and effect on the metabolism of *M. tb* can be deduced. Through this technique, new insights for known antibiotics that are currently useful for the treatment of tuberculosis can be obtained. This will also help in identification of mode of action and prediction of various targets of new drugs. This technique also provides information related to changes in transcriptome profile after treatment with antibiotic [41]. It also provides information related to capability of bacteria to escape the effect of antibiotics. So it will be helpful in identification of genes responsible in adaptive responses and tolerance to drugs. Transcriptome profiling helps in identification of genes differentially expressed between drug-sensitive *M. tb* and multidrug-resistant clinical isolates. Clinical strains of *M. tb* are different from H37Rv, more specifically clinical drug-resistant strains. By the help of transcriptome sequencing multidrug-resistant strains of *M. tb*, attributes of resistance and virulence were depicted [42]. There was an enrichment in biosynthesis of arginine, biosynthesis of fatty acid, and pathways of metabolism which was depicted through the DEGs of MDR strains. Type VII secretion system was downregulated in MDR strains.

7.6 Conclusions

Characteristic miRNAs, expressed at different stages of tuberculosis, might give useful insights on the nature of immune responses generated against this elusive pathogen. The advantage of using miRNA profile as a diagnostic biomarker is their

stability in the circulating body fluids. However, inconsistent results due to variability in miRNA expression levels, lack of optimal experimental models, and correlation of the results with the actual pathologies limit their use, and further validation is required to conclude clearly on the role of miRNAs as biomarkers in TB diagnosis and treatment. Nevertheless, we can conclude that (1) miRNAs were differentially expressed between active TB and LTBI patients, (2) the results of in vitro and ex vivo studies can vary considerably and can contradict each other, (3) PTB and EPTB can provide different miRNA signatures, and (4) treatment to TB patients highlights changes in miRNA profiles, indicating their potential use as a measure of efficacy to anti-TB therapy.

The advent of next-generation sequencing technologies that enabled transcriptomic investigations revolutionized the field of mycobacterial research at an unprecedented scale. However, the application of the transcriptomics-based investigation methods in mycobacterial research is still in its infancy and is yet to reach its zenith in the years to come. Once the full potential of these methods is realized and applied, the field will definitely be endowed with the information and insights on enigmatic mycobacterial lifestyle in its favored niche that is still largely elusive.

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