

# MicroRNAs in the Vitreous Humor of Patients with Retinal Detachment and a Different Grading of Proliferative Vitreoretinopathy: A Pilot Study

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**Purpose:** Although the expression of microRNAs (miRNAs) in retinal pigment epithelial (RPE) cells undergoing epithelial-mesenchymal transition (EMT) is involved in the pathogenesis of proliferative vitreoretinopathy (PVR), its expression in the vitreous of patients with primary retinal detachment (RD) and different PVR grading has not yet been investigated. We assessed the expression of miRNAs in the vitreous humor (VH) of patients diagnosed with RD and different grading of PVR.

**Methods:** The VH was extracted from the core of the vitreous chamber in patients who had undergone standard vitrectomy for primary RD. RNA was extracted and TaqMan Low-Density Arrays (TLDA) were used for miRNA profiling that was performed by single TaqMan assays. A gene ontology (GO) analysis was performed on the differentially expressed miRNAs.

**Results:** A total of 15 eyes with RD, 3 eyes for each grade of PVR (A, B, C, and D) and 3 from unaffected individuals, were enrolled in this prospective comparative study. Twenty miRNAs were altered in the comparison among pathological groups. Interestingly, the expression of miR-143-3p, miR-224-5p, miR-361-5p, miR-452-5p, miR-486-3p, and miR-891a-5p increased with the worsening of PVR grading. We also identified 34 miRNAs showing differential expression in PVR compared to control vitreous samples. GO analysis showed that the deregulated miRNAs participate in processes previously associated with PVR pathogenesis.

**Conclusions:** The present pilot study suggested that dysregulated vitreal miRNAs may be considered as a biomarker of PVR and associated with the PVR-related complications in patients with RD.

**Translational Relevance:** The correlation between vitreal miRNAs and the pathological phenotypes are essential to identify the novel miRNA-based mechanisms underlying the PVR disease that would improve the diagnosis and treatment of the condition.

## Introduction

Proliferative vitreoretinopathy (PVR) is a multifactorial and complex clinical syndrome common to a variety of clinical disorders, including retinal detachment (RD).<sup>1</sup> The frequency of PVR remains largely unchanged in primary RD, with the incidence ranging from 5.1% to 11.7% of all rhegmatogenous RDs, and it is believed to be the leading cause of RD surgery failure accounting for 75% of retinal redetachment surgeries.<sup>1,2</sup> PVR is characterized by pre-, sub-, or intra-retinal fibrosis (scarring) that grows on the membrane surface of the detached retina and posterior hyaloids causing foreshortening of the retina, traction, and recurrent detachment mostly within the first 6 to 8 weeks after surgery.<sup>1</sup> Typically, PVR with recurrent RDs requires additional surgical interventions and is associated with poor visual recovery.<sup>2–8</sup>

Although the pathogenesis is not elucidated,<sup>8–10</sup> previous studies suggested that the epithelial-mesenchymal transition (EMT)<sup>11–13</sup> of the retinal pigment epithelial (RPE) cells and the inflammatory response-associated pathways might be involved in the pathogenesis underlying PVR.<sup>13–18</sup> However, to date, there are no effective medications for the prevention and treatment of PVR and an urgent approach is demanded.<sup>19–22</sup>

MicroRNAs (miRNAs) are small endogenous noncoding RNAs that negatively regulate gene expression within all cell types. The miRNAs play a key role in cellular physiology and various biological pathways in specific cell types and tissues.<sup>23–25</sup> Previously, abnormal miRNA expression has been reported in cellular and extracellular compartments with respect to cancers and other diseases, such as cardiac, neurological, and ocular,<sup>26–32</sup> and previous studies have shown that specific miRNAs induce/inhibit EMT in other fibroblast-like cells.<sup>33–35</sup>

The aberrant expression of miRNAs in RPE cells undergoing EMT is involved in the pathogenesis of PVR.<sup>36–44</sup> Takayama et al. demonstrated the involvement of miR-148a-3p in the regulation of migration ability of RPE cells,<sup>36</sup> and the same function was reported by Wang et al. for miR-182.<sup>44</sup> Nevertheless, the characteristics and the distinct role of miRNAs in PVR and their expression in the vitreous of patients with primary RD with different PVR grading have been poorly investigated, with only few papers published.<sup>37,45</sup>

In this pilot study, 754 miRNAs were subjected to real-time PCR expression profiling in order to identify the differentially expressed miRNAs in the vitreous of

patients diagnosed with primary RD and a different grading of PVR.

## Materials and Methods

This prospective pilot study included consecutive eyes undergoing pars plana vitrectomy for the treatment of primary RD with and without PVR.

All surgery procedures were performed by the same surgeon at the Department of General Ophthalmology, Medical University of Lublin (Poland) between January and June 2018.

The exclusion criteria were as follows: patients with diabetes mellitus, known rheumatic and autoimmune diseases, systemic treatments involving corticosteroids or immunomodulatory drugs, vitreous hemorrhage, uveitis, glaucoma, or any concomitant retinal pathology, a previous ocular trauma, a diagnosed eye tumor, or who had undergone intraocular surgery or treatment within 6 months after the diagnosis of RD. These systemic or ocular comorbidities might influence the mechanisms underlying ocular fibrosis.

The present patient study was approved by the Ethics Committee of the Medical University of Lublin (KE-0254/277/2019) in compliance with the Declaration of Helsinki. Written informed consent was obtained from each participant allowing the use of their biological materials and clinical data.

## PVR Grading and Patient Grouping

Based on the severity of the PVR, the patients were classified into four stages: A (minimal), B, C, and D (massive) according to the “Retina Society Terminology Committee.”<sup>46</sup>

As proposed by Zandi et al.,<sup>18</sup> in the current study, the risk of developing postoperative PVR in patients with RD with low PVR severity (grades A or B) or without PVR was found to be similar. Thus, PVR grade C was included until three quadrants were seen with visible PVR membrane formation. However, the severity was grade D if all four quadrants were affected.

Because advanced PVR is challenging for accurate grading, all patients underwent indirect fundus ophthalmoscopy with scleral indentation prior to surgery. Two masked expert retinal specialists (M.D.T. and K.N.) investigated the fundus and assigned the PVR score; the discrepancies were resolved by a third investigator (R.R.).

Control vitreous samples (CTRL) were obtained from patients who underwent vitrectomy for primary symptomatic idiopathic floaters.

## Handling of Vitreous Fluid Samples

A 3-port 23-gauge vitrectomy was performed on all the patients while they were under local anesthesia. The Resight 700 (Carl Zeiss Meditec AG, Jena, Germany) wide-angle viewing system or the Binocular Indirect Ophthalmic-Microscope wide-angle viewing system (BIOM; Oculus Inc., Wetzlar, Germany) was used. Sclerotomy was carried out at 3.5 mm parallel to the limbus at 30°. Then, 2 mL vitreous sample extracted from the core of the vitreous cavity before vitrectomy was subjected to centrifugation at  $700 \times g$  for 10 minutes to exclude any circulating cells or debris. The pellets were stored at  $-80^{\circ}\text{C}$  until further analysis.

## miRNA Expression Profiling in Vitreous Humor by TLDA

Total RNA was isolated from 400  $\mu\text{L}$  vitreous humor (VH) using miRNeasy Mini Kit (Qiagen), according to the manufacturer's protocol. The amount and purity of RNA were assessed using NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific). The expression of 754 miRNAs was evaluated by real-time PCR using the TaqMan Low-Density Arrays (TLDA) from 15 VH samples (3 patients for each grade of the disease and 3 unaffected individuals). About 30 ng of RNA was transcribed using TaqMan MicroRNA Reverse Transcription Kit and Megaplex RT Primers Human Pool A version 2.1 and Pool B version 3.0 (Thermo Fisher Scientific) and pre-amplified by TaqMan PreAmp Master Mix Kit and Megaplex PreAmp Primers using the Human Pool A version 2.1 and Pool B version 3.0 (Thermo Fisher Scientific). The products were loaded in TaqMan Human MicroRNA Array version 3.0 A and B (Thermo Fisher Scientific), and the real-time PCR reactions were carried out on a 7900 HT Fast Real-Time PCR System (Applied Biosystems) using TaqMan Universal Master Mix II without uracil-DNA N-glycosylase UNG (Thermo Fisher Scientific), according to the manufacturer's instructions. Expression fold changes of differentially expressed (DE) miRNAs were calculated by applying the  $2^{-\Delta\Delta\text{Ct}}$  method.<sup>47</sup>

## Statistical Analysis

The expression data were subjected to significance analysis of microarrays (SAMs), computed by Multi Experiment Viewer version 4.8.1 (<http://mev.tm4.org>) using the multiclass tests and 1-way analysis of variance (ANOVA) test ( $P < 0.05$ ) among  $\Delta\text{Ct}$ s. The endogenous control was selected based on the global median normalization method, which allowed us to

identify the miRNAs with the most stable expression in the samples.<sup>24</sup> We used three different endogenous controls for each TLDA panel (comparisons among PVR stages: panel A: miR-197, U6, and median of  $\Delta\text{Ct}$ s; panel B: miR-1285, U6, and median of  $\Delta\text{Ct}$ s; PVR versus CTRL: panel A: median of  $\Delta\text{Ct}$ s, miR-146a-5p, and miR-28-3p; panel B: median of  $\Delta\text{Ct}$ s, miR-625-3p, and miR-30e-3p; A + B versus CTRL: panel A: miR-29a-3p, miR-20b-5p, and miR-24-3p; panel B: miR-30-3p, median of  $\Delta\text{Ct}$ s, and miR-30a-5p; C + D versus CTRL: panel A: miR-17-5p, miR-320a, and miR-28-3p; panel B: U6, median of  $\Delta\text{Ct}$ s, and miR-30e-3p), considering differentially expressed only those miRNAs that were deregulated according to two endogenous control. Gene ontology (GO) analysis was performed on the DE miRNAs through DIANA-miRPath version 3.0 (<http://snf-515788.vm.okeanos.grnet.gr/>).<sup>48</sup>

## Results

### miRNA Expression Profile in the VH of Patients With PVR

The expression of 754 miRNAs in the VH of 15 patients, including 3 patients for each grade of the disease (A, B, C, and D) and 3 unaffected individuals, was analyzed by TLDA profiling. When comparing the different disease stages, statistical analysis of profiling results was performed by grouping A and B samples, characterized by absent or minimal proliferation. We identified 20 miRNAs with altered expression in one or more pathological groups, according to at least two out of three endogenous controls. Specifically, let-7b-5p, miR-100-5p, miR-1300, miR-143-3p, miR-152-3p, miR-16-5p, miR-19b-3p, miR-203a, miR-21-5p, miR-218-5p, miR-223-5p, miR-224-5p, miR-30b-5p, miR-335-5p, miR-340-5p, miR-361-5p, miR-452-5p, miR-486-3p, miR-891a-5p, and miR-99a-5p showed differential expression in different comparisons (Table 1, Fig. 1).

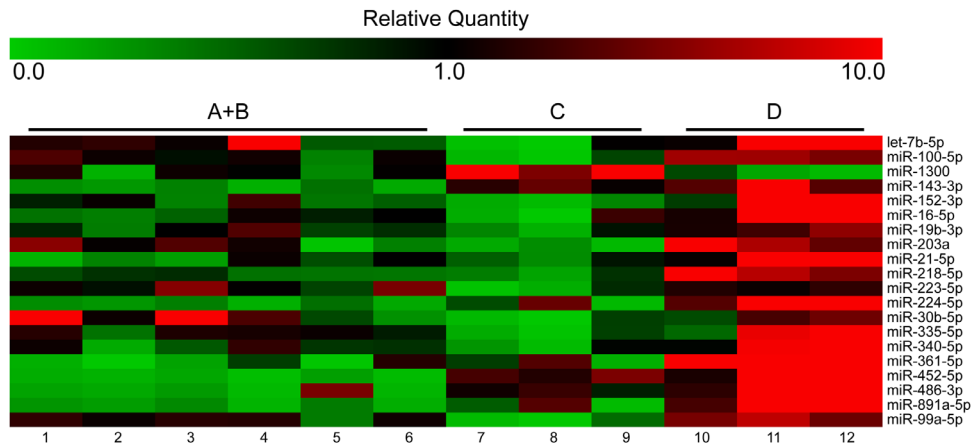
Interestingly, miR-143-3p, miR-224-5p, miR-361-5p, miR-452-5p, miR-486-3p, and miR-891a-5p expression aggravated the disease, suggesting a possible application of these miRNAs as biomarkers for PVR (Fig. 2).

In addition, we compared the vitreous of patients with PVR to unaffected individuals. This analysis was performed in three steps, grouping all PVR samples, samples from stages A and B, and samples from stages C and D, respectively. This additional analysis showed the deregulation of different sets of miRNAs in each comparison (Fig. 3, Tables 2, 3, 4).

**Table 1.** TLDA Profiling Showed the Differential Expression of 20 MiRNAs in the Different Comparisons

miRNA	ANOVA	C Versus A + B		D Versus A + B		D Versus C	
	P Value	FC	P Value	FC	P Value	FC	P Value
let-7b-5p	<b>0.022</b>	-16.85	<b>0.021</b>	3.76	0.33	<b>28.76</b>	<b>0.009</b>
miR-100-5p	<b>0.003</b>	-13.21	<b>0.008</b>	4.9	0.06	<b>24.76</b>	<b>0.001</b>
miR-1300	<b>0.003</b>	<b>12.59</b>	<b>0.005</b>	-3.45	0.1	-23.53	<b>0.001</b>
miR-143-3p	< <b>0.0001</b>	<b>9.93</b>	<b>0.0005</b>	<b>26.41</b>	< <b>0.0001</b>	2.65	0.08
miR-152-3p	<b>0.01</b>	-5.28	0.1	<b>12.03</b>	<b>0.024</b>	<b>33.68</b>	<b>0.003</b>
miR-16-5p	<b>0.026</b>	-4.94	0.22	<b>16.77</b>	<b>0.035</b>	<b>32.68</b>	<b>0.009</b>
miR-19b-3p	<b>0.019</b>	-2.79	0.1	<b>3.59</b>	<b>0.049</b>	<b>10.03</b>	<b>0.006</b>
miR-203a	<b>0.033</b>	-5.64	0.13	8.95	0.07	<b>30.51</b>	<b>0.011</b>
miR-21-5p	<b>0.011</b>	1.19	0.83	<b>22.15</b>	<b>0.004</b>	<b>18.56</b>	<b>0.013</b>
miR-218-5p	< <b>0.0001</b>	-1.45	0.27	<b>15.28</b>	< <b>0.0001</b>	<b>22.3</b>	< <b>0.0001</b>
miR-223-5p	<b>0.016</b>	-11.65	<b>0.008</b>	1.14	0.85	<b>13.3</b>	<b>0.013</b>
miR-224-5p	<b>0.003</b>	2.46	0.38	<b>36.26</b>	<b>0.001</b>	<b>28.97</b>	<b>0.01</b>
miR-30b-5p	<b>0.039</b>	-26.91	<b>0.017</b>	-1.02	0.98	<b>26.33</b>	<b>0.034</b>
miR-335-5p	<b>0.025</b>	-8.72	<b>0.033</b>	3.09	0.22	<b>26.99</b>	<b>0.009</b>
miR-340-5p	<b>0.031</b>	-2.91	0.25	<b>8.36</b>	<b>0.039</b>	<b>24.38</b>	<b>0.012</b>
miR-361-5p	<b>0.003</b>	4.82	0.26	<b>36.81</b>	<b>0.001</b>	<b>18.78</b>	<b>0.014</b>
miR-452-5p	<b>0.0002</b>	<b>19.17</b>	<b>0.001</b>	<b>33.38</b>	< <b>0.0001</b>	5.6	0.07
miR-486-3p	<b>0.011</b>	6.06	0.08	<b>32.98</b>	<b>0.004</b>	5.43	0.14
miR-891a-5p	<b>0.002</b>	2.46	0.4	<b>35.38</b>	<b>0.0007</b>	<b>21.01</b>	<b>0.005</b>
miR-99a-5p	<b>0.001</b>	-21.67	<b>0.002</b>	3.82	0.1	<b>32.84</b>	<b>0.0006</b>

The average FC and the P value derived from multiple comparisons for each miRNA are shown. The P value of the ANOVA test between all groups is also shown. Significant P values are highlighted in bold. FC, fold change.

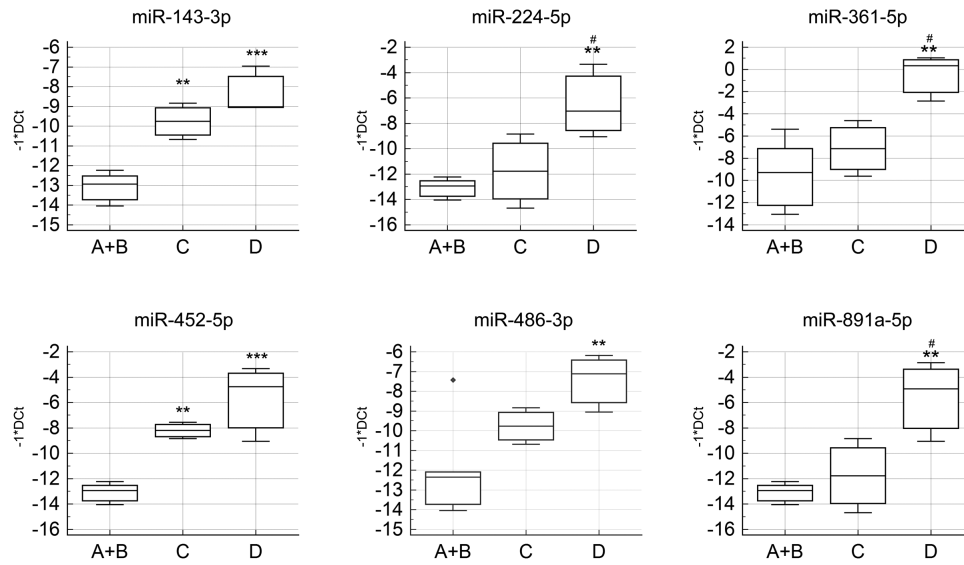


**Figure 1.** Heatmap showing the expression of miRNAs analyzed through TLDA profiling. The miRNA expression is represented as relative quantity (RQ), calculated with respect to the average of  $\Delta$ Cts of all the analyzed samples.

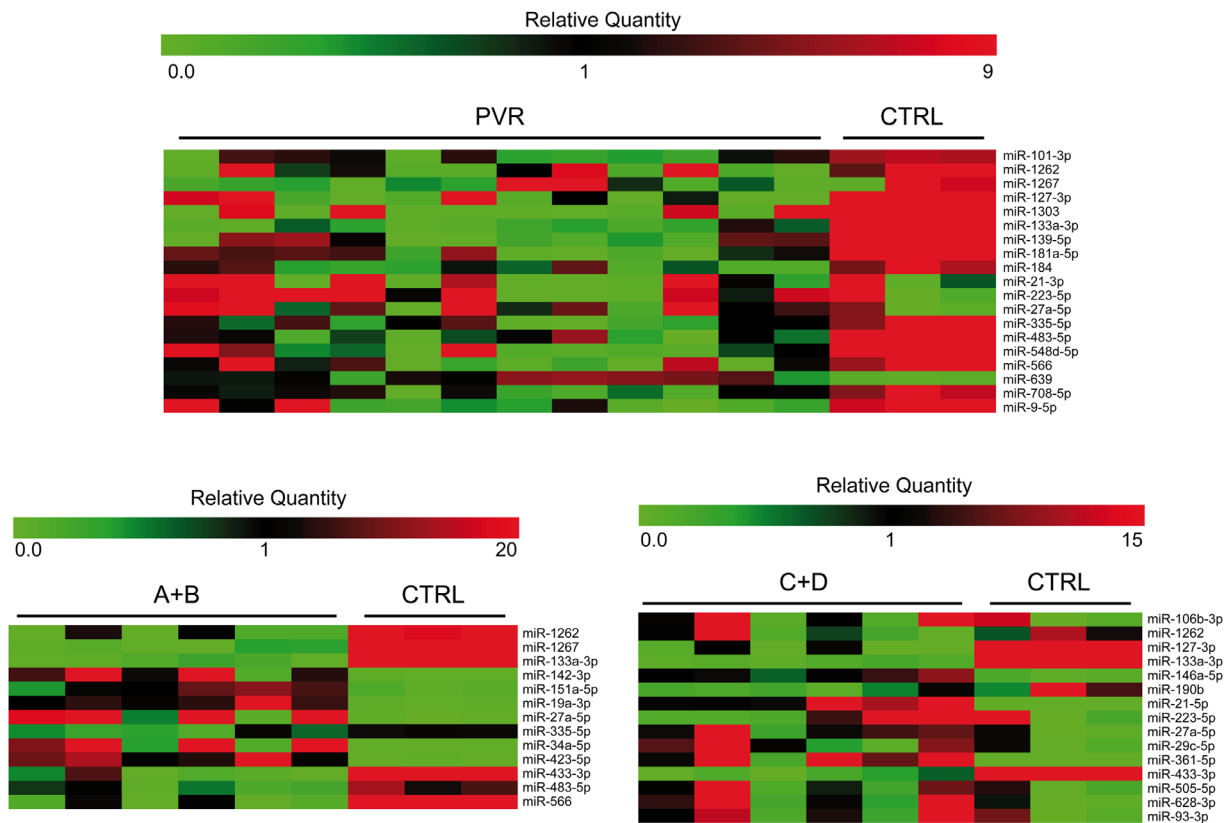
**GO analysis**

GO analysis was performed on the six miRNAs showing increased expression with PVR worsening. GO results showed that miR-143-3p, miR-224-5p, miR-361-5p, miR-452-5p, miR-486-3p, and

miR-891a-5p participate in the biological processes involved in PVR pathogenesis, such as cell cycle regulation, adhesion to the extracellular matrix (ECM), and regulation of actin cytoskeleton<sup>10,49-51</sup> (Fig. 4).

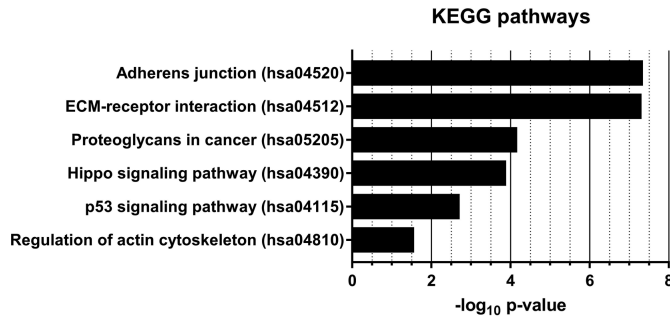


**Figure 2.** Boxplots showing the expression of miR-143-3p, miR-224-5p, miR-361-5p, miR-452-5p, miR-486-3p, and miR-891a-5p in the three pathological groups. \*\* *P* value versus A + B < 0.005; \*\*\* *P* value versus A + B < 0.0005; # *P* value versus C < 0.05.



**Figure 3.** Heatmap showing the expression of miRNAs analyzed through TLDA profiling in the comparisons of patients with PVR compared to unaffected individuals. The miRNA expression is represented as relative quantity (RQ), calculated with respect to the average of  $\Delta$ Cts of all the analyzed samples.





**Figure 4.** PVR-related Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways regulated by miR-143-3p, miR-224-5p, miR-361-5p, miR-452-5p, miR-486-3p, and miR-891a-5p. The X-axis represents the  $-\log_{10}$  of the *P* value for each pathway.

Similarly, GO analysis was performed on miRNAs showing altered expression in the three comparisons with respect to unaffected individuals. Again, results showed the involvement of DE miRNAs in biological processes previously associated with PVR pathogenesis<sup>10,49-51</sup> (Fig. 5).

## Discussion

The current pilot study identified altered expression of 20 miRNAs in one or more pathological groups of the VH of patients with primary RD and a different grading of PVR. The analysis revealed that the expression of six miRNAs (miR-143-3p, miR-224-5p, miR-361-5p, miR-452-5p, miR-486-3p, and miR-891a-5p) increased with the worsening of the disease; similarly, expression profiles of vitreal miRNAs in patients with PVR were compared with unaffected individuals, showing that several miRNAs may be used as molecular biomarkers for the disease. According to the GO analysis, these miRNAs participated in the biological processes involved in PVR pathogenesis, such as cell cycle regulation, adhesion to ECM, and regulation of actin cytoskeleton.

PVR is the main cause of retinal surgical failure.<sup>52</sup> The PVR is primarily treated using vitrectomy, systematic peeling and dissecting epiretinal membranes, and retinal tamponade with silicone oil or gas.<sup>53-55</sup> However, recurrent traction proliferation causes postsurgical retinal re-detachment.<sup>52</sup>

Currently, the adjuvant therapy for the treatment of PVR includes agents, such as anti-inflammatory drugs, growth factor inhibitors, and antioxidants.<sup>2,22</sup> Recently, a series of low-dose methotrexate injections seem to be beneficial for treating complex retinal detachment caused by PVR,<sup>56,57</sup> however, all these therapies are quite elusive and an urgent approach is demanded. Thus, additional studies are essential to

**Table 2.** The miRNAs Showing Altered Expression in all Patients With PVR Compared to Unaffected Individuals

miRNA	PVR versus CTRL	
	FC	<i>P</i> Value
miR-101-3p	-6.94	0.001
miR-1262	-24.41	3.00E-05
miR-1267	-54.44	0.001
miR-127-3p	-49.79	6.00E-05
miR-1303	-45.25	2.00E-05
miR-133a-3p	-61.18	1.00E-05
miR-139-5p	-23.79	1.00E-04
miR-181a-5p	-13.55	0.002
miR-184	-7.37	2.00E-04
miR-21-3p	22.23	0.001
miR-223-5p	57.22	1.00E-06
miR-27a-5p	53.55	0.001
miR-335-5p	-10.61	0.002
miR-483-5p	-15.59	2.00E-04
miR-548d-5p	-13.3	7.00E-04
miR-566	-33.43	1.00E-05
miR-639	13.24	7.00E-06
miR-708-5p	-7.68	4.00E-04
miR-9-5p	-13.95	0.001

The average FC and the *P* value derived from the *t*-test are shown for each miRNA.

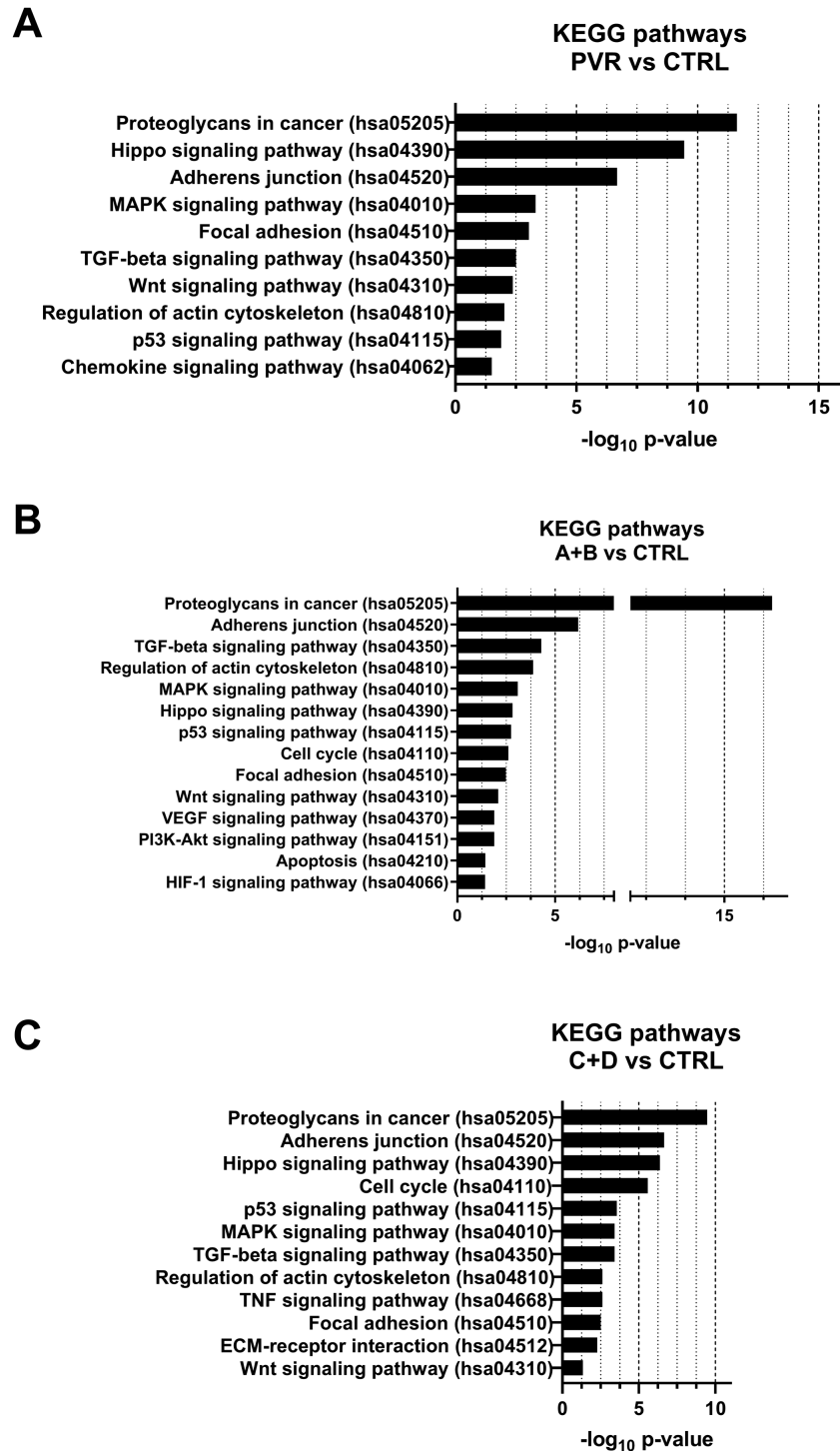
FC, fold change.

**Table 3.** The MiRNAs Showing Altered Expression in Stage A + B Patients Compared to Unaffected Individuals

miRNA	A + B versus CTRL	
	FC	<i>P</i> Value
miR-1262	-27.56	0.003
miR-1267	-59.32	0.001
miR-133a-3p	-63.19	1.00E-05
miR-142-3p	32.66	0.001
miR-151a-5p	18.31	1.00E-03
miR-19a-3p	25.32	1.00E-04
miR-27a-5p	48.41	0.003
miR-335-5p	-5.75	0.007
miR-34a-5p	38.92	0.003
miR-423-5p	45.42	6.00E-05
miR-433-3p	-41.76	0.002
miR-483-5p	-10.67	0.003
miR-566	-38.37	0.002

The average FC and the *P* value derived from the *t*-test are shown for each miRNA.

FC, fold change.



**Figure 5.** PVR-related Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways regulated by miRNAs showing altered expression in the comparisons PVR versus CTRL, A + B versus CTRL, and C + D versus CTRL. The X-axis represents the  $-\log_{10}$  of the *P* value for each pathway.

elucidate the mechanisms regulating the early phases and development of PVR.

The development and progression of fibrotic lesions, including proliferative diabetic retinopathy (PDR) and PVR are caused by EMT. In addition, wound healing and stimulation of inflammatory

cytokines lead to EMT, thereby forming pre- or subretinal fibrous membranes.<sup>1</sup>

Importantly, RPE cells play a vital role in the development of fibrosis on the retina and constitute the largest cellular component of epiretinal membranes in addition to hyalocytes, retinal Müller glial cells,

**Table 4.** The MiRNAs Showing Altered Expression in Stage C + D Patients Compared to Unaffected Individuals

miRNA	C+D vs CTRL	
	FC	P-Value
miR-106b-3p	42.68	0.001
miR-1262	-21.96	0.008
miR-127-3p	-56.47	0.003
miR-133a-3p	-45.23	6.00E-06
miR-146a-5p	11.88	0.005
miR-190b	-24.04	7.00E-05
miR-21-5p	43.92	6.00E-04
miR-223-5p	47.08	0.003
miR-27a-5p	53.79	0.001
miR-29c-5p	28.51	0.001
miR-361-5p	52.31	0.002
miR-433-3p	-44.76	0.005
miR-505-5p	28.64	0.002
miR-628-3p	31.01	0.001
miR-93-3p	39.46	0.001

The average FC and the *P* value derived from the *t*-test are shown for each miRNA.

FC, fold change.

fibroblasts, and macrophages.<sup>1</sup> RPE cells are usually quiescent in healthy condition. Interestingly, trauma or intraocular diseases damage the RPE or cause retinal detachment. The subsequent repair triggers the loss of cell-cell contact in RPE cells, and, also, the epithelial cells are stimulated to proliferate into motile fibroblast-like cells.<sup>1</sup>

Initially, the transforming growth factor- $\beta$  (TGF- $\beta$ ), promotes various types of fibrotic diseases, including PVR and PDR.<sup>1,54,55,58-64</sup> Subsequently, trans-differentiated RPE cells migrate into the inner retinal layers or vitreous body, produce ECM components, and transform into fibroblast-like cells. This phenomenon results in the formation of epiretinal membranes that contract and cause retinal detachment as well as visual impairment.<sup>1,54,55,58-63</sup>

The miRNAs regulate the complex physiological and pathological processes, such as embryogenesis, organ development, oncogenesis, and angiogenesis.<sup>65,66</sup>

Intriguingly, miRNAs are positive or negative regulators of EMT that target the multiple components of the EMT machinery and exacerbate their critical roles in TGF- $\beta$ 2-induced EMT in human RPE cells.<sup>37,67</sup> In addition, miRNAs regulate fibrosis in several organs.<sup>68</sup>

Although the role of miRNAs in PVR is not yet clarified, no study has investigated their expression in the vitreous of patients with RD with different PVR grades. Nevertheless, most of the miRNAs showing altered expression in the inter-stage comparisons has been previously associated with other ocular diseases, as shown in Table 5.<sup>31,37,69-88</sup>

The only study that assessed the miRNA expression in the VH of patients with PVR diseases, including PDR, was performed by Usui-Ouchi and co-workers.<sup>37</sup> These authors used quantitative real time (qRT)-PCR to identify miR-21-5p in the VH as a potential disease-modifying agent. Furthermore, the expression of miR-21-5p is enhanced by the disease-associated expression of TGF- $\beta$ 2 and/or high glucose conditions, which could be crucial in the fibroproliferative response of RPE cells during the development of retinal fibrotic disorders. In addition, the cell migration and proliferation of RPE cells was increased markedly. Also, the level of miR-16-5p was upregulated in the vitreous of the same eyes. Consistent with this report, the current data showed an increased expression of miR-21-5p and miR-16-5p in vitreous patients with PVR.

Among the miRNAs previously associated with EMT, miR-223-5p was shown to be upregulated after TGF- $\beta$  treatment of RPE cells, whereas all the other differentially expressed miRNAs (except for miR-1300 and miR-891a-5p) regulate the EMT in cancer.<sup>40</sup> Further, several studies reported the involvement of differentially expressed miRNAs in angiogenesis in eye-associated diseases (let-7b-5p, miR-152-3p, miR-21-5p, miR-218-5p, and miR-30b-5p)<sup>37,69,74,78,79,81,82,89</sup> or various cancer models (miR-891a-5p).<sup>90</sup> Similarly, among differentially expressed miRNAs, 12 out of 20 (let-7b-5p, miR-152-3p, miR-16-5p, miR-19b-3p, miR-203a, miR-21-5p, miR-224-5p, miR-335-5p, miR-340-5p, miR-486-3p, miR-891a-5p, and miR-99a-5p) are associated with fibrosis regulation.<sup>91-102</sup>

The present pilot study, which we believe has been done for the first time, described a putative correlation between miRNAs and fibrotic phenomena in patients with PVR following RD. Nevertheless, the present study has some limitations: the low number of samples and the modality of grading of PVR. The grading of PVR, even if assigned based on the classification system established by the "Retina Society Terminology Committee,"<sup>46</sup> is still conducive to a subjective clinical choice of the retinal disease specialists and not on an objective diagnostic method. Despite the small number of patients enrolled in this pilot study, our results suggest that vitreal miRNAs could be considered PVR biomarkers. Needless to say, further studies on larger cohorts, possibly including vitreous samples



**Table 5.** Previous Studies Reporting the Association of DE miRNAs Deregulated in Inter Stage Comparisons With Ocular Diseases

miRNA	Disease	Reference
let-7b-5p	Diabetic retinopathy, age-related cataract	69,70
miR-143-3p	Glaucoma, human subconjunctival fibrosis, oxygen-induced retinopathy	71–73
miR-152-3p	Diabetic retinopathy, age-related macular degeneration, oxygen-induced retinopathy	73–75
miR-16-5p	Proliferative vitreoretinopathy, glaucoma, age-related cataract	37,76,77
miR-19b-3p	Idiopathic epiretinal membrane and macular hole	31
miR-21-5p	Proliferative vitreoretinopathy, ischemic retina, diabetic retinopathy	37,78,79
miR-223-5p	Noninfectious uveitis	80
miR-224-5p	Uveal melanoma	81
miR-30b-5p	Diabetic retinopathy	82
miR-335-5p	Age-related macular degeneration	83
miR-340-5p	UVB-mediated retinal pigment epithelium cell damage	84
miR-361-5p	Age-related macular degeneration, retinoblastoma	85,86
miR-452-5p	Cataract	87
miR-486-3p	Retinoblastoma	88

from unaffected individuals, will be useful to validate miRNAs as disease biomarkers.

All authors reviewed and approved the manuscript for submission toward publication.

## Conclusions

In conclusion, a set of DE miRNAs were identified in PVR conditions. Despite the small cohort, our study suggests that specific miRNAs could be considered good candidates as biomarkers for PVR. Furthermore, elucidating the role of other miRNAs in EMT in RPE cells in vitro and in PVR in vivo would provide an in-depth insight into the EMT-related gene expression. Thus, additional studies on the correlation between vitreal miRNAs and the pathological phenotypes are essential to identify the novel miRNA-based mechanisms underlying the PVR disease that would improve the diagnosis and treatment of the condition.

The authors alone are responsible for the content and writing of the paper.

The present study was approved by the Ethics Committee of the Medical University of Lublin (KE-0254/277/2019) in compliance with the Declaration of Helsinki.

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