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Review Article

The Role of HSA21 Encoded Mirna in Down Syndrome Pathophysiology: Opportunities in miRNA-Targeted Pharmacotherapy and Diagnosis of the Down Syndrome

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Abstract

Trisomy 21 is the most prevalent aneuploidy disorder among live-born children worldwide. It results from the presence of an extra copy of chromosome 21 which leads to a wide spectrum of pathophysiological abnormalities and intellectual disabilities. Nevertheless human chromosome 21 (HSA21) possess protein non-coding regions where HAS-21 derived-microRNA genes are transcribed from. In turn, these HSA21-derived miRNAs curb protein translation of several genes which are essential to meet memory and cognitive abilities. From the genetics and molecular biology standpoints, dissecting the mechanistic relationship between DS pathology/symptoms and five chromosome 21-encoded miRNAs including miR-99a, let-7c, miR-125b-2, miR-155 and miR-802 seems pivotal for unraveling novel therapeutic targets. Recently, several studies have successfully carried out small molecule inhibition of miRNAs function, maturation, and biogenesis. One might assume in the case of DS trisomy, the pharmacological inhibition of these five overexpressed miRNAs might open new avenues for amelioration of the DS symptoms and complications. In this review, we primarily elucidated the role of HSA21-encoded miRNAs in the DS pathology which in turn introduced and addressed important therapeutic targets. Moreover, we reviewed relevant pharmaceutical efforts that based their goals on inhibition of these pathological miRNAs at their different biogenesis steps. We have also discussed the challenges that undermine and question the reliability of miRNAs as none-invasive biomarkers in prenatal diagnosis.

Introduction

Down syndrome (DS) was recognized by John Langdon Down for the first time in 1866 and around one decade later, it was ascribed to existing of an extra copy of human chromosome 21 (HSA21).[1,2] HSA21(21q), as the smallest human chromosome, was reported to contain approximately 700 genes. The overexpression of some of these genes are the well-recognized culprits of DS pathology.[3] The size of the extra copy can range from 3-4 Kb to the whole chromosome 21.[4] Depending on the size of the extra copy, the trisomy can be categorized into three groups including complete trisomy, microtrisomy and partial trisomy. This survivable congenital chromosomal abnormality originates from the failure of chromosome pairs to separate appropriately through the cell division. This genetic d isorder occasionally happens during the meiotic division I and II. In particular the maternal parent cells are more prone to this event.[5-7] Additionally, the increasing DS incidence rate were found to be correlated with the maternal age.[1] DS is the most prevalent genetic malady among diseases in which phenotypes are accompanied with intellectual disability. It affects 0.1% and 0.14% of live-births in the EU and the United States, respectively.[8-11] According to the clinical observations that addressed manifestations in DS, DS symptoms and complications resemble the pathological hallmarks of cell senescence. Epigenetic factors like DNA methylation and miRNA silencing were discovered to contribute to DS onset or progression.[12] The miRNAs with broad distribution can be found in both plant and animal kingdoms.[13] They serve predominantly as negative regulators of protein expression

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through base-pair interactions with the target mRNA. Strikingly, each miRNA can simultaneously downregulate several protein expressions and eventually modulate multiple signaling pathways. Their significant roles in the pathophysiological process of neurodegenerative disorders, various types of cancers, inflammatory abnormalities, cardiovascular diseases, and so forth are extensively uncovered.[14] One of the understudied and overlooked factors in delineating pathophysiological alterations in DS is the Chromosome 21-encoded miRNAs, in which triplication and overexpression have been deemed to in part contribute to progress or onset of DS.[15,16] we shed lights on biological mechanisms and related signaling through which chromosome 21-encoded miRNAs including miR-99a, let-7c, miR-125b-2, miR-155, miR-802 impart to onset and progression of DS symptoms. In the light of the fact that HSA21-encoded miRNAs meet the criteria as putative therapeutic targets in DS pathology, we brought up and debated the advantages and the drawbacks of miRNA-targeted drug discovery. Encapsulated miRNAs in exosomes circulating in extracellular medium can be detected in a highly sensitive manner within multitude of tissues; we reviewed and discussed the prenatal DS diagnostic studies based on biological HSA-21-encoded miRNA analysis.

Down Syndrome

Since the chromosomal anomaly results in DS phenotypes, the "gene-dosage" hypothesis gains value. The "gene-dosage" hypothesis claims that 50% increase in the expression level of the RNA transcripted from trisomic genes, imbalance in critical genes would take place and thus, DS phenotypes manifest.[3,17-19] In contrast to with the "gene-dosage" hypothesis, the "amplified developmental instability" instability hypothesis argue that trisomy 21 result from a nonspecific disturbance of chromosome balance.[20] According to this hypothesis, the size of the triplicated chromosomal region is anticipated to correlate with the levels of cognitive malfunctions.[21,22]

However, the identification of full trisomy patients with milder intellectual disability casts doubts on this hypothesis.[23] Toward a comprehensive understanding of the phenotype-genotype relationship in DS, nearly 80 phenotypes were predicted to associate with DS.[24,25]

Bioinformatics studies have established that HSA21 harbors five microRNA (miRNAs) genes.

Interestingly, the post-mortem dissections of DS brains have indicated the presence of extracellular plaques, made of Aβ protein. Aβ protein overexpression and their abnormal fibrillation and amylogenesis have been considered as hallmarks and the roots of the underlying neurodegenerative mechanisms in Alzheimer Diseases (AD). This phenotypic crosstalk has been ascribed to the fact that the amyloid precursor protein APP gene is located on the HSA21 chromosome in DS and AD. Previous findings on DS transgenic mouse models elucidated triplication of specific genes, like APP, leads to the disruption of nerve growth factor (NGF) axonal transportation in cholinergic neurons, located in basal forebrain to the hippocampus. The impaired cholinergic pathology in partial trisomy Ts65Dn model has been reversed and recovered when the extra copy of APP was genetically deleted from the HSA21 chromosome, meaning the AD phenotypes associate and parallel the trisomy-centric DS pathology.[24,26]

The intracellular neurofibrillary tangles, containing hyperphosphorylated tau, are another hallmark of AD onset. The APP gene is not only AD pathology-associated gene that undergoes triplication in DS trisomy. The DYRK1A gene also lays on the HSA21 chromosome. Its gene triplication and protein overexpression influence alternative splicing of tau. Resultantly, it might eventually cause tau hyper phosphorylation. The DYRK1A-mediated tau priming for abnormal hyperphosphorylation can contribute to AD-like cognitive malfunctions in DS patients.[27]

Another important notion is the fact that the overexpression of the APP gene and other proteins which are involved in APP gene expression, post-translational regulation (SUMO3, DYRK1A, SNC27, and miR-155), and APP protein processing and clearance (PICALM, SORL1, BACE1, and BACE2) are considered to improve the association and deposition of amyloid beta (Aβ) plaques.[28-32]

Research shows that selective deactivation of HSA21-derived miRNAs can be useful as a new therapeutic concept in the treatment of DS.

Additionally, due to the dysfunction of several neurotransmitter-based systems of DS patients including norepinephrine synapses at the locus coeruleus and serotonergic neurons of the raphe nuclei in the brainstem, therapeutic strategies based on the cytogenetic studies and mouse models are well-developed for further inquiries. So far, the Ts65Dn mouse model is widely employed for preclinical investigations purposes.[33,34] The wider perspective on the impacts of the trisomy genes encoding proteins involved in ubiquitin signaling, signal transduction, immune response, and endosomal trafficking attracted many scientists to investigate these cellular mechanisms.[33] Multitude of clinical trials with different modalities of interventions including, but not limited to, diets and physical exercise have been conducted. Although from the regulatory, clinical and translational standpoints, none of their interpretations have been able to repeatedly replicate the endpoint outcomes. This inconsistency within the context of the behavioral examinations was ascribed to the variability of the instrumental analytical tools, whereby the cognitive memory, learning, and language ability of individuals with DS are measured.[35] As a result, devise and development of a validated instrument that could be readily operated for reproducible assessment of the cognitive and language skills in DS with different ages is the mantra. For instance, the Arizona Cognitive Test Battery (ACTB) was exclusively devised to examine the cognitive phenotypes in DS. It is made up of examinations

of overall cognitive ability in the context of hippocampal and cerebellar function and its cross-site usage, data-driven consistency, and precise phenotypic profiling. ACTB has been endorsed by a clinical trial where 74 DS participants and 50 mental age-matched controls were recruited.[36] One potential future study would be to perform clinical trials on patients under 40 years old and to use DS patients as the target group for pre-clinical anti-AD drug therapy, as the rate of the incidence of the disease increases after the age of 40.

To reduce clinical complications, selective inactivation of HSA21-derived miRNAs by the administration is a method in the treatment of DS. In vivo silencing of miR-155 or -802 by antagomir intra-ventricular injection led to the normalization of appropriate miRNA, MeCP2, CREB1, and MEF2C expressions. These results suggest that the neurochemical abnormalities in the brains of DS persons may be resulted from the incorrect repression of MeCP2, secondary to trisomic overexpression of HSA21-derived miRNAs.[37]

Recent studies show the development of nanotechnology-based delivery system, with the advancement of stem cell researches, and optimistically exploiting nanoparticles has become a central issue. Therefore, nano-formulated of HSA21-derived miRNAs could be a major factor improving the cognitive function of individuals with intellectual disability (ID) in DS in the near future.[38]

miRNAs from Biogenesis to Cellular Mechanisms of Diseases

The miRNAs are ~21-nucleotide long, nonprotein-coding RNAs. They play key roles in post-transcriptional modification through complementary-based silencing of the distinctive messenger RNAs (mRNAs).[39] It has been reported that around 2000 miRNAs are present in the mammalian genome with conserved sequences and more interestingly, one-third of human genes are regulated by The biogenesis of miRNA starts with the step in which the RNA polymerase II transcribes miRNA genes and the pri-miRNA is produced. The pri-miRNAs are huge transcripts, containing multiple miRNA sequences and they fold into hairpin structures. In the next step, the nuclear microprocessor, composed of the RNase III enzyme Drosha and the DGCR8 protein, converts the pri-miRNA into the pre-miRNA. After that, through involvement of exportin-5 complex, the pre-miRNA is transported from the nucleus to the cytoplasm. The cytoplasmic endonuclease Dicer, in a complex with TAR RNA-binding protein (TRBP) and PKR-activating protein (PACT), cleaves the stem-loop in the pre-miRNA. Then, the miRNA duplex is unwound and the passenger strand is degraded, while the mature miRNA is released. Upon this event silencing complex (RISC) containing Argonaute (AGO) protein is recruited to furnish base-pair match the mRNA and mature miRNA. If the miRNA and the target mRNA are exactly paired match or approximately complementary to each other, the target mRNA will be degraded and thus the target gene becomes silenced (Figure 1).[24-26]

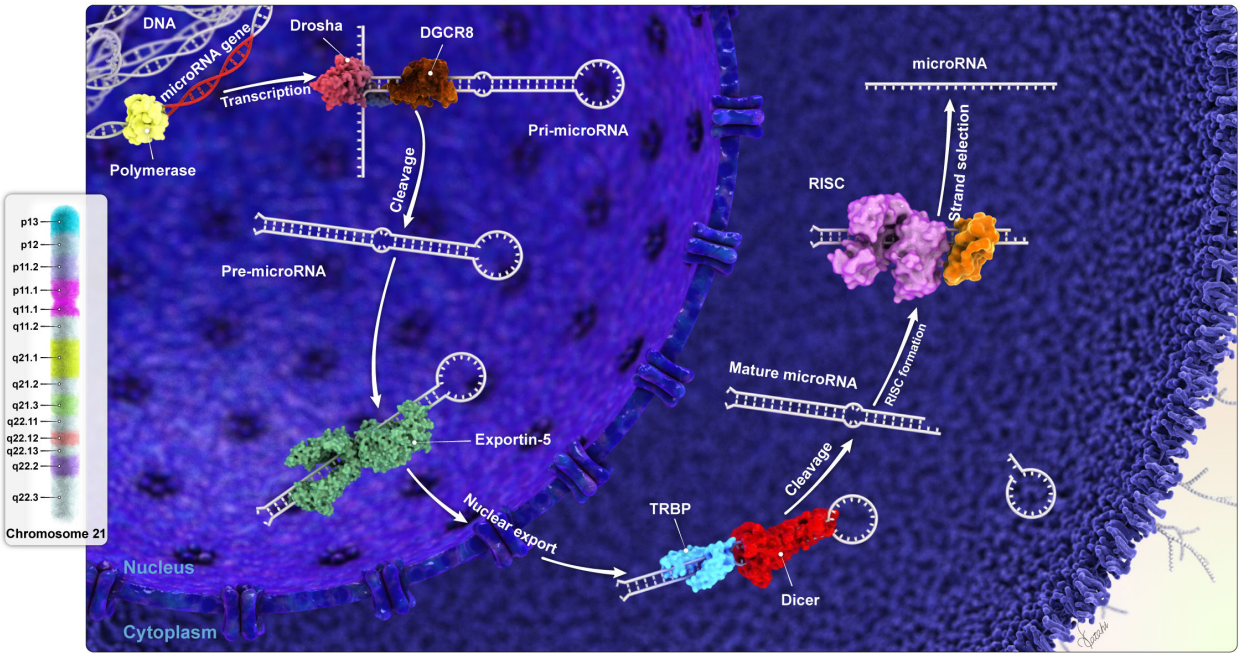

Figure 1. The schematic illustration of the miRNA biogenesis from transcription to mature single-stranded form. The miR-155 gene positions in somewhere between the q21.2 and q21.3 on Chromosome 21. The polymerase enzyme transcribes the has-miRNA genes and produces the immature version of the miRNA as the pri-miRNA. Later, the Drosha and DGCR8 proteins using unique cleavage capability generate the pre-miRNA. Then, the exportin protein adheres to the pre-miRNA and conducts the loaded-complex through the nuclear pore into the cytosol. Once the complex enters the cytosol, the TRBP and Dicer proteins are recruited in order to remove the hairpin from the cytosolic pre-miRNA and produce the double-stranded form of the miRNA as the mature version. Eventually, the RISC complex distinguishes and separates the single-strand guide RNA from the other strand.

An attractive aspect of miRNA genes results from their genome position. MiRNA genes might be found as a single unit or might be organized in gene clusters. The miRNA is an important regulator in biological processes, including development, adaptation to stress, and cell fate determination, proliferation, differentiation, immune reaction, apoptosis and hence their statue in the diabetes type 1 and 2, diabetic retinopathy, microvascular complications, cancer, and kidney diseases were investigated.[40-42]

Roles of HSA21 miRNAs in Onset and Progression of DS

Based on bioinformatics annotations, it has been uncovered that chromosome 21 as the smallest human chromosome possesses at least five miRNA genes with the 1.5 ratios of transcription level. These miRNAs comprise miR-99a, let-7c, miR-125b-2, miR-155 and miR-802.[43] The abnormal expression levels of the miRNAs encoded from the HSA21 in DS were found to correlate with symptoms onsets and in turn it may affect specific haploinsufficiency-related genes.[44] People with DS are more susceptible to AD owing to the copies of the chromosome 21 genes, like DYRK1A and APP. These copies cause the formation of amyloid beta) Aβ(peptide and hyperphosphorylation of tau. DYRK1A localized to nucleus where it may interfere with tau splicing acts upon hyperphosphorylation of tau. In mice models of DS, tau pathology is related to overexpression of DYRK1A. The literature has emphasized the importance of miRNAs to mitigate tau and Aβ pathology in DS. The association between miRNAs miR-17, -20a, -101, -106b, -199b, -26a, and 26b and some of their target mRNAs such as APP, DYRK1A, and BDNF, and the levels of hyperphosphorylated tau in the hippocampus mice model of trisomy 21 (Ts65Dn) have been studied. These results suggested that miR-17, -20a, -26a/b, -101, -106b, and -199b could be important components for decreasing tau and Aβ damage in DS.[45]

Prior studies have noted the importance of several miRNAs in adjusting synaptic plasticity. Regulation of synaptic function mediated by miRNA contributes to the pathophysiology of plasticity-related diseases, such as Alzheimer's and frontotemporal dementia. Also it accounts synaptic activity. On the other hand, miRNAs play a key role in the pathogenesis of AD.[46] They could affect Aβ metabolism in persons with DS, not only through action on 3'UTR of BACE1, ABCA1, APP and other related genes, but also through indirect regulation of other factors. Some evidence indicates that aberrantly expressed miRNAs such as let-7,[47] miR-155[48] have been caused by the changes of both Aβ formation and tau phosphorylation. These molecules increase in the brains of people with DS and are vital in the pathogenesis of AD.[49,50]

It is believed miR-125b has been increased in AD and DS. In initial neurons, tau hyperphosphorylation and the upregulation of p35, cdk5, and p44/42-MAPK signaling are due to the overexpression of miR-125b. Tau hyperphosphorylation has been prompted by the Knockdown of phosphatases DUSP6 and PPP1CA and the anti-apoptotic factor Bcl-W. Together these results provide important insights into the role of these phosphatases mediating the effects of miR-125b on tau.[51]

DNA polymerase beta and MeCP2 expression are downregulated by the miR-155.[52] The miR-155 prohibits the complement factor H mRNA (CFH). Consequently the CFH decrease has been noticed in DS tissues. Neurons are protected from complement opsonization and leukocyte infiltration by the CFH in the brain parenchyma. So overexpression of miR-155 has been widely studied in the brain pathology of DS patients.[53] Since miRNA-155 targets angiotensin II type 1 receptor which was found to contribute to the cardiac pathology, the rate of cardiovascular disease among DS patients becomes about zero. In the DS mouse model Ts65Dn, miR-155 and miR-802 were considerably more than control. Overexpression of these miRNAs has shown hippocampal deficits in DS phenotypes. Hippocampus are generating considerable interest in terms of learning, memory, and long-term synaptic plasticity.[54] Overexpression of the miR-99a/let-7c cluster was proposed to associate with congenital heart defects in DS.[55] The miR125b-2 was demonstrated to be an oncogenic miRNA that contributes to acute megakaryoblastic leukemia (DS-AMKL) in children with trisomy.[56] MiR-100, miR-125b, miR-335, miR-146, and miR-99a have been the most expressed miRNAs noted in pediatric acute myeloid leukemia (AML) patients.[57]

The solid tumor development in patients with DS is far less likely to occur and the reason arises from overexpression of miRNA let-7 and miR-99. The miRNA let-7 and miR-99 inhibits the proliferation of breast and prostate cancer cells respectively[58-60] (Table 1).

DS-Phenotype-Associating miRNAs Encoded from Other Chromosomes

It was demonstrated that four miRNAs from other chromosomes including miR-10b, miR-542-5p, miR-654, and miR-615 were overexpressed in DS placentas.[61] However, their target genes on the chromosome 21 were unknown and another research contradicted these results. Lim *et al.* reported that miR-1973 and miR-3196 in the placenta of trisomy 21 were upregulated and accordingly, the regulation of genes contributing to the improvement of the nervous system was affected by this event. Additionally, another investigation on cord blood samples from normal and DS fetuses indicated the overexpression of three miRNAs including miR-27b, miR-27a, and miR-329 as well as over-translation of several proteins, for example, thymosin β10 and mitogen-activated protein. Depending on the tissues of interest as well as times of extracting bio-sample, a variance in data is expected.[62]

Therapeutic Avenues Addressing HSA21-encoded miRNAs Crisis in DS Pathophysiology

Adopting the HSA21chromosme as a putative target has gained therapeutic values, in particular, when it comes to

Table 1. The HSA21-encoded miRNAs whose overexpression in trisomy contributes to the DS symptoms and complications.

miRNA	Under-expressed mRNAs (genes) associating with DS pathophysiology	Pathophysiological effects in DS complications	Ref.
miR-155	Complement factor H mRNA	The neuronal loss of protection against complete opsonization and leukocyte infiltration into the penumbra	52,53,54
	Angiotensin II type 1 receptor	Less cardiac pathology so that the rate of cardiovascular disease among DS patients become about zero	54
miRNA let-7	Protein Ezh2	Congenital heart defects in DS	55
miR-99a	Nucleosome-remodeling factor Smarca5	Weakening Nodal/Smad2 signaling, DS fetal heart	55
miR-802	Ship1 (inositol phosphatase)	Malfunctional hippocampal synaptic plasticity	54
miR-125b-2	DICER1, ST18	Acute megakaryoblastic leukemia (DS-AMKL) in pediatric DS	56

explore promising strategies for restoring the intellectual disabilities in DS patients. The precise and practical gene edition, offered by the CRISPR-Cas9 which could be genetically optimized to revise the DS-pathology-relevant HSA21-encoded genes on the extra copy of the chromosme21, has been already hypothesized. This Hypothesis in the light of development of safe and blood-brain barrier permeable nanoparticles on which, CRISPR-Cas9 protein cocktails could be encapsulated, become more achievable.[63]

Pharmacological modulations of the HSA21-encoded miRNAs are anticipated to mitigate the translation of several under-expressed proteins. Modulation of the HSA21-encoded miRNAs are anticipated to mitigate the translation of several under-expressed proteins and thereby might lead to phenotypical recovery.[64] The DS pathogenesis in part can be assumed to be dictated through the overexpression of several HSA21-encoded miRNAs. These set of miRNAs serves as key players in silencing several mutual or non-mutual target proteins through which important signaling pathways are modulated.

Coding miR-155 and miR-125b, HSA21 has been thought of as a key factor in the immune response[64,65] and an increase of their expression has been noted in the cells of DS persons. Farroni *et al.*[66] reported the overexpression of miR-155 and miR-125b in tonsillar memory B cells. Remarkably, miR-125b was more than the expected amount in plasma cells. They noticed a significant reduction of activation-induced cytidine deaminase (AID) protein, a miR-155 target, in MBCs of tonsils of DS children. MiR-155 and miR-125b were importantly overexpressed in PBMCs. They worked on the sorted cells and estimated the expression of miR-155 and miR-125b in them. Then their activity was hindered in culture with antagomiRs. Their study outlines that it is possible to modify the activity of miR by applying antagomiRs. Therefore, anatagomiRs have been identified as being pharmacological helpful tools in the treatment of DS.

A recent review of the literature found that the additional copy of chromosome 21 in Down's syndrome leads to overexpression of miR-155. Thanks to this overexpression, C/EBPb is negatively adjusted and the production of sorting nexin 27 (SNX27) in the brain is decreased which causes synaptic dysfunction. SNX27 has been found to be a vital protein for memory and learning. The studies on miR-155 finds that its inhibition upregulates SNX27 protein in the brain of Down's syndrome mice and rescues cognitive and synaptic deficits. Thus memory could be returned.[67]

Another study reveals that miR-155 is crucially upregulated in DS brain. A decrease in the abundance of the miR-155 mRNA affects the complement factor H (CFH), an important repressor of the innate immune response. Stressed primary human neuronal-glial cells show an increase in miR-155. Curiously, there is a significant correlation between miR-155 upregulation and CFH downregulation. One interesting finding is an anti-miR-155 regulates miR-155 abundance resulting in returning CFH to homeostatic levels.[68]

In a recent research, the Argonaute 2 (Ago2) protein which conducts silencing of the target mRNA was adopted as the therapeutic target.[69] Following the virtual screening of the OTAVA_CNS_library against the miR-155/Ago2 complex, a potent small molecule inhibitor was rationally selected. Employing molecular dynamic simulations, the pharmacological activity of the selected small molecule under influence of virtual mutations including Thr526 to isoleucine and Gln545 to alanine was explored and found to fairly depending on the involvement of Thr526 and Gln545 in the first essential contacts between the miR-155 and Ago2.[69] Not only the function but also the maturation and biogenesis of the miR-155 can be regarded as feasible and approachable targets.

For example, in one study driven revealing the binding and inhibition of pre-miRNA with few peptides and linear peptides (poly-N-substituted glycine), a diverse library made of combinatorial macrocyclic γ-AA-peptides was designed and synthesized.[70] Then, this library against affinity binding to pre-miRNA, using the fluorescence polarization (FP) assay was screened. One potent candidate was chosen and the interaction between pre-miRNA and the ligand was endorsed through the performing gel-electrophoresis.

Breast cancer cell lines MCF-7, which had been already proven to overexpress the miR-155. The pharmacological activity of candidate small molecules through the influence on the downstream regulations of miR-155 was examined while lacking alteration in expression of other miRNAs confer specificity to the candidate molecule.[71] In another

team-work research, the cluster miRNA let-7c/99a/125b-2 was suppressed through the androgen-induced repression of at the transcriptional level. Upon the androgen treatment, the androgen binds to the cluster host gene as a receptor and then polycomb protein EZH2 is recruited. After these events, the transcriptions of these miRNAs were repressed. This pharmacological discovery has laid groundwork for the translational scientists who are constantly seeking new targets with less off-site toxicity and more specificity. The curiosity to identify therapeutic targets in the nucleus where the miRNA biogenesis initiates through transcription of their gene may lead to more impactful phenotypic manifestation.[72] However, in this research, the mentioned cluster posit on the different chromosomes rather than chromosome 21 and the androgen-androgen receptor complex needs to be deeply interrogated (Figure 2).

Here we have explained the most recent and creative studies performed in this field where the small molecule inhibition of the overexpressed miRNAs including the miR-155, let-7c, miR-99a, miR-125b-2 accompanied with the double-verified upregulation of downstream proteins were briefly discussed. Nevertheless, lacking both in-vitro and in-vivo pharmacological inhibition of these miRNAs on chromosome 21 whose mechanism of action are being tailored to a DS model, challenges the translatability of this approach.

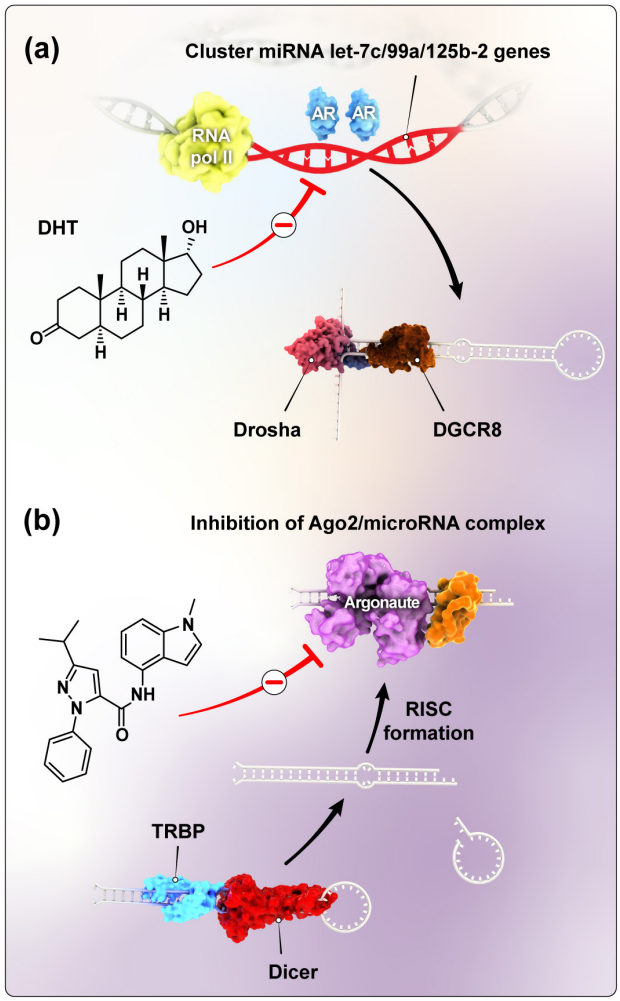

Figure 2. The schematic representation of two pharmacological inhibitors and their mechanism of actions. (a) The transcription of the cluster let-7c/99a/125b-2 miRNAs on a non-HSA21 chromosome is negatively repressed by androgen receptor agonists like dihydrotestosterone DHT (b) The pharmacological inhibition of Ago2/miR-155 complex which is the responsible machinery for the miRNAs function.

Prenatal Diagnosis of DS

The American College of Obstetricians and Gynecologists suggest screening tests for pregnant women that regardless of their gestational age confer with the probability of carrying DS fetuses. These standard tests include the first trimester combined test, the integrated screening test and the cell-free fetal DNA analysis. The first test is the maternal blood level of pregnancy-associated plasma protein-A (PAPP-A) and human chorionic gonadotropin (HCG) while the second one is the ultrasound able to measure the specific area on the back of the fetus neck. Biological fluids including amniotic fluid, semen, milk, saliva, serum, plasma, urine and bronchial lavage contain extracellular nucleic acids including such as miRNAs.[73] The entrance of fetal miRNAs to maternal circulation (blood) is hypothetically justified by three mechanisms. The first one is cellular released exosomes,[74] apoptotic bodies and microvesicles which contain waste materials and extracellular nucleic acids excreted out in a selective manner.[75,76]

The second one is the inter-villous space that sustains the placenta with nutrients and oxygen and the third mechanistic hypothesis relies on the connection between placenta, mother and fetus.[77] As the pathology of trisomy 21 is attributed to the presence of an extra copy of HSA21 harboring several DS pathology-associating miRNAs, one might envision on exploiting these overexpression patterns whereby, DS fetuses would be spotted and legally terminated.[78,79] Furthermore, among extracellular nucleic acids, miRNAs tend to be more stable in maternal circulation,[80] due to the resistance against various pH conditions and enzymatic degradation. Thereby these HSA21 miRNAs could constitute a potential biomarker for diagnosis of some diseases.[81]

Placenta-specific miRNA in maternal blood is detected by many methods including microarray, deep sequencing, and quantitative RT-PCR.[74,82,83] Here we reviewed miRNAs as a non-invasive novel maternal biomarker for diagnosis of DS. In 2014 the microarray-based genome-wide expression profiling was undertaken in order to assess the expression of miRNA in maternal whole blood and placenta sample. Some other studies revealed maternal plasma levels of miR-99a and miR-3156 were significantly higher in pregnant women carrying DS affected fetuses.[84] Additionally, four upregulated HSA21-encoded miRNAs including miR-99a, Let-7c miR-125b-2, and miR-155 were significantly found to be higher in a pregnant woman compared to none-pregnant woman although they didn't

differ between euploid and trisomy21 bearing pregnancies, therefore, they couldn't be considered reliable biomarkers for none-invasive diagnosis of DS.[85] Until now based on most of the clinical investigations, the HSA-21 encoded miRNAs cannot be perfect biomarkers for diagnosis of DS.

Conclusion

Down's syndrome is a genetic disorder characterized by the presence of a third copy of chromosome 21. Given together, several HSA21-encoded miRNAs, including miR-155, miR-125b-2, miR-802, miR-99, and let-7c are overexpressed and they lead to underexpression of their specific target proteins whose physiological functions are essential in preventing/reversing DS complications. The miR-155 is well-documented to associate with neuropathology. The miRNA-802 mediates the neuropathology, while the cluster composed of miR-99 and let-7c serve in the favor of congenital heart defects. The miR-99 and let-7c were also implicated in a low rate of solid tumor development, generally observed among DS patients.

Also, miRNAs in heart tissues from DS fetuses, displays miR-99a-5p, miR-155-5p, and let-7c-5p were significantly overexpressed in trisomic hearts. MiR-125b-2, let-7c, and miR-99a are the three HSA21-derived miRNAs which their orthologs were seen in the mouse inner ear. To reduce tau and Aβ damage in DS, MiRNAs-17, -20a, -26a/b, -101, -106b and -199b would be one of the major objectives. Studies have stressed the importance of several miRNAs in adjusting synaptic plasticity. MiRNA-mediated regulation of synaptic function is responsible for Alzheimer's and frontotemporal dementia. The miR125b-2 was demonstrated to be an oncogenic miRNA contributing to acute megakaryoblastic leukemia (DS-AMKL) that affects children with trisomy.

One of the methods for the reduction of clinical complications is selective inactivation of HSA21-derived miRNAs by the administration of antagomiRs in the treatment of DS. Another way is using nanotechnology-based delivery system.

From the experimental therapeutic standpoint, investments on the design, screening, and synthesis of the small molecules with the excellent potency to halt the HSA21-encoded miRNA overexpression are worthwhile. The pharmacological mechanism of action for these miRNA inhibitors could be classified based on the stage where the proposed small molecule binds to stage-specific RNA-binding protein complexes with miRNA or its precursors. Thereby we can conclude that in order of the normal biochemical events through which the miRNA becomes biologically functional, the initial transcription, biogenesis, maturation and eventually function of the miRNA are the target sites for therapeutic interventions. One noticeable challenge in the pharmacology of these classes of small molecules is their unpredictable specificity and accordingly, future outreach must take this hurdle into their accounts. So far, no pre-clinical DS model study has investigated the efficacy and safety of this niche of small molecules. Additionally, with insightful questions seeking the answer through the overlooked factors might finally establish precious translational and clinical breakthroughs regarding DS therapeutic and management.

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Author Contributions

ShM: Participated in the design of the study and contribution in writing the manuscript, SS: Assistance in writing, YF: Preparing the figures, MA: Supervising and revising the manuscript critically for important intellectual content before submission; editing the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare there is no conflict of interest in this study.

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