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Review Article

Role of RNA Methylation and Non-Coding RNAs in Pathobiology of Autism Spectrum Disorders

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Abstract: Autism Spectrum Disorders (ASD) are a multifaceted set of neurodevelopmental disorders caused by diverse genetic, epigenetic and environmental factors. Epigenetic mechanisms such as DNA methylation regulate gene expression without changing the genomic DNA sequence, but changing how genomic information is interpreted. The identification of RNA molecules such as non-coding RNAs and its modifications such as RNA methylation as separate functional entities caused a paradigm shift in the epigenetic field. Recent advances have demonstrated that epigenetic mechanisms involving RNA molecules could be immensely contributing to the complex ASD pathobiology. Indirect evidence suggests that methylation of mRNA could be functioning as a regulatory switch in maintaining a balance between mRNA turnover and protein synthesis in autistic patients. Moreover, many studies provide supporting evidence that alterations to 'the methylation cycle', which extend to the methionine-homocysteine pathway, folate cycle, and the redox-homeostasis pathway could underwrite the reduced methylation capacity in ASD. This, in turn, may reduce the RNA methylation status in autistic patients. While implications of RNA methylation in ASD is intriguing, the direct role of RNA methylation in ASD pathogenesis is yet to be explored in depth. In contrast, the functional aspects of non-coding RNAs – both microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) – in ASD have been investigated. MiRNAs found in the autistic brain, blood, saliva or lymphoblast cell lines have shown differential expression as well as deregulation of their target genes. These miRNAs and target genes are associated with synaptic processes, synaptic plasticity, memory, neuronal morphology as well as many cell signaling pathways which could be contributing to the pathobiology of ASD. Similarly, many lncRNAs are differentially expressed in autistic patients and are involved in the deregulation of neuronal connectivity, synaptic functions, and imprinting. Interestingly, some of these lncRNAs are associated with increased risk of ASD. Collectively, epigenetic mechanisms provide codes for maintenance of proper cellular functions. When these epigenetic mechanisms are miscoded, the altered expression of genes, cellular processes and functions contribute towards the pathogenesis of ASD. Therefore, understanding these miscoded RNA epigenetic mechanisms will hold promise for future therapeutic developments for ASD.

Keywords: RNA Methylation, MicroRNA, Non-coding RNA

1. Introduction

Autism Spectrum Disorders (ASD) are a complex set of neurodevelopmental disorders characterized by social, cognitive and behavioral impairments and stereotyped behaviors [1]. Extensive research work based on novel high-throughput sequencing technologies have exposed the complex genetic contribution to the etiology of ASD [2]. Yet, factors beyond genetics have also been implicated in the complex *spectrum* of autism. Epigenetic factors, transcriptome or gene expression changes, as well as many environmental influences, have been shown to interplay with genetic factors [1, 3]. On the other hand, environmental insults influence the epigenome, which ultimately results in gene expression changes underwriting the phenotypes observed. This review summarizes one major aspect of epigenetics associated with RNA molecules in ASD.

The term epigenetics basically refers to DNA methylation

and modifications on histone proteins which occur on the DNA and DNA-bound proteins, without altering the genomic DNA sequence [4]. Yet, the discovery of non-coding RNAs such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), as well as modifications to RNA, such as RNA methylation have caused a paradigm shift in the epigenetic field. The involvement of epigenetic mechanisms associated with RNA molecules in the central dogma of molecular biology has shed light on complex gene expression regulation and disease mechanisms.

RNA methylation and RNA epigenetics: Common RNA modifications include RNA methylation and RNA editing. Among them, RNA methylation has claimed the limelight as the most prevalent modification of messenger RNA (mRNA), with dynamic functions of mainly post-transcriptional gene regulation, such as RNA turnover, stability and splicing [5, 6]. This is in contrast to the major functions of DNA methylation in regulation of transcription, co-transcriptional splicing, and chromatin architecture [4, 7]. Additionally, ribosomal RNA (rRNA), transfer RNA (tRNA), small nuclear RNA (snoRNA), miRNA and lncRNAs have also been shown to be methylated [8]. The N^6-methyladenosine (m^6A) modification of RNA (Fig. 1) has been implicated in the modulation of mRNA splicing, mRNA stability and/or degradation, the stability of RNA duplexes, and ultimately protein translation. As a reversible epigenetic modification, m^6A RNA methylation serves in the fine tuning of the epi-transcriptome. Moreover, its oxidized forms N^6-hydroxymethyladenosine (hm^6A) and N^6-formyladenosine (f^6A) (Fig. 1) seem to add a further layer of complexity into epigenetics [8]. The conversion of m^6A to other types of methyl modifications and back to unmodified A is considered to be oxidative RNA demethylation, which is carried out by ALKBH5 and FTO [6, 9]. Generally, these RNA modifications and their functions belong to the field of RNA epigenetics [10].

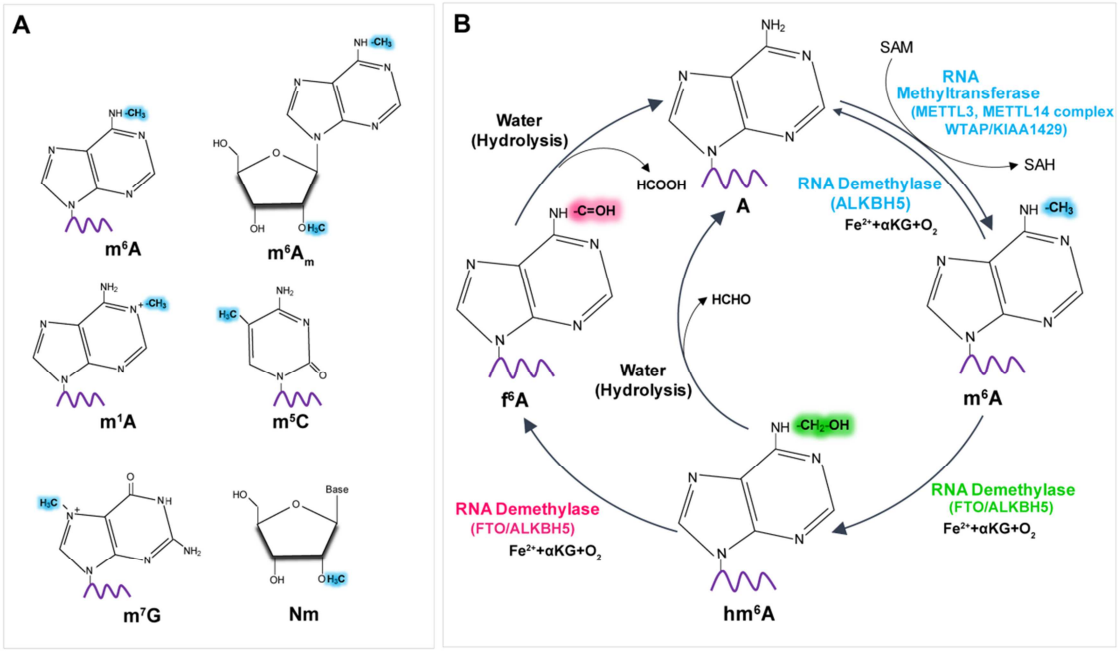

Figure 1. *RNA methylation*

A) The most common RNA methyl modification occurs at the 6th position of Adenosine, called N^6-methyladenosine or m^6A methylation. This modification is further methylated at its ribose moiety to generate m^6A_m. Adenosine on RNA can also be methylated at position 1 (m^1A). Other types of RNA methyl modifications occur on 5th position of the Cytosine (m^5C) or 7th position of Guanosine (m^7G). The methylation of the Ribose moiety itself is considered as an RNA modification referred to as Nm.

B) The methylation and oxidative demethylation of m^6A RNA are shown here. RNA methylation is a reversible RNA modification carried out by multi-complex Methyltransferases -METTL3 and METTL14. These methyltransferases convert the methyl donor S-adenosylmethionine (SAM) to S-adenosylhomocysteine (SAH), transferring a methyl group to RNA. Therefore, this RNA modification is Further oxidation of m^6A generates N^6-hydroxymethyladenosine (hm^6A) and N^6-formyladenosine (f^6A) by the RNA Demethylase, α-ketoglutarate-dependent dioxygenase (FTO). The methyl group removal or demethylation of m^6A is carried out by AlkB Homolog 5, RNA Demethylase (ALKBH5). The hm^6A and f^6A modifications are demethylated to A by hydrolysis.

Non-coding RNAs and epigenetics: Non-coding RNAs can be of different kinds depending on their length, namely, miRNAs, lncRNAs, snoRNs and piwi-interacting RNAs (piRNAs). MiRNAs are typically 21-23 nucleotides long and belong to the category of small non-coding RNAs. They play crucial roles in the regulation of post-transcriptional gene expression, either through regulation of mRNA stability and/or efficiency of protein translation through binding to the

3'untranslated regions (3'UTR) of mRNAs [11]. In contrast to miRNAs, lncRNAs are larger (>200 nucleotides) non-protein-coding RNAs or more than 2kb RNAs which have the potential to be translated into protein with less than 100 amino acids [12]. These lncRNAs encompass RNA molecules with a wide range of functions extending beyond transcriptional regulation. They have been shown to regulate transcriptional activation through enhancer action or suppression and post-transcriptional regulation of gene expression [12]. Moreover, they regulate chromatin remodeling through recruitment of chromatin remodeling enzymes [13]. Additionally, the X-inactive specific transcript (*Xist*) which is a known lncRNA, is involved in X-chromosome inactivation [14].

The role of DNA methylation-associated epigenetic deregulation in ASD is well established and deeply studied [15-17]. In contrast, the influence of altered epigenetics involving RNA molecules in ASD is still under investigation at the moment. In this review, priorities will be given to the discussion of the role of RNA methylation, miRNAs, and lncRNAs in the pathobiology of ASD. The review summarizes the known literature on this topic, simultaneously highlighting the importance of continued investigation of epigenetics involving RNA molecules in ASD pathogenesis.

2. Potential Role of m^6A RNA Methylation in ASD

In order to maintain proper cellular functions within the brain, it is necessary to have a balance among mRNA transcription, processing (splicing and editing), degradation which are generally referred to as transcript turnover and then protein translation and protein turnover. Trivedi and Deth proposed a hypothesis that a regulatory switch within the mRNA life cycle is contributing to the neurological and behavioral phenotypes in neurological disorders such as ASD [18]. They hypothesized that RNA methylation may act as a regulatory switch in mRNA life cycle. As indicated in the introduction, mRNA methylation has been implicated in the modulation of mRNA processing and maintaining its stability [8]. In many neurodevelopmental disorders, for instance, ASD and Rett Syndrome, imbalances between mRNA levels and protein translation have been observed [19, 20]. Defects in the mRNA decay pathways in ASD have also been reported before, which have been implicated in neurocognitive phenotypes of ASD [21, 22]. It is of wide repute that miRNAs are involved in mRNA decay. Hundreds of miRNA molecules show altered expression profiles in ASD, which will be discussed in a later section of the review. However, to date, only indirect evidence of the role of RNA methylation in deregulating mRNA life cycle in ASD is available.

Previous studies have shown correlations between the methylation capacity of the cells and mRNA methylation [23], mRNA splicing as well as localization of mRNA into neuronal synapses [24]. Hence, it is possible that the depletion of methylation capacity in neurons, as seen in autistic patients [25, 26], may affect the mRNA splicing and localization within the neuronal compartments.

Moreover, NSun2 is an RNA methylase which mainly carries out tRNA methylation, while it also does methylate neuronal mRNA. Mutations and loss of Nsun2 lead to autistic features such as intellectual disability and neurodevelopmental phenotypes [27]. Therefore, it is likely that the loss of NSun2 leads to impaired mRNA methylation as well as tRNA methylation which could contribute to the neurological autistic phenotypes observed. As both mRNA and tRNA required for protein translation might be affected by the loss of NSun2, it is possible that Nsun2 might be able to explain imbalances between mRNA turnover and protein translation in certain autistic disorders.

Additionally, methylation at the 5^{th} position of the cytosine of RNA molecules has also been reported, albeit at much lower levels [28]. This modification is called m^5C, similar to the 5-methylcytosine DNA methylation [7]. Both m^6A and m^5C RNA methylation occur at internal positions of mRNA. On the other hand, N^7-methylguanosine (m^7G), N^6-methyl-2'-O-methyladenosine (m^6Am) and 2'-O-methylation (Nm) methylations occur at the 5'-cap of mRNA molecules. The m^7G modification at the 5'-cap of mRNA is important in mRNA translation [28] (Fig. 1). Nonetheless, it is unknown whether these methyl marks of RNA play any roles in ASD.

3. Does the 'Methylation Cycle' Play a Role in RNA Methylation and ASD

The cycle which involves generation and transfer of methyl groups ($-CH_3$) for RNA methylation, DNA methylation, and protein post-translational methylation is generally referred to as the 'methylation cycle'. It includes the methionine-homocysteine pathway, folate cycle and the redox-homeostasis pathway (Fig. 2). The methyl donor for both RNA and DNA methylation is S-adenosyl methionine (SAM) (Fig. 1). Similar to DNA methylation, RNA methylation occurs in the presence of Serine metabolism which provides ATP required for methionine cycle [29]. Many studies provide strong evidence on the involvement of methylation cycle in RNA methylation [18, 29-31]. Therefore, it is plausible to anticipate that any changes to the enzymes or components within the methylation cycle would influence the methylation of RNA, and thereby ultimately affect the downstream functions of methylated RNA in ASD (Fig. 2).

In line with this notion, deficiencies and/or alterations in the components and players of the methionine-homocysteine pathway have been reported in autistic patients. The reduced levels of homocysteine, methionine, the methyl donor SAM, increased levels of S-adenosylhomocysteine (SAH) and adenosine were implicated in the reduced capacity of methylation in ASD patients [25, 26]. The reduced ability of cells to carry out methylation would impact DNA methylation, represented by DNA hypomethylation [25, 32], and possibly RNA methylation (Fig. 2). Folate cycle and

methionine-homocysteine pathway are connected by methionine synthase enzyme and Vitamin B12. In autistic patients, reduced serum folate levels, Vitamin B12 as well as the activity of the methionine synthase have been observed [33, 34], and postulated that they also could be contributing factors to hypomethylation status or reduced methylation capacity in these patients [25, 32]. Another research group observed prematurely reduced transcription of methionine synthase mRNA in the cerebral cortex of autistic patients. Examining the mRNA and protein levels of methionine synthase in autistic patients at different ages suggested alterations in methionine synthase turnover, which is further associated with the methylation cycle [35].

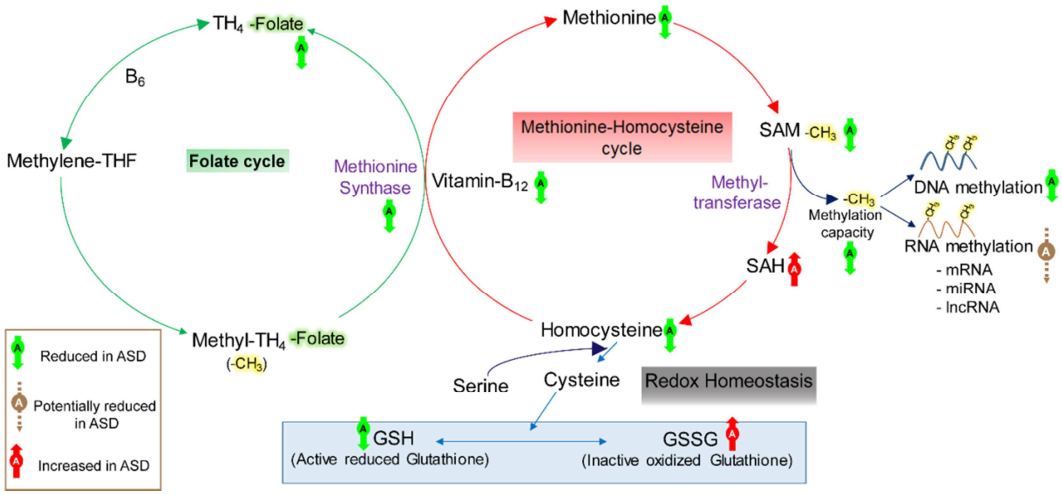

Figure 2. *The alterations of methylation cycle in Autism Spectrum Disorders (ASD) and its potential effects on RNA methylation.*

The methylation cycle basically contains 3 interconnected pathways, Methionine-Homocysteine cycle/pathway, folate cycle and redox homeostasis pathway. The Methionine-Homocysteine cycle involves the conversion of methionine to homocysteine in a series of reactions involving the conversion of S-adenosylmethionine (SAM) to S-adenosylhomocysteine (SAH). This conversion by methyltransferases generates methyl (-CH3) groups which are used in DNA methylation as well as RNA methylation. Homocysteine is then converted back to methionine by methionine synthase utilizing Vitamin B12. Methionine synthase and Vitamin B12 also connects folate cycle to the Methionine-Homocysteine cycle/pathway. Conversion of homocysteine to cysteine connects Methionine-Homocysteine cycle/pathway to the redox homeostasis where the active form of Glutathione (GSH) is oxidized to inactive form GSSG. Many of these factors are altered (either reduced or induced) in autistic patients. The overall impact of these alterations is to reduce the cellular methylation capacity. This would impact DNA methylation and RNA methylation. In the case of RNA methylation, the methylation status of mRNA, microRNA (miRNA and long-noncoding-RNA (lncRNA) may be altered.

Moreover, an imbalance in active glutathione (GSH) and oxidized glutathione which suggest an imbalance in redox homeostasis in relation to the methylation cycle has also been demonstrated (Fig. 2) [36]. This concept is also referred to as the *redox/methylation hypothesis*, during which oxidative stress caused by environmental factors lead to impaired methylation, and ultimately add up to the neurological phenotypes seen in ASD [37]. It has been suggested that under oxidative stress conditions seen in ASD, methionine synthase activity is reduced along with reduced cysteine and GSH levels. Not only the methionine synthase activity but also its expression and turnover are reduced by oxidative stress in ASD patients [35]. Altered ratio of GSH/GSSG an indication of oxidative stress, and in many studies reduced GSH/GSSG ratio have been reported in autistic patients [38, 39]. Interestingly, the oxidative stress caused by glutathione imbalance seem to be occurring in a brain region-specific manner in autistic patients, where more prominent defects are seen in temporal cortex in comparison to the cerebellum [40]. Certain genetic factors also contribute to the alterations of the redox/methylation hypothesis in ASD. A prime example is that mutations of the Adenylosuccinate lyase (*ASL*) gene cause a reduction in methyl-folate used by methionine synthase, affecting the subsequent steps of the methylation cycle [41]. Therefore, mutations in *ASL* gene are associated with infantile autism [42, 43].

Collectively, the impact of the aberrations in the components in the methionine-homocysteine pathway, folate cycle, and redox homeostasis, seem to reduce the cellular methylation capacity in ASD patients. This would include both reduced DNA methylation and RNA methylation. The reduced capacity of RNA methylation could ultimately influence the functions of methylated RNA. This provides us future avenues to investigate into whether the functions of methylated RNA such as mRNA splicing, maintaining the stability of mRNAs and regulating protein translation are influenced by aberrant RNA methylation in ASD.

4. MicroRNAs in ASD

Expression of miRNAs: MiRNAs are forefront regulators of brain development and proper brain functions, and therefore have been associated with many neurological disorders including ASD [44]. Whole genome transcriptome and microarray approaches have been able to demonstrate altered expression profiles of hundreds of miRNAs and cellular pathways that are compromised in autistic patients. To name a few, in many autistic patient brains, there was a significant

reduction in the expression of genes encoding synaptic proteins [45, 46]. In contrast, these studies showed an induction of genes encoding proteins associated with immune response in autistic patients [45, 46]. Interestingly, other studies have demonstrated that altered miRNA expression is able to explain the aforementioned changes to synaptic and immune system-related mRNA expression [47].

Similarly, many other studies have conducted miRNA profiling in either autistic brain samples, serum samples or lymphoblast cell lines. These studies also identify mRNA targets of these miRNAs as well as the molecular pathways that are associated. A summary of some of the studies conducted using autistic patient brain, blood, saliva and lymphoblast cell lines are summarized in Table I. These studies have provided important implications in cellular processes and molecular pathways that are altered in ASD, and how they can influence the pathogenesis. Based on these studies, it has been discovered that synaptic processes, immune system regulation, biomolecular pathways such as TGFβ signaling, MAPK signaling, Hedgehog signaling, Wnt signaling and mTOR signaling, and regulation of the integrity of the cytoskeleton are affected by the altered expression of miRNAs in autistics patients.

Table I. *Role of microRNAs in Autism Spectrum Disorders.*

microRNA	Target genes	Associated pathways/cellular functions	Type of autistic patient sample	References
miR-132, miR-212	*MECP2, SPRED1*	Synaptic plasticity, Immune responses, Neuronal morphology	Cerebellum	[51]
miR-128b	*CREB, SP1, SRC*	CREB signaling associated with synaptic signaling	Cerebellum, Blood, Lymphoblast cell line	[51, 77, 78]
miR-195	*BDNF, RELN*	Synaptic plasticity, CREB signaling, Synaptic maturation, Neuronal connectivity	Blood	[77]
miR-21	*PTEN*	Phosphoinositide 3-kinase [PI3K]/protein kinase B signalling	Cerebellum	[79]
miR-27a, miR-101, miR-106b, miR-130a, miR-151a, miR-181b, miR-195, miR-19b, miR-320a, miR-328*, miR-433, miR-489, miR-572*	600 genes [not specified]	Axon guidance, TGFβ signaling, MAPK signaling, Hedgehog and Wnt signaling, mTOR signaling, Oxidative phosphorylation, Actin cytoskeleton regulation	Serum blood	[55]
miR-29b, miR-219	*ID3, PLK2*	Memory, Synaptic plasticity	Lymphoblasts	[78]
miR-146a, miR-221	*MAP1B, UHRF1, FMR1, LASS2, FOXO3A*	Cognitive functions, Neuronal morphology, Astrocyte glutamate uptake, Netrin signaling, Il8 signaling, Axonal guidance	Olfactory stem cells from living patients	[63]
miR-3e*, miR-7*, miR-14*, miR-23a*, miR-28*, miR-27a*, miR-32*, miR-127*, miR-191*, miR-218*, miR-335*, miR-628*, miR-2467*, miR-3529*	*MGEA5, KIF3B, ST6GALNAC3, ZMYM4, PPP3R1, CELSR3, PAPPA, TAF5, TMED5, FCHSD2, GLCE, ATF7, CD69, NFYA*	Brain development, Adaptive behavior, Gene transcription	Saliva	[56]
miR-139	Myomegalin [*CDK5RAP2*]	embryonic development, estrogen receptor signaling and gastrointestinal diseases/digestive system development and functions	lymphoblast	[52]
miR-29b	*ID3, ARNTL, ATF2, DUSP2, PER1, PER3, VIP*	Circadian rhythm signaling, skeletal and muscular disorders/skeletal and muscular system development and functions		

*Candidate Biomarkers

Genome-wide expression profiling studies have been a great asset in deciphering the roles of miRNA in ASD pathogenesis. One such study identified *miR-486* and *miR-181b* to be associated with neurological phenotypes of ASD [48]. The copy numbers of 24 miRNA genes found within chromosomes 1, 2 and 22 were reported to be deregulated in a similar genome-wide study [49]. This study highlighted two novel miRNAs *miR-436 b-1* and *miR-4436b-2* as novel ASD miRNAs which have functions in Notch signaling, cytotoxicity, drug metabolism, and regulation of two autism candidate genes *2NSD1* and *AMT*. A high throughput microarray analysis of autism samples from China identified *miR-34b* as a potential player in the male bias of ASD [50]. While *miR-34* has functions within the nervous system, it also plays roles in male sexual and gonad development, which might be a contributing factor for the male bias of ASD [50].

Abu-Elneel *et al* studied the expression of miRNAs as well as the distance of these miRNAs to the genomic location of known genetic markers for ASD [51]. They found that *miR-139*, *miR-423*, *miR-365-2*, and *miR-10a* were significantly closer to the ASD marker loci D11S1314, D17S1294, D17S1800, and D17S2180, respectively. However, their study did not reveal any expression changes in these four miRNAs within the autistic brain samples they studied. Yet, *miR-139* was found to be downregulated in lymphoblast from autistic patients in another study, potentially targeting Myomegalin (*CDK5RAP2*) and involved in embryonic development, estrogen receptor signaling and gastrointestinal diseases/digestive system development and functions [52].

The same study by Sarachana *et al* showed *miR-29b* was significantly upregulated in autistic lymphocyte samples in associated with the reduction of its target *ID3*. Additionally, *miR-29b* gets *ARNTL*, *ATF2*, *DUSP2*, *PER1*, *PER3*, *VIP* to modulate Circadian rhythm signaling, skeletal and muscular disorders/skeletal and muscular system development and functions It was proposed that *miR-29b* could be causal to some of the muscular phenotypes found in ASD patients [52]. Moreover, when Sarachana *et al* in 2014 compared autistic patients to their healthy twins and siblings, *miR-139-5p* was downregulated while *miR-29b* was upregulated [53]. These results were in agreement with the previous studies by Sarachana *et al* in 2010 [52]. Collectively, these reports suggest dysregulation of miRNAs in autism could be tissue/cell type-specific.

While the complex pathophysiology makes it challenging to determine reliable biomarkers for ASD, studies on circulating miRNAs have shed some light on the potential of using miRNAs as predictive, diagnostic or prognosis biomarkers for ASD [54, 55]. Some of the miRNAs which hold promise for ASD biomarker applications are listed in Table I. Among them *miR-23* was found to be a common miRNA reported in many studies using different bio-accessible tissues such as saliva [56], lymphocyte [52, 57], as well as brain [58]. The *miR-23a* and *miR-23b* regulate the expression of autism susceptibility genes or autism-associated genes such as *PTEN*, *FMR1*, *AUT2*, *NRXN1-3* and *HTR2C* [57]. Ingenuity pathway analysis identified *miR-23a* and *miR-23b* are associated with neurological diseases/ nervous system development and functions; skeletal and muscular disorders/ skeletal and muscular system development and functions and embryonic development [52]. However, *miR-23* is already being used as a diagnostic biomarker for many other disorders such as diabetes [59] and thus, the reliability of miRNAs biomarkers in autism diagnosis has to be thoroughly evaluated.

miRNA methylation: Interestingly, studies have shown that m^6A methylation of miRNAs may also change their processing, stability, localization and functions [6]. For instance, diminution of the RNA methylase METTL3 led to the accumulation of pre-miRNAs due to reduced pre-miRNA methylation and interfered processing [60]. Moreover, reduced levels of FTO demethylase leads to altered expression of many miRNAs in HEK293 cells, without affecting the genes that are involved in miRNA biogenesis (Eg. *DICER, DROSHA, DGCR8,* and *ADAR*). The *miR-125b* which was downregulated by FTO knockdown was also shown to be highly m^6A methylated [61]. Expression of *miR-125b* had been shown to be upregulated in Down syndrome patient brains [62]. Several other miRNAs were found to be highly methylated and their expression was also reported to be altered in autistic patient samples. Examples include *miR-28* in saliva [56], *miR-146* in Olfactory stem cells [63] and *miR-320* in blood serum [55]. Unfortunately, there is a knowledge gap in whether miRNA methylation is altered in ASD patients and whether such modifications are involved in altered miRNA expression in ASD.

5. Long Non-Coding RNAs in ASD

Expression of lncRNAs: Many lncRNAs are highly expressed in the brain, and are linked to brain development and neuronal functions. For instance, *BDNFOS*, a lncRNA which regulates Brain-derived neurotrophic factor (*BDNF*) expression in brain was decreased in neocortical regions of the brain in seizure patients along with an induction of its target *BDNF* [64]. Moreover, *ASFMR1* and *FMR4* are lncRNAs of major genes associated with Fragile X Mental Retardation, which may be contributing to the pathobiology of Fragile X syndrome [13]. Prader–Willi syndrome is a neurological disorder which shows phenotypes of autism and a study reported the role of lncRNA 116HG in brain energy expenditure regulation in this disorder [65]. However, until recent advances, the role of lncRNAs in ASD had been rather unclear. Some of the lncRNAs discovered to be associated with ASD since then are listed in Table II.

Table II. *Long-non-coding RNAs (lncRNAs) altered in Autism Spectrum Disorders.*

lncRNA	Target genes	Associated pathways/cellular functions	Type of autistic patient sample	References
82 (cortex) *143* (cerebellum)	*C9orf85, SLC4A2, UBE3A, RBM8a, ARL17A, KLF6*	Imprinting, cell migration, neuronal connectivity, synaptogenesis	Prefrontal Cortex and cerebellum	[66]
LINC00087, RP11-4K16.2	Not reported	Synaptic transmission, ASD risk	Brain	[69]
MSNP1AS	*MSNP1*	Autistic susceptibility	Temporal cortex	[68]
ST7OT1-4	*RAY1/ST7*	Gene regulation in autism	Lymphocytes	[70]
SYNGAP1-AS	*SYNGAP1*	Synaptic functions and cognition	prefrontal cortex and superior temporal gyrus	[72]

Filling this knowledge gap, Ziats and Rennert reported that the prefrontal cortex and the cerebellum of autistic patients show differential expression of 82 and 143 lncRNAs, respectively, in contrast to age-matched healthy controls [66]. These differentially expressed lncRNAs were implicated in cell migration and also targeted by *miR-103/107*, which might be linked to neuronal connectivity in ASD. Additionally, many of these lncRNAs were located within imprinting loci of *C9orf85, SLC4A2,* and *UBE3A*, which have strong implications in neurodevelopmental disorders such as Angelman syndrome and share many overlapping phenotypes with ASD. Using peripheral blood samples of Chinese autistic patients, Wang and colleagues identified 13 synaptic lncRNAs which is involved in synaptic functions and may contribute to synaptic dysfunctions in ASD [67]. Moreover, they found 19 lncRNAs associated with known autistic genes including *HOX, SHANK2,* and *BDNF*, which may shed light on the role of lncRNA epigenetics in ASD pathobiology.

Over the years, many studies have found risk factors and genes associated with ASD risk. Recent studies show differential expression of lncRNAs also being associated with ASD risk. For instance, *MSNP1AS* lncRNA which targets moesin pseudogene 1 (*MSNP1*) was found to be ~13-fold induced in the autistic patient temporal cortex. Interestingly, its expression was elevated ~22-fold in autistic patients with rs4307059 risk allele, which is a known autistic susceptibility gene. Therefore, this study suggested that *MSNP1AS* lncRNA could be associated with increased ASD risk [68]. Moreover, a study conducted using 27 brain regions of ASD patients within BrainSpan Developmental Transcriptome Dataset discovered two major lncRNAs, *LINC00087,* and *RP11-4K16.2* being deregulated in ASD brains and are associated with synaptic transmission [69]. These two lncRNAs belonged to a set of genes which show the highest expression changes during mid-fetal and early post-natal periods which are high ASD risk periods during embryonic development [69]. *RAY1/ST7* is a known autism locus located within the chromosome 7q31 associated with autism susceptibility. The lncRNAs *ST7OT1-4* are located within this locus and supposedly regulate the expression of *RAY1/ST7* [70]. As these rare variants were found only in autistic patients but not in healthy individuals, it is assumed to be associated with autism [70, 71]. *SYNGAP1-AS* is another lncRNA that is located within autism risk gene loci and targets *SYNGAP1* involved in synaptic functions and cognition. This lncRNA was significantly upregulated prefrontal cortex and superior temporal gyrus but not in the cerebellum of autistic patients [72].

Methylation of lncRNA: Similar to mRNA, lncRNAs are also modified by m^6A methylation [73, 74] and FTO carries out the demethylated of m^6A lncRNA [75]. Additionally, lncRNAs also harbor m^5C modifications as shown with two major lncRNAs *XIST* and *HOTAIR* [76]. The m^5C methylation of these two lncRNAs caused changes to their functions in terms of chromatin interactions. It is of interest to study whether there is any methylation occurring at lncRNAs in ASD patients and what their functions would be.

6. Concluding Remarks and Future Directions

With the recognition of RNA molecules and its modifications as functional entities of the central dogma, the concept of *epi-transcriptomics* expanded immensely. The influence of RNA methylation, miRNA, and lncRNAs in ASD were discussed in this review and are summarized in Fig. 3. I discussed the implications of RNA methylation in mRNA life cycle and its contribution to ASD. Then again, there are a plethora of mechanisms by which RNA methylation may contribute to ASD pathobiology which was not discussed here. These mechanisms include the changes to the writers, readers, and erasers of RNA methylation, who establish, identify and remove RNA methylation, respectively. RNA methylation determines the type of proteins that are bound to RNA, and thereby the functional consequences of the absence or alterations of RNA methylation. The significance of these writers, readers and erasers of RNA methylation in ASD will be an interesting avenue to continue future research on to discover more pathways to understanding the pathology of ASD and treatment strategies.

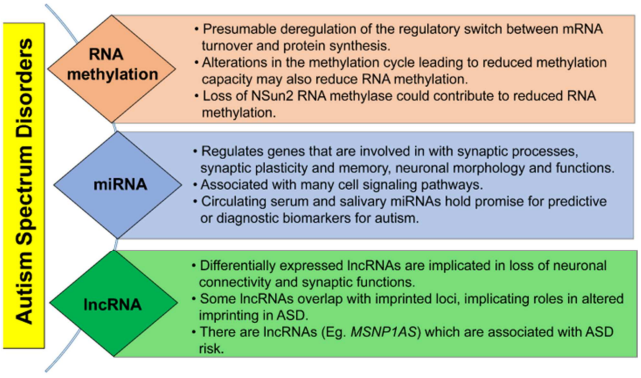

Figure 3. *Summary of epigenetic mechanisms associated with RNA molecules in Autism Spectrum Disorders (ASD).*

Epigenetic mechanisms associated with RNA molecules include RNA methylation and non-coding RNAs such as microRNAs (miRNA) and long non-coding RNAs (lncRNAs). The figure summarizes how these three aspects of epigenetics may contribute to the pathobiology of ASD.

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