MicroRNA 125b overexpression in pseudoexfoliation syndrome

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Abstract

Background MicroRNAs (miRs) are small non-coding RNAs. Qin et al. described miR 125b was downregulated in cataract tissue when compared to a transparent lens. The aim of study was (1) to establish the expression of miR 125b in cataracts complicated by pseudoexfoliation syndrome /PEX/, glaucoma or pseudoexfoliation glaucoma and (2) to determine whether any environmental factors influence miR 125b expression. **Methods** Anterior lens capsule were obtained from 150 patients. The patients were selected for three groups: with PEX, primary open angle glaucoma, pseudoexfoliation glaucoma, with matched controls in the term of gender (cataract alone). qPCR expression of microRNA 125b was examined in every groups. **Results** Mean age of 150 patients was 75.18years (SD +/-9.12years). Investigation indicates for the first time, that miR 125b expression is significantly increased 3.33x in PEX group ($p=0,046$). A lower level of miR 125b ($p=0,01$) was found in patients who smoked compared to those who did not. **Conclusions** Our data reveal the possible role of miR 125b in pseudoexfoliation syndrome development. There are two possible interpretations of these results. Either the co-existence of PEX acts as a moderator of miR 125b expression in the anterior lens, or the increased expression of miR 125b can be a cause of PEX.

Background

MicroRNAs (miRs) are a group of small endogenous non-coding RNAs, generally consisting of 21 to 23 nucleotides. They regulate posttranscriptional gene expression through complementary or partial complementary binding with the 3' untranslated region of target mRNA (1). miRs represent prominent class of gene regulators (2-4). A single miR may modulate the expression of several mRNAs, and conversely, a single mRNA strand may interfere with multiple miRs (5). Mature miRs have long half-lives, are not rapidly degraded and can influence different biological pathways by binding to different mRNAs (5). While miRs usually act as negative modulators of gene expression, they can also stimulate translation (6). Previous studies have demonstrated that expression of miRs, such as miR-760, miR-186, miR-20a, miR-24, miR-34a, miR-106a, and miR-449a, can be involved in senescence or age-related diseases including neurological disorders, diabetes, degenerative arthritis, and cataract (2,7).

Distinct expression profiles of miRs in the central epithelium of transparent human lenses and age-related cataract changed human lenses were reported previously (8). In transparent lenses, a high expression of miR 184, miR 1826, miR 125b, miR 1308, miR 26a, miR 638, miR 923, and let 7b was observed. In age-related cataract changed lenses other miRs were identified, including miR 184, miR 1826, let 7b/c, miR 24, mir 23b, miR 23a, miR 923 (8). It is possible that all identified miRs are involved in multifactorial pathogenesis of cataract formation, in which processes of senescence, as well as multiple genetic and environmental factors have been implicated. Specifically, miR 34a may play an important role in lens senescence, given that its expression in lens epithelium is positively correlated with age and the severity of cataract (9). There are probably also different exclusively age-specific gene expression changes in human lenses (10).

The pathogenesis of a cataract is still poorly understood, but Li et al. described lens epithelial cell apoptosis as a common cellular process in cataract development (11). Specifically, the p53 gene is closely associated with lens epithelial cell apoptosis (12). Qin et al. described that micro-RNA 125b (miR 125b) directly targets p53. To this end, miR 125b was downregulated in age-related cataract tissue, when compared with healthy transparent lens tissue and inversely related to the level of p53 (13).

With its monolayer structure and direct exposure to ultraviolet radiation, the lens capsule epithelium seems to be an important model for investigation of complex factors playing role in cellular aging, including genetics and environmental influences (10). Senescence has been implicated in cataract, pseudoexfoliation syndrome (PEX), and primary open angle glaucoma, but disease-specific mechanisms remain elusive.

Pseudoexfoliation syndrome is a common, multifactorial, age-related, genetically determined elastosis. Pathologic pseudoexfoliation material (PEXM) accumulates as a result of excessive production and abnormal netting of elastic fibres, which result in the accumulation of elastic fiber aggregates in tissues of the eye and other organs (14).

Our study aimed to (1) establish the expression of miR 125b in cataract complicated by other eye diseases and to (2) determine whether any environmental factors have any influence on miR 125b expression. For the first time we quantified the expression of miR 125b in lens capsule of patients with cataract, and PEX, primary open angle glaucoma and pseudoexfoliation glaucoma.

Methods

Fresh specimens of anterior lens capsule were obtained from 150 patients between 46 and 92 (mean age 75.18 years, SD +/- 9.12 years) during cataract phacoemulsification surgery. All lens samples were obtained by intact continuous curvilinear capsulorhexis of 5 to 6 mm diameter.

Each patient underwent a complete ophthalmological examination comprising an interview, anterior segment slit lamp biomicroscopy before and after dilation of the pupil, intraocular pressure (IOP) and fundoscopic examination. Patients, who had previously undergone eye surgery or have other ophthalmic diseases (eg. diabetes retinopathy, uveitis, traumas) were excluded.

The patients were consecutively selected and assigned to one of the 4 groups:

patients with cataract and PEX (PEXg) – 38 patients

patients with cataract and primary open angle glaucoma (Gg) – 37 patients

patients with cataract, PEX and glaucoma (pseudoexfoliation glaucoma) (PEXGg) – 30 patients

patients with cataract – control group (Cg) – 45 patients

The severity of cataract changes has been similar in each study group.

Information regarding current ophthalmic medication usage (timolol, brymonidine, prostaglandin analogue, dorzolamide), and lifestyle data, such as smoking habits were elucidated through a patient interview prior to surgery.

RNA isolation and relative real-time RT-PCR

Fragments of lens capsule from patients with cataract were homogenized using MagNA Lyser Green Beads (Roche, Mannheim, Germany) in a lysis buffer. Total RNA was isolated using the NucleoSpin RNA II kit (Macherey-Nagel, Düren, Germany) following the manufacturer's protocol. Reverse transcription reaction (RT) was performed using 3 µl of extracted total RNA, TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific, Foster City, CA, USA) and specific RT primers for RNU6B (RT: Thermo Fisher Scientific) and miR 125b (RT: 000449, Thermo Fisher Scientific) in a final volume of 15 µl according to manufacturer's recommendation. Kapa PROBE Master Mix (Kapa Biosystems, Boston, MA, USA) and specific TaqMan MicroRNA Assays (Thermo Fisher Scientific) were used to the indication of microRNA expression according to recommendations of both manufacturers. 1.33 µl of twice diluted RT product was added into single real-time PCR. All reactions were made in triplicate in a 96-well plate under following thermal cycling conditions: 20 s at 95°C followed by 40 cycles of 1 s at 95°C and 20 s at 60°C. The reactions run in the 7900HT Fast Real-Time PCR System (Thermo Fisher Scientific) and the threshold cycle data (Ct) were collected by SDS 2.2 software (Thermo Fisher Scientific). The relative expression of miR 125b in each sample was calculated using the comparative Ct method ($2^{-\Delta\Delta C_t}$). Patients samples were normalized to RNU6B, calibrated to the control group and presented as a relative quantity (RQ).

Statistical analysis

The qualitative analysis (chi-square test) used for formation of categorized table and quantitative analysis (student's t-test) were performed. The ANOVA analysis with post-hoc (LSD-test) evaluation has been used in defining the differences between each group. The partial analysis was performed in defined by chi-square method intervals of RQ (and logRQ). In addition, the correlation between miR 125b and the age of the patients were assessed. The logistic regression was used to assess the probability of influence of RQ in each specified group. The results were considered statistically significant at a p-value less than 0.05. All calculations were carried out using STATISTICA 12 software. (StatSoft Inc., Tulsa, OK, USA) and MedCalc.

Results

In order to determine the expression level of miR 125b in every group relative real-time PCR was performed. The level of miR 125b expression was increased in each of the study groups (PEXg, Gg and PEXGg) compared to the control group (Figure 1)

The qualitative analysis revealed statistically significant 3.33 time higher miR 125b levels in PEXg cohort as compared to the Cg cohort ($p=0.046$, chi-square test, table 1). There were no statistically significant differences in miR 125b levels between the samples from the Gg and Cg cohorts.

Table 1 shows the mean and standard deviations of RQ values, however the RQ distributions are right-skewed. Therefore the ANOVA test was used after the RQ logarithm transformation. An LSD test as post hoc test was used after ANOVA. No differences in sex distribution between groups has been found ($p=0.4822$).

In the PEXg vs all others (Gg and PEXGg), the number of patients with the RQ value >0.6 was increased by 10%, while 10% fewer patients had an RQ value < -0.3 . This further supports the statistical difference between the described groups (Figure 2).

The quantitative analysis of miR 125b expression conducted between PEXg+PEXGg (combined group of all the patients that have the pseudoexfoliation syndrome with or without glaucoma) and Cg revealed a statistically significant difference ($p=0.04$, t-test) (Figure 3).

Additionally the logistic regression model (Figure 4) revealed the statistically significant correlation (probability $>50\%$) between level of RQ and defined in the study diagnosis (A) existing PEX with or without glaucoma, (B) existing Glaucoma with or without PEX; the PEXG - a secondary glaucoma is combined with primary open angle glaucoma; (C) only PEX, (D) only Glaucoma vs control group. The PEXG group is an additional factor that reverses the 95%CI RQ values from predominantly positive in the PEXg and Gg into the predominantly negative in the combined groups such as PEXg+PEXGg and PEXGg+Gg. This analysis with wide 95%CI range should be specified in further studies. The p-values and 95%CI ranges are: 0.0013, -6.94 – 2.17; 0.0056, -14.32 – 2.09; 0.0002, 1.35-7.90; 0.0027, 0.95 – 12.89 respectively for PEXg+PEXGg, PEXGg+Gg, PEXg and Gg.

Factors that may affect miR 125b expression

There were no statistically significant correlations between the levels of miR 125b and the age of the patients.

During the assesment of relationships between levels of miR 125b and important factors obtained from patients' history records (smoking, drops for glaucoma) only one statistically significant difference was found. A lower level of RQ miR 125b ($p=0.01$) was found in patients who smoked compared to those who did not (Table 2).

Discussion

We present, for the first time, the correlation between the level of miR 125b and pseudoexfoliation syndrome in patients with cataracts and PEX compared to patients with cataracts alone.

miR 125b is demonstrated to be inversely related to the level of p53 in age-related cataract tissue and is involved in DNA damage response-induced aging, which is mainly governed by P53 pathway (13). Some confirmed risk factors for cataract formation, like chronic ultraviolet light exposure and oxidative stress cause DNA damage as a result of the cumulative effects of reactive oxygen species (ROS) (9).

In our study, we observed no differences in expression of miR 125b in lens capsules of patients with cataracts alone and with both cataract and primary open angle glaucoma. The main finding of our study is that miR 125b is significantly increased in lens capsules of patients with PEXg. The results obtained by us and from prior studies allow us to assume that in the examined population, the co-existence of PEX was the main moderator of miR 125b expression in lens capsules and that the increased expression of miR 125b can be a cause of PEX. It should be emphasized that our research, reflecting miR 125b expression level close to that seen *in vivo*, was carried out on a large number of sampled capsules, a patient population that was relatively homogenous in terms of age and disease severity.

Numerous mechanisms, such as disruptions in the proteolytic balance of the extracellular matrix, chronic inflammatory processes of small intensity, chronic or recurrent ischemia/hypoxia, regulation disturbances at a cellular level, and oxidative stress, have all been implicated in the formation of PEXM complexes (4,15-17). These factors interplay in creating conditions specific for PEX and the upregulation of miR-125b in capsular lens tissue can be either a cause or effect of these processes.

Altered expression of miR 125b has been demonstrated in a number of chronic diseases and malignancies, including prostate, breast and pancreatic cancer (18,19). miR 125b expression is associated with classic phenotypic and genotypic sequelae of reduced P53 expression including altered sensitivity to genotoxic agents and reduced expression of P53 related transcriptional targets such as miR 34a/b/c (20).

Excessive oxidative stress was shown to augment expression of miR 125b (20). Upregulation of miR 125b expression was identified in acute cerebral ischemia (21, 22). Also, miR 125b was shown to increase in settings of hypoxia (23). Thus, oxidative stress and hypoxia appear to play a major role in the modification of gene expression.

The central fragment of the anterior lens capsule was the material subjected to examination specifying miR 125b level. The lens capsule is an ideal source of information about PEX, as it is constantly exposed to damaging factors which participate in the disease pathogenesis, such as UV rays. It is a region in which molecules comprising PEXM are deposited and bound in insoluble structures. The lens capsule is avascular and is washed by aqueous fluid. The components of the aqueous fluid can impact the lens capsule. According to the protein sink model, the anterior chamber of PEX eyes reflects a breakdown of the blood-eye barrier (14). Chronic inflammatory changes of the aqueous humour may lead to the dysregulation of miRNAs expression, influence P53 activity leading to the transcription of numerous genes responsible for senescence, or apoptosis by activation of target mRNA (24).

Conclusions

An interesting and innovative aspect of our preliminary study was the correlation of PEX and miR 125b expression in examined eye tissue. Further studies to determine the role of miR in eye pathologies are needed. Dysregulation of miR expression and activity can contribute to stress-related chronic diseases, like PEX. Even with advances in the last decade, effective novel and therapeutic approaches are still

lacking for PEX. An improved understanding of the impact of impact of epigenetic factors such as DNA hypermethylation and histone modifications in the regulation of miR expression is needed. With these results, miR indicates a potential future avenue for effective diagnosis and therapeutic strategies for PEX.

Abbreviations

MicroRNAs (miRs)

micro-RNA 125b (miR 125b)

pseudoexfoliation syndrome (PEX)

pseudoexfoliation material (PEXM)

intraocular pressure (IOP)

polymerase chain reaction (PCR)

reverse transcription reaction (RT)

relative quantity (RQ)

Declarations

Ethics approval and consent to participate: This prospective, controlled study was approved by the Regional Ethical Review Board and was conducted in accordance with the principles set forth in the Guidelines for Good Clinical Practice and the Declaration of Helsinki (and its amendments) (KB 272/2017 from 26/04/2017). Written informed consent was obtained from every study participants.

Consent for publication: Not applicable

Availability of data and material: The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests

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Authors' contributions:

All authors have participated directly in planning and execution of the work and have approved the final version of the manuscript.

MTS, JPD, ATK took samples, analysed and interpreted patient data.

DB performed microRNA analysis and interpreted patient data.

MTS was the main factor contributing to the writing of the manuscript

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References

1. Dogini DB, Pascoal VD, Avansini SH, Vieira AS, Pereira TC, Lopes-Cendes I. The new world of RNAs. *Genet Mol Biol.* 2014;37(1 Suppl):285-93.
2. Lee YH, Kim SY, Bae YS. Upregulation of miR-760 and miR-186 is associated with replicative senescence in human lung fibroblast cells. *Mol Cells.* 2014;37(8):620-7.
3. Chen K, Rajewsky N. The evolution of gene regulation by transcription factors and microRNA's. *Nat Rev Genet.* 2007;8:93-103.
4. Peng CH, Liu JH, Woung LC, Lin TJ, Chiou SH, Tseng PC, Du WY, Cheng CK, Hu CC, Chien KH, Chen SJ. MicroRNAs and cataracts: a correlation among let-7 expression, age and severity of lens opacity. *Br J Ophthalmol.* 2012;96:747-751.
5. Kowlru RA, Kowlru A, Mishra M, Kumar B. Oxidative stress and epigenetic modifications in the pathogenesis of diabetic retinopathy. *Progress in Retinal and Eye Res.* 2015;48:40-61.
6. Vasudevan S, Tong Y, Steitz JA. Switching from repression to activation: microRNAs can up-regulate translation. *Science.* 2007;318:1931-34.
7. Khee SG, Yusof YA, Makpol S. Expression of senescence-associated microRNAs and target genes in cellular aging and modulation by tocotrienol-rich fraction. *Oxid Med Cell Longev.* 2014:725929. doi: 10.1155/2014/725929. Epub 2014 Jul 14.
8. Wu C, Lin H, Wang Q, Chen W, Luo H, Chen W, Zhang H. Discrepant expression of MicroRNAs in transparent and cataractous human lenses. *Invest Ophthalmol Vis Sci.* 2012;53:3906-3912.
9. Chien KH, Chen SJ, Liu JH, Chang HM, Woung LC, Liang CM. Correlation between microRNA-34a levels and lens opacity severity in age-related cataracts. *Eye.* 2013;27:883-888.
10. Hawse JR, Hejtmancik JF, Horwitz J, Kantorow M. Identification and functional clustering of global gene expression differences between age-related cataract and clear human lenses and aged human lenses. *Exp Eye Res.* 2004;79(6): 935-940.
11. Li WC, Kuszak JR, Dunn K, Wang RR, Ma W, Wang GM, Spector A, Leib M, Cotliar AM, Weiss M, et al. Lens epithelial cell apoptosis appears to be a common cellular basis for non-congenital cataract development in humans and animals. *J Cell Biol.* 1995;130(1):169-81.
12. Geatrell JC, Gan PM, Mansergh FC, Kisiswa L, Jarrin M, Williams LA, Evans MJ, Boulton ME, Wride MA. Apoptosis gene profiling reveals spatio-temporal regulated expression of the p53/Mdm2 pathway during lens development. *Exp Eye Res.* 2009;88(6):1137-51.

13. Qin Y, Zhao J, Min X, Wang M, Luo W, Wu D, Yan Q, Li J, Wu X, Zhang J. MicroRNA-125b inhibits lens epithelial cell apoptosis by targeting p53 in age-related cataract. *Biochim Biophys Acta*. 2014;1842(12 Pt A):2439-47.
14. Lee RK. The molecular pathophysiology of pseudoexfoliation glaucoma. *Curr Opin Ophthalmol*. 2008;19:95-101.
15. Wu C, Liu Z, Ma L, Pei C, Qin L, Gao N, Li J, Yin Y. MiRNAs regulate oxidative stress related genes via binding to the 3' UTR and TATA-box regions: a new hypothesis for cataract pathogenesis. *BMC Ophthalmol*. 2017;17(1):142.
16. Zenkel M, Kruse FE, Naumann GO, Schlötzer-Schrehard U. Impaired cytoprotective mechanisms in eyes with pseudoexfoliation syndrome/glaucoma. *Invest Ophthalmol Vis Sci*. 2007;48: 5558-66.
17. Wang S, Guo C, Yu M, Ning X, Yan B, Zhao J, Yang A, Yan H. Identification of H₂O₂ induced oxidative stress associated microRNAs in HLE-B3 cells and their clinical relevance to the progression of age-related nuclear cataract. *BMC Ophthalmol*. 2018;18(1):93.
18. Shi XB, Xue L, Yang J, Ma AH, Zhao J, Xu M, Tepper CG, Evans CP, Kung HJ, deVere White RW. An androgen-regulated miRNA suppresses Bak1 expression and induces androgen-independent growth of prostate cancer cells. *Proc Natl Acad Sci USA*. 2007; 104(50):19983-8.
19. Thomson JM, Newman M, Parker JS, Morin-Kensicki EM, Wright T, Hammond SM. Extensive post-transcriptional regulation of microRNAs and its implications for cancer. *Genes Dev*. 2006;20(16):2202–7.
20. Selbach M, Schwanhäusser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N. Widespread changes in protein synthesis induced by microRNAs. *Nature*. 2008; 455(7209):58–63.
21. Lukiw WJ, Pogue AI. Induction of specific micro RNA (miRNA) species by ROS-generating metal sulfates in primary human brain cells. *J Inorg Biochem*. 2007;101:1265e1269.
22. Sepramaniam S, Tan JR, Tan KS, DeSilva DA, Tavintharan S, Woon FP, Wang CW, Yong FL, Karolina DS, Kaur P, Liu FJ, Lim KY, Armugam A, Jeyaseelan K. Circulating microRNAs as biomarkers of acute stroke. *Int J Mol Sci*. 2014;15:1418e1432.
23. Kulshreshtha R, Ferracin M, Wojcik SE, Garzon R, Alder H, Agosto-Perez FJ, Davuluri R, Liu CG, Croce CM, Negrini M, Calin GA, Ivan M. A microRNA signature of hypoxia. *Mol Cell Biol*. 2006;27:1859e1867.
24. Liu B, Chen Y, Clair DK. ROS and p53: a versatile partnership. *Free Radic Biol Med*. 2008;44(8):1529–35

Tables

Table 1. Mean expression of microRNA 125b in every group (LSD-test as post-hoc test).

Group	Number of patients	Mean age [†]	Sex proportion in each group (F:M) [†]	Mean RQ	SD	p-value (tested group vs Cg)
PEXg	38	77	15:4	5.11	6.92	0.015
Gg	37	72	25:12	4.30	6.44	0.14
PEXGg	30	76	22:8	2.78	4.10	0.45
Cg	45	73	29:16	1.53	1.38	x
All groups	150	75	53:22	3.37	5.27	x

[†] no statistically significant differences between groups (p>0.05)

Table 2. Summary of additional factors in the analysis of miR125.

Medical history information		Mean		Number of valid cases		Standard deviation		p
		no	yes	no	yes	no	yes	
Medications	Timololi maleas	3.46	2.80	131	19	5.43	4.15	0.57
	Brimonidine	3.35	3.52	131	19	5.40	4.46	0.96
	Prostaglandins	3.36	3.49	137	13	5.27	5.52	0.77
Habits	Smoking	0.90	3.63	136	14	1.01	5.47	0.01
Comorbidities	DM	3.34	3.50	119	31	5.51	4.34	0.52
	Heart failure	3.33	3.44	91	59	5.28	5.31	0.95
	HT	3.72	3.19	52	98	6.28	4.68	0.90
	Dementia	3.44	1.30	145	5	5.35	0.72	0.69
	Vertigo	3.37	3.44	138	12	5.23	6.04	0.60
	Hearing impairment	3.42	0.84	147	3	5.31	0.92	0.27

DM – diabetes mellitus; HT – hypertension; LBP – low blood pressure

Figures

Figure 1

Comparison of miRNA 125b expression level in PEXg, Gg, PEXGg and Cg. Observed values were higher in study groups with an ophthalmic disease compared to the control group, but only in PEXg compared to Cg there is a statistically significant difference - $P=0.046$ (median – horizontal line inside of the 25-75% box - box with horizontal line , minimum and maximum - box with vertical line, plotted data – circle, extreme values – red square)

Figure 2

MiR 125b expression analysis in PEXg, Gg, PEXGg and Cg with subdivision into the different value ranges of logRQ (number of patients in each group on the bar)

Figure 3

Comparison of miR 125b (logRQ values) expression in all the patients with pseudoexfoliation syndrome compared to control group (mean logRQ PEXg+PEXGg = 0,203; Cg=-0,0228, P=0,04)

Figure 4

Logistic regression analysis for different categorical outcomes (A) existing PEX with or without glaucoma, (B) existing Glaucoma with or without PEX; the PEXG - a secondary glaucoma is combined with primary open angle glaucoma; this analysis with wide 95%CI range should be specified in further studies, (C) only PEX, (D) only Glaucoma vs control group. The PEXG group is an additional factor that reverse the 95%CI RQ values from predominantly positive in the PEXg and Gg into the predominantly negative in the combined groups such as PEXg+PEXGg and PEXGg+Gg.