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REVIEW ARTICLE

Current insight into the functions of microRNAs in common human hair loss disorders: a mini review

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Abstract

Alopecia areata (AA) and Androgenic alopecia (AGA) are the most common multifactorial hair loss disorders that have a serious psychological impact on the affected individuals, while frontal fibrosing alopecia (FFA) is comparatively less common. However, due to the unknown etiology and the effect of many adverse factors, the prognosis of these conditions is challenging to predict. Moreover, no approved therapy has been available to date to prevent or treat these disorders. MicroRNAs (miRNAs) are a group of evolutionary conserved small non-coding RNA molecules with significant roles in the posttranscriptional gene regulation either through mRNA degradation or translational repression. A number of biological processes are controlled by these molecules, including cell growth and differentiation, proliferation, inflammation, immune responses, and apoptosis. Recently, a handful of studies have demonstrated the impact of miRNAs on common hair loss-related disorders; however, the exhaustive molecular mechanisms are still unclear. In this review, we discussed the functional implications of miRNAs in common hair loss-related disorders and addressed their efficacy to be used for theranostic purposes shortly.

Keywords MicroRNA · Alopecia · T lymphocytes · Biomarker · Theranostic

Abbreviations

AA	Alopecia areata
AGA	Androgenic alopecia
AGO2	Argonaute 2
APP	Amyloid-beta precursor protein
ASK1	Apoptosis signal-regulating kinase 1
BAK1	Brassinosteroid insensitive 1–associated kinase 1
BCL2L10	B-cell lymphoma/leukemia 2-like protein 10
BNIP2	B-cell lymphoma 2 interacting protein 2
CTLA4	Cytotoxic T lymphocyte antigen 4
CXCL11	C-X-C motif chemokine 11
DTH	Delayed-type hypersensitivity
DYRK1A	Dual-specificity tyrosine phosphorylation-regulated kinase 1A
FFA	Frontal fibrosing alopecia
FOXO1	Forkhead transcription factor 1
FPHL	Female pattern hair loss
HF	Hair follicle
HDPC	Human dermal papilla cells
HS	Hair shaft
ICOS	Inducible co-stimulatory protein
IFNG	Interferon-gamma
IL2RA	Interleukin-2 receptor alpha
JAK/STAT	Janus kinase/signal transducers and activators of transcription
MAPK	Mitogen-activated protein kinase
miRNA	MicroRNA
MPB	Male pattern baldness
NFAT	Nuclear factor of activated T cell

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p27/kip1	Cyclin-dependent kinase inhibitor 1B
p57/kip2	Cyclin-dependent kinase inhibitor 1C
pre-miRNA	Precursor microRNA
pri-miRNA	Primary microRNA
RISC	RNA-induced silencing complex
SCF/c-kit	Stem cell factor/tyrosine-protein kinase KIT
SFRP1	Secreted frizzled-related protein 1
STAT1	Signal transducer and activator of transcription 1
STX17	Syntaxin 17
TAP	Transporter associated with antigen processing
TAP2	Transporter associated with antigen processing 2
TGF	Transforming growth factor
TNKS2	Tankyrase-2
TNXB	Tenascin XB
UTR	Untranslated region
VDR	Vitamin D receptor
XPO5	Exportin 5

Introduction

The hair follicle (HF) is a mini-organ located in the dermal layer of mammalian skin and consists of 20 distinct types of cells. Lifelong cycling of the HF maintains the hair healthy and intact; thus, regulation of the hair cell cycle is a crucial part of the physiology of human HF as changes in proliferation dynamics during responses to growth or stress factors may create perturbations in HF pathologies [1, 2]. HF undergoes three growth cycles: anagen (active growing phase), catagen (transition phase), and telogen (resting phase) to supply new hairs continuously throughout human life [3]. During anagen, the HF produces an entire hair shaft (HS) through matrix cell proliferation and differentiation at the base of the HF, while the catagen phase is a brief phase of transition that indicates the culmination of active hair growth through the apoptosis of epithelial cells that leads to the HF regression. Finally, in the telogen cycle, the follicles remain dormant for a long time before starting a new cycle (telogen-to-anagen transition) [4]. Understanding the HF cycle at the molecular level may lead to more persuasive treatment options for alopecia patients [3, 5].

MicroRNAs (miRNAs) are small non-coding endogenous single-stranded RNA molecules ranging from 19 to 24 nucleotides in length that regulate numerous biological functions in animals and plants [6–14]. They are known to play critical regulatory roles in developing a variety of human diseases, including neurodegenerative disorders, autoimmune diseases, and cancer [10–12, 15, 16]. MicroRNAs commonly act as negative gene expression regulators at the posttranscriptional level and have been reported to be involved in controlling more than 50% of the human protein-coding genes [17–19]. To date, 2,654 unique mature human miRNAs are available in miRBase (http://www.mirbase.org/), supporting the notion of their participation in complex regulatory networks.

The miRNA biogenesis starts in the nucleus when RNA polymerase II/III transcribes the miRNA gene into a long primary transcript (pri-miRNA). Afterwards, the pri-miRNA transcript is processed by a nuclear RNase III enzyme Drosha, and the RNA-binding protein DGCR8 generates the characteristic stem–loop precursor miRNA (pre-miRNA). Then, the pre-miRNA is transferred to the cytoplasm by the Exportin5 (XPO5)/RanGTP complex, where it is further cleaved by the Dicer/TRBP complex producing a short miRNA/miRNA* duplex with one passenger strand and one mature strand [20, 21]. To be functional, the mature miRNA is then loaded into the RNA-induced silencing complex (RISC) coupled with Argonaute 2 (AGO2) protein and interacts with the 3′-untranslated region (3′-UTR) of the target mRNA sequence [19, 22, 23]. Gene silencing might occur either by mRNA degradation or translation inhibition [24] (Supplementary File 1).

Previous reports have described the regulatory impact of miRNAs in the normal functioning of HFs; thus, the aberrant expression of miRNAs is suggested to affect the pathogenesis of common hair loss disorders, such as AA, AGA, and FFA [25, 26]. In the present review, we have discussed the miRNA dysregulations associated with the initiation and progress of common hair loss disorders that might be useful for disease forecasting and develop novel therapeutic strategies.

Alopecia areata (AA)

Over the past years, miRNAs have emerged in various biological processes involved in autoimmune responses as crucial posttranscriptional gene regulators [27]. For instance, a common autoimmune human hair loss disorder known as Alopecia areata (AA), with a prevalence rate of 0.1% worldwide, is associated with irregular levels of miRNAs in the skin and T lymphocyte cells attack the follicle as compared with healthy subjects [27–29] (Fig. 1). The most common symptom of AA is circular patches of hair loss that may spread further, affecting the whole scalp [29]. During AA development, aberrantly activated T lymphocytes infiltrate around the bulb region of the anagen HF and break down its immune privilege [30]. AA is challenging to prevent, and most of the available therapeutic options are unsatisfactory or under clinical trials [29, 31]. Moreover, differential miRNAs expression has been identified in various autoimmune disorders, including rheumatoid arthritis and type 1 diabetes, that share similar kind of pathogenic pathways to AA [27].

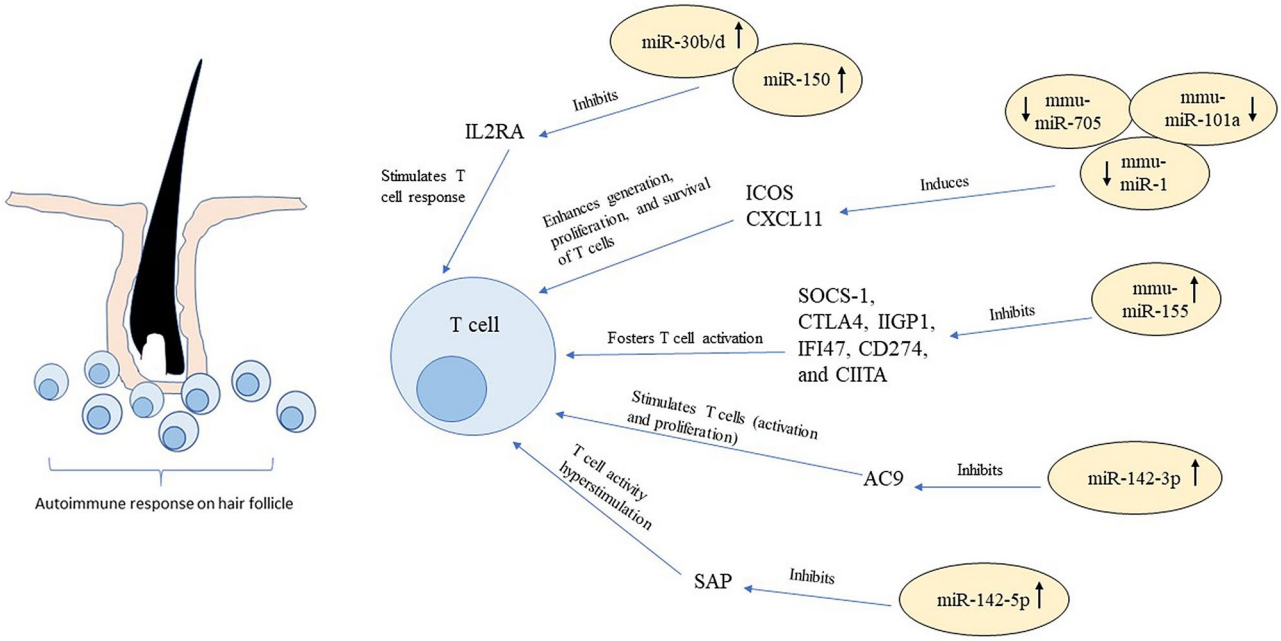

Fig. 1 Dysregulated miRNAs and their impact on T cells during AA pathogenesis. AA is a T cell-mediated autoimmune disease. This figure shows the effects of dysregulated miRNAs on several relevant target proteins and their consequences in T cells during AA pathogenesis. The AA-associated miRNAs, such as miR-142-5p, miR-142-3p, mmu-miR-155, miR-30b/d, and miR-150, are upregulated and inhibit the expression of their respective target proteins, while downregulated miRNAs, such as mmu-miR-705, mmu-miR-101a, and mmu-miR-1, induce the expression of their target proteins. This dysregulated miRNA-target interaction triggers abnormal T cell activation, which in turn damage hair follicles and leads to AA (↑: upregulated; ↓: downregulated)

The C3H/HeJ mouse model is considered one of the most characteristic animal models for AA research and has been extensively used as a preclinical drug testing model for this disorder [32]. To demonstrate the differential miRNA expression in the skin during AA development, Wang et al. [27] conducted a microarray experiment using C3H/HeJ mice that spontaneously developed AA compared to healthy mice. Their results demonstrated a significant alteration of the expression of around 100 miRNAs in AA mice that play key roles in AA pathogenesis, regulating JAK-STAT signaling, apoptosis, antigen presentation, and interferon signaling. Among these differentially expressed miRNAs, some of the potential AA-associated miRNAs, such as upregulated mmu-miR-31, mmu-miR-155, mmu-miR-329, and downregulated mmu-miR-100, mmu-miR-1, mmu-miR-26b, mmu-miR-29c, mmu-miR-30b, mmu-miR-101a, mmu-miR-133a, mmu-miR-365, mmu-miR-451, and mmu-miR-705, were identified. Table 1 displayed the role of the aforesaid AA-associated miRNAs, their relevant targets, and tissue-specific expression pattern in several biological tissues observed during AA development. During miRNA-target network analysis, Wang et al. also noticed a significant association between mmu-miR-155 and the T cell activity inhibitor CTLA4 during AA pathogenesis, while two other important AA-associated proteins ICOS (a T cell activity stimulator), and CXCL11 are overexpressed by downregulation of mmu-miR-1, mmu-miR-101a, and mmu-miR-705, which resulted in an enhanced T cell activity (Fig. 1). Overexpression of CXCL11 promotes excessive T cell recruitment, while the miR-101 performs the Roquin-mediated degradation of ICOS mRNA (Fig. 1) [33]. Moreover, the production of interferon-gamma (IFNG) is considered as one of the main consequences of T cell activation, which signals via the pathway of JAK-STAT, and the significant upregulation of STAT1 target gene in AA mice due to the downregulation of its corresponding miRNA mmu-miR-100 might be associated with AA pathogenesis. Nevertheless, artificial miRNA-mediated inhibition of the JAK-STAT pathway could effectively treat AA in the near future [27].

In the same study, mmu-miR-26b- and mmu-miR-29c-mediated suppressions of BAK1 (apoptosis inducer) gene in AA mice were also identified. By reducing these miRNA's expression, the *BAK1* gene could activate the granzyme B apoptosis pathway. In addition, it has been reported that anomalous activation of T cells during AA is linked to the TAP molecules since they mediate T cell recognition of autoantigen epitopes in the HF [34]. An underexpression of mmu-miR-30b and mmu-miR-365 was noticed targeting the *TAP2* gene resulted in a cytotoxic T lymphocyte-mediated autoimmune response against HF [27]. Furthermore,

Table 1 miRNAs and their respective target genes involved in common hair loss disorders

miRNA	Disorder	Expression pattern	Target gene/protein	Tissue source	Consequences	Reference
miR-30b/d	AA	Upregulated	IL2RA and STX17	Mice skin	Autoimmunity development and T cell response increase	[39–41]
miR-142-3p	AA	Upregulated	AC9	Human skin	T cells stimulatory effects (activation and proliferation)	[46]
miR-142-5p	AA	Upregulated	SAP	Human skin	T cell activity hyperstimulation	[44, 47]
miR-150	AA	Upregulated	IL2RA	Human Skin	Autoimmunity development and T cell response increase	[44, 45]
miR-185-5p	AA	Downregulated	-	Human Blood	Inflammatory regulation	[37]
miR-186-5p	AA	Downregulated	FOXO1	Human Blood	AA enhancement from cellular metabolism, cell cycle, and apoptosis affection	[37]
miR-210	AA	Upregulated	FOXP3	Human blood	Regulatory T cells weakening	[37, 62]
miR-223	AA	Upregulated	IGF-1R	Human Blood	Suppression of IL-10 production	[63]
miR-629-5p	AA	Upregulated	-	HeLa cells	Angiogenesis proliferation and inflammatory response	[38]
miR-1246	AA	Upregulated	DYRK1A	Murine CD4 + CD62L + naïve T cells and human peripheral mononuclear cells	Regulatory T cells levels increase	[37, 64]
mmu-miR-1 and mmu-miR-705	AA	Downregulated	ICOS and CXCL11	The skin of C3H/HeJ mice	Proliferation and survival of T-Cells; T cells recruitment increase	[27]
mmu-miR-26b and mmu-miR-29c	AA	Downregulated	BAK1	The skin of C3H/HeJ mice	Activation of granzyme B apoptosis pathway	[27, 34]
mmu-miR-30b	AA	Downregulated	TAP2	Skin from C3H/HeJ mice	Autoimmune response against HF	[27]
mmu-miR-31	AA	Upregulated	GPRC5A	Skin of mice	Irregular development of peripheral regulatory T cells	[27, 36]
mmu-miR-100	AA	Downregulated	STAT1	Skin from C3H/HeJ mice	T cell activation	[27]
mmu-miR-101a	AA	Downregulated	ICOS	The skin of C3H/HeJ mice	Survival of regulatory T cells and enhanced T cell response	[27, 65]
mmu-miR-133a and 329	AA	Downregulated	–	The skin of C3H/HeJ mice	Abnormal heart morphology	[27]
mmu-miR-155	AA	Upregulated	SOCS-1, CTLA4, IIGP1, IFI47, CD274, and CIITA	The skin of C3H/HeJ mice	Development of Foxp3 + tTreg cells; and T cell activation	[27, 66]
mmu-miR-365	AA	Downregulated	TAP2	The skin of C3H/HeJ mice	Autoimmune response against HF antigens	[27]
mmu-miR-451	AA	Downregulated	NF-kB	Skin of mice	Prolonged inflammation	[27]
miR-21	AGA	Upregulated	–	Human skin	Unknown	[44]

Table 1 (continued)

miRNA	Disorder	Expression pattern	Target gene/protein	Tissue source	Consequences	Reference
miR-106b	AGA	Upregulated	TGF-β	Human dermal papillae	Activation of tumorigenicity process	[26]
hsa-miR-125b/miR-125b-5p	AGA	Upregulated	Vitamin D receptor (VDR)	Mice skin	Suppression of hair differentiation	[67]
hsa-miR-133b	AGA	Upregulated	Wnt	Human hair follicles	Inhibition of human dermal papilla cells proliferation	[51]
hsa-miR-141-5p	AGA	Upregulated	–	Human hair follicles	–	[51]
miR-197-3p	AGA	Upregulated	–	–	–	[25]
miR-221	AGA	Upregulated	Receptor tyrosine kinase, c-kit, p27/kip1, and p57/kip2	Human dermal papillae	Decrease of androgenetic alopecia hair color and pigmentation	[26]
miR-324-3p	AGA	Upregulated	TGF-β, MAPK	Human hair follicles	Migration and proliferation reduction of keratinocytes	[52]
miR-340	AGA	Downregulated	-	Human dermal papillae	Unknown	[26]
miR-365b-5p	AGA	Upregulated	–	–	–	[25]
miR-410	AGA	Upregulated	–	Human dermal papillae	Unknown	[26]
hsa-miR-520d-5p	AGA	Upregulated	–	Human hair follicles	–	[51]
miR-548	AGA	Upregulated	–	Human dermal papillae	Unknown	[26]
miR-570	AGA	Downregulated	–	Human dermal papilla cells	–	[26]
hsa-miR-652-5p	AGA	Upregulated	–	Human hair follicles	–	[51]
hsa-miR-1247-5p	AGA	Upregulated	–	Human hair follicles	–	[51]
miR-3613-3p	AGA	Upregulated	–	–	–	[25]
miR-92a-1-5p	FPHL	Upregulated	MAPK8 and FAS	Human HTR-8/SVneo cells	Reduction of EGF-mediated MMP-9/TIMP1 ratio and invasion	[25, 68]
miR-328-3p	FPHL	Upregulated	CAMK4	Spleen and lymph nodes from mice	Increase in IL-2 production, suppression of T cell activation, and increase in Tregs levels	[25, 69]
hsa-let-7d-5p	FFA	Downregulated	AGO1, DICER1, and HMGA1	Scalp skin and plasma from humans	miRNA biogenesis and gene expression alteration	[54, 56]
hsa-miR-18a-5p	FFA	Upregulated	BCL2L10	Human skin	Keratinocytes apoptosis	[57]
hsa-miR-19a-3p	FFA	Downregulated	TNF-α	Human skin	Suppression of hair follicle growth	[54, 60, 61]
hsa-miR-20a-5p	FFA	Downregulated	HDAC4, BNIP2, APP, ASK1, and TNKS2	Human HMC-1 cells and human skin	Allergic inflammation	[54, 59]

an upregulation of mmu-miR-329 and a downregulation of mmu-miR-133a were evidenced to be linked with cardiovascular disorders [27]. Previously, it was demonstrated that C3H/HeJ affected with AA present irregular heart morphology, so it is suggested that this aberrant heart morphology is related to deregulation of mmu-miR-329 and 133a [27, 35]. Also, an essential dysregulation of mmu-miR-451 and mmu-miR-31 in AA mice was noticed. Specifically, an underexpression of mmu-miR-451 could promote NF-kB activity by leading to prolonged inflammation that increases AA progression. On the other hand, the upregulation of mmu-miR-31 could lead to the uncontrolled development of peripheral regulatory T cells during AA [27, 36].

Sheng et al. performed a miRNA microarray experiment using Agilent Human miRNA Microarray (covers 2549 human miRNAs) from blood samples of severe AA patients against healthy controls, and they documented 36 significantly differentially expressed miRNAs (22 upregulated, 14

downregulated) in AA patients as compared to healthy control [37]. Among these 36 altered miRNAs, miR-125b-5p and miR-186-5p were documented to synergistically regulate the biological mechanism of 'organismal injury' in the inflammatory system along with miR-185-5p which might contribute to the AA pathogenesis. The miR-186-5p putatively targets FOXO1, leading to a possible affection during cellular metabolism, cell cycle regulation, and apoptosis, enhancing AA progression. While the target transcripts of miR-210 are linked to immune responses, apoptosis control, and lipid metabolism, their precise regulatory functions in AA pathogenesis are still unclear (Table 1). Interestingly, miR-210 and miR-1246 were characterized as the most potent biomarkers for AA due to their high accuracy. On the other hand, p53 was reported to hinder the expression of DYRK1A by modulating miR-1246 and thereby triggering the nuclear factor of activated T cells (NFAT) that might contribute to AA development. Furthermore, it has been detected that deregulated miR-629-5p might lead to the formation of angiogenesis, proliferative, and inflammatory responses during AA disorder [38].

Another important microRNA reported to be significantly associated with AA pathogenesis is miR-30b/d which controls organelle/vesicle transport and is poorly expressed in the AA patients' HF, and it is documented that its upregulation leads to an increase in T cell response (Fig. 1) [39–41]. AA risk genes, such as *STX17*, *TNXB*, and *IL2RA*, were validated as the most potent targets of miR-30b/d via luciferase assay, and they might control the regulatory T cells contributing towards AA pathogenesis; as well as these three genes play essential roles in hair pigmentation, maintenance of extracellular homeostasis, and T cell differentiation, respectively [39, 42, 43]. On the other hand, Delayed-Type Hypersensitivity (DTH) reactions are reported to be strongly involved in a number of autoimmune disorders, including AA. miR-150, miR-21, miR-142-3p, miR-223, and miR-142-5p have significant implications on DTH disorder; thus, they may also be used to develop novel miRNA-based therapeutics for AA [44]. Specifically, the upregulation of miR-150 and miR-21 promotes the resolution of inflammation during DTH preventing AA progression, respectively [44]. Also, miR-150 upregulation is associated with a T cell response increase (Fig. 1) [45]. Finally, miR-142-3p and miR-142-5p both are linked to T cell stimulation but target AC9 and SAP, respectively (Fig. 1) [44, 46, 47].

Androgenic alopecia (AGA)

Androgenic alopecia (AGA), or Male Pattern Baldness (MPB), is an inheritable disorder characterized by progressive androgen-dependent HF loss [48]. It is demonstrated that androgen target genes can be activated or suppressed in the balding scalp dermal papilla cells by androgens, testosterone, and dihydrotestosterone (DHT), causing significant changes in hair cycles leading to AGA [26]. AGA is a common hair loss disorder that can affect both genders; however, men tend to be more prone to this disorder [49].

Goodarzi et al. compared the expression pattern of 7 selected miRNAs (hsa-miR-221, hsa-miR-125b, hsa-miR-106b, hsa-miR-410, miR-570, miR-548, and miR-340) between balding and non-balding dermal papilla cells collected from 8 individuals with grade IV baldness and found that four out of them (hsa-miR-106b, hsa-miR-125b, hsa-miR-221, and hsa-miR-410) were significantly upregulated in the balding papilla cells as compared with non-balding ones indicating their roles in AGA pathogenesis [26]. They demonstrated that the upregulated miR-221 in the balding papilla cells might control the expression of their functional targets p27/kip1 and p57/kip2, universal cyclin-dependent kinase inhibitors, receptor tyrosine kinase, as well as c-kit, and trigger the AGA pathogenesis; while a relation among the upregulated miR-125b, Vitamin D receptor (VDR) target, and reversible inhibition of hair growth was also documented in the mouse HF stem cells (Fig. 2) [50]. Nevertheless, Goodarzi et al. also reported that there might be a correlation of the miR-106b upregulation in the balding papilla cells with the TGF-beta signaling pathway, the key factor involved in AGA pathogenesis (Fig. 2). Interestingly, all the aforesaid upregulated miRNAs were reported to be involved in various types of cancers, including prostate, pancreatic, thyroid, and gastric cancer, indicating their role in cell proliferation control and apoptosis [26].

In a recent study, Deng et al. [51] showed 43 significantly differentially expressed miRNAs (21 upregulated, 22 downregulated) via microarray analysis in the scalp samples of AGA patients as compared to healthy individuals, and among the upregulated miRNAs hsa-miR-1247-5p, hsa-miR-652-5p, hsa-miR-520d-5p, hsa-miR-141-5p, and hsa-miR-133b were further verified by qRT-PCR to confirm their expression pattern. The target genes of hsa-miR-133b and hsa-miR-520d-5p were predicted to be regulators of signal transduction, cell cycle, and hormone secretion in hair growth, suggesting that they might be involved in the AGA pathogenesis. Specifically, the aberrant expression of hsa-miR-133b in the scalp samples of AGA patients was demonstrated to be significantly involved in the disease pathogenesis by interfering with the Wnt/β-catenin pathway, which is an essential factor in the regulation of HF development, specifically in promoting hair morphogenesis, human dermal papilla cells proliferation and sustaining the long hair growth cycle. In another recent study, Mohammadi et al. observed a sharp underexpression of miR-324-3p in the bald stem cells of AGA patients as compared to regular hair stem [52]. Moreover, miRNA-324-3p was found to regulate the

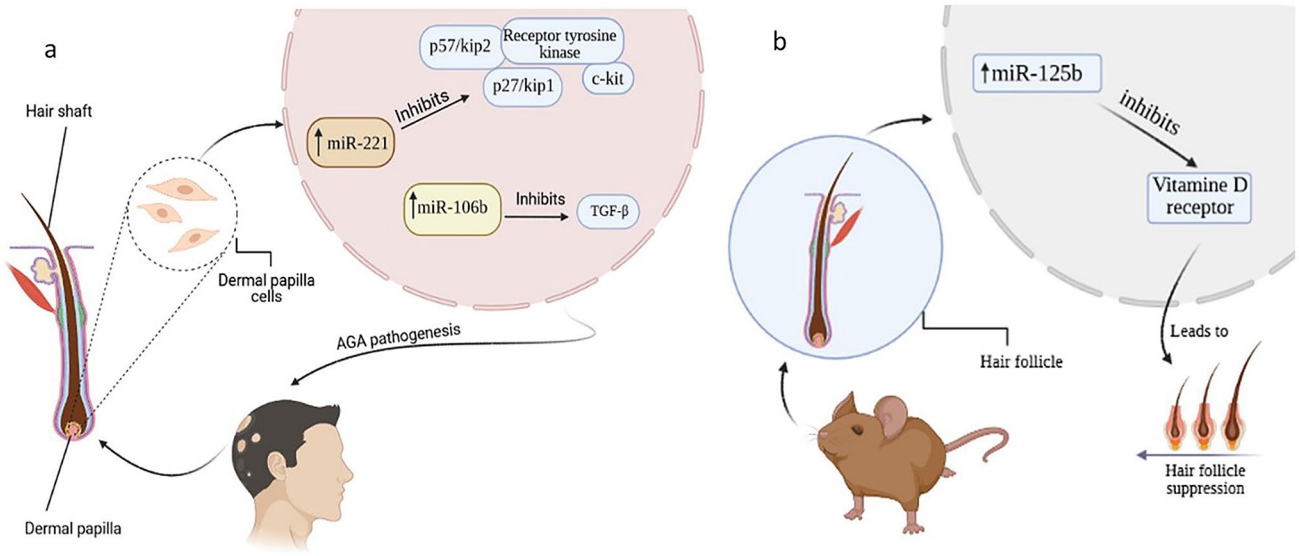

Fig. 2 miRNAs dysregulation and their implications in AGA. MiRNAs are significantly dysregulated in humans and mice affected by AGA. (2a) shows that miR-221 and miR-106b are upregulated in human dermal papilla cells and inhibit multiple target proteins, such as p57/kip2, p27/kip1, receptor tyrosine kinase, and c-kit proteins, and TGF-b leads to the AGA pathogenesis. While in (2b), it has been shown that upregulation of miR-125b inhibits vitamin D receptor (VDR) in mice hair follicles, promoting hair follicle suppression. Therefore, it is suggested that these interactions might explain AGA pathogenesis (↑: upregulated; ↓: downregulated)

differentiation of keratinocytes by modulating the transforming growth factor (TGF)-β signaling and mitogen-activated protein kinase (MAPK) pathways, thus, could be a potential new therapeutic target for AGA (Table 1).

Female Pattern Hair Loss (FPHL) is a type of AGA that commonly affects women and leads to the shrinking of HFs, and subsequently decreasing the hair numbers (particularly in parietal, central, and anterior scalp regions). Unlike male AGA, FPHL is related chiefly to estrogen metabolism [25]. Nonetheless, the frequency rate of FPHL among women increases with age but varies among population groups [53]. Aksenenko et al. performed a miRNA microarray experiment using samples of the average female scalp, FPHL skin, and normal male interscapular skin to demonstrate the differential miRNA expression [25]. Their results showed that a total of 981 and 972 miRNAs were upregulated in FPHL patients as compared to the normal female scalp and normal male interscapular skin, respectively. Significant upregulation of miR-197-3p, miR-328-3p, miR-3613-3p in FPHL in comparison with normal female skin was reported, while miR-92a-1-5p, miR-328-3p, and miR-365b-5p were found to be the most significantly upregulated miRNAs in FPHL against normal male interscapular skin. Notably, the upregulation of miR-92a-1-5p is linked to atypical metabolism and biosynthesis of fatty acids, which is critical since women suffering from FPHL have changes in their lipid profile. Whereas, overexpressed miR-328-3p might dysregulate the thyroid hormone biosynthesis in FPHL patients by targeting SFRP-1, an Wnt signaling inhibitor, as the influence of Wnt signaling capability to mediate thyroid hormones is well known [25] (Table 1).

Frontal fibrosing alopecia (FFA)

Frontal Fibrosing Alopecia (FFA) is a less common type of alopecia that predominantly affects postmenopausal women and is characterized by the presence of scars on the scalp near the forehead and primarily causes immune privilege collapse in the HF bulge [54]. Clinically, FFA's features are recession of the hairline, scarring, perifollicular erythema, and follicular hyperkeratosis; however, in the rare case, it might extend to eyebrows. Although FFA might be associated with hormonal imbalance, the etiology of this disease is still unknown [55]. To investigate the differential expression pattern of circulating miRNAs in the plasma samples of FFA patients, Tziotzios et al. conducted an experiment using Human Fibrosis miRNA PCR Array, and they found that miRNAs hsa-let-7d-5p, hsa-miR-18a-5p, hsa-miR-19a-3p, and hsa-miR-20a-5p are vastly predictive of the state of the disease and could be considered as potential biomarkers [54]. Moreover, all four miRNAs coregulate similar sets of targets. The let-7 miRNA family is an ancient miRNA family representing the most abundant members in the epidermis, and they might function as the foremost regulators of cutaneous differentiation processes [50]. However, hsa-let-7d-5p and FFA relationship is poorly reported in the

literature [54, 56] (Table 1). In comparison, hsa-miR-18a-5p upregulation was reported to produce symptoms like skin erythema or erosion by regulating BCL2L10, commonly noticed in AA patients (Fig. 3). The hsa-miR-20a-5p naturally regulates the inflammatory reactions, the common feature of AA, by targeting Histone Deacetylase 4 (HDAC4), where hsa-miR-20a-5p downregulation increases HDAC4 expression and, consequently, trigger allergic inflammation (Fig. 3). Although other potential targets of hsa-miR-20a-5p, such as BNIP2, APP, ASK1, and TNKS2, are reported, they are not well studied; hence further research is required to elucidate their roles in FFA [54, 57–59]. In another study, Jinnin showed that the upregulation of hsa-miR-19a-3p in the hair root tissue of psoriasis patients leads to the inhibition of TNF-α, a target protein that has an association with AA [60, 61]. It is also reported that TNF-α is a standard inhibitor of hair follicle growth in vitro, so its overexpression might suppress the growth of hair follicles, triggering AGA pathogenesis (Fig. 3) [61]. Moreover, Tziotzios et al. [54] utilized Generally Applicable Gene-set Enrichment (GAGE) analysis to identify pathways allied to FFA, and they found that endocytosis, focal adhesion, and mitogen-activated protein kinase (MAPK) signaling pathways were down-regulated in the co-targeting genes networks of the aforesaid four miRNAs. Nevertheless, more experimental data are needed to elucidate the regulatory roles of miRNAs in FFA pathogenesis fully.

Conclusion and future perspective

Several discoveries in biomedicine and clinical research have been done since the elucidation of the Human Genome Project. Notably, emerging miRNA technologies offer new promise in challenging health disorders. For future novel epigenetic therapies, recognition of the molecular, as well as the biological mechanisms underlying the initiation and progression of alopecia-related disorders, is expected to contribute to elucidating its pathophysiology. Therefore, increasing knowledge of the miRNA-controlled regulatory networks will play a crucial role in a better comprehension of the development of the disease as they are the critical gene expression regulators in humans, and their aberrant expression would lead to the development of several diseases. Potential techniques in epigenetic and gene expression profiling will change how clinical procedures are performed from early diagnosis/prognosis to treatment response as potential biomarkers will be necessary to be identified. So, the application of machine learning in bioinformatics programs for determining miRNA-binding sites in target genes and their related biological pathways, in vitro and in vivo preclinical research models, data analysis, and patient's medical history will significantly accelerate the clinical utility of miRNAs.

Nonetheless, this novel tool is still in its infancy, so in-depth research of its mechanism and functional significance within the disease is still required for clinical management. In the coming years, acquired biological knowledge about miRNAs, especially in biomedical research, is expected to

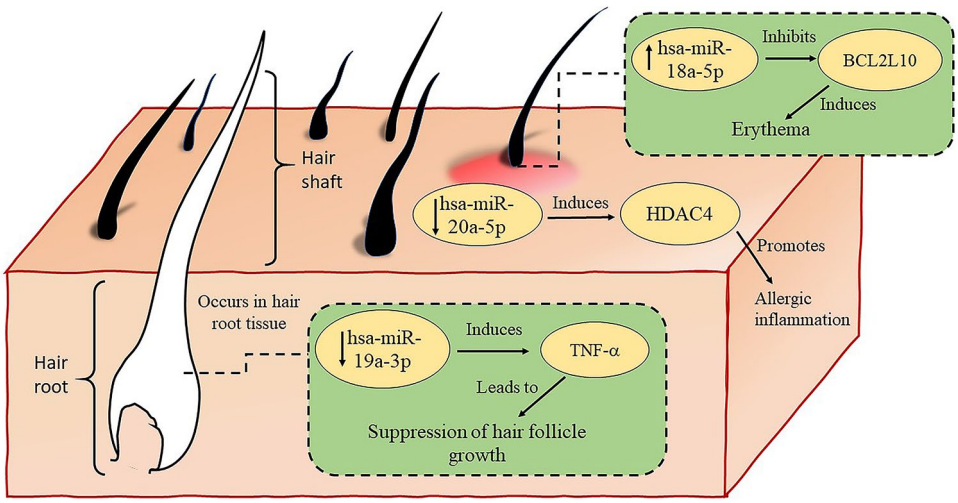

Fig. 3 Regulatory mechanism of miRNA expression in FFA. MiRNAs have several roles in frontal fibrosing alopecia (FFA) pathogenesis. For instance, hsa-miR-18a-5p upregulation is associated with erythema symptoms. Hsa-miR-20a-5p is a natural regulator of inflammatory reactions, but its downregulation induces HDAC4 protein, leading to allergic inflammation. Besides, hsa-miR-19a-3p downregulation induces TNF-a protein which suppresses hair follicle growth. All of the above-mentioned miRNAs-targets interaction leads to the FFA (↑: upregulated; ↓: downregulated)

be extensively interpreted in common hair loss disorders as miRNAs have a tremendous potential to be used as a new class of theranostic tool. The discovery of more disease-specific miRNAs with highly specialized functions might be expected to contribute to more successful guidance to the physicians for the early diagnosis of chronic diseases, such as alopecia and developing a novel class of therapeutic tools.

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Declarations

Conflicts of interest The authors declare that there are no conflicts of interest.

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