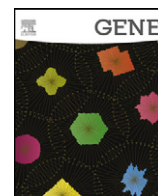Contents lists available at ScienceDirect

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Review

Integrative computational mRNA–miRNA interaction analyses of the autoimmune-deregulated miRNAs and well-known Th17 differentiation regulators: An attempt to discover new potential miRNAs involved in Th17 differentiation

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A R T I C L E   I N F O

A B S T R A C T

Th17 cells are a lineage of $CD4^+$ T helper cells in immune system which differentiate from naïve $CD4^+$ T cells and have demonstrated to play a critical role in the pathogenesis of different autoimmune disorders. miRNAs are a novel group of non-coding RNAs which participate in post-transcriptional regulation of gene expression mostly by pairing with 3′UTR of their mRNA targets and inhibition of its translation. It has been demonstrated that miRNAs function in various cellular processes such as differentiation, proliferation, and apoptosis. By now, several signaling pathways and their downstream positive and negative regulators involve in Th17 differentiation have been discovered. Several studies have reported the aberrant miRNA expression profile in patients with autoimmune disease called autoimmune-deregulated miRNAs. Here, using integrative miRwalk database which assembles the data gathered from ten different bioinformatics databases designed to predict miRNA-target interaction, we analyzed possible targeting effect of "autoimmune–deregulated miRNAs" on prominent positive and negative regulators of Th17 differentiation. Our resulting mRNA-miRNA network simply nominated several miRNAs with strong possibility which probably may have inducing (miR-27b, miR-27a, miR-30c, miR-1, and miR-141) or inhibitory (miR-20b, miR-93, miR-20a, miR-152, miR-21, and miR-106a) role in Th17 differentiation by targeting negative or positive regulators of Th17 differentiation, respectively.

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Contents

1. Introduction

Autoimmune disorders refer to a group of diseases arising due to aberrant immune responses against self-antigens. Like immune responses against foreign antigens, autoimmune responses recruit the same major components of immune system including innate (i.e. macrophage, neutrophils, monocytes) and adaptive immune cells (such as B cells and $CD4^+/CD8^+$ T cells). These responses against self-antigens could involve either the whole body or a specific organ resulting in systemic or organ-specific autoimmunity, respectively (Cotsapas and Hafler, 2013; Vyse and Todd, 1996).

T helper 17 (Th17) lineage is a well-known $CD4^+$ T cell lineage which is characterized by secreting inflammatory cytokines such as IL-17A, IL-17F, IL-22, and IL-21 and also expressing lineage-specific transcription factor, RORC. On the other hand, induced-regulatory T (iTreg) lineage is an anti-inflammatory $CD4^+$ T cell lineage which differentiates from the same naïve $CD4^+$ T cells. This lineage participates in regulation of immune responses through suppressing differentiation and function of other effector $CD4^+$ T cells such as Th17 cells. In addition, iTreg cells play a central role in induction of self-antigen tolerance. ITreg cells are characterized by the expression of master transcription factor, FOXP3 and secretion of anti-inflammatory cytokines including IL-10 and TGF-β (Chen et al., 2003; Davidson et al., 2007; Burchill et al., 2008; Yao et al., 2007).

Recently, increasing number of studies have revealed a pathogenic role of Th17 lineage in development or progression of different organ-specific autoimmune diseases including, multiple sclerosis (MS), rheumatoid artists (RA), psoriasis, systemic lupus erythematosus (SLE), inflammatory bowel disease (IBD), allergy and asthma (Waite and Skokos, 2011). Accordingly, numerous studies have been carried out to shed light on the precise molecular mechanisms and signaling pathways involved in induction of pathogenic Th17 differentiation and to find the best therapeutic targets for the suppression of its differentiation. Furthermore, it is beneficial to find therapeutic targets that induce iTreg differentiation, while suppressing pathogenic Th17 differentiation, since it has been observed that iTreg cells population decreases during pathogenesis of above-mentioned autoimmune diseases (Afzali et al., 2007). Nevertheless, the first step to get to this goal is to attain a precise and comprehensive knowledge of signaling pathways and molecular mechanisms to determine the fate of differentiation to Th17 and/or iTreg lineages.

MicroRNAs are a class of endogenous small (18–22 nucleotide) noncoding RNA which play a crucial role in regulation of various cellular processes such as cell cycle, mitosis, apoptosis, differentiation and so forth (Bartel, 2004). Hence, up/down-regulation of miRNA expression could result in numerous abnormalities and dysfunction of cellular activities (Bartel, 2004; Garzon et al., 2010). Until now, several studies have reported miRNAs up/down-regulation during pathogenesis of different autoimmune diseases. Interestingly, a number of these miRNAs have been accounted for differentiation and pathogenesis of Th17 cells. Although, in recent years many studies have been carried out to decipher the molecular mechanism of Th17 differentiation, little is known about the precise role of miRNAs in differentiation of Th17 cells. Few studies have investigated the possible role of miRNAs in
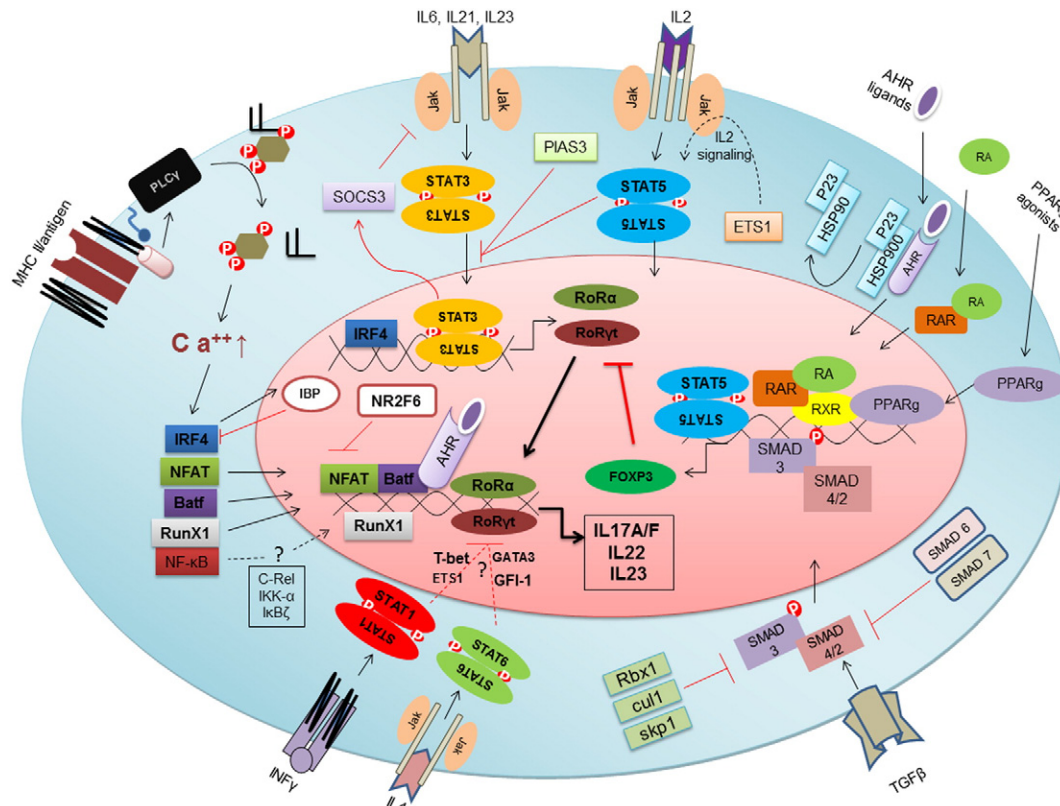

Fig. 1. Non-metabolic signaling pathways of naïve CD4 + T cells differentiation to Th17 lineage namely, TCR signaling pathway, retinoic acid receptor signaling pathway, cytokine signaling pathway, and AHR receptor signaling pathway.
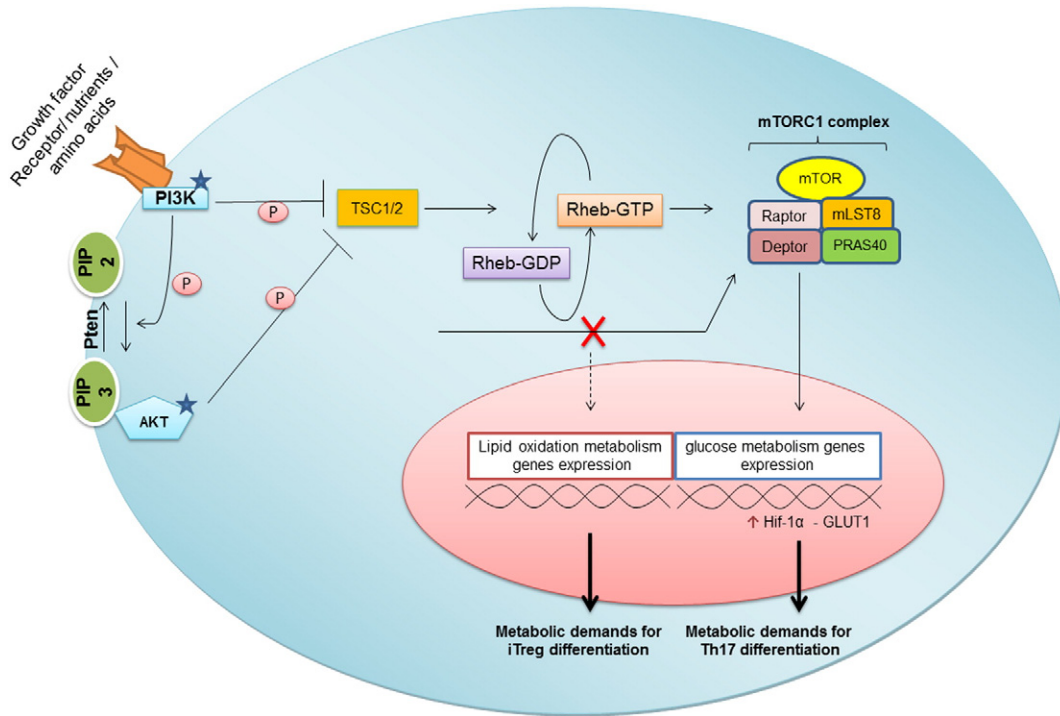
Fig. 2. Metabolic pathway of Th17 differentiation. This pathway is associated with glucose metabolism in the cells and it involves Hif1-α and AKT/PI3K/mTORC1 pathway.

differentiation and pathogenesis of Th17 cells so far. These studies have shown that miR-326 (Du et al., 2009), miR-301a (Mycko et al., 2012) and miR-20b (Zhu et al., 2014) play an inductive role in differentiation and pathogenesis of Th17 cells. Since, each miRNA is capable of regulating numerous mRNA targets (Selbach et al., 2008), a vast analysis of miRNA-mRNA interactions should be performed experimentally to validate such targets. These analyses are both troublesome and laborious. It seems that an integrative computational mRNA-miRNA interaction analysis is capable to predict most putative target genes and nominate most reliable miRNAs involved in Th17 differentiation. To date, several databases for prediction of mRNA-miRNA interactions have been already established based on different algorithms (Lewis et al., 2003; Maziere and Enright, 2007). Not surprisingly, the output of such databases are remarkably dissimilar (Xiao et al., 2009; Dweep et al., 2011). One reliable solution to this problem is to combine all of expression profiles of miRNAs with an integrative computational analysis. Recently, several integrative prediction databases have been established (Xiao et al., 2009; Dweep et al., 2011). MiRwalk is one of these databases that has its own prediction algorithm besides of accessing to the outcomes of other prediction algorithms as well (Dweep et al., 2011).

We performed an in-silico study to make an integrative computational mRNA-miRNA interaction analyses on the literature-derived autoimmune-deregulated miRNAs and mRNAs of Th17 differentiation regulators. Our goal was to find and nominate novel miRNAs with positive or negative regulatory role in Th17 differentiation.

2. Methods

2.1. Literature mining

In order to perform data mining, a manually shaped approach was used in which several key words related to microRNA expression, auto-immune diseases and T helper 17 were combined with a validated methodological filter, as previously described (Vosa et al., 2013). Systematic literature mining was carried out for English papers which were published until August 2014 including PubMed, Web of science, Science direct and MEDLINE databases. We also incorporated the

reference lists of relevant articles and reviews into our searching strategy. However, unpublished results, on-going studies and conference abstracts were not included in our systematic review.

Table 1
List of well-known positive and negative regulators of Th17 differentiation.

Positive regulators of Th17 differentiation	Negative regulators of Th17 differentiation
IL17A	IFN-γ
IL17F	IL4
IL23	IL12
IL23R	IL12R
IL6R	STAT5a
IL6	STAT5b
IL21	FOXP3
IL22	ETS1
IL21R	PPARγ
CCR6	PIAS3
IL1R	GFI1
CD161	TCF1
RORC	NR2F6
STAT3	T-bet
IRF4	GATA3
Batf	STAT1
AHR	STAT4
Runx1	STAT6
IKK-α	BCL6
NFKBIZ	SMAD3
RelB	SMAD4
c-maf	SMAD2
ROR-α	RUNX3
Hif1a	SOCS3
RBX1	RXR
Cul1	RAR
SKP1	TSC1
SMAD6	TSC2
SMAD7	FOXO1
Smurf1	FOXO3
Rheb	
mTOR	
Raptor	
MLST8	

2.2. Eligibility criteria

Regarding miRNA profile studies in autoimmune diseases, following criteria were considered: a] Studies with emphasis on expression profile of miRNAs in organ specific autoimmune diseases at which pathogenic role of Th17 lineage had been observed (including MS, IBD, SLE, Psoriasis, Diabetes and RA). b] Studies on body derived specimen (whole blood, PBMCs, lymphocyte, brain plaque and so forth) in which Th17 cells were detected. No restriction was considered regarding the journal type or patients nationality. Furthermore, data were extracted according to operator's experience upon complete evaluation of full-text contents.

2.3. miRNA–mRNA interaction analyses

All miRNA-mRNA prediction analyses were conducted by miRwalk database which is an integrative mRNA-miRNA prediction database (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/) (Dweep et al., 2011). In addition, in order to search for validated interactions by previous studies, we recruited miRtarbase (http://mirtarbase.mbc.nctu.edu.tw/) database which provides experimentally validated mRNA-miRNA interactions from all previously performed studies (Hsu et al., 2011). The final results were visualized in Cytoscape version 3.

3. Results

3.1. Positive and negative regulators of Th17 differentiation

Hitherto, several studies have been conducted to investigate and establish signaling pathways involved in Th17 differentiation. Therefore,

the most of positive and negative regulators of differentiation to Th17 cells have been characterized so far. Signaling pathways of Th17 differentiation are divided into two main metabolic and non-metabolic pathways. Non-metabolic pathways include: 1) T-cell receptor (TCR) signaling pathway 2) Retinoic acid receptor (RAR) signaling pathway 3) cytokine signaling pathways and 4) Aryl hydrocarbon receptor signaling pathway (Fig. 1). Metabolic pathways comprise hypoxia-inducible factor1-α (Hif1-α) and AKT/PI3K/mTORC1 pathways (Zhu and Qian, 2012; Bi and Yang, 2012; Sundrud and Koralov, 2011; Hirota et al., 2012; Maddur et al., 2012; Gerriets and Rathmell, 2012; Peters et al., 2011). Combination of all or most of these signaling routes contributes to the induction of Th17 differentiation (Fig. 2). Our literature mining, led us to a list of 64 genes which were involved in positive or negative regulation of Th17 differentiation (Table 1). Noticeably, negative regulators of Th17 differentiation could be considered as positive inducers of other T helper lineages, especially iTreg lineage.

3.2. Aberrant miRNA expression in autoimmune diseases

Through a comprehensive literature mining of studies related to miRNA expression profiling in different autoimmune diseases, a list of miRNAs which had been reported to be deregulated in autoimmune diseases was prepared (Table 2). Although, in the most cases a consistent expression was observed for miRNAs in various studies, a number of miRNAs demonstrated different expression profile which was due to different methodology for miRNA expression analysis (i.e. real time PCR, microarray), heterogeneity of the samples (i.e. whole blood, PBMCs, tissue lesions) and patient's treatment with immune modulatory drugs. However, in order to narrow down the results as far as

Table 2
List of deregulated microRNAs in various autoimmune diseases.

Disease	Methods of miRNA expression profiling	Aberrantly expressed microRNAs		Reference
		Up-regulation	Down-regulation	
MS	MicroArray, qRT-PCR	miR-485-3p, miR-376a-3p, miR-1-3p, miR-18b-5p, miR-493-5p, miR-599-3p, miR-95-3p, miR-193-3p, miR-148a-3p,miR-497-5p, miR-193a-3p, miR-200b-3p, miR-486-5p, miR-155-5p, miR-326-3p,miR-142-3p, miR-146a-5p, miR-146b-5p, miR-21-5p, miR-199a-3p, miR-142-5p, miR-200c-3p, miR-130a-3p, miR-223-3p, miR-22-3p, miR-320-3p, miR-214-3p, miR-148a-3p, miR-152-3p, miR-30a-5p, miR-365-3p, miR-9-5p, miR-95-3p, miR-186-5p, miR-29b-3p, miR-29c-3p,miR-210-3p, miR-19a-3p, miR-324-3p, miR-29a-3p, miR-133b-3p, miR-26a-5p, miR-16-2-3p	miR-27b-3p, miR-184-3p, miR-181c-5p, Let-7 g-5p, miR-7-1-3p, miR-106a-5p, miR-20a-5p,*[Let-7i-5p, miR-93-5p, miR-150-5p, miR-34a-5p, miR-27a-3p, miR-15a-5p, miR-20b-5p, miR-340-5p, miR-126-3p, miR-17-5p]	(Honardoost et al., 2014; Junker et al., 2009; Otaegui et al., 2009; Keller et al., 2009; Cox et al., 2010; Lorenzi et al., 2012; Haghikia et al., 2012; Fenoglio et al., 2011; De Santis et al., 2010; Guerau-de-Arellano et al., 2010, 2011)
SLE	MicroArray, qRT-PCR	miR-126-3p, miR-21-5p, miR-148a-3p, miR-7-5p	miR-17-5p, miR-142-5p, miR-184-3p, miR-95-3p, miR-186-5p, miR-31-5p, miR-141-3p, miR-197-3p, miR-10a-5p, *[mir-142-3p]	(Zhao et al., 2010, 2011; Ding et al., 2012; Tang et al., 2009; Pan et al., 2010)
IBD	qRT-PCR	miR-150-5p, miR-26a-5p, **[miR-126-3p, miR-34a-5p, miR-155-5p, miR-146a-5p, miR-21-5p, miR-199a-3p, miR-15a-5p, miR-200c-3p, miR-130a-3p, miR-223-3p, miR-22-3p, miR-320-3p, miR-214-3p, miR-181c-5p, miR-152-3p, miR-30a-5p, miR-20b-5p, miR-9-5p, let-7 g-5p, miR-106a-5p, miR-29b-3p, miR-29c-3p, miR-19a-3p, miR-324-3p, miR-29a-3p, miR-133b-3p, miR-31-5p, miR-125b-5p, miR-30c-5p, miR-215-5p, miR-7-5p, miR-130b-3p, miR-196a-5p]		(Fasseu et al., 2010; Wu et al., 2010, 2011; Iborra et al., 2012; Paraskevi et al., 2012; Olaru et al., 2011; Coskun et al., 2013)
Psoriasis	MicroArray, qRT-PCR, immunohistochemistry	miR-17-5p, miR-142-3p, miR-146a-5p, miR-146b-5p, miR-21-5p, miR-106a-5p, miR-20a-5p, miR-31-5p, miR-200a-3p, miR-141-3p	miR-326-3p, miR-22-3p, miR-365-3p, miR-133b-3p, miR-125b-5p, miR-197-3p, miR-30c-5p, miR-10a-5p, miR-215-5p	(Sonkoly et al., 2007; Holst et al., 2010; Gu et al., 2011)
RA	MicroArray, qRT-PCR	miR-155-5p, miR-146a-5p, miR-146b-5p, miR-223-3p, miR-150-5p, miR-26a-5p	Let-7a-5p	(Fulci et al., 2010; Stanczyk et al., 2008; Pauley et al., 2008; Xu et al., 2014)
Diabetes	Solexa sequencing, qRT-PCR	miR-326-3p, miR-27a-3p, miR-27b-3p, miR-148a-3p, miR-152-3p, miR-30a-5p, miR-210-3p, miR-29a-3p, miR-26a-5p, miR-200a-3p	miR-21-5p, miR-93-5p	(Zampetaki et al., 2010; Guay and Regazzi, 2013)

*microRNAs showed contradicted expression pattern in different studies. In some studies they showed over expression, while in the other studies showed decrease in their expression.
**miRNAs which their deregulation were reported without additional explanation for up or down-regulation.

possible, those miRNAs which were reported in at least two studies were selected. Finally, 64 deregulated miRNAs were selected and subsequently used in our in-silico mRNA-miRNA interaction analysis.

3.3. Prediction of mRNA-miRNA interaction of autoimmune-deregulated miRNAs and Th17 differentiation regulators

Considering Th17 lineage as a common pathogenic CD4$^+$ T cell lineage in various autoimmune diseases, we proposed a nominated miRNA could participate in Th17 differentiation with the following criteria: a) deregulated expression during pathogenesis of autoimmune diseases and b) targeting of the positive or negative regulators of Th17 lineage. Accordingly, deregulated miRNAs of autoimmune diseases were considered to have a positive or negative role in Th17 differentiation. To examine such possibility, integrative in-silico mRNA-miRNA prediction analysis was performed on autoimmune-deregulated miRNAs and well-known regulators of Th17 lineage. To accomplish this analysis, two comprehensive lists of Th17 regulators and autoimmune-deregulated miRNAs were provided (Tables 1 and 2). miRWalk database was utilized for in-silico miRNA-mRNA prediction analyses (Dweep et al., 2011). miRWalk database represents miRNA-mRNA prediction results of 10 different databases with a score of 1 or 0 for each database which predicted the interaction or not, respectively. Thus, an overall prediction score of 10 demonstrates a strong predicted interaction by all databases (Dweep et al., 2011).

We analyzed 4096 interactions (64 miRNA × 64 regulators) of which 1839 interactions were not predicted by any database (prediction score (PS) = 0); however, 613 interactions were predicted by one database (PS = 1), 564 interactions by two databases (PS = 2), 225 interactions by three databases (PS = 3), 267 interactions by four databases (PS = 4), 387 interactions by five databases (PS = 5), 143 interactions by six databases (PS = 6), 34 interactions by seven databases (PS = 7), 19 interactions by eight databases (PS = 8) and finally 5 interactions acquired prediction score of 9 (PS = 9) (Supplementary Fig. 1). At the first step, to select the strongest candidates, interactions with PS < 5 were ignored. The rest of interactions were subjected into the Cytoscape_v3.0.1 software to visualize miRNA–mRNA network of autoimmune-deregulated miRNA and positive/negative regulators of Th17 differentiation (Fig. 3). In the next step, to narrow down the strongest candidates, interactions with PS < 6 were removed from the

Fig. 3. miRNA–mRNA network considering all interactions with PS of 5 and higher. Autoimmune-deregulated miRNAs are arranged in a circle shape. Positive and negative regulators of Th17 differentiation are placed on the right and left inside the circle, respectively. Inhibitory interactions (i.e. interaction between miRNAs and positive regulator of Th17) are represented with green edges, whereas red edges stand for inducing interactions (that is interactions between miRNAs and negative regulators of Th17). Gradient color of green or red edges show strength of each interaction based on its PS.

Table 3

Inducing or suppressing candidate miRNAs based on their interaction with regulators of Th17 differentiation.

Category	Rank	Th17 inducing miRNAs	Target genes		Expression deregulation in autoimmune disease	Th17 suppressing miRNAs	Target genes		Expression deregulation in autoimmune disease
			Negative regulators (PS)	Positive regulators (PS)			Negative regulators (PS)	Positive regulators (PS)	
Strong	1	miR-27b	FOXO1(8), NR2F6(7), GATA3(7), TSC1(7), Stat5a(6), RXRA(6), PPARG(6)	IRF4(6), RunX1(6)	– MS (peripheral blood) = down – Diabetes (serum) = up	miR-20b	IL12RB2(6), FOXO1(6)	Hif1a(8), SMAD7(8), STAT3(7), SMAD6(7), RUNX1(6), IKK-a(6), RORC(6)	- **MS** (**inactive plaque**) = **up** - **MS**(**whole blood**) = **down** - **IBD** (**biopsies**) = **deregulated**
	2	miR-27a	NR2F6(7), GATA3(7), TSC1(7), FOXO1(7), Stat5a(6), RXRA(6)	IRF4(6), RunX1(6)	– MS (active plaque) = up – MS (peripheral blood) = down – Diabetes (serum) = up	miR-93	SMAD2(7), IL12RB (6)	STAT3(8), SMAD7(8), Hif1a(7), SMAD6(7), RORC(6), RUNX1(6)	- **MS** (**whole blood**) = **down** - **MS** (**Treg**) = **up** - **Diabetes** (**PBMCs**) = **down**
	3	miR-30c	Socs3(8), RARB (8), FOXO3(6), BCL6(6), RARC(6), TSC1(6),	IRF4(6)	– Psoriasis (skin lesions) = down – IBD (biopsies) = deregulated	miR-20a	RARB(7), IL12RB2(6), BCL6(6),	Hif1a(9), SMAD7(8), STAT3(7), SMAD6(7), RUNX1(6)	- **MS** (**treg cells**) = **down** - **Psoriasis** (**skin lesions**) = **up**
	4	miR-1	RARB(8), ETS1(7), PIAS3(6)		– MS (CD4$^+$ T cell) = up	miR-152	ETS1(6)	IKK-a(7), SKP1(8), MLST8(6),	- **MS** (**inactive plaques**) = **up** - **Diabetes** (**serum**) = **up** - **IBD** (**biopsies**) = **deregulated**
	5	miR-141	Stat4(7), RARB(7), Stat5a(6), Stat5b(6), Tbet(6), GATA3(6), TSC1(6)	Rheb (6)	– Psoriasis (skin lesions) = up – Lupus (PBMCs) = down	miR-21	IL12A(6)	STAT3(7), SMAD7(8),	- **MS** (**active plaque**) = **up** - **MS** (**PBMCs**) = **up** - **Psoriasis** (**skin lesions**) = **up** - **Lupus** (**CD4 T cell**) = **up** - **Lupus** (**PBMCs**) = **up** - **Diabetes** (**PBMCs**) = **down** - **IBD** (**biopsies**) = **deregulated**
	6					miR-106a	IL12RB(6), TSC1(6)	SMAD7(8), STAT3(7), Hif1a(6)	- **MS** (**whole blood**) = **down** - **Psoriasis** (**skin lesions**) = **up** - **IBS** (**biopsies**) = **deregulated**
Moderate	1	miR-223	FOXO3(7), Stat1(6), FOXO1(6)		– MS (active plaque) = up – MS (whole blood) = up – MS (Treg cell) = up – Psoriasis (skin lesion) = down – IBD (biopsies) = deregulated	miR-376		NFKBIZ(7), IKK-a(6)	- **MS** (**CD4 T cell**) = **up**
	2	miR-142-3p	FOXO1(7), TSC1(6)		– MS (active plaque) = up – MS (whole blood) = up – Psoriasis (skin lesion) = up) – Lupus (PBMC) = down	miR-497	TSC1(7)	SMAD7(7), IKK-a(6)	- **MS** (**CD4+ Tcell**) = **up** - **MS** (**Inactive plaque**) = **up**
	3	miR-29a/b/c	RARB(8), IFNg(6), IL12B(6)	AHR(6)	miR-29a: – MS (Treg cell) = up – Diabetes (serum) = up – IBD (biopsies) = deregulated) miR-29b: – MS (CD4$^+$ T cell) = up – Diabetes (serum) = up	miR-214	TCF1(6), Stat6(6)	RBX1(8), IL1R(6), mTOR(6), MLST8(6),	- **MS**(**active plaque**) = **up** - **MS** (**inactive plaque**) = **up** - **IBD** (**biopsies**) = **deregulated**

	#	miRNA	Targets	Targets	Expression	miRNA	Targets	Targets	Expression
					– IBD (biopsies) = deregulated miR-29c: – MS (Treg cell) = up – IBD (biopsies) = deregulated				
	4	Let-7i	TSC1(7)	RORC(6)	– MS (whole blood) = down – MS (Treg) = up	miR-130b	RUNX3(6), TSC1(6)	SKP1(8), IKK-a(6)	- **Lupus** (**PBMCs**) = **down** - **IBD** (**biopsies**) = **deregulated**
	5	miR-19a	Socs3(8), Stat5b(6), Runx3(6),	IL1R(6), Hif1a(6)	– MS (Treg cell) = up – IBD (biopsies) = deregulated				
	6	miR-15a	RARB(7), SMAD3(6), TSC1(6),	SMAD7(9), IL1R (6)	– MS (active plaque) = up – MS (whole blood) = down – MS (Treg cell) = up – IBD (biopsies) = deregulated				
Weak	1	miR-9	PIAS3(6), Stat4(6), BCL6(6), RXRA(6), TSC1(6), FOXO1(6),		– MS (inactive plaque) = up – IBD (biopsies) = deregulated	miR-17		Stat3(6), hif1a(6), SMAD7(6),	- **MS** (**CD4 T cell**) = **up** - **MS** (**peripheral blood**) = **down** - **Psoriasis** (**skin lesion**) = **up** - **Lupus** (**PBMCs**) = **down**
	2	miR-155	ETS1(6), RXRA(6), FOXO3(6)		– MS (active plaque) = up – RA (PBMCs) = up – RA (synovium tissue) = up – RA (IL17 producing T cell) = up – IBD (biopsies) = deregulated	miR-142-5p		IL17f(6), IL-22(6), AHR (6),	- **MS** (**active plaque**) = **up** - **Lupus** (**PBMCs**) = **down**
	3	miR-150	Stat5b(6), GATA3(6), Stat4(6)		– RA (PBMCs) = up – RA (synovium tissue) = up – RA (IL17 producing T cell) = up – IBD (biopsies) = up	miR-7		SKP1(6), mTOR(6)	- **Lupus** (**PBMCs**) = **up** - **IBD** (**biopsies**) = **deregulated**
	4	miR-10a	GATA3(6), BCL6(6), TSC1(6)		– Psoriasis (skin lesions) = down – Lupus (PBMCs) = down	miR-197		IL1R(6), IKK-a(6)	- **Psoriasis** (**skin lesion**) = **down** - **Lupus** (**PBMCs**) = **down**
	5	miR-200a	Tbet(6), GATA3(6), RARB(6)	Rheb(6)	– Psoriasis (skin lesions) = up – Diabetes (serum) = up	miR-340	SMAD2(6)	IL-22(6), RORC(6),	- **MS** (**active plaque**) = **down** - **MS** (**memory CD4 T cell**) = **up**
	6	miR-181c	ETS1(6), PIAs3(6), BCL6(6)	SMAD7(9)	– MS (active plaque) = down – MS (CSF) = deregulated – IBD (biopsies) = deregulated	miR-31	IL12RB2(6),	IL1R(6)	- **Psoriasis** (**skin lesion**) = **up** - **Lupus** (**PBMCs**) = **down** - **Lupus** (**CD4 T cell**) = **down** - **IBD** (**biopsies**) = **deregulated**
	7	miR-200c	ETS1(6), SMAD2(6),	NFKBIZ (6)	– MS (active plaque) = up – MS (inactive plaque) = up – IBD (biopsies) = deregulated				
	8	miR-199a	PIAS3(6), RARB(6)	Hif1a(6)	– MS (active plaque) = up – IBD (biopsies) = deregulated				
	9	miR-196a	RARB(6), TSC1(6)	SMAD6(7)	– Lupus (PBMCs) = down – IBD (biopsies) = deregulated				
	10	miR-26a	SMAD4 (6)	RORC (6)	– RA (PBMCs) = up – RA (synovium tissue) = up – RA (IL17 producing T cell) = up – Diabetes (serum = up) – IBD (biopsies = deregulated)				
	11	miR-200b	ETS1(6)	NFKBIZ(6)	– MS (CD4 T cell) = up				
	12	miR-324-3p	TSC1(6)	SMAD7(7)	– MS (Treg) = up – IBD (biopsies) = deregulated				

network (Supplementary Fig. 2). Finally, selected miRNAs were categorized into three groups of strong, moderate and weak candidates according to the following criteria with priority order of: a) the number of strong interactions with target genes. b) the number of inconsistent interactions with the predicted role of miRNAs and c) the PS of each interaction. However, the deregulated expression direction of miRNAs in autoimmune disease was not included for this ranking due to the heterogeneity of the included studies. This approach led us to identify high potential miRNAs involved in differentiation of Th17 cells. Final list of these candidates and their putative targets and deregulated expression direction of these miRNAs in autoimmune diseases is represented (Table 3).

4. Discussion

In the current study, mRNAs–miRNAs interaction analysis on autoimmune-deregulated miRNAs and well-known regulators of Th17 differentiation was implemented to explore novel targets of miRNAs which might play a vital role in Th17 differentiation. Therefore a network of mRNAs–miRNAs was diagrammed. Based on this network, several miRNAs were nominated with strong possibility to induce (miR-27b, miR-27a, miR-30c, miR-1, and miR-141) or inhibit (miR-20b, miR-93, miR-20a, miR-152, miR-21, and miR-106a) Th17 differentiation through interaction with the negative or positive regulators of this pathway, respectively. In this approach, several miRNAs were predicted to be involved which were positioned as moderate or weak effectors (Table 3).

Among our strongly nominated miRNAs, miR-20b, was ranked at first location in our list. The suppressing role of miR-20b in Th17 differentiation has been recently validated experimentally by Zhu and colleagues (Zhu et al., 2014). They have represented that miR-20b is able to ameliorate pathogenesis of experimental autoimmune encephalomyelitis by targeting $RoR\gamma t$ and $STAT3$ genes (Zhu et al., 2014). Interestingly, these validated targets were also predicted by our mRNA–miRNA network with PS of 6 and 7, respectively. Furthermore, our network predicts other possible aspects of suppressing function of miR-20b in Th17 differentiation which might be through targeting of $Hif1a$ (PS = 8), $SMAD7$ (PS = 7), $SMAD6$ (PS = 7) or TGF-β signaling pathways. In addition, recently we reported up-regulation of miR-26a in RR-MS patients during relapsing phase in comparison with remitting phase and healthy subjects (Honardoost et al., 2014). We also investigated the possible role of miR-141 and miR-200a in differentiation of Th17 cells and showed their inducing role in differentiation of Th17 cells (Naghavian et al., 2015), which is consistent with predicted inducing role for these miRNAs in our current mRNA-miRNA network. Consistently, according to the results of miRtar database, several interactions which were predicted in our network with PS ≥ 7 had been previously validated in other cells and tissues other than Th17 cells (data not shown). Interestingly, deregulated expression levels of some nominated inducing (miR-1, miR-29a/b/c, miR-19a, miR-150, miR-155, miR-200a, miR-199a, miR-26a) or suppressing miRNAs (miR-197, miR-130b, miR-20a/b, miR93) were completely compatible with their predicted role in Th17 differentiation. All of these observations confirm the fidelity and accuracy of our mRNA-miRNA network in nominating and discovery of miRNAs which might participate in Th17 differentiation. However, several discrepancies in our mRNA–miRNA network were evident which are needed to be addressed more.

Firstly, two other previously validated miRNAs in Th17 differentiation (i.e. miR-326 and miR-301a) were not present in our nominated miRNA list. miR-301a was excluded from our list as its up/down-regulation was reported in a singular study. Meanwhile, miR-326 was also disqualified as respective PS was not highly scored despite its potential to suppress several negative regulators of Th17 (SMAD3, SMAD4, RUNX3, RARC, RXRA. STAT5b, NR2F6). Inductive role of miR-326 in Th17 differentiation was already reported by of Du et al.

(Du et al., 2009). Hence, we concluded despite of the beneficial aspect of our mRNA–miRNA network to provide neumerous possible results, it only predicts the most important miRNAs involved in Th17 differentiation program and does not necessarily introduce all miRNAs participating in differentiation of this lineage.

Secondly, some of nominated miRNAs showed unreliable interactions in their predicted role in Th17 differentiation. For instance, miR-20b, which suppresses several positive regulators of Th17 differentiation, was also predicted to have a suppressing role on negative regulators of Th17 differentiation. Such kinds of discrepancies could be explained by several studies aiming to discover precise molecular mechanisms of Th17 differentiation. Recently, Peters et al. have categorized Th17 lineage in two pathogenic and non-pathogenic types. Pathogenic Th17 cells are generated in the absence of TGF-β signaling pathway and mostly present in autoimmune conditions while, non-pathogenic Th17 cells are produced by induction of TGF-β signaling pathway (Peters et al., 2011). Considering of this evidence, we concluded that TGF-β signaling pathway is not necessarily an inducing pathway for Th17 differentiation as lack of this signaling pathway was apparent in differentiation of pathogenic Th17 lineage. This fact is arisen by opposite interactions which are relevant to TGF-β signaling pathway in our mRNA–miRNA network similar to what we reported for miR-26a in our previous study (Honardoost et al., 2014).

Another explanation for such kinds of discrepancies is provided by a recent study of Yosef and colleagues (Yosef et al., 2013). They combined a high-resolution transcriptional time course profiling as a novel method for reconstructing transcriptional network of Th17 differentiation in order to introduce a dynamic regulatory network controlling differentiation of this lineage. They defined three distinct transcriptional phases with discrete gene expression profile during in vitro differentiation of naïve CD4 T cells into Th17 lineage, specifically at early or induction phase (up to 4 h), intermediate phase where acquiring of phenotype and amplification occurs (4–20 h), and finally late or stabilization phase which is associated with IL-23 signaling. Furthermore, they represented that some known negative regulators of Th17 (such as SOCS3, STAT2, STAT1) are needed to be expressed in the first stage while some positive regulators (including IRF4, SOCS1, Batf) are expressed in later phases (Yosef et al., 2013). According to these results, we concluded that Th17 differentiation is a multi-stage process and a specific set of positive or even negative regulators are required to be expressed in each stage in order to achieve functional Th17 lineage.

Thus, one miRNA could not be considered as an absolute inducer or suppressor of Th17 differentiation. In fact, to fulfill transcriptional requirement in each stage, a collection of several miRNAs are needed to exert a regulatory role in Th17 differentiation process. This phenomenon clarifies why for a number of miRNAs, both positive and negative roles are supposed in differentiation of Th17 cells.

Thirdly, beside of those nominated miRNAs which showed compatible expression direction with their predicted role in Th17 differentiation, few cases miRNAs with incompatible and illogical expression direction were noticed. Such discordant note could be due to the heterogeneity of previous miRNA profiling studies which were performed with different samples or methodology and possible consumption of immune-modulatory responses in patients.

Notably, our categorization of nominated miRNAs (strong, median, and weak groups) was only based on bioinformatics criteria. Thus it is not surprising that further experimental studies reveal a miRNA from the weak category to exert a crucial role similar to strong miRNAs in Th17 differentiation.

5. Conclusion

We utilized miRWalk database for integrative computational mRNA-miRNA interaction prediction aiming to assess interaction of

autoimmune deregulated miRNAs with well-known regulators of Th17 differentiation. By analyzing of 64 miRNAs and 4096 interactions, a network of mRNAs-miRNAs interactions was diagrammed which predicted 11 miRNAs with strong possibility to induce or suppress Th17 differentiation. These results are preliminary steps to unravel the novel mRNAs–miRNAs interactions. Obviously, additional in vitro and in vivo experiments are required to validate these predictions in Th17 differentiation as are currently on going by our colleagues.

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