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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 common chromosomal aneuploidy disorder among live-born labors 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. The triplication and, resultantly the overexpression of chromosome 21-encoding proteins stand out as main causes and key players of Down syndrome (DS) pathology. Among chromosome 21-encoding genes, microRNA-encoding genes in turn were found to impair protein translation of several genes, whose main physiological functions are essential for bearing memory and cognitive abilities. From the genetic and molecular biology standpoint, dissecting the mechanistic relationship between DS pathology/symptoms and five chromosome21-encoded microRNAs including miR-99a, let-7c, miR-125b-2, miR-155, miR-802 seems pivotal for unraveling novel therapeutic targets. As several recent studies successfully carried out small molecule inhibition of miRNA's function, maturation, and biogenesis, one might assume that 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 the study, we primarily elucidated role of HSA21-encoded microRNAs in the DS pathology which in turn introduced and address 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 challenge that undermines and questions the reliability of microRNAs as none-invasive biomarkers in prenatal diagnostics.

Keywords: Down syndrome, parthenogenesis, trisomy 21, microRNAs, prenatal diagnostic.

Introduction

Down syndrome (DS) for the first time was recognized by John Langdon Down in 1866 and around one decade later, Lejeune et al ascribed the DS etiology to existing of an extra copy of human chromosome 21 (HSA21).^{1,2} HSA21 as the smallest human chromosome includes ~1.5% of the human genome and the long-arm of this chromosome (21q) was reported to contain approximately 700 genes. The overexpression of some these genes are 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 while depending on the size of the extra copy, they could be categorized into three groups including complete trisomy, microtrisomy and partial trisomy. This survivable congenital chromosomal abnormality originates from chromosome pairs failure to separate appropriately through the cell division. This disorder usually occurs during the meiotic division I and II especially when it comes to the maternal parent.⁵⁻⁷ Additionally, the increasing DS incidence rate were found to correlate with the age of maternal parents.¹ DS is the most prevalent genetical malady among diseases whose 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 In accordance with the knowledge that clinical manifestations in DS are similar to the aging process, epigenetic factors like DNA methylation and miRNA silencing were discovered to meddle in DS onset or progression.¹² The miRNAs comprise a evolutionarily conserved group of endogenous small non-protein-coding RNAs with broad distribution in both the plant and animal kingdoms.¹³ They serve predominantly as negative regulators of protein expression through base-pair interaction 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

numerous diseases including neurodegenerative disorders, various types of cancer, cardiovascular diseases, inflammatory abnormalities 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, whose triplication and overexpression, have been deemed to in part contribute to progress or onset of DS.^{15,16} In the present review, we shed light on the biological mechanisms through which chromosome 21-encoded miRNAs including miR-99a, let-7c, miR-125b-2, miR-155, miR-802 are reported to contribute 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 drawbacks of miRNA-targeted drug discovery. As miRNAs, encapsulated in exosomes are circulating in biological fluids and could be extracellularly detected in a highly sensitive manner within multitude of tissues, we reviewed and discussed the prenatal DS diagnostic studies whose biological analyte were HSA21-encoded miRNAs.

Down syndrome

Since the chromosomal anomaly results in DS phenotypes and manifestations, the “gene-dosage” hypothesis is strengthened. The “gene-dosage” hypothesis claims that following 50% increase in the expression level of the RNA, related to the trisomic genes, imbalance in critical genes would occur and therefore, DS phenotypes appear.^{3,17-19} In contrast with the “gene-dosage” hypothesis, the ‘amplified developmental instability’ hypothesis asserts that DS phenotypes 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 A β protein. A β protein overexpression and their abnormal fibrillation and amylogenesis, have been considered as hallmarks and roots of underlying neurodegenerative mechanisms in Alzheimer Diseases (AD). This phenotypic crosstalk has been ascribed to fact that the amyloid precursor protein APP gene located on the HSA21 chromosome in DS and AD. Previous findings on DS transgenic mouse models elucidated that triplication of specific genes like (App) led to 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 associates and parallels the trisomy-centric DS pathology.^{24,26}

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

Another important point is that, the overexpression of the APP gene and factors 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 A β plaques, therefore further affecting the age of onset of AD in DS.²⁸⁻³²

Research shows that selective inactivation of Hsa21-derived miRNAs may provide a new therapeutic tool in the treatment of DS. Additionally, due to dysfunction of several neurotransmitter-based systems including norepinephrine synapses at the locus coeruleus and serotonergic neurons of the raphe nuclei in the brainstem of DS, 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 in the favor of preclinical investigations.^{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 the Diets and Physical exercise has been conducted, though from the regulatory, clinical and translational standpoint, 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 is 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) ACTB was exclusively devised to examine the cognitive phenotype in DS. It is made 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, have been endorsed by a clinical trial where 74 DS

patient participants and 50 mental age-matched controls were recruited.³⁶ One critical future way would be to perform clinical trials in patients before 40 years of age, and to use DS patients as a target group for pre-clinical anti-AD drug therapy, because of the high incidence of disease after the age of 40.

An important method for reductions of clinical complications, selective inactivation of HSA21-derived miRNAs by the administration of antagomiRs in the treatment of DS. Such as, in vivo silencing miR-155 or -802, by antagomir intra-ventricular injection, resulted in the normalization of appropriate miRNA, MeCP2, CREB1, and MEF2C expression. These results indicate that incorrect repression of MeCP2, secondary to trisomic overexpression of HSA21-derived miRNAs, may contribute to the abnormalities in the neurochemistry observed in the brains of DS persons.³⁷

There have been many recent studies that show, the development of nanotechnology-based delivery system could be advancement of stem cell researches with targeting miRNAs involvement in improving the cognitive function of individuals with intellectual disability (ID) in DS in near future.³⁸

miRNAs: from Biogenesis to cellular mechanisms of diseases

The miRNAs are ~21-nucleotide long, nonprotein-coding RNAs 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 while one-third of human genes are regulated by miRNAs.

The biogenesis of miRNA starts from the first step in which the RNA polymerase II transcribes miR genes and the pri-miRNA is produced. The pri-miRNAs are huge transcripts, containing multiple miRNA sequences and, in this step, they are folded 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, by the 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), cleaved the stem-loop in the pre-miRNA. After cleavage by the Dicer complex, the miRNA duplex is unwound and the passenger strand is degraded, while the guide strand or mature miRNA is released and loaded to mRNA. Upon this event silencing complex (RISC) containing Argonaute (AGO) protein is primed to the mRNA target. 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

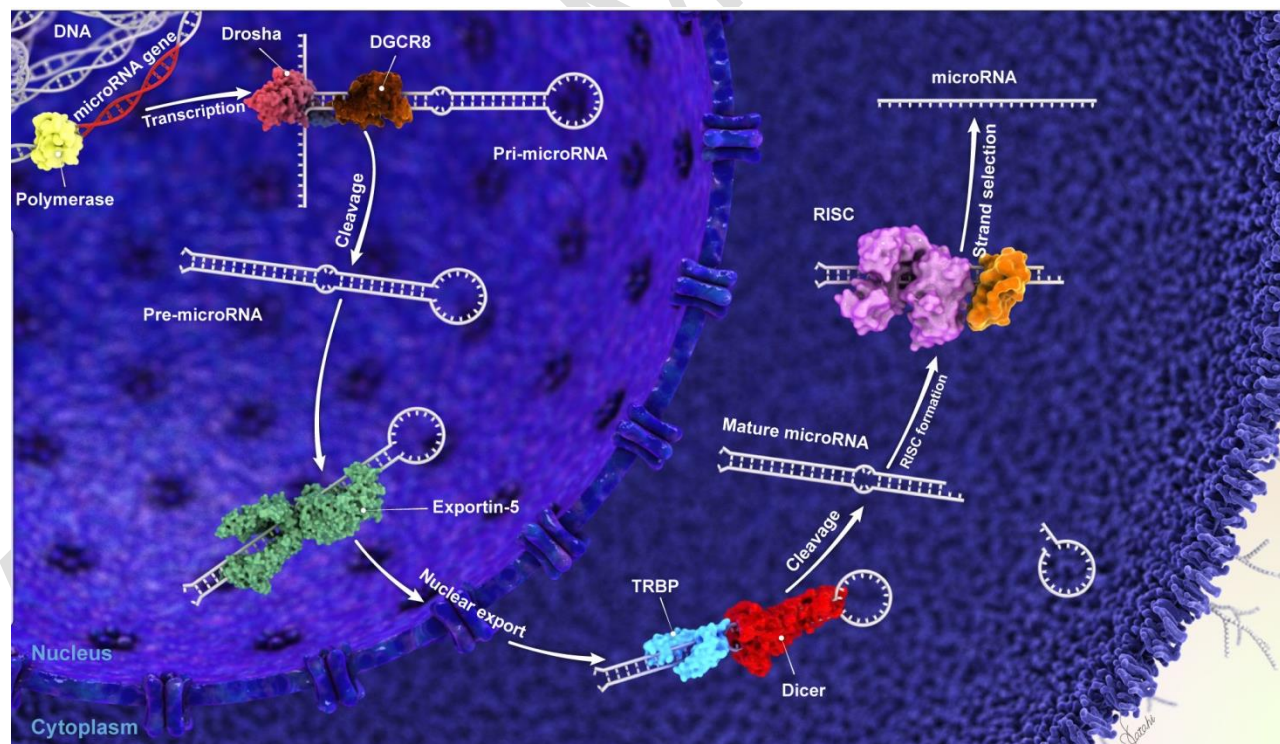


Figure 1: The schematic illustration of the miRNA biogenesis from transcription to mature single-stranded form. The miR-155 gene positions in somewhere between the q21.2 and q21.3 on Chromosome 21. The polymerase enzyme transcribes the has-miRNA genes and produces the immature version of the miRNA as 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 miR genes results from their genome position. miRNA's 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, hence their statue in the Diabetes Type 1 & 2, diabetic retinopathy, microvascular complications, cancer, and kidney diseases were investigated.⁴⁰⁻⁴²

HSA21 miRNAs roles 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 microRNA genes with the 1.5 ratios of transcription level. These microRNAs 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

Three copies of the chromosome 21 gene, such as DYRK1A and App, in people with DS make them more susceptible to AD and leading to formation of amyloid beta (A β) peptide and hyperphosphorylation of Tau. DYRK1A acts upon hyperphosphorylation of Tau, but is also localized to nucleus where it may interfere with Tau splicing. Overexpression of DYRK1A contributes to Tau pathology in mice model of DS.

A recently published article suggests that miRNAs might be interesting targets to mitigate Tau and A β pathology in DS. They studied the association between miRNAs miR-17, -20a, -101, -106b, -199b, -26a, 26b and some of their target mRNAs such as APP, DYRK1A and BDNF, as well as the levels of hyperphosphorylated Tau in the hippocampus mice model of trisomy 21 (Ts65Dn). Their results show that miR-17, -20a, -26a / b, -101, -106b and -199b could be one of the main targets for reducing Tau and A β damage in DS.⁴⁵

Studies have shown that several miRNAs play main roles in adjusting synaptic plasticity. MiRNA-mediated regulation of synaptic function is responsible for synaptic activity and involved in the pathophysiology of plasticity-related diseases, such as Alzheimer's, frontotemporal dementia. As well as, miRNAs could affect A β metabolism in people with DS, counting their through action on 3'UTR of BACE1, ABCA1, APP and other related genes, also indirect regulation through other factors, and played important roles in the pathogenesis of AD.⁴⁶

Some evidence indicates that aberrantly expressed miRNAs such as let-7⁴⁷, miR-155⁴⁸ which increases in the brains of people with DS, were closely related to the changes of both A β formation and Tau phosphorylation, which were vital in the pathogenesis of AD.^{49,50}

Also miR-125b, elevated in AD and DS. In initial neurons, overexpression of miR-125b reasons tau hyperphosphorylation and an upregulation of p35, cdk5, and p44/42-MAPK signaling. Knockdown of phosphatases DUSP6 and PPP1CA and the anti-apoptotic factor Bcl-W induces tau hyperphosphorylation, suggesting that they mediate the effects of miR-125b on tau.⁵¹

The miR-155 downregulate DNA polymerase beta as well as MeCP2 expression.⁵² The miR-155 also silences the complement factor H mRNA (CFH) that reportedly was found to decline in DS tissues. Since the CFH protects neurons from complement opsonization, and leukocyte infiltration in the brain parenchyma, overexpression of miR-155 was highlighted 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. Both miR-155 and miR-802 were significantly higher than the control, in the DS mouse model Ts65Dn, and following overexpression of these miRNAs, hippocampal deficits in DS phenotypes were observed. It is noteworthy that the hippocampus plays notable roles in learning and memory and in long-term synaptic plasticity.⁵⁴ Overexpression of the miR-99a/let-7c cluster and thus, underexpression of their target proteins in fetal DS heart tissue were observed. Thereby this cluster was proposed to associate with congenital heart defects in DS.⁵⁵ The miR125b-2 was demonstrated to be an oncogenic miRNA contributing to acute megakaryoblastic leukemia (DS-AMKL) that affects children with trisomy.⁵⁶ The most expressed miRNAs found in pediatric acute myeloid leukemia (AML) patients were miR-100, miR-125b, miR-335, miR-146, and miR-99a.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 increases tumor formation capacity of breast cancer stem cells while the miR-99 inhibits the proliferation of prostate cancer cells.⁵⁸⁻⁶⁰

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	References
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

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 development of the nervous system was affected by this event. Additionally, another investigation on cord blood samples from DS and normal fetuses indicated overexpression of three miRNAs including miR-27b, miR-27a, and miR-329 as well as over-translation of several proteins, for example, thymosin β 10 and mitogen-activated protein. Depending on the tissues of interest as well as times of extracting bio-sample, a variance in data is expected.

Therapeutic avenues addressing HSA21-encoded miRNAs crisis in DS pathophysiology

Adopting the HSA21 chromosome as a putative target has become a very critical question, when it comes to 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 chromosome21, 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.⁶²

With similar rational basis whose therapeutic target has been directed onto HSA21-encoded genes, the pharmacological modulation of the HSA21-encoded miRNAs are anticipated to mitigate the translation of several under-expressed proteins and thereby might lead to phenotypical recovery, thereby appreciation of small molecules modulating the miRNA function, biogenesis and maturation drew scientific attention.⁶³ 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.

HSA21 encodes miR-155 and miR-125b, play an important role in the immune response.^{64,65} and their expression has been found increased in cells of DS persons. Farroni et al. investigated the over expression of miR-155 and miR-125b in tonsillar memory B cells and miR-125b was also higher than wait in plasma cells. They showed that activation-induced cytidine deaminase (AID) protein, a miR-155 target, was significantly reduced in MBCs of tonsils of DS children. MiR-155 and miR-125b were significantly overexpressed in PBMCs. They evaluated the expression of miR-155 and miR-125b in sorted and inhibited their activity in culture with antagomiRs. Their study shows as miR activity can be modulated by the administration of antagomiRs as a result represent pharmacological tools helpful for the treatment of DS.⁶⁶

In an earlier study published in Nature Medicine, an international team of scientists discovered that the additional copy of chromosome 21 in Down's syndrome causes overexpression of miR-155, that negatively regulates C/EBP β , reduces the production of sorting nexin 27 (SNX27) in the brain and results in synaptic dysfunction. SNX27 is an essential protein for memory and learning. They showed that, inhibition of miR-155 seems a promising way to upregulate SNX27 protein in the brain of Down's syndrome mice rescues cognitive and synaptic deficits and memory could be restored therefore.⁶⁷

Also in another study, it shows that miR-155 was found to be significantly upregulated in Down's syndrome brain and a decrease in the abundance of the miR-155 mRNA target complement factor H (CFH), an important repressor of the innate immune response. Stressed primary human neuronal-glia cells increases in miR-155 correlated with CFH downregulation, and an anti-miR-155 was shown to quench miR-155 abundance and restore CFH back 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 were adopted as feasible approachable targets.

For example, in one study driven by some concrete piece of evidences 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 and one potent candidate was chosen. 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 receptor binds to the cluster of host gene 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.

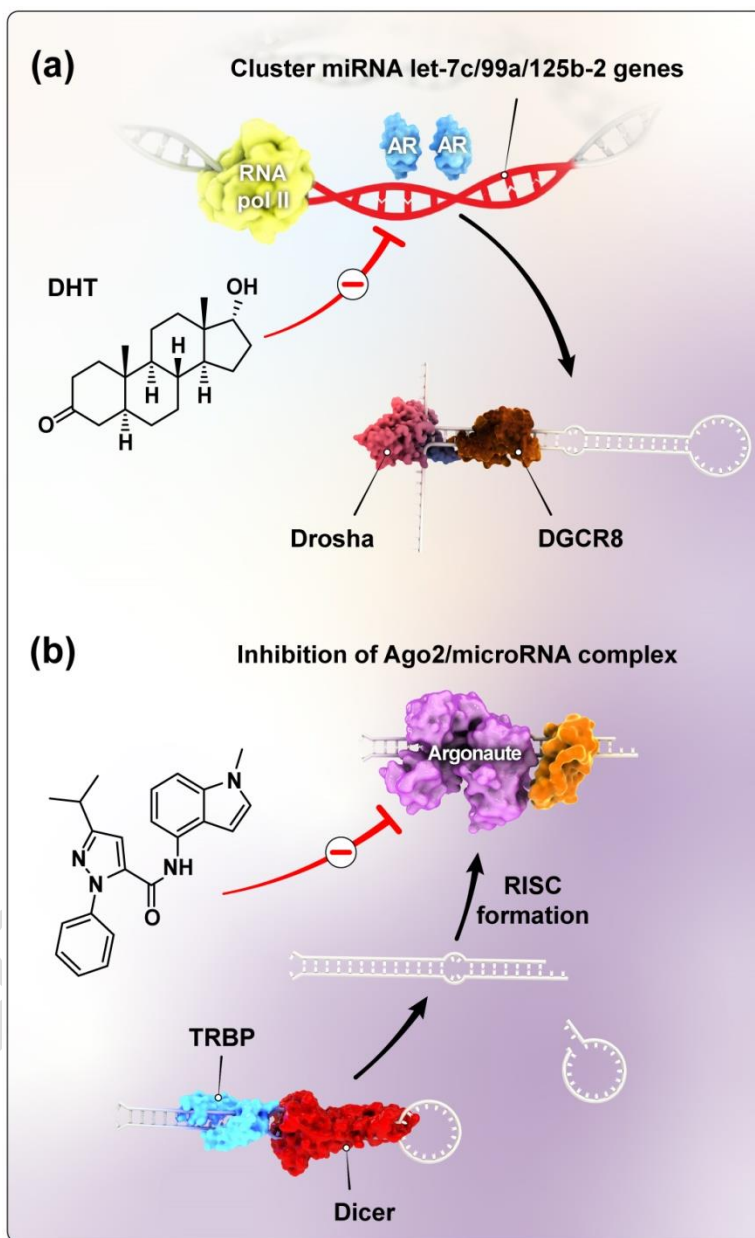


Figure 2: The schematic representation of two pharmacological inhibitors and their mechanism of actions. (a) The transcription of the cluster let-7c/99a/125b-2 miRNAs on a non-HSA21 chromosome is

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 miRNA's 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 trimester is combined of two steps. 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 serum, plasma, amniotic fluid, semen, milk, saliva, urine, and bronchial lavage contain extracellular nucleic acids including but not limited to the miRNAs.⁷³ The entrance of fetal microRNAs 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, microRNAs 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 microRNA in maternal blood is detected by many methods including microarray, deep sequencing, and quantitative RT-PCR.^{74,82,83} Here we reviewed microRNAs 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 microRNA 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 microRNAs 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 microRNAs cannot be perfect biomarkers for diagnosis of DS.

Conclusion and prospective:

Down's syndrome is a genetic condition characterized by the presence of an additional copy of chromosome 21. Given together, several HSA21-encoded miRNAs, including miR-155, 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.

As well as, miRNAs in heart tissues from DS fetuses, displaying that 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 orthologs were found expressed in the mouse inner ear. miRNAs-17, -20a, -26a / b, -101, -106b and -199b could be one of the main targets for reducing Tau and A β damage in DS. Studies have shown that several miRNAs play main roles in adjusting synaptic plasticity. MiRNA-mediated regulation of synaptic function is responsible for Alzheimer's, 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 reductions of clinical complications, selective inactivation of HSA21-derived miRNAs by the administration of antagomiRs in the treatment of DS. Another way, nanotechnology-based delivery system could be advancement of stem cell researches would make the goal of known as miRNAs involvement in intellectual disability (ID) and development with special focus on DS.

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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Conflict of interest

The authors declare that they have no conflicts of interest with the contents of this review.

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