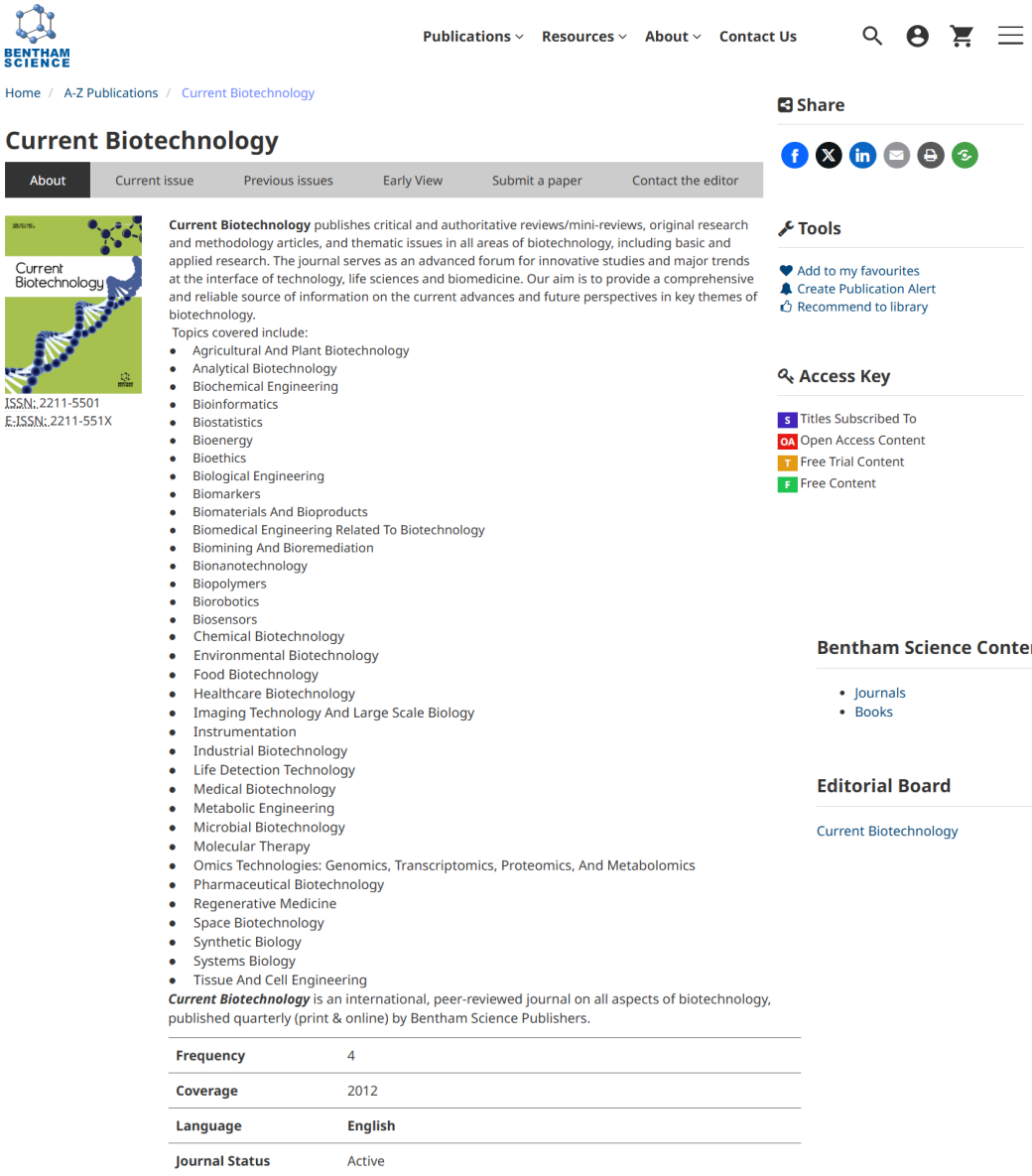
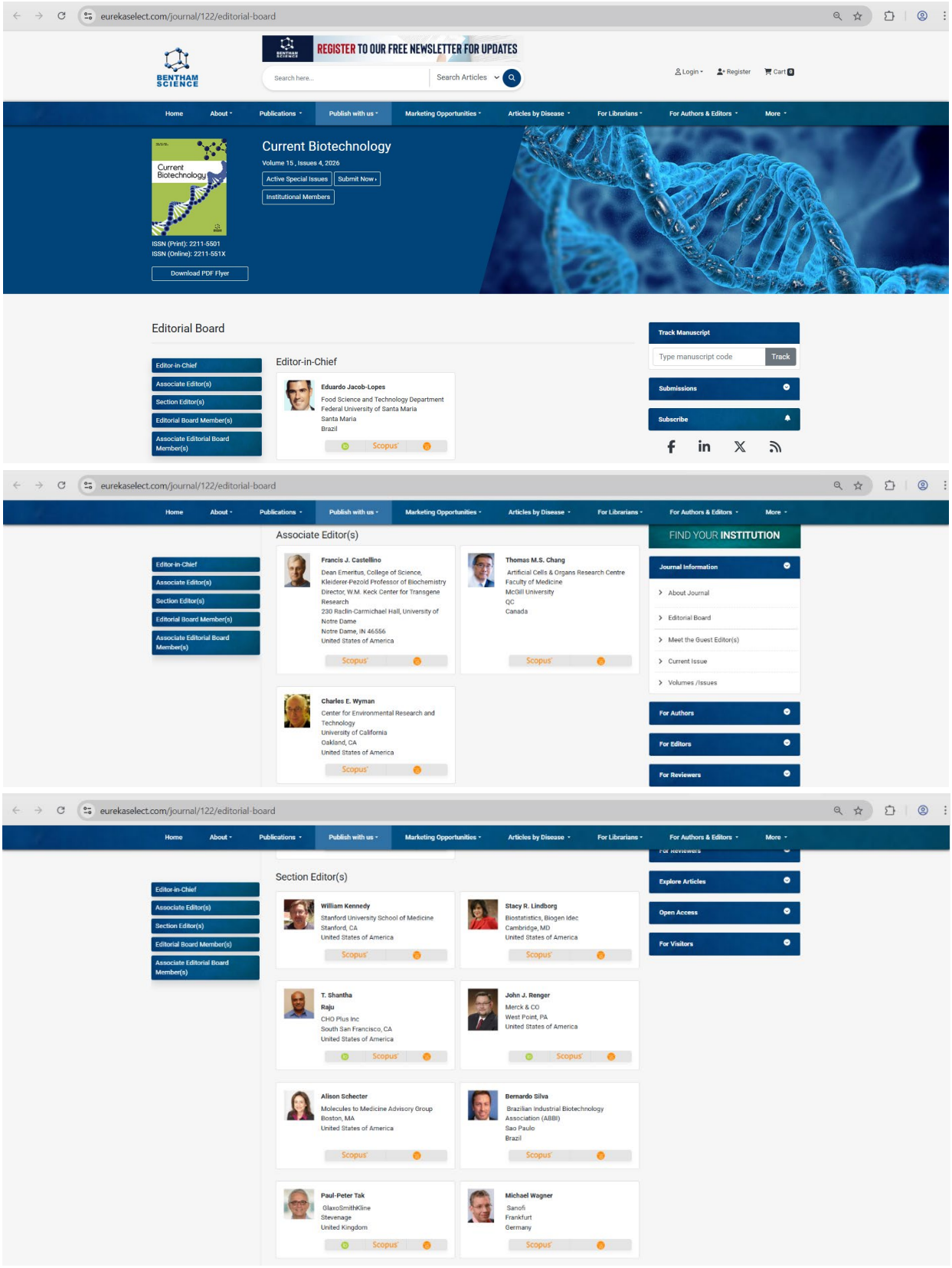
Current Biotechnology – Sampul depan/Cover

https://www.benthamdirect.com/content/journals/cbiot

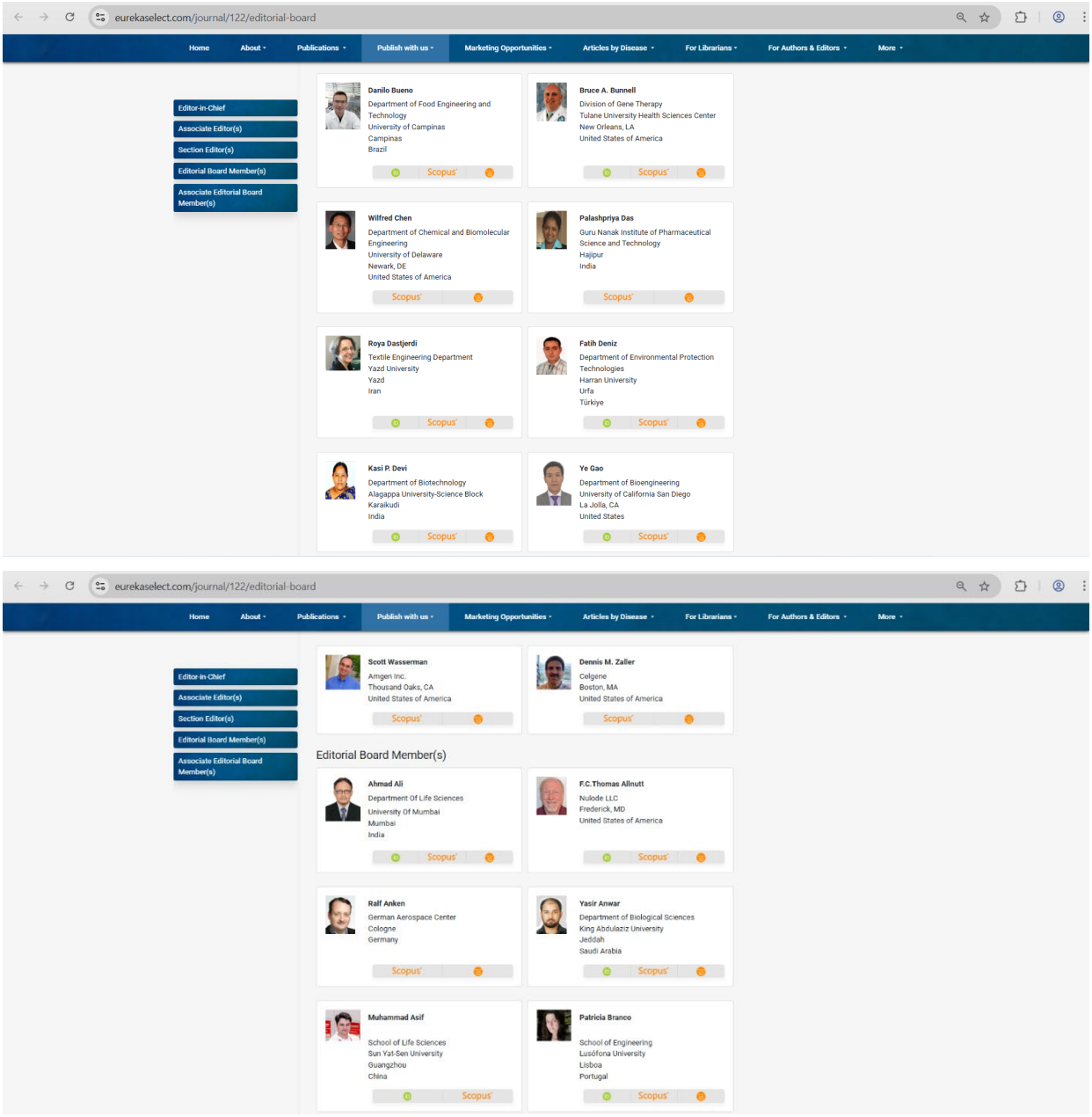


Current Biotechnology – Daftar isi

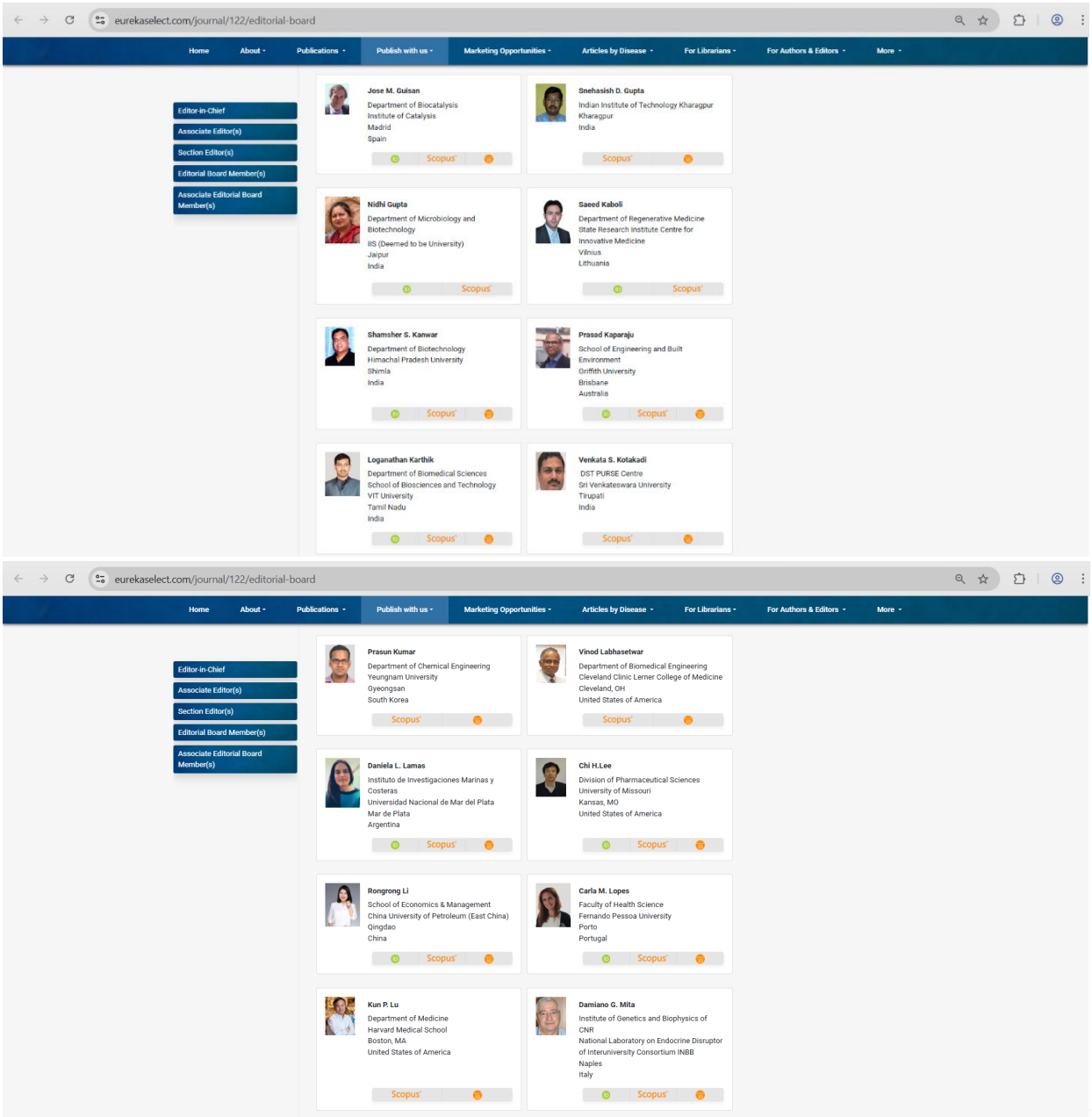
Current Biotehcnology – Editorial Board (hal 1)



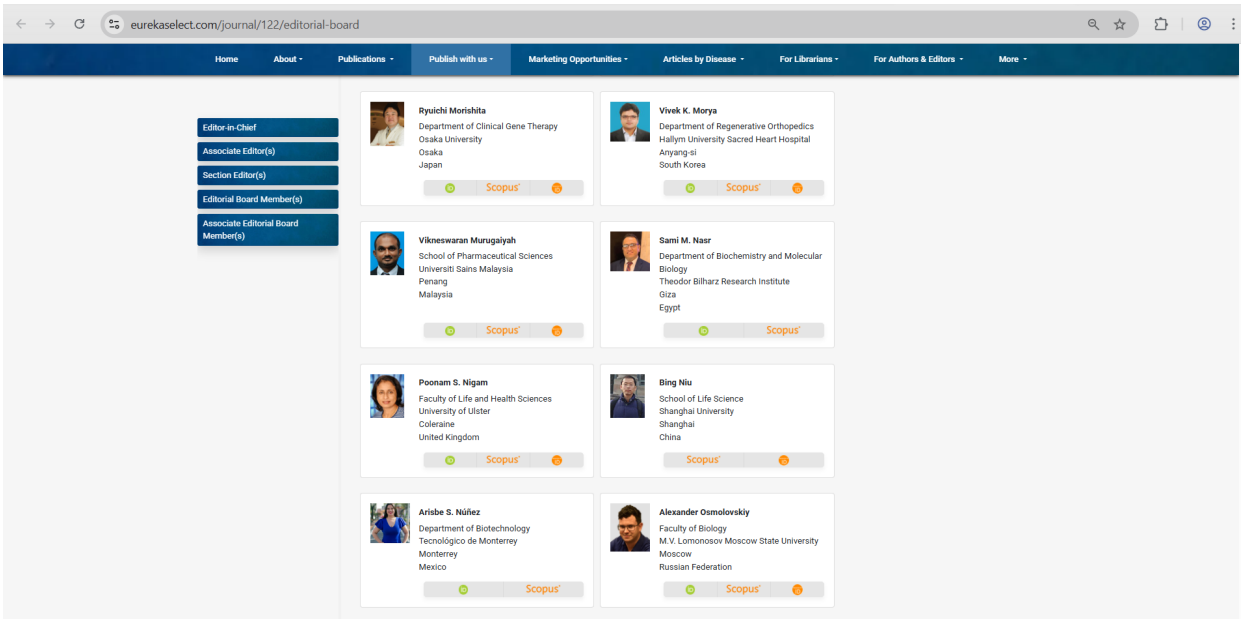
Current Biotehcnology – Editorial Board (hal 2)

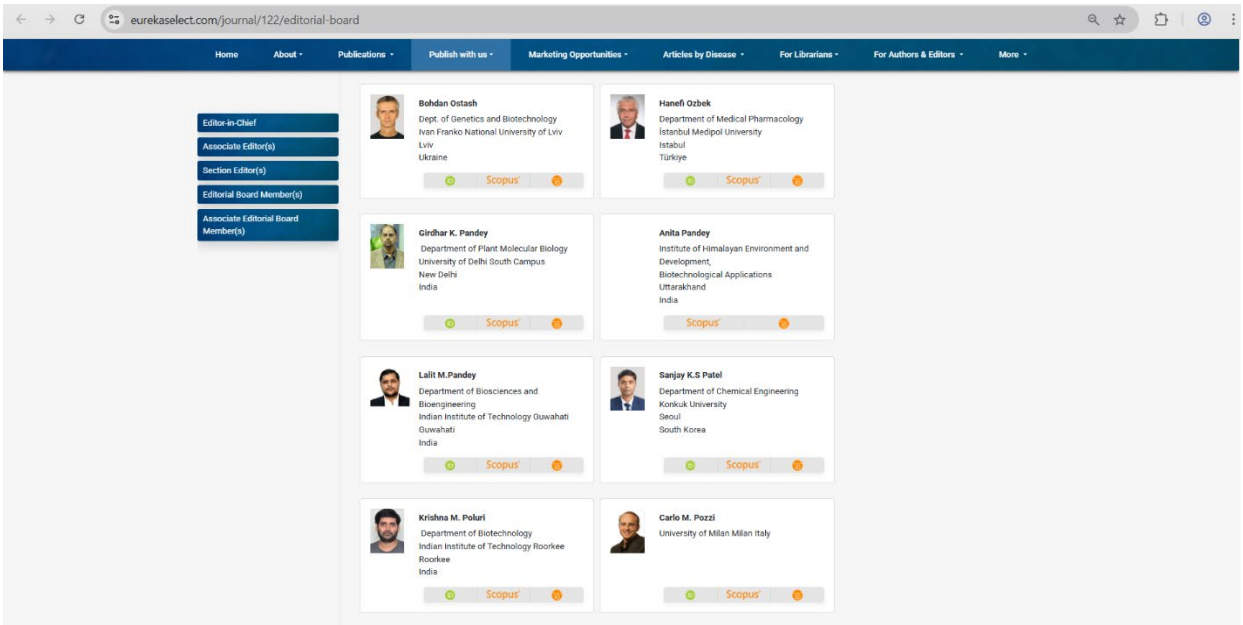


Current Biotehcnology – Editorial Board (hal 3)



Current Biotehcnology – Editorial Board (hal 4)





Suriyani Tan_MS text.docx

by Suriyanitan FK

Submission date: 24-Feb-2025 04:31PM (UTC+0700)
Submission ID: 2597192364
File name: Suriyani_Tan_MS_text.docx (259.79K)
Word count: 6106
Character count: 34850

MINI REVIEW ARTICLE

Title: MicroRNA miR-155 and miR-21 as Biomarker in active Pulmonary Tuberculosis and Healing Process: A Mini Review

Suriyani Tan[1], Machrumnizar Machrumnizar [1], Yuliana Yuliana[1], Rina Kusumaratna[2], Eveline Margo[3], Jipri Suyanto[4]

1 Parasitology Department, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia
2 Public Health and Community Medicine Department, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia
3 Physiology Department, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia
4 Faculty of Health Science, Dehasen University, Bengkulu, Indonesia

ARTICLE HISTORY

Received:
Revised:
Accepted:

DOI:

Abstract: Pulmonary tuberculosis (TB) caused by Mycobacterium tuberculosis is still a great challenge in the public health domain until nowadays. Sputum collection from TB patients followed by an examination of acid-fast bacilli (AFB) is a common diagnostic tool routinely done, yet it could lead to false negative results as the patient excrete saliva instead of sputum, meanwhile bacterial culture which stands as the gold standard is time and labor consuming. MicroRNAs (miRNAs) are one type of RNA that is small (18-25 nucleic acids) and controls the function of messenger RNA (mRNA). MicroRNA is the 6th and most recent cell communication pathway discovered because the secreted miRNAs are encased in exosomes and could circulate through-out the body and can be found in any body fluids including sputum. MiRNAs of TB patients associated with TB infection can be expressed as increased or decreased according to the severity of the infection. MiRNA-155 and 21 are miRNAs with increased expression in active pulmonary TB and decreases in the healing process so both miRNAs hold the potency to be used as biomarkers to monitor the level of disease activity and the healing process.

Keywords: biomarker, miRNA-155, miRNA-21, *Mycobacterium tuberculosis*, pulmonary Tuberculosis, sputum

1. INTRODUCTION

Pulmonary tuberculosis (TB) caused by the bacterium *Mycobacterium tuberculosis* (*M. tuberculosis*) is an old story. This bacterium has developed ways of invading and manipulating the human immune system, thus surviving inside human body; similar to the human parasite. Pulmonary TB was first identified 1 century ago by Robert Koch, and ironically, this disease remains a challenge in public health problems until the 21st century though anti-TB drugs are available extensively (1, 2). Although the mortality rate from tuberculosis is decreasing by 22% for 15 years from 2000 to 2015, WHO stated that tuberculosis was still the 10th biggest cause of death worldwide in 2016 (3). TB infection causes devastating effects, as in 2017 only, there were approximately 1.3 million and 300.000 mortality cases of pulmonary TB infection in HIV-positive and negative patients. There is an estimation of 10 million new cases of TB infection arising annually or 133 new cases per 100.000 people (4) thus, TB remains a key priority globally and is one of the SDGs as of this point (Sustainability Development Goals) (5, 6).

*Address correspondence to this author at the Parasitology Department, Faculty of Medicine, Universitas Trisakti,Jakarta, Indonesia. Phone +62-215672731. Email:suriyani@trisakti.ac.id

Only in 2020, as many as 1.5 million people worldwide died from TB according to WHO, including those living with HIV, as this fact shows that TB is the second greatest cause of death worldwide after Covid-19 (7). These data also imply that deaths caused by TB are even higher than those from HIV-TB. TB cases in 2020 reached 10 million people, consisting of 8.9 million adults and 1.1 million children. How powerful is the fact that TB is, thus ironically, this disease can be prevented and even cured with prompt TB-positive people can be treated and cured using a regimen of only 1-6 months, including the four first-line drugs, namely isoniazid, rifampin, ethambutol and pyrazinamide, and the treatment success rate is 85%. Also in 2020, WHO reported that 30 countries are bearing the heaviest TB burden, and sharing 86% of new TB cases, while 8 countries, including India, Indonesia, China, Pakistan, and the Philippines accounted for a third or 56% of new TB cases (8). Some countries with the heaviest burden of TB has misdiagnosed other lung disease, such as paragonimiasis, with TB infection, as those cases will obscure the real TB incidence (9, 10).

Another problem arises during the Covid-19 pandemic as TB infection was out shadowed by Covid-19, resulting in increasing TB cases due to hampered TB control, diagnosis, and treatment (11, 12). As those problems arise, then there is an urgent need to find a good biomarker for early diagnosis and prompt treatment.

MicroRNAs are a family of regulatory RNAs with 18-30 nucleotides (average of 22 nucleotides) long that control the expression of post-transcriptional genes in specific sequences utilizing base pairing, usually in the 3'-untranslated regions (3'-UTRs) of target messenger RNA (mRNA) and reduced expression of the gene can be reached. These miRNAs play important roles in broad aspects of cellular processes such as cell signaling processes, cell development and apoptosis, cellular metabolism, and many more. Any disturbance in miRNAs function will lead to excess or decreased mRNA expression that causes diseases, such as cancer and other diseases (13).

2. BIOGENESIS AND FUNCTION OF MICRORNA

MicroRNA was discovered more than 25 years ago, but this type of RNA remains an interesting topic to explore since we know only a small part of this RNA (14). RNA is divided into 2 major par: coding RNA and non-coding RNA (15). Messenger RNA is the only family of coding RNAs, while the non-coding RNAs family, so-called regulatory RNAs, consists of several types, such as tRNA, rRNA, miRNA, snRNA, snoRNA, siRNA, piRNA, 7SL, and other lncRNAs (16). The first microRNA lin-4 was discovered in 1993 in a free-living nematode, *Caenorhabditis elegans*, in the collaboration of Victor Ambros's and Gary Ruvkun's laboratories while the second, let-7, was found 7 years after the first discovery, also in the same species (17, 18). The lin-4 was found not to encode any functional protein but otherwise encode for small regulatory RNAs that consist only of 22 nucleotides (19). Lin-4 then showed to be capable of making a base-pairing with lin-14 (20). The lin-4 miRNA turned out to accumulate during the early larval development of *Caenorhabditis elegans*, attached to target regions in the LIN-14 mRNA, causing a significant drop in lin-4 protein abundance (21). This phenomenal finding of miRNAs led to the understanding that only lower organisms use these tiny RNAs (22). The discovery of the second miRNA, let-7, which had a major role in cancer, first suggested the importance of miRNAs as a regulatory molecule (23, 24). The discovery of hundreds of miRNAs from worms, flies, and humans revealed that miRNAs do, in fact, represent a huge set of hitherto undiscovered regulatory molecules. Soon after the second miRNAs finding, let-7, the scientists started to realize the importance of miRNAs as the regulatory molecule as miRNAs were conserved across species (25). Since the discovery of the first miRNA (lin-4) in the nematode worm, 38589 entries of miRNAs (or pri-RNAs) from the plant, animal, bacterial, and viral species have been found or characterized (Release 22.1, miRBase https://www.mirbase.org).

Biogenesis of miRNAs happen in a short time and effective, as the transcription process takes only several minutes to produce mature miRNAs. There are 2 pathways of miRNA biogenesis, comprised of canonical, which is also known as the classical pathway, and the non-canonical pathway (26). The canonical miRNAs pathway starts from the transcription process in the nucleus from DNA sequences by RNA polymerase II (POL II) producing a long-primary miRNA or pri-miRNA. These pri-miRNAs consist of hundreds of nucleotides and also possess local hairpin structures in which miRNA sequences are embedded. The advance process of pri-miRNA is 5'-capped, while splicing and polyadenylation will follow, this process results in two or more hairpins, and each of the hairpin contains a different mature miRNAs species. The enzyme RNA-polymerase II can determine the fate of long pri-miRNAs, either becoming a precursor of messenger RNA (mRNA), small nuclear RNA (snRNA), or mi-RNA. The pri-miRNAs will subsequently be processed in two steps; the first takes place in the nucleus which is processed by the RNase III enzyme so-called Drosha, and the second process happens in the cytoplasm by Dicer, the other RNase III enzyme. These two enzymes are in charge of converting the stem-loop precursor of miRNAs into mature miRNAs (27, 28). Drosha and DGCR8 are responsible for the cleavage process of pri-miRNA unto precursor miRNA (pre-miRNA). Pre-miRNA which has double-stranded with a stem-loop is then exported to the cytoplasm by Exportin 5 enzyme. In the cytoplasm, pre-miRNA is once again processed by Dicer into the double-stranded miRNA/miRNA* duplex, a mature miRNA form. After the unwinding process, mature miRNA with only 1 strand is loaded into RNA-induced silencing complex (RISC). MicroRNA target recognition indicates that miRNA is bound and presented by the RNA-induced silencing complex (RISC), known as miRISC. The miRISC then joins with a target messenger RNA (mRNA), generally within its 3'UTR, and suppresses the target mRNA's protein production (29).

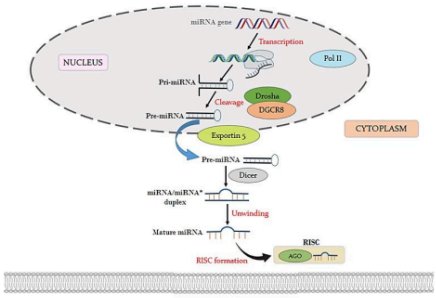


Fig. (1). Biogenesis of miRNA in the canonical pathway

There is also known another deviation from the canonical pathway, known as the non-canonical pathway. Non-canonical miRNA biogenesis routes are emerging as a highlight in some miRNA biogenesis processes, either Drosha-independent or Dicer-independent. Deep sequencing methods have identified multiple types of RNA molecules that mirror miRNAs structurally and functionally. However, they do skip one or more steps in the normal biogenesis process (30, 31). These miRNAs are therefore named non-canonical miRNAs. Several pathways included in non-canonical pathways are: 1) miRtrons, as certain non-canonical pri-miRNAs are encoded in coding genes' introns.; 2) Dicer-Independent miRNAs, as only one miRNA known, which is Pre-miR-451 being processed by this pathway; 3) Small nucleolar RNA (snoRNA)-Derived miRNAs; 4) Transfer (tRNA)-Derived miRNAs. There is ample evidence that both snoRNA and tRNA can be alternative source of miRNAs (31-34).

MicroRNAs are indeed able to reduce gene expression by multiple pathways and modes. It has been proven that a single miRNA has the ability to rule the expression of several target mRNAs. In contrast, multiple miRNAs can govern just only one mRNA (35). Because of extensive experimental data, the 5' region of miRNA is known to aid in terms of selectivity and activity in binding targets. MicroRNAs regulate target expression by perfectly or nearly perfectly complementarity base pairing to sequence motifs in the 3'UTR of mRNAs. A study of miRNA target sites revealed that the longer the 3'UTRs of one gene, the greater the density of miRNA-binding sites, which play a role in developmental control, at the opposite, genes with shorter 3'UTRs have lower density towards miRNA-binding sites, indicating that they are connected in basic cellular functions. All of these data demonstrated the significance of 3'UTR interaction to miRNAs (36, 37).

Earlier research showed that miRNAs predominantly affected target mRNA translation, however recent evidence suggests that joining of miRISC with the target mRNAs also results in direct destruction of target mRNA. MicroRNAs repress protein expression in three distinct mechanisms, which are 1) Initiation mechanism; 2) Post initiation mechanism; and 3) miRNA-mediated mRNA decay. In the initiation mechanism; miRNAs work in the initiation part of mRNA formation, meanwhile in the post initiation; miRNAs work to repress the translation elongation process, also in the termination or even involve in the degradation and sequestration process of protein (38, 39).

3. MICRORNAS AND TUBERCULOSIS

M. tuberculosis is an immemorial organism and has developed an adaptation in human host macrophages to preserve its existence and survival inside the host. It is estimated that more than one-third of the whole population globally carries the infection of *M. tuberculosis,* and even though only one-tenth of those infected with *M. tuberculosis* eventually develop as active tuberculosis cases, but the morbidity dan mortality is beyond imagination (40, 41). Until now, there is no single effective vaccine for total protection of infection of *M. tuberculosis* (42). As the bacteria enter human body for the first time, macrophages are activated as the first line of innate immunity. During the invasion of pathogens, macrophages would encounter with inflammatory response, which are pattern-recognition receptors (PRRs) for recognition of the bacteria leading lead to the clearance of bacteria, disappearance of inflammation process, replacement and repairment of damaged tissue (43). As in the infection of *M. tuberculosis*, macrophages deploy several mechanisms such as phagocytosis or inflammatory responses in regard to eliminating the mycobacteria, resulting in clearance of the *M. tuberculosis* infection, or repressing the infection as latent status (44). The latent status could stay for a period, either short or long and about 5-15% of those would progress into active infection depending on several factors, such as host-bacterial interaction, host immune and nutrition status (45).

Polarization into classically activated-macrophage (CAM) or alternatively activated macrophage (AAM) during the infection process is affected by miRNAs. The working mode of both CAM and AAM is contrast to each other. CAM is arising to respond in intracellular bacteria invasion, releasing pro-inflammatory cytokine, ROS and NOS, and the expression of several miRNAs such as miR-155, miR-181, miR-451 are also increasing in this

situation. In the other side, AAM is responding to the extracellular foreign, such as parasites, and the expression of miR-146a, miR-125b, and miR-127 is increasing (46, 47).

There is a limitation of knowledge about regulation of host miRNAs regarding the response of macrophages as the first phagocytic immune cell in the lung microenvironment to mycobacterial during tuberculosis infection. Bacteria influence multiple host biological pathways and their functions to ensure survival and multiplication. Regulation of miRNAs expression by bacterial pathogens shortly after infection occurs is a principal aspect of the host response to infection, as this is an innovative strategy of the bacteria to evade host immune system. MicroRNAs plays an important role for the both innate and adaptive immune system. In innate stage, miRNAs regulate the main functions of macrophages, dendritic cells, and Natural Killer Cells (NKCs) (48, 49), and in adaptive stage, activation up to polarization of T-cell is tightly regulated by miRNAs (50).

Many studies showed either up-regulated or down-regulated miRNAs expression during TB infection as miRNAs regulated immunity in the infection process. Several miRNAs are up-regulated in active TB infection causing the bacteria to survive in host by suppressing the inflammatory pathway of the host or through apoptosis inhibition, meanwhile on the other side, some miRNAs are down-regulated and could also leading to host susceptibility to TB or clearance of the bacteria (51). The diagnostic value of miRNAs in whole blood or serum sample could not rely in 1 single miRNA, but the combination of several miRNAs could serve as good diagnostic tool, such as miR-155 and miR-21 (52). Meanwhile, in sputum, the most highlighted miRNA is miR-155 as a promising biomarker for diagnostic (53).

3.1. miR-155 and Tuberculosis

The origin of miR-155 is from B-cell integration cluster (BIC), also known as *MIR155HG* (54) that can be found in human chromosome 21, but this homologous gene also found in mouse (55). This microRNA is a well-known miRNA that involved in inflammatory processes and its expression is up-regulated in most of cancers through several pathway, including inflammation or Vascular Endothelial Growth Factor (VEGF) pathway inducing tumor angiogenesis and further metastasis (56). MiR-155 also involved in lung disorder as this miRNA showed to be in high expression in lungs of TB-infected mice (57).

The expression of miR-155 varies in different cell types and tissue environments, but the most common organs expressed this miRNA are spleen and thymus and is regulated in a coordinated manner by multiple pathways in response to cellular signals (58). Both of spleen and thymus are the major organ involved in immunology process. Several studies have shown increased expression of miR-155 in various activated immune cells in innate and adaptive immunity and this suggests an important role of miR-155 in the immune response (59). Three of well-known microRNA, miR-146a, miR-21, and miR-155 are those microRNA that hold an important role governing the inflammatory process especially in myeloid cells (43).

MicroRNA-155 plays important role as the regulator of the innate immune response in general, but interestingly, miR-155 has dual role in active TB infection. In the early innate immune phase, miR-155 is up-regulated, especially in macrophages, dendritic cells (DCs), and in several tissue, leading to the inhibition of host innate immunity. Several modulations in host by miR-155 including 1) host metabolic and energy pathways; 2) autophagy process of infected cells; 3) cell apoptosis and 4) immune response. For the function of miR-155 in host metabolic and energy pathways, miR-155 might involve indirectly in the cholesterol metabolism, as miR-155 has a conserved binding site of ABCA1 (ATP Binding Cassette Subfamily A Member 1). ABCA1 is a protein coding gene, and this gene produces a membrane-associated protein that belongs to the superfamily of ATP-binding cassette (ABC) transporters. The ABC transporters protein performs as a cholesterol efflux pump in the cellular lipid elimination process, using cholesterol as its substrate (60).

In a variety of physiological situations, the autophagy mechanism performs a fundamentally significant basic housekeeping function. *M. tuberculosis* is a well-known bacteria that can reside inside immune cells while modulating and inhibiting the autophagy process of those cells. Through the increasing expression of miR-155, *M. tuberculosis* then lowering Atg3 protein, an essential enzyme for the starting of autophagy, thus prolong the bacteria lifetime inside the host (61, 62).

Normally, all the cells will undergo the apoptosis process, as apoptosis is one of the methods used by human body to clean out the aged or abnormal cell, so this process is being controlled tightly. There are 2 mechanisms of apoptosis; the extrinsic pathway and the intrinsic pathway Extrinsic pathway is initiated by death receptors, such as FAS or tumor necrosis factor, activating caspase- 8 through caspase-3, leading to apoptosis, meanwhile the intrinsic pathway happens in mitochondria through several chemistry process. MicroRNA-155 targeting the transcription process of FOXO3 (Forkhead box O3) directly in the 3′-untranslated regions (3′-UTRs), thus inhibited the apoptosis of monocyte. The forkhead family of transcription factors, which includes this FOXO3 gene, is distinguished by a unique forkhead domain. Upon stress stimuli to the cell, FOXO3 can relay signal to mitochondria, and the response can be either cellular

homeostasis, adaptation to the stress or apoptosis, depends on the stressor types (63). In active infection of *M. tuberculosis*, the bacteria manipulating and lowering the miRNA-155 level leading to increasing immune cell apoptosis. The overexpressed miR-155 also connected with the decreasing NO synthesis in macrophages infected by *M. tuberculosis,* and leading to the survival of the bacteria (64).

In adaptive immunity phase, up-regulation of miR-155 convey a protective against TB infection by encouraging the survival of both infected macrophage and dendritic cell and thus promoting the bacterial clearance (51). Other studies highlight the importance of miR-155 in innate immune response regarding T cells, such as Th17 and Th9, as the differentiation of those T-helper cells are regulated by miR-55 (65).

Research by Ying H et al. showed that the expression miRNA-155 is increased in cell cultures infected with *M. tuberculosis* and in sputum of patients with active pulmonary TB, so miRNA-155 has the potential to be used as a biomarker of pulmonary TB (66). These findings also supported by a lot of studies, such as Wu J et al. that showed miR-155 is up-regulated in active TB patients compared to healthy controls group (67), similar to study conducted by Huang J, et al. that showed *M. tuberculosis* can induce up-regulation of the miR-155 expression (68). According to results of several studies, up-regulation of miR-155 in active TB infection leads to several pathway, such as increasing apoptosis, decreasing bacterial survival in host and higher immune response, and all of those pathways come to one conclusion, which is clearance of *M. tuberculosis* (69).

Controversially, some other studies regarding miR-155 stated that miR-155 expression in serum sample was low in active TB cases, and increasing during the TB therapy, even though in natural killer (NK) cells from TB patients have overexpressed miR-155 but those cells lack of inflammatory cytokine production such as TNF-α (70, 71). Study by using mice model with miR-155 -/- showed that they could not clear out the *M. tuberculosis* and have small number of CD4+ T cells if compared to wild types (57), thus promoting the replication of mycobacterial inside the macrophage (72).

All those results showed that miR-155 is one of potential microRNA proposed be used as diagnostic tools for active TB infection and biomarker of healing process as the expression will be disrupted during the active infection phase whether its expression is up-regulated or down-regulated if compared to uninfected person. A systematic review and meta-analysis study by Li X, et al. concluded that miR-155 has high accuracy value for active TB infection and could be used to distinguish between TB-infected people from healthy one (53). As in TB therapy process, the expression of miR-155 will gradually return to normal value even though the expression will never touch the normal standard value as in the uninfected person (69).

3.2. miR-21 and Tuberculosis

MicroRNA-21 is a well-known oncomiR which was discovered earlier than other miRNAs. MiR-21 is targeting tumor suppressors and its expression is usually up-regulated in many types of cancer, such a, malignancy in hematology aspect, sarcoma or adenocarcinoma types (73, 74), but also in both innate and adaptive immune cells (75). Location of miR-21 gene in human is in chromosome 17q23.2, as the sequence of miR-21 is 5′-UAGCUUAUCAGACUGAUGUUGA, as miR-21 is positively regulated by Activation Protein-1 (AP-1), Signal Transducer and Activator of Transcription (STAT-3), and Nuclear Factor (NF-κB), meanwhile B-cell lymphoma 6 (Bcl-6), Growth Arrest Specific 5 (GAS5), and PAP associated domain containing 5 (PAPD5) negatively regulates miR-21 (76). MiR-21 is involved in the reduction of host T-helper (77), pulmonary fibrosis through TGB-β pathway, in inflammation process by targeting TLR4 (78), and also in apoptosis through the pathway NF-κB-MTP64-Bcl-2 [55]. Information about miR-21 during active TB infection is limited. A study by Abd-El-Fattah, et al. stated that miR-21 was overexpressed in lung cancer patients in accordance with the role of miR-21 as oncomiR, but not in TB patients (79). On the other hand, several studies showed that miR-21 was up-regulated in innate immune cells after challenges with antigen of mycobacterial (79-81).

MicroRNA-21 has opposing role during TB infection, as in one hand, up-regulation of miR-21 causing down-regulation of Bcl-2 and TLR-4 leading to apoptosis and bacterial survival in host. Overexpression of miR-21 dampening activation of macrophage, and also impaired Th1 as downstream effect. Wu, et al. also stated that IL-12 and Bcl-2 are targets of miR-21 and up-regulation of miR-21 is leading to impairment of IL-12 and Bcl-2, and thus hampering host immunity to M. tuberculosis (77). Meanwhile, on the opposite, miR-21 also induced after macrophage activation as the downstream is up-regulation of Bcl-2 thus apoptosis is inhibited, as stated by Zhao et al (82). Results of study by Duffy et al. clearly state that miR-21 was up-regulated in serum samples of healthy household contacts of TB cases (83), but Kleinsteuber, et al showed that the expression of miR-21 was decreasing in active TB patients (84). Either the expression of miR-21 is up-regulated or down-regulated, all of those suggestions of using miR-21 as biomarker during infection or recovery process.

CONCLUSION

As stated in SGD 3.3 that tuberculosis epidemics should be ended in 2030, it is an urgent issue to discover an

effective and efficient biomarker for early diagnosis for precise treatment for reducing misdiagnosis cases. MicroRNAs could serve as good biomarker in diagnosis and healing process, as miRNAs could be found in many kind of body fluid, such as sputum, serum or even urine, making miRNAs as effective and non-invasive biomarker. Nowadays, there are so many miRNAs that have been explored in studies, so the challenge should be choosing one out of those miRNAs that could serve as the precise biomarker to differentiate the acute, chronic or even multi drug resistant TB infection.

CONSENT FOR PUBLICATION

Not applicable

FUNDING

This work was financially supported by Universitas Trisakti (Grant no 334/A.1/LPPMP/USAKTI/XI/2022).

Figure 1 already obtained copyright (number EC00202263198 / 9th September 2022) under Directorate General of Intellectual Property, Indonesian Ministry of Law and Human Rights. This work also financially supported by Universitas Trisakti (Grant no 334/A.1/LPPMP/USAKTI/XI/2022).

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Not applicable

REFERENCES

[1. Loddenkemper R, Murray JF. History of tuberculosis. Essential Tuberculosis: Springer; 2021. p. 3-9.
2. Tripathy S, Srivastava K, Kant S, Sarin R. History of Tuberculosis. National Medicos Organisation Journal (एन एम ओ जर्नल)(ISSN-2348-3806). 2018;12(01):14-8.
Chakaya J, Khan M, Ntoumi F, Aklillu E, Fatima R, Mwaba P, et al. Global Tuberculosis Report 2020–Reflections on the Global TB burden, treatment and prevention efforts. International Journal of Infectious Diseases. 2021;113:S7-S12.
4. Menzies NA, Quaife M, Allwood BW, Byrne AL, Coussens AK, Harries AD, et al. Lifetime burden of disease due to incident tuberculosis: a global reappraisal including post-tuberculosis sequelae. Lancet Glob Health. 2021;9(1[illegible]:e1679-e87.
5. Xue Y, Zhou J, Wang P, Lan JH, Lian WQ, Fan YY, et al. Burden of tuberculosis and its association with socio-economic development status in 204 countries and territories, 1990-2019. Front Med (Lausanne). 2022;9:905245.
6. Fukunaga R, Glaziou P, Harris JB, Date A, Floyd K, Kasaeva T. Epidemiology of tuberculosis and progress toward meeting global targets—worldwide, 2019. Morbidity and Mortality Weekly Report. 2021;70(12):427.
7. Petersen E, Al-Abri S, Chakaya J, Goletti D, Parolina L, Wejse C, et al. World TB Day 2022: Revamping and reshaping global TB control programs by advancing lessons learnt from the COVID-19 pandemic. International Journal of Infectious Diseases. 2022.
8. Klinton JS, Heitkamp P, Rashid A, Faleye BO, Win Htat H, Hussain H, et al. One year of COVID-19 and its impact on private provider engagement for TB: A rapid assessment of intermediary NGOs in seven high TB burden countries. Journal of Clinical Tuberculosis and Other Mycobacterial Diseases. 2021;25:100277.
9. Poudyal BS, Paudel B, Bista B, Shrestha GS, Pudasaini P. Clinical, Laboratory and Radiological Features of Paragonimi-asis Misdiagnosed as Pulmonary Tuberculosis. Iranian Journal of Parasitology. 2022;17(3):410-4.

10. Kong L, Hua L, Liu Q, Bao C, Hu J, Xu S. One delayed diagnosis of paragonimiasis case and literature review. Respirology Case Reports. 2021;9(5):e00750.
11. Alene KA, Wangdi K, Clements ACA. Impact of the COVID-19 Pandemic on Tuberculosis Control: An Overview. Tropical Medicine and Infectious Disease. 2020;5(3):123.
12. Zimmer AJ, Klinton JS, Oga-Omenka C, Heitkamp P, Nawina Nyirenda C, Furin J, et al. Tuberculosis in times of COVID-19. Journal of Epidemiology and Community Health. 2022;76(8):310-6.
13. Li C, Ni YQ, Xu H, Xiang QY, Zhao Y, Zhan JK, et al. Roles and mechanisms of exosomal non-coding RNAs in human health and diseases. Signal Transduct Target Ther. 2021;6(1):383.
14. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993;75(5):843-54.
15. Saliminejad K, Khorram Khorshid HR, Soleymani Fard S, Ghaffari SH. An overview of microRNAs: biology, functions, therapeutics, and analysis methods. Journal of cellular physiology. 2019;234(5):5451-65.
16. Rajesh N, Gupta MK, Donde R, Sabarinathan S, Gouda G, Dash GK, et al. Expression Profiling and Discovery of microRNA. Bioinformatics in Rice Research: Springer; 2021. p. 459-86.
17. Almeida MI, Reis RM, Calin GA. MicroRNA history: discovery, recent applications, and next frontiers. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2011;717(1-2):1-8.
18. Mahapatra N, Dash K, Bhuyan L, Behura S, Mishra P, Panda A. Biogenesis of microRNAs and its implication in head and neck pathologies: A Narrative Review. Journal of International Oral Health. 2021;13(2):101-7.
19. Hammond SM. An overview of microRNAs. Advanced drug delivery reviews. 2015;87:3-14.
20. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Frontiers in endocrinology. 2018;9:402.
21. Lu Y, Deng M, Wang K, Peng Y, Ouyang M. The Regulatory Effects of MicroRNAs on Tumor Immunity. BioMed Research International. 2022;2022:2121993.
22. Gebert LFR, MacRae IJ. Regulation of microRNA function in animals. Nature Reviews Molecular Cell Biology. 2019;20(1):21-37.
23. Hashemi A, Gorji-Bahri G. MicroRNA: Promising roles in cancer therapy. Current Pharmaceutical Biotechnology. 2020;21(12):1186-203.
24. Ma Y, Shen N, Wicha MS, Luo M. The Roles of the Let-7 Family of MicroRNAs in the Regulation of Cancer Stemness. Cells. 2021;10(9):2415.
25. Dexheimer PJ, Cochella L. MicroRNAs: From Mechanism to Organism. Frontiers in Cell and Developmental Biology. 2020;8.
26. Ergin K, Çetinkaya R. Regulation of microRNAs. miRNomics: Springer; 2022. p. 1-32.
27. Pozniak T, Shcharbin D, Bryszewska M. Circulating microRNAs in Medicine. Int J Mol Sci. 2022;23(7).
28. Rojas-Pirela M, Andrade-Alviárez D, Medina L, Castillo C, Liempi A, Guerrero-Muñoz J, et al. MicroRNAs: master regulators in host-parasitic protist interactions. Open biology. 2022;12(6):210395-.
29. Starega-Roslan J, Koscianska E, Kozlowski P, Krzyzosiak WJ. The role of the precursor structure in the biogenesis of microRNA. Cell Mol Life Sci. 2011;68(17):2859-71.
30. Bartel DP. Metazoan MicroRNAs. Cell. 2018;173(1):20-51.
31. Stavast CJ, Erkeland SJ. The Non-Canonical Aspects of MicroRNAs: Many Roads to Gene Regulation. Cells. 2019;8(11).
32. Abdelfattah AM, Park C, Choi MY. Update on non-canonical microRNAs. Biomol Concepts. 2014;5(4):275-87.
33. Miyoshi K, Miyoshi T, Siomi H. Many ways to generate microRNA-like small RNAs: Non-canonical pathways for microRNA production. Molecular genetics and genomics : MGG. 2010;284:95-103.
34. Michlewski G, Cáceres JF. Post-transcriptional control of miRNA biogenesis. Rna. 2019;25(1):1-16.
35. Dragomir M, Mafra ACP, Dias SMG, Vasilescu C, Calin GA. Using microRNA Networks to Understand Cancer. International Journal of Molecular Sciences. 2018;19(7):1871.
36. Davey MG, Davies M, Lowery AJ, Miller N, Kerin MJ. The Role of MicroRNA as Clinical Biomarkers for Breast Cancer Surgery and Treatment. International Journal of Molecular Sciences. 2021;22(15):8290.
37. Jia Y, Wei Y. Modulators of MicroRNA function in the immune system. International Journal of Molecular Sciences. 2020;21(7):2357.
38. Duchaine TF, Fabian MR. Mechanistic insights into microRNA-mediated gene silencing. Cold Spring Harbor perspectives in biology. 2019;11(3):a032771.
39. Gierlikowski W, Gierlikowska B. MicroRNAs as Regulators of Phagocytosis. Cells. 2022;11(9).
40. Behr MA, Edelstein PH, Ramakrishnan L. Revisiting the timetable of tuberculosis. Bmj. 2018;362.
41. MacNeil A, Glaziou P, Sismanidis C, Maloney S, Floyd K. Global Epidemiology of Tuberculosis and Progress Toward Achieving Global Targets - 2017. MMWR Morbidity and mortality weekly report. 2019;68(11):263-6.
42. Huang L, Russell DG. Protective immunity against tuberculosis: what does it look like and how do we find it? Current Opinion in Immunology. 2017;48:44-50.
43. Curtale G, Rubino M, Locati M. MicroRNAs as Molecular Switches in Macrophage Activation. Frontiers in Immunology. 2019;10.
44. Tsolaki AG, Varghese PM, Kishore U. Innate Immune Pattern Recognition Receptors of Mycobacterium tuberculosis: Nature and Consequences for Pathogenesis of Tuberculosis. In: Kishore U, editor. Microbial Pathogenesis: Infection and Immunity. Cham: Springer International Publishing; 2021. p. 179-215.
45. Kiazyk S, Ball TB. Latent tuberculosis infection: An overview. Canada communicable disease report = Releve des maladies transmissibles au Canada. 2017;43(3-4):62-6.

46. Kundu M, Basu J. The role of microRNAs and long non-coding RNAs in the regulation of the immune response to mycobacterium tuberculosis infection. Frontiers in Immunology. 2021;12:687962.
9. Behrouzi A, Alimohammadi M, Nafari AH, Yousefi MH, Riazi Rad F, Vaziri F, et al. The role of host miRNAs on Mycobacterium tuberculosis. ExRNA. 2019;1(1):40.
48. Looney M, Lorenc R, Halushka MK, Karakousis PC. Key macrophage responses to infection with Mycobacterium tuberculosis are co-regulated by microRNAs and DNA methylation. Frontiers in immunology. 2021;12:685237.
3. Sampath P, Periyasamy KM, Ranganathan UD, Bethunaickan R. Monocyte and Macrophage miRNA: Potent Biomarker and Target for Host-Directed Therapy for Tuberculosis. Frontiers in Immunology. 2021;12:667206.
11 Naqvi RA, Datta M, Khan SH, Naqvi AR, editors. Regulatory roles of MicroRNA in shaping T cell function, differentiation and polarization. Seminars in cell & developmental biology; 2021: Elsevier.
51. Sinigaglia A, Peta E, Riccetti S, Venkateswaran S, Manganelli R, Barzon L. Tuberculosis-Associated MicroRNAs: From Pathogenesis to Disease Biomarkers. Cells. 2020;9(10).
12 Latorre I, Leidinger P, Backes C, Domínguez J, de Souza-Galvão ML, Maldonado J, et al. A novel whole-blood miRNA signature for a rapid diagnosis of pulmonary tuberculosis. European Respiratory Journal. 2015;45(4):1173-6.
53. Li X, He J, Wang G, Sun J. Diagnostic value of microRNA-155 in active tuberculosis: A systematic review and meta-analysis. Medicine (Baltimore). 2021;100(46):e27869.
54. Witten L, Slack FJ. miR-155 as a novel clinical target for hematological malignancies. Carcinogenesis. 2019;41(1):2-7.
55. Mashima R. Physiological roles of miR-155. Immunology. 2015;145(3):323-33.
56. Hu J, Huang S, Liu X, Zhang Y, Wei S, Hu X. miR-155: An Important Role in Inflammation Response. J Immunol Res. 2022;2022:7437281.
57. Iwai H, Funatogawa K, Matsumura K, Kato-Miyazawa M, Kirikae F, Kiga K, et al. MicroRNA-155 knockout mice are susceptible to Mycobacterium tuberculosis infection. Tuberculosis (Edinb). 2015;95(3):246-50.
58. Zingale VD, Gugliandolo A, Mazzon E. MiR-155: An Important Regulator of Neuroinflammation. International Journal of Molecular Sciences. 2022;23(1):90.
59. Vigorito E, Kohlhaas S, Lu D, Leyland R. miR-155: an ancient regulator of the immune system. Immunological Reviews. 2013;253(1):146-57.
60. He P, Gelissen IC, Ammit AJ. Regulation of ATP binding cassette transporter A1 (ABCA1) expression: cholesterol-dependent and – independent signaling pathways with relevance to inflammatory lung disease. Respiratory Research. 2020;21(1):250.
61. Paik S, Kim JK, Chung C, Jo E-K. Autophagy: A new strategy for host-directed therapy of tuberculosis. Virulence. 2019;10(1):448-59.
62. Etna MP, Sinigaglia A, Grassi A, Giacomini E, Romagnoli A, Pardini M, et al. Mycobacterium tuberculosis-induced miR-155 subverts autophagy by targeting ATG3 in human dendritic cells. PLOS Pathogens. 2018;14(1):e1006790.
63. Fasano C, Disciglio V, Bertora S, Lepore Signorile M, Simone C. FOXO3a from the Nucleus to the Mitochondria: A Round Trip in Cellular Stress Response. Cells. 2019;8(9):1110.
64. Qin Y, Wang Q, Zhou Y, Duan Y, Gao Q. Inhibition of IFN-γ-Induced Nitric Oxide Dependent Antimycobacterial Activity by miR-155 and C/EBPβ. Int J Mol Sci. 2016;17(4):535.
65. Jafarzadeh A, Naseri A, Shojaie L, Nemati M, Jafarzadeh S, Bannazadeh Baghi H, et al. MicroRNA-155 and antiviral immune responses. International Immunopharmacology. 2021;101:108188.
66. Ying H, FengYing S, YanHong W, YouMing H, FaYou Z, HongXiang Z, et al. MicroRNA-155 from sputum as noninvasive biomarker for diagnosis of active pulmonary tuberculosis. Iran J Basic Med Sci. 2020;23(11):1419-25.
3. Wu J, Lu C, Diao N, Zhang S, Wang S, Wang F, et al. Analysis of microRNA expression profiling identifies miR-155 and miR-155* as potential diagnostic markers for active tuberculosis: a preliminary study. Human immunology. 2012;73(4):31-7.
68. Huang J, Jiao J, Xu W, Zhao H, Zhang C, Shi Y, et al. miR-155 is upregulated in patients with active tuberculosis and inhibits apoptosis of monocytes by targeting FOXO3. Mol Med Rep. 2015;12(5):7102-8.
69. Ruiz-Tagle C, Naves R, Balcells ME. Unraveling the role of microRNAs in Mycobacterium tuberculosis infection and disease: advances and pitfalls. Infection and Immunity. 2020;88(3):e00649-19.
70. Wagh V, Urhekar A, Modi D. Levels of microRNA 10R-16 and miR-155 are altered in serum of patients with tuberculosis and associate with responses to therapy. Tuberculosis (Edinb). 2017;102:24-30.
71. Zhang C, Xi X, Wang Q, Jiao J, Zhang L, Zhao H, et al. The association between serum miR-155 and natural killer cells from tuberculosis patients. International Journal of Clinical and Experimental Medicine. 2015;8(6):9168.
72. Sabir N, Hussain T, Shah SZA, Peramo A, Zhao D, Zhou X. miRNAs in tuberculosis: new avenues for diagnosis and host-directed therapy. Frontiers in microbiology. 2018;9:602.
73. Buscaglia LE, Li Y. Apoptosis and the target genes of microRNA-21. Chin J Cancer. 2011;30(6):371-80.
74. Huang Y, Yang YB, Zhang XH, Yu XL, Wang ZB, Cheng XC. MicroRNA-21 gene and cancer. Medical Oncology. 2013;30(1):376.
75. Wang S, Wan X, Ruan Q. The MicroRNA-21 in Autoimmune Diseases. International Journal of Molecular Sciences. 2016;17(6):864.
76. Li X, Wei Y, Wang Z. microRNA-21 and hypertension. Hypertension Research. 2018;41(9):649-61.

77. Wu Z, Lu H, Sheng J, Li L. Inductive microRNA-21 impairs anti-mycobacterial responses by targeting IL-12 and Bcl-2. FEBS Lett. 2012;586(16):2459-67.
78. Pattnaik B, Patnaik N, Mittal S, Mohan A, Agrawal A, Guleria R, et al. Micro RNAs (miRNAs) as potential biomarkers in tuberculosis: A systematic review. Non-coding RNA Research. 2022.
4. Abd-El-Fattah AA, Sadik NAH, Shaker OG, Aboulftouh ML. Differential MicroRNAs Expression in Serum of Patients with Lung Cancer, Pulmonary Tuberculosis, and Pneumonia. Cell Biochemistry and Biophysics. 2013;67(3):875-84.
80. Harapan H, Fitra F, Ichsan I, Mulyadi M, Miotto P, Hasan NA, et al. The roles of microRNAs on tuberculosis infection: meaning or myth? Tuberculosis. 2013;93(6):596-605.
81. Kumar R, Halder P, Sahu SK, Kumar M, Kumari M, 10a K, et al. Identification of a novel role of ESAT-6-dependent miR-155 induction during infection of macrophages with M ycobacterium tuberculosis. Cellular microbiology. 2012;14(10):1620-31.
82. Zhao Z, Hao J, Li X, Chen Y, Qi X. MiR-21-5p regulates mycobacterial survival and inflammatory responses by targeting Bcl-2 and TLR4 in Mycobacterium tuberculosis-infected macrophages. FEBS letters. 2019;593(12):1326-35.
83. Duffy FJ, Thompson E, Downing K, Suliman S, Mayanja-Kizza H, Boom WH, et al. A serum circulating miRNA signature for short-term risk of progression to active tuberculosis among household contacts. Frontiers in immunology. 2018;9:661.
84. Kleinsteuber K, Heesch K, Schattling S, Kohns M, Sander-Jülch C, Walzl G, et al. Decreased expression of miR-9, miR-26a, miR-29a, and miR-142-3p in $CD4^+$ T cells and peripheral blood from tuberculosis patients. PLoS One. 2013;8(4):e61609.

Suriyani Tan_MS text.docx

ORIGINALITY REPORT

14%	12%	13%	10%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to South Asian University Student Paper	2%
2	Submitted to University of Western Australia Student Paper	1%
3	www.frontiersin.org Internet Source	1%
4	www.tanaffosjournal.ir Internet Source	1%
5	Priyanka Mehta. "miRNAs: The new dawn of tuberculosis", Indian Journal of Tuberculosis, 2020 Publication	1%
6	pmc.ncbi.nlm.nih.gov Internet Source	1%
7	journals.plos.org Internet Source	1%
8	stemcellres.biomedcentral.com Internet Source	1%
9	oaji.net Internet Source	1%
10	dspace.library.uu.nl Internet Source	1%
11	Raza Ali Naqvi, Manali Datta, Samia Haseeb Khan, Afsar R. Naqvi. "Regulatory roles of MicroRNA in shaping T cell function, differentiation and polarization", Seminars in Cell & Developmental Biology, 2021 Publication	1%
	www.jneonatalsurg.com	

12	Internet Source	1 %
13	Tjam Diana Samara, Heri Wibowo, Isabella Kurnia Liem, Ani Retno Prijanti, Andrijono Andrijono. "Comparison of matrix metalloproteinase-9 and E-cadherin expression in early- and late-onset preeclampsia", Universa Medicina, 2021 Publication	1 %
14	www.imrpress.com Internet Source	1 %
15	Guruswamy Mahesh, Roopa Biswas. "MicroRNA-155: A Master Regulator of Inflammation", Journal of Interferon & Cytokine Research, 2019 Publication	1 %
16	Keita Miyoshi, Tomohiro Miyoshi, Haruhiko Siomi. "Many ways to generate microRNA-like small RNAs: non-canonical pathways for microRNA production", Molecular Genetics and Genomics, 2010 Publication	1 %
17	abiocode.com Internet Source	1 %
18	www.mdpi.com Internet Source	1 %

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		

Current Biotechnology, 2025, *14*, 147-154

MINI-REVIEW ARTICLE

MicroRNA miR-155 and miR-21 as Biomarkers in Active Pulmonary Tuberculosis and the Healing Process: A Mini Review

Suriyani Tan[1,*], Machrumnizar Machrumnizar[1], Yuliana Yuliana[1], Rina Kusumaratna[2], Eveline Margo[3] and Jipri Suyanto[4]

[1]*Department of Parasitology, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia;* [2]*Department of Public Health and Community Medicine, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia;* [3]*Department of Physiology, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia;* [4]*Faculty of Health Science, Dehasen University, Bengkulu, Indonesia*

ARTICLE HISTORY

Received: February 24, 2025
Revised: May 02, 2025
Accepted: May 09, 2025

DOI:
10.2174/0122115501395403250908114647

Abstract: Pulmonary tuberculosis (TB) caused by Mycobacterium tuberculosis is still a great challenge in the public health domain to this day. Sputum collection from TB patients followed by an examination of acid-fast bacilli (AFB) is a common diagnostic tool routinely done; however, it could lead to false negative results when the patient excretes saliva instead of sputum. Meanwhile, bacterial culture, which is the gold standard, is time- and labor-consuming. MicroRNAs (miRNAs) are a type of RNA that is small (18-25 nucleotides) and controls the function of messenger RNA (mRNA). MicroRNA is the 6th and most recent cell communication pathway discovered, as the secreted miRNAs are encased in exosomes and can circulate throughout the body and can be found in any body fluids including sputum. MiRNAs in TB patients associated with TB infection can be expressed as increased or decreased according to the severity of the infection. MiRNA-155 and 21 are miRNAs with increased expression in active pulmonary TB and decrease in the healing process, so both miRNAs hold the potency to be used as biomarkers to monitor the level of disease activity and the healing process.

Keywords: Biomarker, miRNA-155, miRNA-21, *Mycobacterium tuberculosis*, pulmonary Tuberculosis, sputum.

1. INTRODUCTION

Pulmonary tuberculosis (TB) caused by the bacterium Mycobacterium tuberculosis (M. tuberculosis) is an old story. This bacterium has developed ways of invading and manipulating the human immune system, thus surviving inside the human body, similar to a human parasite. Pulmonary TB was first identified a century ago by Robert Koch, and ironically, this disease remains a challenge in public health until the 21st century, though anti-TB drugs are widely available [1, 2]. Although the mortality rate from tuberculosis decreased by 22% over 15 years from 2000 to 2015, the WHO stated that tuberculosis was still the 10th biggest cause of death worldwide in 2016 [3]. TB infection causes devastating effects; in 2017 alone, there were approximately 1.3 million cases and 300,000 mortality cases of pulmonary TB infection in HIV-positive and -negative patients. There is an estimation of 10 million new cases of TB infection arising annually, or 133 new cases per 100,000 people [4]. Thus, TB remains a key priority globally and is one of the SDGs at this point (Sustainable Development Goals) [5, 6].

Only in 2020, as many as 1.5 million people worldwide died from TB according to WHO, including those living with HIV, which shows that TB is the second greatest cause of death worldwide after Covid-19 [7]. These data also imply that deaths caused by TB are even higher than those from HIV-TB. TB cases in 2020 reached 10 million people, consisting of 8.9 million adults and 1.1 million children. How powerful is the fact that TB is, and thus ironically, this disease can be prevented and even cured if TB-positive people are treated promptly using a regimen lasting only 1-6 months, including the four first-line drugs, namely isoniazid, rifampin, ethambutol, and pyrazinamide; the treatment success rate is 85%. Also in 2020, WHO reported that 30 countries bear the heaviest TB burden, sharing 86% of new TB cases, while 8 countries, including India, Indonesia, China, Pakistan, and the Philippines, accounted for a third or 56% of new TB cases [8]. Some countries with the heaviest burden of TB have misdiagnosed other lung diseases, such as paragonimiasis, as TB infection, which obscures the real TB incidence [9, 10]. Another problem arose during the Covid-19 pandemic, as TB infection was overshadowed by Covid-19, resulting in increasing TB cases due to hampered TB control, diagnosis, and treatment [11, 12]. As these problems arise, there is an urgent need to find a good biomarker for early diagnosis and prompt treatment.

MicroRNAs are a family of regulatory RNAs with 18 to 25 nucleotides (average of 22 nucleotides) in length that control the expression of genes post-transcriptionally in specific sequences utilizing base pairing, usually in the 3'-untranslat-

*Address correspondence to this author at the Department of Parasitology, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia;
E-mail: suriyani@trisakti.ac.id

2211-551X/25 $65.00+.00

ed regions (3'-UTRs) of target messenger RNA (mRNA), resulting in reduced expression of the gene. These miRNAs play important roles in a broad range of cellular processes such as cell signaling, cell development and apoptosis, cellular metabolism, and many more. Any disturbance in miRNA function will lead to excessive or decreased mRNA expression that causes diseases, such as cancer and other diseases [13].

2. STUDY DESIGN AND METHODOLOGY

Supporting literature searches were conducted using online databases such as Pubmed, Pubmed Central (PMC), Google Scholar, and Web of Science, published within the last 10 years, with the keywords "microRNA", "miRNA", "miR-155", "miR-21", "Tuberculosis", "Pulmonary tuberculosis". All of the sources used were articles in English. All publications in languages other than English have been excluded.

3. BIOGENESIS AND FUNCTION OF MICRORNA

MicroRNA was discovered more than 25 years ago, but this type of RNA remains an interesting topic to explore since we know only a small part about it [14]. RNA is divided into 2 major parts: coding RNA and non-coding RNA [15]. Messenger RNA is the only family of coding RNAs, while the non-coding RNA family, so-called regulatory RNAs, consists of several types, such as tRNA, rRNA, miRNA, snRNA, snoRNA, siRNA, piRNA, 7SL, and other lncRNAs [16]. The first microRNA, lin-4, was discovered in 1993 in a free-living nematode, Caenorhabditis elegans, through the collaboration of Victor Ambros's and Gary Ruvkun's laboratories, while the second, let-7, was found 7 years after the first discovery, also in the same species [17, 18]. Lin-4 was found not to encode any functional protein but instead to encode small regulatory RNAs that consist only of 22 nucleotides [19]. Lin-4 was then shown to be capable of base-pairing with lin-14 [20]. The lin-4 miRNA turned out to accumulate during the early larval development of Caenorhabditis elegans, attaching to target regions in the LIN-14 mRNA, causing a significant drop in LIN-14 protein abundance [21]. This phenomenal finding of miRNAs led to the understanding that only lower organisms use these tiny RNAs [22]. The discovery of the second miRNA, let-7, which had a major role in cancer, first suggested the importance of miRNAs as regulatory molecules [23, 24]. The discovery of hundreds of miRNAs from worms, flies, and humans revealed that miRNAs do, in fact, represent a huge set of hitherto undiscovered regulatory molecules. Soon after the discovery of the second miRNA, let-7, scientists started to realize the importance of miRNAs as regulatory molecules, since miRNAs were conserved across species [25]. Since the discovery of the first miRNA (lin-4) in the nematode worm, 38,589 entries of miRNAs (or pri-RNAs) from plant, animal, bacterial, and viral species have been found or characterized (Release 22.1, miRBase https://www.mirbase.org).

Biogenesis of miRNAs happens in a short time and is effective, as the transcription process takes only several minutes to produce mature miRNAs (Fig. **1**). There are 2 pathways of miRNA biogenesis, comprised of the canonical pathway, also known as the classical pathway, and the non-canonical pathway [26]. The canonical miRNA pathway starts from the transcription process in the nucleus from DNA sequences by RNA polymerase II (POL II), producing a long primary miRNA or pri-miRNA. These pri-miRNAs consist of hundreds of nucleotides and also possess local hairpin structures in which miRNA sequences are embedded. The processing of pri-miRNA involves 5'-capping, followed by splicing and polyadenylation; this process results in two or more hairpins, and each hairpin contains a different mature miRNA species. The enzyme RNA polymerase II can determine the fate of long pri-miRNAs, which may become precursors of messenger RNA (mRNA), small nuclear RNA (snRNA), or miRNA. The pri-miRNAs will subsequently be processed in two steps: the first takes place in the nucleus, where they are processed by the RNase III enzyme called Drosha, and the second process happens in the cytoplasm by Dicer, the other RNase III enzyme. These two enzymes are responsible for converting the stem-loop precursor of miRNAs into mature miRNAs [27, 28]. Drosha and DGCR8 are responsible for the cleavage process of pri-miRNA into precursor miRNA (pre-miRNA). Pre-miRNA, which is double-stranded with a stem-loop, is then exported to the cytoplasm by the Exportin 5 enzyme. In the cytoplasm, pre-miRNA is once again processed by Dicer into the double-stranded miRNA/miRNA* duplex, the mature form of miRNA. After the unwinding process, mature miRNA with only one strand is loaded into the RNA-induced silencing complex (RISC). MicroRNA target recognition occurs when miRNA is bound and presented by the RNA-induced silencing complex (RISC), known as miRISC. The miRISC then joins with a target messenger RNA (mRNA), generally within its 3' UTR, and suppresses the target mRNA's protein production (Fig. **1**) [29].

There is also another deviation from the canonical pathway, known as the non-canonical pathway. Non-canonical miRNA biogenesis routes are emerging as a highlight in some miRNA biogenesis processes, being either Drosha-independent or Dicer-independent. Deep sequencing methods have identified multiple types of RNA molecules that mirror miRNAs structurally and functionally. However, they skip one or more steps in the normal biogenesis process [30, 31]. These miRNAs are therefore named non-canonical miRNAs. Several pathways are included in non-canonical pathways: (1) miRtrons, as certain non-canonical pri-miRNAs are encoded in coding genes' introns; (2) Dicer-independent miRNAs, with only one miRNA known, which is Pre-miR-451, being processed by this pathway; (3) Small nucleolar RNA (snoRNA)-derived miRNAs; (4) Transfer RNA (tRNA)- derived miRNAs. There is ample evidence that both snoRNA and tRNA can be alternative sources of miRNAs [31-34].

MicroRNAs are indeed able to reduce gene expression by multiple pathways and modes. It has been proven that a single miRNA has the ability to regulate the expression of

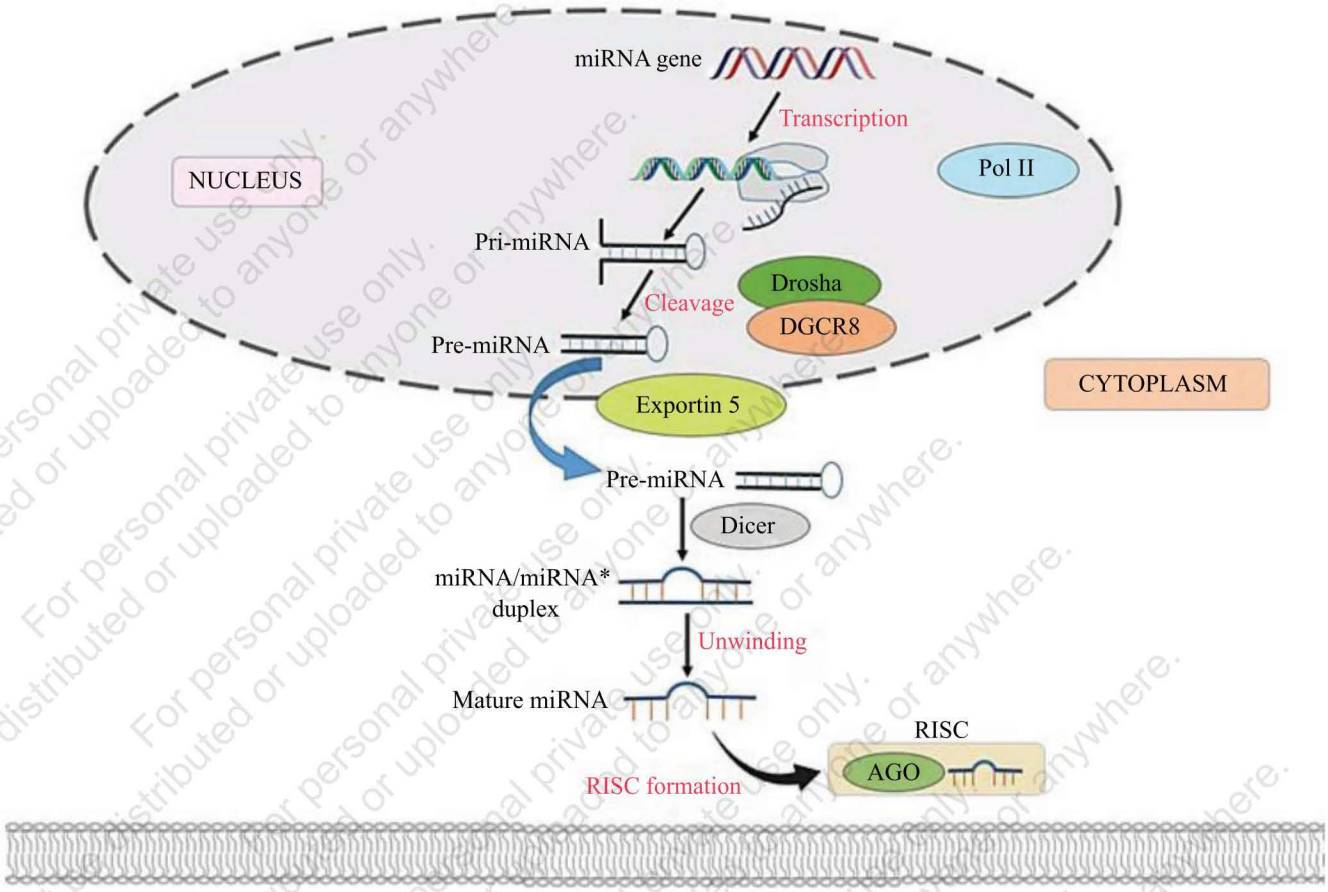


Fig. (1). Biogenesis of miRNA in the canonical pathway. (obtained copyright number EC0020 2263198 / 9th September 2022, under Directorate General of Intellectual Property, Indonesian Ministry of Law and Human Rights. The copyright owner is Universitas Trisakti). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

several target mRNAs. In contrast, multiple miRNAs can govern just one mRNA [35]. Extensive experimental data show that the 5' region of miRNA aids in selectivity and activity in binding targets. MicroRNAs regulate target expression by perfectly or nearly perfectly complementary base pairing to sequence motifs in the 3' UTR of mRNAs. A study of miRNA target sites revealed that the longer the 3' UTRs of a gene, the greater the density of miRNA-binding sites, which play a role in developmental control. In contrast, genes with shorter 3' UTRs have lower density of miRNA-binding sites, indicating that they are connected to basic cellular functions. All of these data demonstrate the significance of 3' UTR interaction with miRNAs [36, 37].

Earlier research showed that miRNAs predominantly affected target mRNA translation; however, recent evidence suggests that the binding of miRISC with the target mRNAs also results in direct destruction of target mRNA. MicroRNAs repress protein expression through three distinct mechanisms: (1) Initiation mechanism; (2) Post-initiation mechanism; and (3) miRNA-mediated mRNA decay. In the initiation mechanism, miRNAs work during the initiation phase of mRNA translation. Meanwhile, in the post-initiation mechanism, miRNAs repress the translation elongation process and also affect termination or even involve the degradation and sequestration of protein [38, 39].

4. MicroRNA AND TUBERCULOSIS

M. tuberculosis is an ancient organism and has developed adaptations in human host macrophages to preserve its existence and survival inside the host. It is estimated that more than one-third of the global population carries the infection of M. tuberculosis, and even though only one-tenth of those infected with M. tuberculosis eventually develop active tuberculosis cases, the morbidity and mortality are beyond imagination [40, 41]. Until now, there is no single effective vaccine for complete protection against infection by M. tuberculosis [42]. As the bacteria enter the human body for the first time, macrophages are activated as the first line of innate immunity. During the invasion of pathogens, macrophages encounter an inflammatory response, which involves pattern-recognition receptors (PRRs) for recognition of the bacteria, leading to the clearance of bacteria, disappearance of the inflammation process, and replacement and repair of damaged tissue [43]. In the infection of M. tubercu-

losis, macrophages deploy several mechanisms such as phagocytosis or inflammatory responses to eliminate the mycobacteria, resulting in clearance of the M. tuberculosis infection or suppressing the infection as latent status [44]. The latent status can persist for a period, either short or long, and about 5-15% of those cases would progress into active infection depending on several factors, such as host-bacterial interaction, host immune status, and nutrition status [45].

Polarization into classically activated macrophages (CAM) or alternatively activated macrophages (AAM) during the infection process is affected by miRNAs. The working modes of both CAM and AAM are contrasting to each other. CAM arises to respond to intracellular bacterial invasion, releasing pro-inflammatory cytokines, ROS, and NOS, and the expression of several miRNAs such as miR-155, miR-181, and miR-451 also increases in this situation. On the other side, AAM responds to extracellular foreign agents, such as parasites, and the expression of miR-146a, miR-125b, and miR-127 increases [46, 47].

There is limited knowledge about the regulation of host miRNAs regarding the response of macrophages, the first phagocytic immune cells in the lung microenvironment, to mycobacteria during tuberculosis infection. Bacteria influence multiple host biological pathways and their functions to ensure survival and multiplication. Regulation of miRNA expression by bacterial pathogens shortly after infection occurs and is a principal aspect of the host response to infection, as this is an innovative strategy used by bacteria to evade the host immune system. MicroRNAs play an important role in both the innate and adaptive immune systems. In the innate stage, miRNAs regulate the main functions of macrophages, dendritic cells, and Natural Killer Cells (NKCs) [48, 49], and in the adaptive stage, activation and polarization of T-cells are tightly regulated by miRNAs [50].

Many studies have shown either up-regulated or down-regulated miRNA expression during TB infection, as miRNAs regulate immunity in the infection process. Several miRNAs are up-regulated in active TB infection, helping the bacteria survive in the host by suppressing the inflammatory pathway or through apoptosis inhibition. Meanwhile, some miRNAs are down-regulated and could also lead to host susceptibility to TB or clearance of the bacteria [51]. The diagnostic value of miRNAs in whole blood or serum samples cannot rely on a single miRNA, but the combination of several miRNAs could serve as a good diagnostic tool, such as miR-155 and miR-21 [52]. In sputum, the most highlighted miRNA is miR-155, which shows promise as a biomarker for diagnosis [53].

4.1. miR-155 and Tuberculosis

The origin of miR-155 is from the B-cell integration cluster (BIC), also known as MIR155HG [54], which is found on human chromosome 21, but this homologous gene is also found in mouse [55]. This microRNA is a well-known miRNA involved in inflammatory processes, and its expression is up-regulated in most cancers through several pathways, including inflammation or the Vascular Endothelial Growth Factor (VEGF) pathway, which induces tumor angiogenesis and further metastasis [56]. MiR-155 is also involved in lung disorders, as this miRNA has been shown to be highly expressed in the lungs of TB-infected mice [57].

The expression of miR-155 varies in different cell types and tissue environments, but the most common organs expressing this miRNA are the spleen and thymus, and it is regulated in a coordinated manner by multiple pathways in response to cellular signals [58]. Both the spleen and thymus are major organs involved in the immunological process. Several studies have shown increased expression of miR-155 in various activated immune cells in innate and adaptive immunity, which suggests an important role of miR-155 in the immune response [59]. Three well-known microRNAs, miR-146a, miR-21, and miR-155, are those microRNAs that hold an important role in governing the inflammatory process, especially in myeloid cells [43].

MicroRNA-155 plays an important role as a regulator of the innate immune response in general, but interestingly, miR-155 has a dual role in active TB infection. In the early innate immune phase, miR-155 is up-regulated, especially in macrophages, dendritic cells (DCs), and in several tissues, leading to the inhibition of host innate immunity. Several modulations in the host by miR-155 include (1) host metabolic and energy pathways; (2) autophagy process of infected cells; (3) cell apoptosis; and (4) immune response. Regarding the function of miR-155 in host metabolic and energy pathways, miR-155 might be involved indirectly in cholesterol metabolism, as miR-155 has a conserved binding site on ABCA1 (ATP Binding Cassette Subfamily A Member (1). ABCA1 is a protein-coding gene that produces a membrane-associated protein belonging to the superfamily of ATP-binding cassette (ABC) transporters. The ABC transporter proteins function as cholesterol efflux pumps in the cellular lipid elimination process, using cholesterol as their substrate [60].

In a variety of physiological situations, the autophagy mechanism performs a fundamentally significant basic housekeeping function. M. tuberculosis is a well-known bacterium that can reside inside immune cells while modulating and inhibiting the autophagy process of those cells. Through the increased expression of miR-155, M. tuberculosis lowers Atg3 protein, an essential enzyme for the initiation of autophagy, thus prolonging the bacteria's lifetime inside the host [61, 62].

Normally, all cells undergo the apoptosis process, as apoptosis is one of the methods used by the human body to eliminate aged or abnormal cells, so this process is tightly controlled. There are 2 mechanisms of apoptosis: the extrinsic pathway and the intrinsic pathway. The extrinsic pathway is initiated by death receptors, such as FAS or tumor necrosis factor, activating caspase-8 through caspase-3, leading to apoptosis, while the intrinsic pathway happens in mitochondria through several chemical processes. MicroRNA-155 targets the transcription process of FOXO3 (Forkhead box O3) directly in the 3′-untranslated regions (3′-UTRs), thus inhibiting the apoptosis of monocytes. The fork-

head family of transcription factors, which includes this FOXO3 gene, is distinguished by a unique forkhead domain. Upon stress stimuli to the cell, FOXO3 can relay signals to mitochondria, and the response can be either cellular homeostasis, adaptation to the stress, or apoptosis, depending on the stressor type [63]. In active infection with M. tuberculosis, the bacteria manipulate and lower the miRNA-155 level, leading to increased immune cell apoptosis. The overexpressed miR-155 is also connected with decreased NO synthesis in macrophages infected by M. tuberculosis, leading to the survival of the bacteria [64].

In the adaptive immunity phase, up-regulation of miR-155 conveys protection against TB infection by encouraging the survival of both infected macrophages and dendritic cells, thus promoting bacterial clearance [51]. Other studies highlight the importance of miR-155 in the innate immune response regarding T cells, such as Th17 and Th9, as the differentiation of those T-helper cells is regulated by miR-155 [65].

Research by Ying *et al.* showed that the expression of miRNA-155 is increased in cell cultures infected with M. tuberculosis and in sputum of patients with active pulmonary TB, so miRNA-155 has the potential to be used as a biomarker of pulmonary TB [66]. These findings are also supported by many studies, such as Wu *et al.*, who showed miR-155 is up-regulated in active TB patients compared to the healthy control group [67], similar to a study conducted by Huang *et al.*, which showed M. tuberculosis can induce up-regulation of miR-155 expression [68]. According to the results of several studies, up-regulation of miR-155 in active TB infection leads to several pathways, such as increased apoptosis, decreased bacterial survival in the host, and enhanced immune response, and all of these pathways lead to one conclusion: clearance of M. tuberculosis [69].

Controversially, some other studies regarding miR-155 stated that miR-155 expression in serum samples was low in active TB cases and increased during TB therapy, even though natural killer (NK) cells from TB patients overexpressed miR-155, but those cells lacked inflammatory cytokine production such as TNF-α [70, 71]. A study using a mouse model with miR-155 -/- showed that they could not clear M. tuberculosis and had a smaller number of CD4+ T cells compared to wild types [57], thus promoting the replication of mycobacteria inside the macrophage [72].

All those results showed that miR-155 is one of the potential microRNAs proposed to be used as diagnostic tools for active TB infection and as a biomarker of the healing process, as the expression is disrupted during the active infection phase, whether its expression is up-regulated or down-regulated compared to that of an uninfected person. A systematic review and meta-analysis study by Li *et al.* concluded that miR-155 has a high accuracy value for active TB infection and could be used to distinguish between TB-infected people and healthy individuals [53]. During the TB therapy process, the expression of miR-155 gradually returns to normal values, even though it may never fully reach the normal standard value seen in uninfected persons [69].

4.2. miR-21 and Tuberculosis

MicroRNA-21 is a well-known oncomiR which was discovered earlier than other miRNAs. MiR-21 targets tumor suppressors and its expression is usually up-regulated in many types of cancer, such as malignancies in the hematology aspect, sarcoma, or adenocarcinoma types [73, 74], but also in both innate and adaptive immune cells [75]. The location of the miR-21 gene in humans is on chromosome 17q23.2, and the sequence of miR-21 is 5′-UAGCUUAUCAGACUGAUGUUGA. MiR-21 is positively regulated by Activation Protein-1 (AP-1), Signal Transducer and Activator of Transcription (STAT-3), and Nuclear Factor (NF-κB), meanwhile B-cell lymphoma 6 (Bcl-6), Growth Arrest Specific 5 (GAS5), and PAP associated domain containing 5 (PAPD5) negatively regulate miR-21 [76]. MiR-21 is involved in the reduction of host T-helper cells [77], pulmonary fibrosis through the TGF-β pathway, the inflammation process by targeting TLR4 [78], and also in apoptosis through the pathway NF-κB-MTP64-Bcl-2 [55]. Information about miR-21 during active TB infection is limited. A study by Abd-El-Fattah *et al.* stated that miR-21 was overexpressed in lung cancer patients, in accordance with the role of miR-21 as an oncomiR, but not in TB patients [79]. On the other hand, several studies showed that miR-21 was up-regulated in innate immune cells after challenges with mycobacterial antigen [79-81].

MicroRNA-21 plays an opposing role during TB infection. On one hand, up-regulation of miR-21 causes down-regulation of Bcl-2 and TLR-4, leading to apoptosis and bacterial survival in the host. Overexpression of miR-21 dampens macrophage activation and also impairs Th1 responses as a downstream effect. Wu *et al.* reported that IL-12 and Bcl-2 are targets of miR-21, and its up-regulation leads to impairment of IL-12 and Bcl-2, thereby weakening host immunity against M. tuberculosis [77]. On the other hand, miR-21 is also induced after macrophage activation, resulting in up-regulation of Bcl-2 and inhibition of apoptosis, as described by Zhao *et al.* [82]. A study by Duffy *et al.* showed that miR-21 was up-regulated in serum samples of healthy household contacts of TB cases [83], while Kleinsteuber *et al.* demonstrated decreased miR-21 expression in active TB patients [84]. Whether miR-21 expression is up--regulated or down-regulated, these findings suggest its potential as a biomarker during both the infection and recovery processes.

CONCLUSION

MicroRNA-155 (miR-155) and microRNA-21 (miR-21) show promise as tuberculosis (TB) biomarkers, with potential applications in early diagnosis and treatment monitoring. However, miR-155 has limitations, including variability in expression levels across different TB stages, as well as lack of disease specificity due to its involvement in other inflammatory conditions. Further validation in larger, diverse cohorts is needed to standardize detection methods and establish reliable cutoff values. Integrating miR-155 and miR-21 with existing diagnostic tools could enhance TB detection ac-

curacy, especially in resource-limited settings. Thus, while promising, their clinical application requires more extensive research before routine implementation.

AUTHORS' CONTRIBUTIONS

The authors confirm contribution to the paper as follows: study conception and design: ST, MM; data collection: YY, RK; final drafting and proofreading: EM, JS. All authors reviewed the results and approved the final version of the manuscript.

LIST OF ABBREVIATIONS

3'-Utrs	=	3'-Untranslated Regions
AAM	=	Alternatively Activated Macrophage
CAM	=	Classically Activated-Macrophage
Mrna	=	Messenger RNA
Prrs	=	Pattern-Recognition Receptors
TB	=	Pulmonary Tuberculosis

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

This work was financially supported by Universitas Trisakti, Indonesia, (Grant no. 334/A.1/LPPMP/USAKTI/X-I/2022).

CONFLICT OF INTEREST

The authors declare no conflict of interest financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

DECLARATION

Fig. (**1**) already obtained copyright (number EC0020 2263198 / 9th September 2022) under Directorate General of Intellectual Property, Indonesian Ministry of Law and Human Rights.

REFERENCES

[1] Loddenkemper R, Murray JF. History of tuberculosis. Essential Tuberculosis. Springer 2021; pp. 3-9. http://dx.doi.org/10.1007/978-3-030-66703-0_1

[2] Tripathy S, Srivastava K, Kant S, Sarin R. History of Tuberculosis. National Medicos Organisation Journal 2018; 12(01): 14-8.

[3] Chakaya J, Khan M, Ntoumi F, *et al.* Global Tuberculosis Report 2020 – Reflections on the Global TB burden, treatment and prevention efforts. Int J Infect Dis 2021; 113(Suppl 1) (Suppl. 1): S7-S12. http://dx.doi.org/10.1016/j.ijid.2021.02.107 PMID: 33716195

[4] Menzies NA, Quaife M, Allwood BW, *et al.* Lifetime burden of disease due to incident tuberculosis: a global reappraisal including post-tuberculosis sequelae. Lancet Glob Health 2021; 9(12): e1679-87. http://dx.doi.org/10.1016/S2214-109X(21)00367-3 PMID: 34798027

[5] Xue Y, Zhou J, Wang P, *et al.* Burden of tuberculosis and its association with socio-economic development status in 204 countries and territories, 1990–2019. Front Med (Lausanne) 2022; 9: 905245. http://dx.doi.org/10.3389/fmed.2022.905245 PMID: 35935764

[6] Fukunaga R, Glaziou P, Harris JB, Date A, Floyd K, Kasaeva T. Epidemiology of tuberculosis and progress toward meeting global targets—worldwide, 2019. MMWR Morb Mortal Wkly Rep 2021; 70(12): 427-30. http://dx.doi.org/10.15585/mmwr.mm7012a4 PMID: 33764960

[7] Petersen E, Al-Abri S, Chakaya J, *et al.* World TB Day 2022: Revamping and reshaping global TB control programs by advancing lessons learnt from the COVID-19 pandemic. Int J Infect Dis 2022; 124 (Suppl. 1): S1-3. http://dx.doi.org/10.1016/j.ijid.2022.02.057 PMID: 35248715

[8] Klinton JS, Heitkamp P, Rashid A, *et al.* One year of COVID-19 and its impact on private provider engagement for TB: A rapid assessment of intermediary NGOs in seven high TB burden countries. J Clin Tuberc Other Mycobact Dis 2021; 25: 100277. http://dx.doi.org/10.1016/j.jctube.2021.100277 PMID: 34545343

[9] Poudyal BS, Paudel B, Bista B, Shrestha GS, Pudasaini P. Clinical, Laboratory and Radiological Features of Paragonimiasis Misdiagnosed as Pulmonary Tuberculosis. Iran J Parasitol 2022; 17(3): 410-4. http://dx.doi.org/10.18502/ijpa.v17i3.10632 PMID: 36466025

[10] Kong L, Hua L, Liu Q, Bao C, Hu J, Xu S. One delayed diagnosis of paragonimiasis case and literature review. Respirol Case Rep 2021; 9(5): e00750. http://dx.doi.org/10.1002/rcr2.750 PMID: 33959297

[11] Alene KA, Wangdi K, Clements ACA. Impact of the COVID-19 Pandemic on Tuberculosis Control: An Overview. Trop Med Infect Dis 2020; 5(3): 123. http://dx.doi.org/10.3390/tropicalmed5030123 PMID: 32722014

[12] Zimmer AJ, Klinton JS, Oga-Omenka C, *et al.* Tuberculosis in times of COVID-19. J Epidemiol Community Health 2022; 76(3): 310-6. http://dx.doi.org/10.1136/jech-2021-217529 PMID: 34535539

[13] Li C, Ni YQ, Xu H, *et al.* Roles and mechanisms of exosomal non-coding RNAs in human health and diseases. Signal Transduct Target Ther 2021; 6(1): 383. http://dx.doi.org/10.1038/s41392-021-00779-x PMID: 34753929

[14] Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993; 75(5): 843-54. http://dx.doi.org/10.1016/0092-8674(93)90529-Y PMID: 8252621

[15] Saliminejad K, Khorram Khorshid HR, Soleymani Fard S, Ghaffari SH. An overview of microRNAs: Biology, functions, therapeutics, and analysis methods. J Cell Physiol 2019; 234(5): 5451-65. http://dx.doi.org/10.1002/jcp.27486 PMID: 30471116

[16] Rajesh N, Gupta MK, Donde R, Sabarinathan S, Gouda G, Dash GK, *et al.* Expression Profiling and Discovery of microRNA Bioinformatics in Rice Research. Singapor: Springer 2021; pp. 459-86. http://dx.doi.org/10.1007/978-981-16-3993-7_20

[17] Almeida MI, Reis RM, Calin GA. MicroRNA history: Discovery, recent applications, and next frontiers. Mutat Res 2011; 717(1-2): 1-8. http://dx.doi.org/10.1016/j.mrfmmm.2011.03.009 PMID: 21458467

[18] Dash KC, Mahapatra N, Bhuyan L, Behura S, Mishra P, Panda A. Biogenesis of microRNAs and its implication in head and neck pathologies: A narrative review. J Int Oral Health 2021; 13(2): 101-7. http://dx.doi.org/10.4103/jioh.jioh_226_20

[19] Hammond SM. An overview of microRNAs. Adv Drug Deliv Rev 2015; 87: 3-14. http://dx.doi.org/10.1016/j.addr.2015.05.001 PMID: 25979468

[20] O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Front Endocrinol (Lausanne) 2018; 9: 402. http://dx.doi.org/10.3389/fendo.2018.00402 PMID: 30123182

[21] Lu Y, Deng M, Wang K, Peng Y, Ouyang M. The regulatory effects of micrornas on tumor immunity. BioMed Res Int 2022; 2022(1): 2121993. http://dx.doi.org/10.1155/2022/2121993 PMID: 35909469

[22] Gebert LFR, MacRae IJ. Regulation of microRNA function in animals. Nat Rev Mol Cell Biol 2019; 20(1): 21-37. http://dx.doi.org/10.1038/s41580-018-0045-7 PMID: 30108335

[23] Hashemi A, Gorji-bahri G. MicroRNA: Promising roles in cancer therapy. Curr Pharm Biotechnol 2020; 21(12): 1186-203. http://dx.doi.org/10.2174/1389201021666200420101613 PMID: 32310047

[24] Ma Y, Shen N, Wicha MS, Luo M. The roles of the Let-7 family of MicroRNAs in the regulation of cancer stemness. Cells 2021; 10(9): 2415. http://dx.doi.org/10.3390/cells10092415 PMID: 34572067

[25] Dexheimer PJ, Cochella L. MicroRNAs: From mechanism to organism. Front Cell Dev Biol 2020; 8: 409. http://dx.doi.org/10.3389/fcell.2020.00409 PMID: 32582699

[26] Ergin K, Çetinkaya R. Regulation of microRNAs. Methods Mol Biol 2022; 1-32. http://dx.doi.org/10.1007/978-1-0716-1170-8_1 PMID: 34432271

[27] Pozniak T, Shcharbin D, Bryszewska M. Circulating microRNAs in medicine. Int J Mol Sci 2022; 23(7): 3996. http://dx.doi.org/10.3390/ijms23073996 PMID: 35409354

[28] Rojas-Pirela M, Andrade-Alviárez D, Medina L, *et al.* MicroRNAs: Master regulators in host–parasitic protist interactions. Open Biol 2022; 12(6): 210395. http://dx.doi.org/10.1098/rsob.210395 PMID: 35702995

[29] Starega-Roslan J, Koscianska E, Kozlowski P, Krzyzosiak WJ. The role of the precursor structure in the biogenesis of microRNA. Cell Mol Life Sci 2011; 68(17): 2859-71. http://dx.doi.org/10.1007/s00018-011-0726-2 PMID: 21607569

[30] Bartel DP. Metazoan MicroRNAs. Cell 2018; 173(1): 20-51. http://dx.doi.org/10.1016/j.cell.2018.03.006 PMID: 29570994

[31] Stavast C, Erkeland S. The non-canonical aspects of microRNAs: Many roads to gene regulation. Cells 2019; 8(11): 1465. http://dx.doi.org/10.3390/cells8111465 PMID: 31752361

[32] Abdelfattah AM, Park C, Choi MY. Update on non-canonical microRNAs. Biomol Concepts 2014; 5(4): 275-87. http://dx.doi.org/10.1515/bmc-2014-0012 PMID: 25372759

[33] Miyoshi K, Miyoshi T, Siomi H. Many ways to generate microRNA-like small RNAs: non-canonical pathways for microRNA production. Mol Genet Genomics 2010; 284(2): 95-103. http://dx.doi.org/10.1007/s00438-010-0556-1 PMID: 20596726

[34] Michlewski G, Cáceres JF. Post-transcriptional control of miRNA biogenesis. RNA 2019; 25(1): 1-16. http://dx.doi.org/10.1261/rna.068692.118 PMID: 30333195

[35] Dragomir M, Mafra ACP, Dias SMG, Vasilescu C, Calin GA. Using microRNA networks to understand cancer. Int J Mol Sci 2018; 19(7): 1871. http://dx.doi.org/10.3390/ijms19071871 PMID: 29949872

[36] Davey MG, Davies M, Lowery AJ, Miller N, Kerin MJ. The role of MicroRNA as clinical biomarkers for breast cancer surgery and treatment. Int J Mol Sci 2021; 22(15): 8290. http://dx.doi.org/10.3390/ijms22158290 PMID: 34361056

[37] Jia Y, Wei Y. Modulators of MicroRNA function in the immune system. Int J Mol Sci 2020; 21(7): 2357. http://dx.doi.org/10.3390/ijms21072357 PMID: 32235299

[38] Duchaine TF, Fabian MR. Mechanistic insights into microRNA-mediated gene silencing. Cold Spring Harb Perspect Biol 2019; 11(3): a032771. http://dx.doi.org/10.1101/cshperspect.a032771 PMID: 29959194

[39] Gierlikowski W, Gierlikowska B. MicroRNAs as regulators of phagocytosis. Cells 2022; 11(9): 1380. http://dx.doi.org/10.3390/cells11091380 PMID: 35563685

[40] Behr MA, Edelstein PH, Ramakrishnan L. Revisiting the timetable of tuberculosis. BMJ 2018; 362: k2738. http://dx.doi.org/10.1136/bmj.k2738 PMID: 30139910

[41] MacNeil A, Glaziou P, Sismanidis C, Maloney S, Floyd K. Global epidemiology of tuberculosis and progress toward achieving global targets — 2017. MMWR Morb Mortal Wkly Rep 2019; 68(11): 263-6. http://dx.doi.org/10.15585/mmwr.mm6811a3 PMID: 30897077

[42] Huang L, Russell DG. Protective immunity against tuberculosis: what does it look like and how do we find it? Curr Opin Immunol 2017; 48: 44-50. http://dx.doi.org/10.1016/j.coi.2017.08.001 PMID: 28826036

[43] Curtale G, Rubino M, Locati M. Micrornas as molecular switches in macrophage activation. Front Immunol 2019; 10: 799. http://dx.doi.org/10.3389/fimmu.2019.00799 PMID: 31057539

[44] Tsolaki AG, Varghese PM, Kishore U. Innate immune pattern recognition receptors of mycobacterium tuberculosis: Nature and consequences for pathogenesis of tuberculosis. Adv Exp Med Biol 2021; 1313: 179-215. http://dx.doi.org/10.1007/978-3-030-67452-6_9 PMID: 34661896

[45] Kiazyk S, Ball TB. Latent tuberculosis infection: An overview. Can Commun Dis Rep 2017; 43(3-4): 62-6. http://dx.doi.org/10.14745/ccdr.v43i34a01 PMID: 29770066 PMCID: PMC5764738

[46] Kundu M, Basu J. The role of microRNAs and long non-coding RNAs in the regulation of the immune response to mycobacterium tuberculosis infection. Front Immunol 2021; 12: 687962. http://dx.doi.org/10.3389/fimmu.2021.687962 PMID: 34248974

[47] Behrouzi A, Alimohammadi M, Nafari AH, *et al.* The role of host miRNAs on mycobacterium tuberculosis. ExRNA 2019; 1(1): 40. http://dx.doi.org/10.1186/s41544-019-0040-y

[48] Looney M, Lorenc R, Halushka MK, Karakousis PC. Key macrophage responses to infection with mycobacterium tuberculosis are co-regulated by microRNAs and DNA methylation. Front Immunol 2021; 12: 685237. http://dx.doi.org/10.3389/fimmu.2021.685237 PMID: 34140955

[49] Sampath P, Periyasamy KM, Ranganathan UD, Bethunaickan R. Monocyte and macrophage miRNA: Potent biomarker and target for host-directed therapy for tuberculosis. Front Immunol 2021; 12: 667206. http://dx.doi.org/10.3389/fimmu.2021.667206 PMID: 34248945

[50] Naqvi RA, Datta M, Khan SH, Naqvi AR. Regulatory roles of MicroRNA in shaping T cell function, differentiation and polarization. Seminars in cell & developmental biology. Semin Cell Dev Biol 2022; 124: 34-47. http://dx.doi.org/10.1016/j.semcdb.2021.08.003 PMID: 34446356 PMCID: PMC11661912

[51] Sinigaglia A, Peta E, Riccetti S, Venkateswaran S, Manganelli R, Barzon L. Tuberculosis-associated MicroRNAs: From pathogenesis to disease biomarkers. Cells 2020; 9(10): 2160. http://dx.doi.org/10.3390/cells9102160 PMID: 32987746

[52] Latorre I, Leidinger P, Backes C, *et al.* A novel whole-blood miRNA signature for a rapid diagnosis of pulmonary tuberculosis. Eur Respir J 2015; 45(4): 1173-6. http://dx.doi.org/10.1183/09031936.00221514 PMID: 25657026

[53] Li X, He J, Wang G, Sun J. Diagnostic value of microRNA-155 in active tuberculosis. Medicine (Baltimore) 2021; 100(46): e27869. http://dx.doi.org/10.1097/MD.0000000000027869 PMID: 34797326

[54] Witten L, Slack FJ. miR-155 as a novel clinical target for hematological malignancies. Carcinogenesis 2020; 41(1): 2-7. http://dx.doi.org/10.1093/carcin/bgz183 PMID: 31711135

[55] Mashima R. Physiological roles of miR-155. Immunology 2015; 145(3): 323-33. http://dx.doi.org/10.1111/imm.12468 PMID: 25829072

[56] Hu J, Huang S, Liu X, Zhang Y, Wei S, Hu X. miR-155: An important role in inflammation response. J Immunol Res 2022; 2022: 1-13. http://dx.doi.org/10.1155/2022/7437281 PMID: 35434143

[57] Iwai H, Funatogawa K, Matsumura K, *et al.* MicroRNA-155 knockout mice are susceptible to mycobacterium tuberculosis infection. Tuberculosis (Edinb) 2015; 95(3): 246-50. http://dx.doi.org/10.1016/j.tube.2015.03.006 PMID: 25846955

[58] Zingale VD, Gugliandolo A, Mazzon E. MiR-155: An important regulator of neuroinflammation. Int J Mol Sci 2021; 23(1): 90. http://dx.doi.org/10.3390/ijms23010090 PMID: 35008513

[59] Vigorito E, Kohlhaas S, Lu D, Leyland R. miR-155: An ancient regulator of the immune system. Immunol Rev 2013; 253(1): 146-57. http://dx.doi.org/10.1111/imr.12057 PMID: 23550644

[60] He P, Gelissen IC, Ammit AJ. Regulation of ATP binding cassette

transporter A1 (ABCA1) expression: Cholesterol-dependent and – independent signaling pathways with relevance to inflammatory lung disease. Respir Res 2020; 21(1): 250. http://dx.doi.org/10.1186/s12931-020-01515-9 PMID: 32977800

[61] Paik S, Kim JK, Chung C, Jo EK. Autophagy: A new strategy for host-directed therapy of tuberculosis. Virulence 2019; 10(1): 448-59. http://dx.doi.org/10.1080/21505594.2018.1536598 PMID: 30322337

[62] Etna MP, Sinigaglia A, Grassi A, *et al.* Mycobacterium tuberculosis-induced miR-155 subverts autophagy by targeting ATG3 in human dendritic cells. PLoS Pathog 2018; 14(1): e1006790. http://dx.doi.org/10.1371/journal.ppat.1006790 PMID: 29300789

[63] Fasano C, Disciglio V, Bertora S, Lepore Signorile M, Simone C. FOXO3a from the nucleus to the mitochondria: A round trip in cellular stress response. Cells 2019; 8(9): 1110. http://dx.doi.org/10.3390/cells8091110 PMID: 31546924

[64] Qin Y, Wang Q, Zhou Y, Duan Y, Gao Q. Inhibition of IFN-γ-induced nitric oxide dependent antimycobacterial activity by miR-155 and C/EBPβ. Int J Mol Sci 2016; 17(4): 535. http://dx.doi.org/10.3390/ijms17040535 PMID: 27070591

[65] Jafarzadeh A, Naseri A, Shojaie L, *et al.* MicroRNA-155 and antiviral immune responses. Int Immunopharmacol 2021; 101(Pt A): 108188. http://dx.doi.org/10.1016/j.intimp.2021.108188 PMID: 34626873

[66] Ying H, FengYing S, YanHong W, *et al.* MicroRNA-155 from sputum as noninvasive biomarker for diagnosis of active pulmonary tuberculosis. Iran J Basic Med Sci 2020; 23(11): 1419-25. PMID: 33235699

[67] Wu J, Lu C, Diao N, *et al.* Analysis of microRNA expression profiling identifies miR-155 and miR-155* as potential diagnostic markers for active tuberculosis: A preliminary study. Hum Immunol 2012; 73(1): 31-7. http://dx.doi.org/10.1016/j.humimm.2011.10.003 PMID: 22037148

[68] Huang J, Jiao J, Xu W, *et al.* miR-155 is upregulated in patients with active tuberculosis and inhibits apoptosis of monocytes by targeting FOXO3. Mol Med Rep 2015; 12(5): 7102-8. http://dx.doi.org/10.3892/mmr.2015.4250 PMID: 26324048

[69] Ruiz-Tagle C, Naves R, Balcells ME. Unraveling the role of microRNAs in Mycobacterium tuberculosis infection and disease: Advances and pitfalls. Infect Immun 2020; 88(3): e00649-19. http://dx.doi.org/10.1128/IAI.00649-19 PMID: 31871103

[70] Wagh V, Urhekar A, Modi D. Levels of microRNA miR-16 and miR-155 are altered in serum of patients with tuberculosis and associate with responses to therapy. Tuberculosis (Edinb) 2017; 102: 24-30. http://dx.doi.org/10.1016/j.tube.2016.10.007 PMID: 28061948

[71] Zhang C, Xi X, Wang Q, *et al.* The association between serum miR-155 and natural killer cells from tuberculosis patients. Int J Clin Exp Med 2015; 8(6): 9168-72. PMID: 26309574

[72] Sabir N, Hussain T, Shah SZA, Peramo A, Zhao D, Zhou X. miRNAs in tuberculosis: New avenues for diagnosis and host-directed therapy. Front Microbiol 2018; 9: 602. http://dx.doi.org/10.3389/fmicb.2018.00602 PMID: 29651283

[73] Buscaglia LEB, Li Y. Apoptosis and the target genes of microRNA-21. Chin J Cancer 2011; 30(6): 371-80. http://dx.doi.org/10.5732/cjc.30.0371 PMID: 21627859

[74] Huang Y, Yang YB, Zhang XH, Yu XL, Wang ZB, Cheng XC. MicroRNA-21 gene and cancer. Med Oncol 2013; 30(1): 376. http://dx.doi.org/10.1007/s12032-012-0376-8 PMID: 23277281

[75] Wang S, Wan X, Ruan Q. The MicroRNA-21 in autoimmune diseases. Int J Mol Sci 2016; 17(6): 864. http://dx.doi.org/10.3390/ijms17060864 PMID: 27271606

[76] Li X, Wei Y, Wang Z. microRNA-21 and hypertension. Hypertens Res 2018; 41(9): 649-61. http://dx.doi.org/10.1038/s41440-018-0071-z PMID: 29973661

[77] Wu Z, Lu H, Sheng J, Li L. Inductive microRNA-21 impairs anti-mycobacterial responses by targeting IL-12 and Bcl-2. FEBS Lett 2012; 586(16): 2459-67. http://dx.doi.org/10.1016/j.febslet.2012.06.004 PMID: 22710123

[78] Pattnaik B, Patnaik N, Mittal S, *et al.* Micro RNAs as potential biomarkers in tuberculosis: A systematic review. Noncoding RNA Res 2022; 7(1): 16-26. http://dx.doi.org/10.1016/j.ncrna.2021.12.005 PMID: 35128217

[79] Abd-El-Fattah AA, Sadik NAH, Shaker OG, Aboulftouh ML. Differential microRNAs expression in serum of patients with lung cancer, pulmonary tuberculosis, and pneumonia. Cell Biochem Biophys 2013; 67(3): 875-84. http://dx.doi.org/10.1007/s12013-013-9575-y PMID: 23559272

[80] Harapan H, Fitra F, Ichsan I, *et al.* The roles of microRNAs on tuberculosis infection: Meaning or myth? Tuberculosis (Edinb) 2013; 93(6): 596-605. http://dx.doi.org/10.1016/j.tube.2013.08.004 PMID: 24025365

[81] Kumar R, Halder P, Sahu SK, *et al.* Identification of a novel role of ESAT-6-dependent miR-155 induction during infection of macrophages with *mycobacterium tuberculosis*. Cell Microbiol 2012; 14(10): 1620-31. http://dx.doi.org/10.1111/j.1462-5822.2012.01827.x PMID: 22712528

[82] Zhao Z, Hao J, Li X, Chen Y, Qi X. MiR-21-5p regulates mycobacterial survival and inflammatory responses by targeting Bcl-2 and TLR4 in *Mycobacterium tuberculosis* -infected macrophages. FEBS Lett 2019; 593(12): 1326-35. http://dx.doi.org/10.1002/1873-3468.13438 PMID: 31090056

[83] Duffy FJ, Thompson E, Downing K, *et al.* A serum circulating miRNA signature for short-term risk of progression to active tuberculosis among household contacts. Front Immunol 2018; 9: 661. http://dx.doi.org/10.3389/fimmu.2018.00661 PMID: 29706954

[84] Kleinsteuber K, Heesch K, Schattling S, *et al.* Decreased expression of miR-21, miR-26a, miR-29a, and miR-142-3p in CD4+ T cells and peripheral blood from tuberculosis patients. PLoS One 2013; 8(4): e61609. http://dx.doi.org/10.1371/journal.pone.0061609 PMID: 23613882