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ORIGINAL ARTICLE

MicroRNA expression in antiphospholipid syndrome: a systematic review and microRNA target genes analysis

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Abstract

Antiphospholipid antibodies (aPL) are autoantibodies that attack phospholipid through anti-beta 2-glycoprotein 1. The actions of aPL are associated with events leading to thrombosis and morbidity in pregnancy. Antiphospholipid syndrome (APS) is diagnosed when a patient is persistently positive for aPL and also has recognised clinical manifestations such as recurrent pregnancy losses, arterial or venous thrombosis and in a catastrophic case, can result in death. Unfortunately, the pathogenesis of APS is still not well established. Recently, microRNA expressed in many types of diseased tissues were claimed to be involved in the pathological progression of diseases and has become a useful biomarker to indicate diseases, including APS. *Objective*: This systematic review aims to search for research papers that are focussing on microRNA expression profiles in APS. *Method:* Three search engines (Ebcohost, ProQuest and Ovid) were used to identify papers related to expression of specific microRNA in antiphospholipid syndrome. *Results and Discussion*: A total of 357 papers were found and screened, out of which only one study fulfilled the requirement. In this particular study blood samples from APS patients were tested. The microRNAs found to be related to APS were miR-19b and miR-20a. No data was found on specific microRNA being expressed in obstetric antiphospholipid syndrome. Analysis on the microRNA target genes revealed that most genes targeted by miR-19b and miR-20a involve in TGF-Beta Signalling and VEGF, hypoxia and angiogenesis pathways. *Conclusion:* In view of the limited data on the expressions of microRNA in APS we recommend further research into this field. Characterization of microRNA profile in blood as well as in placenta tissue of patients with APS could be useful in identifying microRNAs involved in obstetric APS.

Keywords: antiphospholipid syndrome, antiphospholipid antibody, autoimmune, microRNA, systematic review

INTRODUCTION

Antiphospholipid syndrome (APS) is an autoimmune disease clinically characterized by thrombotic events (venous and arterial) and pregnancy complications that are associated with persistent levels of antiphospholipid antibodies (aPL). The relationship between antiphospholipid antibodies and its clinical characteristics were discovered only 20 years ago. Since then, the understanding of APS has expanded and many other clinical manifestations of APS were characterised including deep vein thrombosis, thrombocytopenia, livedoreticularis, cerebrovascular accident, pulmonary embolism and foetal loss. In the Euro Phospholipid Project Cohort study by Cervera *et al*, the prevalence of preeclampsia, eclampsia, abruptio placentae and postpartum cardiopulmonary syndrome were 9.5%, 4.4%, 2.0% and 0.5% respectively in 590 pregnant women with APS.[1]

One of the reported pathogenesis of APS is the event of autoantibodies attacking a phospholipid-binding protein known as β2-glycoprotein 1 (β2-GP1), which then serve as an important marker for the disease. Other markers used in the detection of APS are Lupus anticoagulant (LA) and anticardiolipin (ACL). It is important to note

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that the diagnosis of APS is being compromised, as the pathogenesis of APS has not been completely elucidated. Currently, the diagnosis of APS follows the latest standardised Sapporo reviewed criteria or Sydney Criteria 2006, a second revision of the original Sapporo criteria. The diagnostic laboratory was criticised for variability in results due to poor standardisation. Three parameters included in the diagnosis of APS are Lupus anticoagulant-clotting time, anticardiolipin and anti-β2-GP1, detectable by enzyme-linked immunosorbent assay (ELISA). The existence of a diversity of antigens and antibodies with the presence of different aPL antibodies in the same patient complicate the standardisation of the method. Thus, a number of workshops have been conducted internationally to counter this standardisation problem.[2]

Further investigation has shown involvement in the regulation of certain genes in the pathogenesis of APS. The activity of the genes involved in innate immune response such as toll-like receptor 8 (TLR8), CD14, signal transducer and activator of transcription 1 (STAT1), 2′-5′-oligoadenylate synthetase 2 (OAS2), tumour necrosis factor ligand superfamily member 13 (TNFSF13) and Phospholipid Scramblase 1 (PLSCR1) are significantly increased in APS patients and therefore identified as signature genes for APS.[3] Recent updates in molecular research have revealed that microRNA is involved in the regulation of genes in cellular processes where the changes in their expressions indicate specific mechanisms of disease.[4] MicroRNA (miRNA) is a short RNA sequence, typically about 21 or 22 nucleotides generated from longer RNA molecules. Dicer, an RNase-III-like enzyme recognizes and digests these longer dsRNA or the stem-loop structures. These miRNAs have a characteristic structure that help in the identification and prediction of the target gene they might regulate. Therefore, studying the miRNA expression profile could offer enlightenment into understanding the pathogenesis of APS. It could also lead to the discovery of biomarkers that will increase specificity and sensitivity of the diagnosis. In this present study, we provide the latest update of data on miRNA expressions profile in APS. The searching strategy was performed based on the guidelines provided by Prisma 2009 checklists.

MATERIALS AND METHODS

Data sources and search strategy

Studies that fulfilled the inclusion criteria for the review were searched through Ebcohost, Ovid and Proquest with unlimited publication date. The search strategy involved a combination or broad indexing terms of each databases. The following keywords were used for Antiphospholipid syndrome (antiphospholipid) and microRNA (microRNA, miRNA, and miR) respectively. The access to the selected articles was through subscription by the library of Universiti Sains Islam Malaysia. Bibliographies of selected manuscripts including relevant reviews were also screened for eligible studies by using snowballing strategy.

Criteria included in the review

Inclusion criteria were as follows: (1) the study investigated miRNA expression in APS (whether APS patient sample or induced APS), (2) sample used from APS patients were either blood or tissue, (3) sample of APS should have comparison with normal sample, (4) total samples used in study were mentioned, (5) information on differentially expressed miRNA (up-regulated or down-regulated) available.

Identification of relevant studies and data extraction

All manuscripts from searches were downloaded into an Endnote library. Articles retrieved from the database searches were screened by the titles (first step), abstracts (second step) and the entire article (third step) to select for relevant studies. The screenings were conducted by two researchers. A third author was consulted when there were disagreements about eligibility between the reviewers. Records of reasons for rejection were kept.

Data was extracted from the included studies into a table by one author. Each study's details including author, year of study, types of sample, platform use for microarray analysis and findings on the differentially expressed miRNA were listed in the table. Data entry was checked for each study after completing data extraction. The selection protocol of studies is summarised in Fig. 1.

Data reporting

Data were presented in descriptive summary and systematic reviews. Report of the study was done according to PRISMA guidelines.

MicroRNA target genes determination and functional analysis

To identify and explore the target genes of

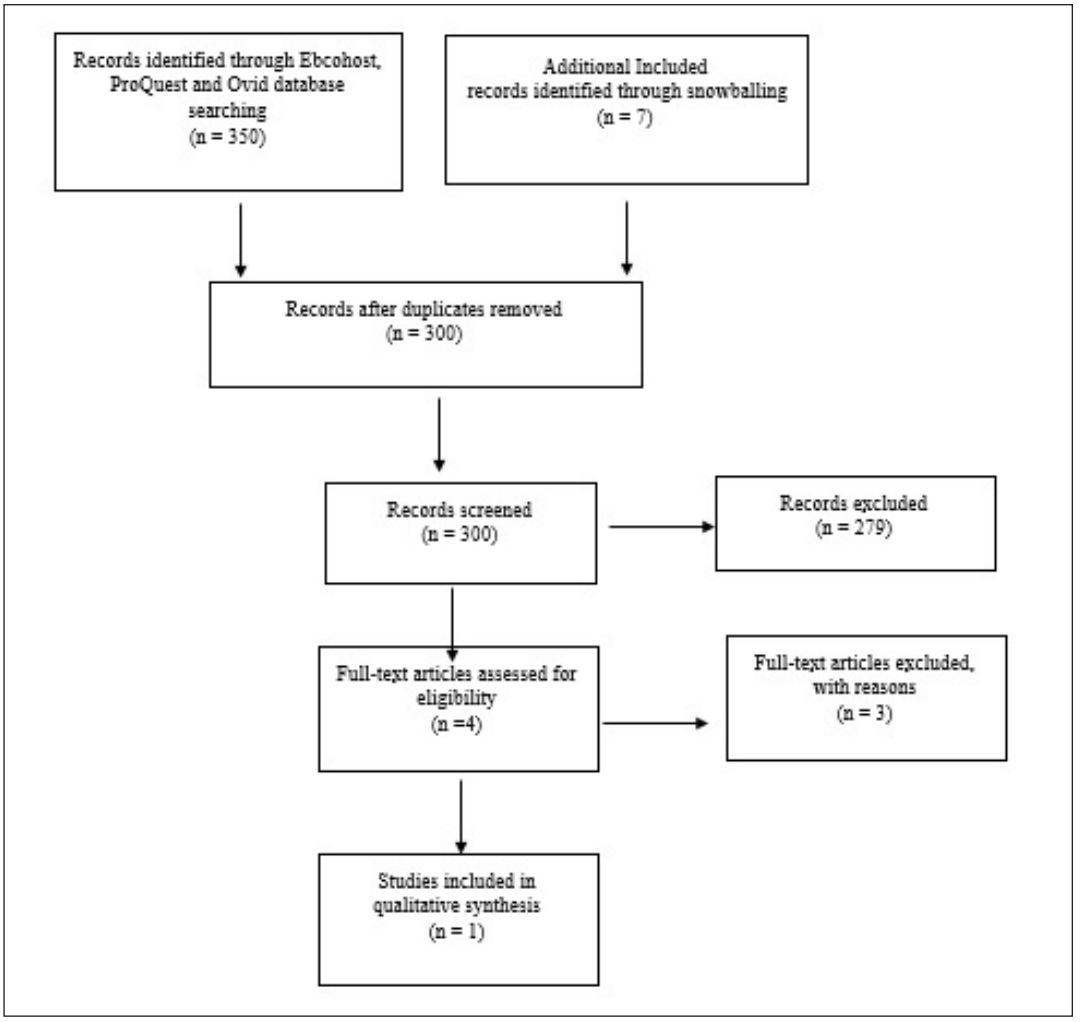

FIG. 1: Flow chart of study selection for systematic review

miRNAs, miRTarbase (mirtarbase.mbc.nctu.edu.tw) was used.[5] It is an established database with 50,000 miRNA-target interactions (MTIs). Collected MTIs in this database were validated experimentally by reporter assay, western blot, microarray and next-generation sequencing experiments.

Molecular functions and pathway analysis of microRNA predicted target gene was done using DAVID Bioinformatic Resources and Kyoto Encyclopedia of Genes and Genome (KEGG). The DAVID Bioinformatics Resources consist of the DAVID Knowledgebase and five integrated web-based functional annotation tool suites including the DAVID Gene Functional Classification Tool and the DAVID Functional Annotation Tool. The pathway maps to explain the functional role of the genes list is generated using KEGG and BioCarta pathways which were integrated in the David Pathway Viewer. DAVID is a database which enables researchers to analyse high throughput gene function and provide biological information from various data annotations in centralised location.[6]

RESULTS

Independent studies on miRNA in APS

Data searched through Ebcohost, ProQuest and Ovid revealed 357 studies closely related to the topic. However, selection of studies based on the inclusion and exclusion criteria included only one independent study for the final review. The data extracted from the study is detailed in Table 1. As the mechanism of APS is still not elucidated, the only study found on the association of miRNA with APS focussed on understanding the effect contributed by miRNAs in the cellular and molecular pathogenesis of APS. The study was on

TABLE 1: Up- and down-regulated miRNA reported in APS study

Author	Year	Type of cells	Platform	No. of differential miRNA	Up-regulated miRNA	Down-regulated miRNA
Teruel et al	2011	White Blood Cells	Taqman probes purchased from Applied Biosystems	2	–	miR-19b, miR-20a.

identification of miRNAs as potential modulators of tissue factor expression in patients with APS and systemic lupus erythematosus (SLE).

Differentially expressed miRNAs reported

Based on the study included, 2 differentially expressed miRNAs in APS were reported, namely miR-19b and miR-20a. Both miRNAs were observed to be down-regulated in APS patients in comparison to normal sample. It is known that tissue factor is the key stimulator of coagulation cascade and its overexpression might provoke the thrombotic occurrence, the hallmark of autoimmune diseases like APS and SLE, which is further indicated by the increase in TF expression in monocytes.

In the study of "Identification of miRNA as potential modulators of tissue factor expression in patients with systemic lupus erythematosus and antiphospholipid syndrome" by R.Teruel, they first did an in silico studies by searching through several web databases (TargetScan and PicTar) and algorithms of miRNA target prediction targeting TF, which resulted in the selection of miR-19b, miR-20a and miR-106 being investigated. Prior to testing on APS and SLE samples, they did *in vitro* testing on breast cancer cell line, MDA-MB-231, a cell line that expressed TF at high levels. Western blotting was performed to measure TF expression with β-actin as control. Both cell lines which were transfected with miR-19b and miR-20a resulted in almost 60% decrease in TF protein expression when further assayed by densitometry. There was also statistically significant correlation between miR-19b and miR-20a expressions and TF mRNA reduction. This finding further verified the effect of miR-19b and miR-20a on TF mRNA transcription in THP-1, a human acute monocytic leukemia cell line. The result showed that the level of TF mRNA decreased by 40-60% following overexpression of miR-19b and miR-20a.

Their next study was on TF levels in monocytes of APS patients (n=12) or SLE (n=23) as compared to a group of healthy individuals (n=17). The monocytes were purified by negative selection using specific antibodies. The levels of miR-19b and miR-20a in monocytes of APS patients showed an approximately seven-fold decrease in comparison with levels in healthy controls. Significant correlation scores of -0.373 for miR-19b and of -0.608 for miR-20a between TF expression on the cell surface and endogenous mature miRNAs was reported, indicating that while one parameter decreases (miRNA) the other increases (TF).[7]

Identifying microRNA target genes

Target genes of miR-19b and miR-20a were identified using miRTarbase. The genes were selected only from the category of "strong evidence" according to the database. 25 and 36 target genes were generated for miR-19b and miR-20a, respectively (Table 2). Four target genes which are phosphatase and tensin homolog (PTEN), transforming growth factor-beta 2 (TGFBR2), bone morphogenetic protein receptor type II (BMPR2) and cyclin D1 (CCND1) were found to be common for both miR-19b and miR-20a.

Gene ontology and pathways analysis of predicted microRNA target genes using DAVID Bioinformatic Resources

A total of 57 target genes for miR-19b and miR-20a were analysed using DAVID Bioinformaticc Resources. Table 3 shows the gene ontology, KEGG and Biocarta for putative target genes. Most significant GO terms involved cellular process, biological regulation, metabolic process and also development process. For KEGG pathway generated through DAVID, approximately 68.4% of putative target genes (n=39) were classified into 25 pathways ($P<0.01$),

TABLE 2: List of target genes for miR-19b and miR-20a

miR-19b	miR-20a
BACE1	HIF1A
PTEN	CCND1
ATXN1	E2F1
HIPK3	CDKN1A
ARID4B	TGFBR2
MYLIP	MAP3K12
ESR1	BCL2
NCOA3	MEF2D
KAT2B	PTEN
SOCS1	APP
BCL2L11	RUNX1
TGFBR2	NRAS
BMPR2	VEGFA
FGFR2	MUC17
CCND1	MYC
KDR	BNIP2
CASP8	THBS1
ITGB8	CCND2
CUL5	E2F3
TLR2	MAPK9
PRKAA1	RB1
PPP2R5E	RBL1
CYP19A1	RBL2
GCM1	WEE1
MYCN	IRF2
	KIT
	EGLN3
	PPARG
	BAMBI
	CRIM1
	MAP2K3
	PURA
	ARHGAP12
	TSG101
	SIRPA

while for Biocarta approximately 56.1% of putative target genes (n=32) were classified into 8 pathways. Two pathways seem likely involved in APS. TGF-Beta signalling pathway generated by KEGG which is shown in Fig. 2, involves genes such as Bone Morphegenetic Protein Receptor type II (BMPR2), Retinoblastoma-like 1 (RBL1), Retinoblastoma-like 2 (RBL2), Thrombospondin 1 (THBS1), Transforming Growth Factor beta receptor II (TGFBR2) and V-myc Myelocytomatosis viral Oncogene Homolog (MYC).[8,9] While Hypoxia Inducible Factor 1 alpha subunit (HIF1A), Kinase insert domain receptor (KDR) and Vascular Endothelial Growth Factor A (VEGFA) were seen to be involved in VEGF, hypoxia, and angiogenesis pathway which is shown in Fig. 3 generated by Biocarta.[10]

DISCUSSION

MicroRNA expression occur in post transcriptional expression. It has been well known for its involvement in most cellular activities including gene regulations during development and differentiation. Many studies have been done to identify the involvement of miRNA in cellular processes of diseases. These findings contributed to the better understanding in the mechanism of the diseases. Cancer research

TABLE 3: GO terms, KEGG and Biocarta Pathways for miR-19b and miR-20a putative genes

	No. of genes included	% of genes included	*P*-value Benjamin adjusted
GO Term for putative target genes			
Reproductive process	21	36.8	5.4E-8
Growth	18	31.6	7.8E-8
Development process	42	73.7	8.7E-8
Biological regulation	56	98.2	1.2E-7
Signaling	42	73.7	3.8E-7
Metabolic process	56	98.2	1.1E-6
Multicellular organismal process	44	77.2	1.0E-6
Immune system process	25	43.9	4.9E-6
Rhythmic process	10	17.5	4.4E-6
Behaviour	13	22.8	1.3E-5
Response to stimulus	46	80.7	1.4E-5
Multi-organism process	21	36.8	1.6E-4
Cellular component organization or biogenesis	36	63.2	2.8E-4
Locomotion	17	29.8	4.1E-4
Reproduction	12	21.1	7.0E-4
Single-organism process	55	96.5	2.9E-3
Localization	31	54.4	2.9E-3
Biological adhesion	12	21.1	9.6E-3
Cellular process	57	100	1.0E-2
KEGG Pathway for putative target genes			
Pathways in cancer	19	33.3	1.7E-10
Bladder cancer	9	15.8	1.5E-8
Chronic myeloid leukemia	9	15.8	1.2E-6
Prostate cancer	9	15.8	3.5E-6
Cell cycle	10	17.5	3.0E-6
Glioma	7	12.3	6.6E-5
Melanoma	7	12.3	1.1E-4
Pancreatic cancer	7	12.3	1.1E-4
Small cell lung cancer	7	12.3	2.4E-4
Focal adhesion	9	15.8	6.3E-4
p53 signaling pathway	6	10.5	8.2E-4
TGF-beta signaling pathway	6	10.5	2.4E-3
Non-small cell lung cancer	5	8.8	3.4E-3
Acute myeloid leukemia	5	8.8	4.1E-3
Thyroid cancer	4	7.0	5.6E-3
Colorectal cancer	5	8.8	1.4E-2
Endometrial cancer	4	7.0	2.7E-2
MAPK signaling pathway	7	12.3	5.0E-2
Renal cell carcinoma	4	7.0	5.3E-2
Wnt signaling pathway	5	8.8	8.4E-2
ErbB signaling pathway	4	7.0	8.5E-2
Toll-like receptor signaling pathway	4	7.0	1.2E-1
Endocytosis	5	8.8	1.3E-1
mTOR signaling pathway	3	5.3	1.6E-1
Insulin signaling pathway	4	7.0	2.0E-1

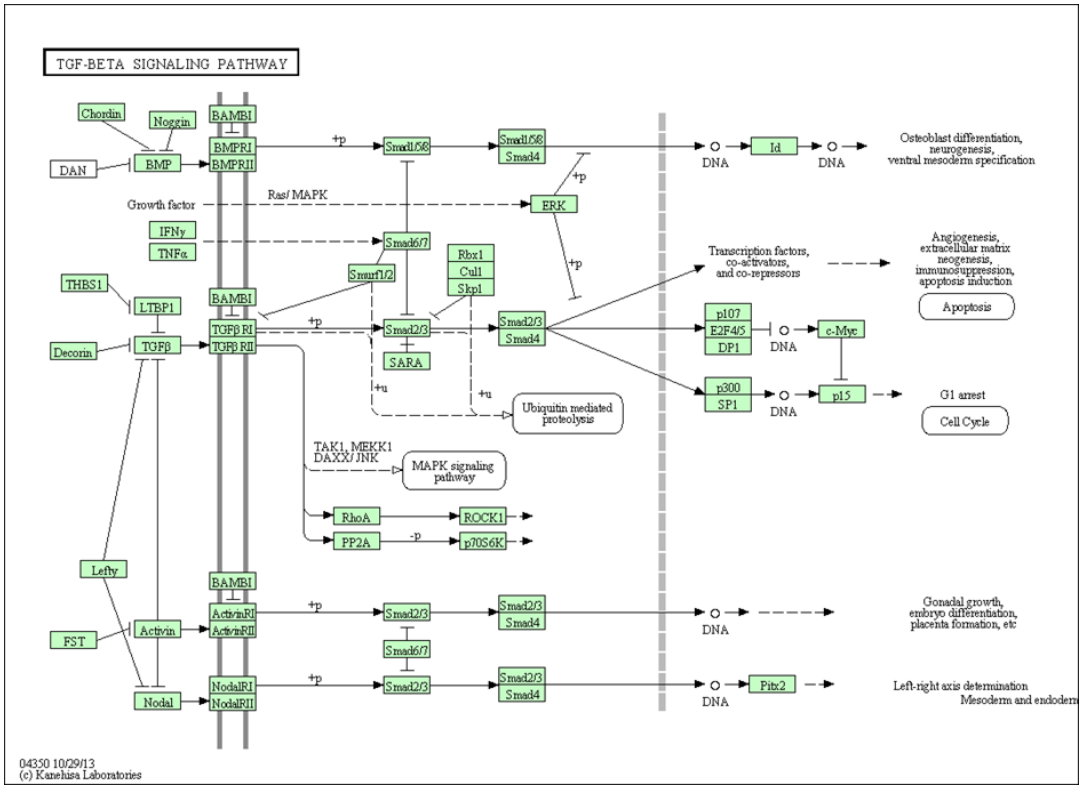

FIG. 2: TGF-Beta Signaling Pathway generated by Kyoto Encyclopedia of Genes and Genome (KEGG). Reproduced with permission from KEGG.

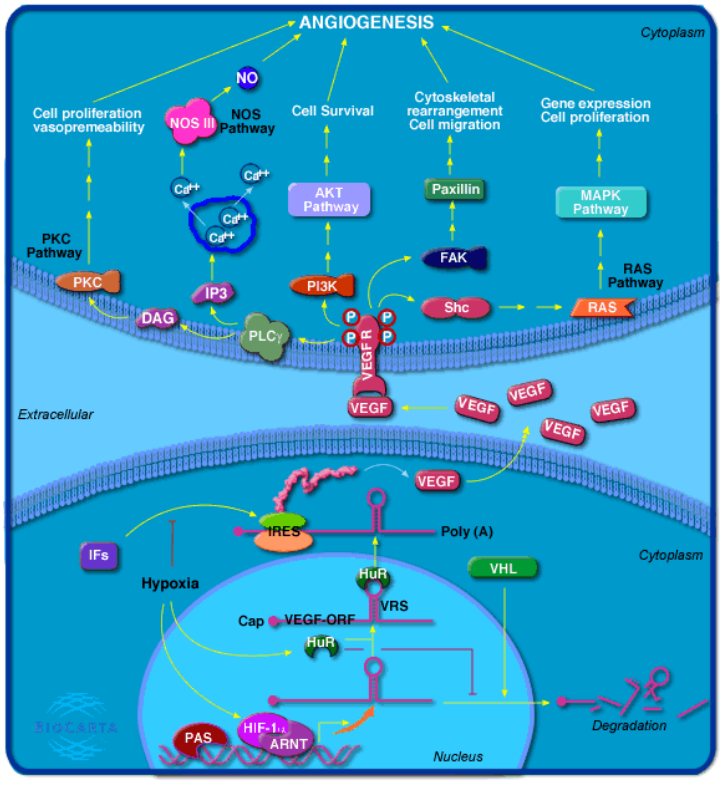

FIG. 3: VEGF, hypoxia, and angiogenesis pathway generated by Biocarta. Reproduced with permission from Biocarta.

for example has benefited from the enhancement of knowledge in this miRNA study. A systematic review conducted by Ma *et al* explored candidate biomarkers for human colorectal cancer, Guan *et al* conducted a meta-analysis of human lung cancer miRNA expression profiling studies comparing cancer tissue with normal tissues, while Shrestha *et al* did a systematic review on human gastric cancer.[11–13] It is our interest to systematically review miRNA studies that has been performed on APS as none has been done on this topic.

This systematic review identified only one relevant study that reported two miRNAs which are involved in APS cellular regulation. Surprisingly, there were only a few studies done on miRNA in APS and only one reported miRNA expression in human APS sample which were miR-19b and miR-20a. This result suggests the importance of detail miRNA profiling to explore other miRNA involved in order to find potential/credible biomarkers which are consistent throughout the studies. Below are reviews of the two reported miRNAs found in APS.

miR 19b

miR-19b plays a role in endothelial cell apoptosis, inflammation and cancer. A recent study by Tang *et al* showed the association of miR-19b with coronary artery disease.[14] Their *in vitro* investigation proved that endothelial cell apoptosis was significantly enhanced by the inhibition of miR-19b. This was in concordance with the findings that miR-17-92 cluster, which also consist of miR-19b, is significantly downregulated in patients with atherosclerosis.[15] A study by Gantier *et al* found that miR19b-coordinated nuclear factor-kappaB (NF-κB) signalling could regulate inflammation activity of rheumatoid arthritis. In a β-cell transformation model, miR19b was detected as one of the main oncogenic component of miRNA cluster.[16] Cluster of miR-17-92 and miR-106-363 carried a function in both normal development and malignant transformation by promoting proliferation and sustain cell survival by carried out plerotropic function.[17] A study by Sun *et al* showed that miR-19b directly targets p53. This is supported by the finding that overexpression of miR-19b in human cancer cells resulted in the downregulation of p53 protein and other downstream components such as Bax and p21. Another study on *in vitro* miR-19b-mediated-p53 reduction correlated with positive effect of cell migration, invasion and cell cycle where it repressed senescence and apoptosis. Moreover, *in vivo* investigation determined that miR-19b could be one of the controlling factor of tumour growth and metastasis.[18] Ohira *et al* on the other hand studied miR-19b in melanoma cells. He found out that most of melanoma cells were miR19b upregulated. This overexpression of miR-19b directly related with downregulation of Paired-like homeodomain I (PITXI) which is identified as a Human Telomerase Reverse Transcriptase (hTERT) suppressor gene. hTERT was demonstrated to have a significant role in the development of cancer. The study further explained that miR-19b regulates hTERT expression and cell proliferation in which miR19b inhibited PITXI mRNA translation through miR-19b binding site of 3' UTR of PITXI mRNA. In addition, increasing miR-19b had been indicated as a new therapeutic strategy for attenuating cellular apoptosis during myocardial ischemia-reperfusion injury;[17] also miR-19b showed an early sustained upregulation in ischemic models of stroke and may be regulating gene expression to control important cellular pathways.[19,20] These findings gave an important reflect to the APS field, as APS is known to be associated with arterial thrombosis, clinical manifestations involved in cerebral circulation such as stroke, myocardial infarction and transient ischemic attack.[21] Looking at the involvement of miR-19b in gene regulation, further investigations on the role of miR-19b in APS patients is needed to characterise any associated expression pattern.

miR 20a

miR-20a is located at chromosomal no 13q31.3. Interestingly, miR-20a was reported to be involved in carcinoma, hypoxia and stem cell regulation. A study by Chang *et al* determined miR-20a to be associated with local invasion and distant metastasis. High expression of miR-20a correlated with the increase of TGF-βI levels in gallbladder carcinoma (GBC) which was subsequently responsible for metastasis enhancement in GBC cells. *In vivo* and *in vitro* analysis showed that miR-20a induced epithelial-mesenchymal transition and enhanced metastasis. They also observed that blockage of miR-20a restored expression of Smad 7 and attenuated TGF-β-induced cell metastasis.[22] In other study, miR-20a high expression played a role in promoting osteogenic differentiation of human mesenchymal stem cells (hMSCs) by targeting osteoblast antagonist markers which

were Bambi and Crim1 in negative regulation of BMP signalling.[23] Recently, a novel mechanism involving miR-20a in regulation of hypoxic stress, aberrant ERK phosphorylation and angiogenesis was discovered. This study demonstrated miR-20a contribution in the development of endometriosis, where it stimulated endothelial and endometrial cell proliferation through prostaglandin-E-induced expression of fibroblast growth factor-9 by upregulation of miR-20a.[24] The involvement of miR-20a in endometrium and endothelia was interesting, as endothelial cells activation by antibody binding was suggested as one of the pathogenic mechanisms leading to prothrombotic state.[25]

MicroRNA target gene functional analysis

Genes targeted by miR-19b and miR-20a are notified to be involved in binding, catalytic activity and protein binding factor activity. Those findings were supported by the study that stated the relationship of aPL with the alteration of endothelial adhesion molecule and upregulation of nitric oxide (NO) and tissue factor (TF) in triggering pathogenesis of APS.[26] Apart from thrombosis, inflammatory immune response had been associated with the second hit to the pathogenesis of APS. Pro-inflammatory and anti-inflammatory balance in immune response is important for normal pregnancy. A tip towards pro-inflammatory event will trigger complement, tumour necrosis factor (TNF) and CC (beta) chemokines in initiating APS.[27] This could be in concordance to involvement of the miRNA target genes in the immune system process as classsified by the DAVID system.

Meanwhile, two pathways generated for miR-19b and miR-20a target genes are "VEGF, Hypoxia and Angiogenesis pathway" and TGF-Beta Signaling pathway. Interestingly, the two pathways generated were directed towards the angiogenesis event. Angiogenesis is a process of new blood vessel formation as a response to the thrombotic event that are associated with the APS manifestations. The increased levels of angiogenic cytokines in APS patients further supported the possible association of angiogenesis with the pathogenic effect of aPL. An elevation in the level of angiogenic factor was observed in a study where they demonstrated how aPL by its proinflammatory phenotype can react with decidual cells, in the newly formed tissue of placenta which was undergoing active angiogenesis.[28] A study by Simone *et al* described the effect of aPL on human endometrial endothelial cell (HEEC) resulting in decreased angiogenesis, whereby aPL inhibited the activation of NF-κB in HEEC. NF-κB is responsible for the expression of matrix metalloproteinases (MMPs) which secrete zinc dependent endopeptides that are required for angiogenesis, cell invasion and ECM degradation.[29] In APS, the upregulation of angiogenic factor level suggested a counter regulatory mechanism for thrombus formation by promoting endothelial cells (ECs) and endothelial progenitor cells (EPCs) migration and the expression of endothelial damage and repair markers including endothelial microparticles (EMPs), circulating ECs and EPCs.[30]

miRNA as potential biomarkers in APS

miRNA provides a potential biomarker as the expression of miRNAs provide important information on the underlying pathogenesis of diseases through the regulation of genes and protein synthesis. The advantage of miRNA is that it is very stable and sampling is made consistent by virtue of being easily acquired from several sources such as tissue, blood and body fluids making it ideal as biomarkers. Currently, studies on miRNA in APS are very limited. With reference to our systematic review result, presumably the exploration of miRNA involvement in APS only started recently. This is more so in obstetric APS where not a single study has been done. It is an open gap for exploration as dependence on current diagnostic practice can be time-consuming, yielding inconsistent results. The availability of archived human placenta from APS patients can provide stable samples for miRNA profiling. We hypothesise significant differences in expressions of miRNAs which can be developed into predictive biomarkers. Thus, more studies should be done to reveal the miRNAs profile in APS which can bring new understanding on the pathogenesis of APS. In terms of miRNA involvement in the pathogenesis of diseases, autoimmune diseases like rheumatoid arthritis, multiple sclerosis, Type-1 diabetes mellitus and systemic lupus erythematosus are more vastly reported.

APS was mistakenly diagnosed under the umbrella of SLE before acquiring its own identity. A profiling microarray analysis conducted by Dai *et al* in 23 SLE patients (10 normal controls), found that seven miRNAs were downregulated whereas nine were upregulated.[31] This finding provided new understanding in the pathogenesis of SLE whereby DNA methylation

play a crucial role in this disease process. Further on, Pan *et al* found that two miRNAs are involved namely, miR-148a and miR-21. miR-148a directly downregulated DNA methyltranferase I (DNMT I) by targeting protein coding region and its transcript whereas miR-21 indirectly downregulated the same DNMT I by targeting gene RAS Guanyl Releasing Protein I(RASGRD I). It was later suggested that inhibition of miR-21 and miR-148a expression in CD4+ T cells would attenuate DNA hypomethylation.[32]

Multiple sclerosis (MS) is an autoimmune disease in which our own immune system attack the protective myelin sheath covering nerves. This will cause inflammation leading to intermittent neurological disorder and disability. A study by Keller *et al* on miRNA expression profiling in blood, where they compared patients with relapsing-remitting MS (RRMS) and healthy controls, found that 165 miRNAs were differentially expressed in patients with RRMS. The most significant miRNA marker found was hsa-miR-145.[33]

Rheumatoid Arthritis (RA) is an autoimmune disease, a chronic inflammation that affects joint lining causing bone and cartilage destruction.[34] A recent study by Nakamachi *et al* found the relationship between apoptotic regulation in RA and miRNA-dependence. They found that miR-124a was significantly decreased in synoviocytes of RA patient as compared to patients with osteoarthritis. miR-124a was also shown to significantly suppress proliferation with arrested cycle at G1 phase. It also significantly suppressed cyclin-dependent kinase 2 (CDK-2) and monocyte chemoattractant protein 1 (MCP-1) proteins in RA synoviocytes.[35]

Type 1 diabetes mellitus (T1DM) is a problem marked by destruction of insulin producing β-cells in human pancreas which further lead to failure in insulin production. miR-375 and miR-25 were reported to be involved in the pathogenesis of T1DM. High expression of miR-375 in pancreatic islets was correlated to normal glucose homeostasis while in miR-375 knockdown mice, hyperglycemia, increased alpha-cells and increased gluconeogenesis were demonstrated.[36] A study found that 12 miRNAs were upregulated in T1DM with some of the miRNAs were associated to apoptosis and beta-cell pathways. Interestingly, miR-25 was suggested to be a tissue-specific marker for glycaemic control in new onset T1DM children.[37]

Conclusion

In conclusion, only two miRNAs associated with APS were reported from one study we identified through our systematic review, which are miR-19b and miR-20a. Both miR-19b and miR-20a were found to be downregulated. Genes that were targeted by both miRNAs were found to involve in TGF-Beta Signalling and VEGF, hypoxia and angiogenesis pathway. The findings may provide the overview of the current study on involvement of microRNA in the pathogenesis of APS and suggest candidate microRNAs that may be used as potential biomarkers in diagnosis of the disease. However, more investigations are needed to characterise the miRNA expression profile in APS to suggest the involvement of this new marker candidate in the mechanism of the disease especially in obstetric APS.

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