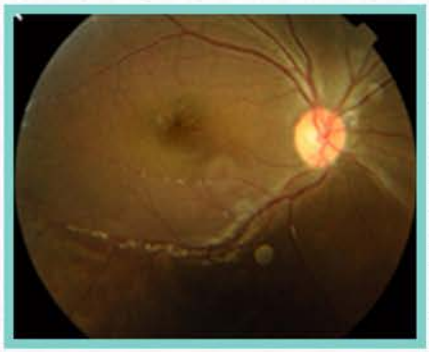
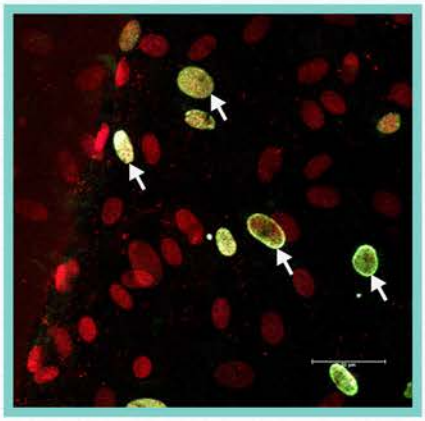
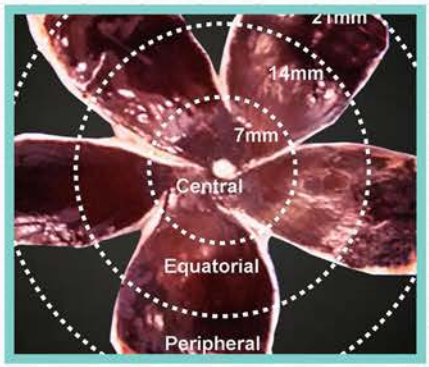
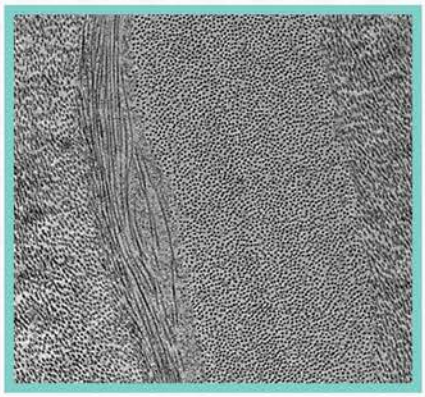
RESEARCH IN OPHTHALMIC SCIENCES

Aravind Medical Research Foundation









ANNUAL REPORT 2022 - 2023

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MISSION

To eliminate needless blindness by providing evidence through research and evolving methods to translate existing evidence and knowledge into effective action.

RESEARCH IN OPHTHALMIC SCIENCES

Dr. G. Venkataswamy Eye Research Institute

Annual Report 2022 - 2023

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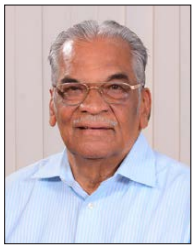

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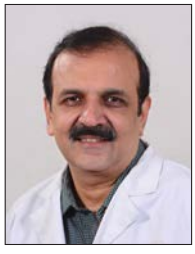

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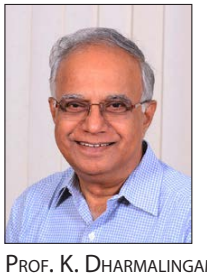

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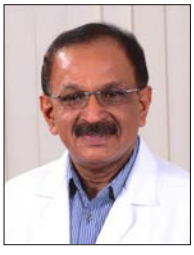

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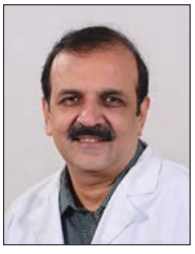

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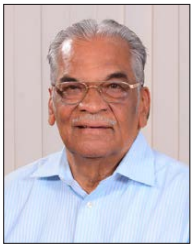

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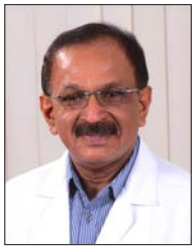

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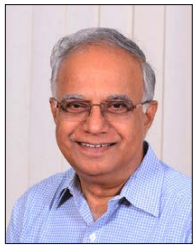

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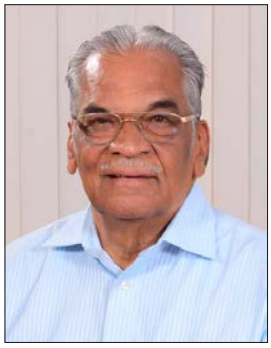
FOREWORD



AMRF is making significant progress in the promotion of existing research projects. It is equally interested in exploring the possibilities of establishing innovative measures to enhance research activities involving both clinicians and scientists.

The multi-centric collaborative study on biomarkers for the prediction of the progression of diabetes to diabetic retinopathy has been completed and published in JAMA. Interestingly the study showed that some of the markers have the potential to predict the progression in both the Indian and UK population. At the same time, some of the markers examined are useful in predicting the disease progression in the Indian population only. The utility of these markers in clinical settings will allow one to prioritise screening and follow-up of the patients.

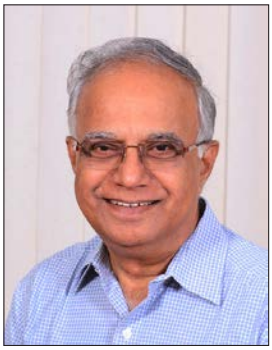
Regional Research Centers at Tirunelveli, Pondicherry and Coimbatore are fully functional, and several programmes have been initiated. Some of the proposals were finalised and submitted for funding to granting agencies. More participation from the clinicians will improve our research base at the clinician-scientist level.

AMRF supported the participation of eight investigators in the ARVO meeting held at New Orleans and presented papers/posters in the symposia. Improving our infrastructure, research base, and research output are our continuing goals and I congratulate the scientists and investigators involved in this process.

AMRF and Aravind Eye Care System will continue to support and nurture research to achieve the goal of our founder, Dr. G. Venkataswamy.

- Dr. P. Namperumalsamy
President, AMRF

INTRODUCTION



A major initiative of AMRF, the Exosome Innovation Centre is now fully functional, and this facility already attracted users from other organisations. The extracellular vesicles (EVs) are emerging as the frontier areas of fundamental research, and the therapeutic potential of these nanoparticles is immense. AMRF has initiated this study on several eye disorders. In the recently held ARVO, the team could see several papers exploring biology of the EVs and their translational potential.

Another area to be noted is the gene correction techniques. Some gene-edited systems are in clinical trials phase. AMRF will expand its technical capability and be equipped for the next phase of this new therapeutic avenue.

The details of the research activities and the progress are given in the following pages. We hope our efforts in research will greatly improve the quality of eye care delivery.

Prof. K. Dharmalingam
Director - Research

CONTENTS

MOLECULAR GENETICS

Genetic diagnosis in ocular disease offers unique advantages for clinicians and patients, including a better understanding of the disease's pathophysiology and an improved correlation between genotype and phenotype. Moreover, a precise genetic diagnosis has been demonstrated to have a positive impact on disease management as well as clinical outcomes. Nowadays, there is increasing use of NGS technology in many clinical settings for prescribing precision treatment for many conditions associated with systemic diseases. In recent years, the lab has utilised the NGS platform to understand the genetic factors underlying a wide variety of eye diseases, including Leber's hereditary optic neuropathy (LHON); Leber's congenital amaurosis (LCA) , and Juvenile X-linked retinoschisis (JXLR). Consequently, in vitro models were developed to understand the functional impact of the candidate genes through siRNA-mediated knockdown and site-directed mutagenesis by over-expressing a mutant gene in HEK293 cells to understand their biological significance to disease progression.

A siRNA-mediated knockdown on 661W mRGC cells to demonstrate the functional effects of genomic variants found in LHON.

Investigators	:	Dr. P. Sundaresan Dr. S. Mahesh Kumar
Research scholar	:	C. Prakash
Funding Agency	:	ICMR – Senior Research Fellowship

Introduction

RNA interference (RNAi) is one of the most widely used technologies for inducing gene-specific RNA degradation. Currently, this study has established an in-vitro model to demonstrate the impact of the genetic variants uncovered by NGS in LHON disease pathogenesis. This study utilised a 661W cell line, the precursor of mouse retinoganglion cells, which may represent an ideal model for studying the molecular mechanisms involved in RGC degeneration. Therefore the current study investigated the function of the identified candidate gene via siRNA-mediated gene knockdown in the present study. 661W mRGC's cells were procured from Dr. Ghansyam Swarup, CCMB after getting proper consent from Muayyad Al-Ubaidi, University of Houston. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 1 g/L glucose and l-glutamine, supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells were incubated at 37°C in humidified 5% CO2. The mouse siRNA oligo duplexes were obtained from Origene for the following genes: NDUFS2 (Locus ID 226646); MTFMT (Locus ID 69606); SLC25A46 (Locus ID 67453); SLC25A3 (Locus ID 18674) and PDSS1 (Locus ID 56075). Each kit contains three unique 27mer siRNA duplexes (2 nmol each) targeting the candidate gene, and 1 Universal Scrambled Negative Control siRNA duplex (2 nmol). The lyophilised siRNA was reconstituted in the RNAse-free siRNA Duplex Resuspension Buffer and stored at -20°C. Approximately, one million cells were seeded in a 6-well plate for 24 h in a complete medium

(DMEM/10% FBS) one day prior to the experiment. As soon as the cells reached 70 to 80% confluency, the complete medium was replaced with 1ml of opti-MEM medium / 5% FBS, and 10uM of siRNA was transfected using Lipofectamine RNAiMAX. After 4 hours, the opti-MEM medium was replaced with 1ml of fresh complete DMEM medium and cultured for 3 days to evaluate the functional effect of the genetic knockdown. The NDUFS2 and MTFMT siRNA were primarily transfected into mouse 661W RGC cells to study their functional significance in disease pathogenesis. Furthermore, total RNA was isolated from the control, and siRNA transfected 661W cells at 48 hrs. post-transfection. The concentration of the RNA samples was estimated through a Nanodrop spectrophotometer and qubit fluorometer. 1µg of total RNA was reverse transcribed into cDNA using the Revert aid cDNA synthesis kit. Real-time PCR was performed using SYBR green master mix. The specificity of the amplifications was ensured with melting curves. The relative mRNA expression and their fold change was determined with the $2^{-\Delta\Delta}$ct method.

Results and Conclusion

siRNA transfection of NDUFS2 and MTFMT genes showed significant down regulation compared with scrambled siRNAs in mouse 661W RGCs. However, three different siRNAs were received for each gene along with a universal control scrambled siRNA. It was transfected using a Lipofectamine RNAimax reagent. Furthermore, a single siRNA with superior performance has been selected for expression studies, and the samples will be sent to RNA sequencing for the identification of dysregulated pathways. Western blot analysis displayed a significant reduction of the electron transport complex I expression in NDUFS2 treated 66w RGC compared to the control. According to string analysis, NDUFS2 interacts strongly with NDUFB8. Hence, genetic knockdown of NDUFS2 subsequently affects NDUFB8 expression.

Functional analysis of GUCY2D mutant: An intrinsic cause of LCA

Investigators : Dr. P. Sundaresan
Dr. P. Vijayalakshmi
Dr. Rupa Anjanamurthy
Research scholar : A. S. Sriee Viswarubhiny

Funding Agency : Lady Tata Memorial Trust (LTMT) – Senior Research Fellowship

Introduction

Leber's Congenital Amaurosis (LCA) is a group of early-onset retinal diseases, accounts for at least 5% of all inherited retinal dystrophies. Currently, mutations in 29 genes are associated with LCA. The distribution of pathogenic genes varies considerably among different populations; however, GUCY2D is one of the most common genes, accounting for approximately 10%–20% of cases worldwide. The study also reported the highest prevalence (26%) of GUCY2D gene mutation from its cohort.

GUCY2D encodes retinal guanylyl cyclase 1 (RetGC-1), which is expressed in rod and cone cells. GUCY2D plays an important role in phototransduction by catalysing the synthesis of 3',5'-cyclic guanosine monophosphate (cGMP). When a photon of light activates rhodopsin, it initiates a cascade of events, resulting in the reduction of cGMP level and the closure of the cGMP-gated channel. Continued expulsion of Ca2+ by Na+/Ca2+-K+ exchangers results in the activation of RetGC-1 by GCAP. RetGC-1 replenishes intracellular cGMP, which reopens cGMP-gated allowing for Ca2+ influx and a return of the photoreceptor to its dark-adapted state. Mutations in the GUCY2D gene can cause cyclase activity to be absent or reduced, thus resulting in photoreceptor cell death and irreversible vision loss.

Overall, 29 LCA patients were positive for GUCY2D, among them 18 patients had missense mutations. The functional impact of these missense mutations is unknown. It is necessary to elucidate the effect of missense mutations upon RetGC-1 activity to understand how it leads to the disease phenotype and progression of visual loss. Thus, the present study aimed to examine the effects of same point mutations on RetGC-1 activity by utilising the site–directed mutagenesis for a better understanding of disease mechanisms and to establish genotype-phenotype correlation.

Results

GUCY2D missense mutation was found in eighteen patients (Table 1), of which, six patients had a homozygous mutation in c.T524G, three patients were detected with c.G169A mutation, other two patients had mutations in c.G2927 and the rest of 7 patients had unique missense mutations. All these mutations were predicted to be deleterious by the in-silico functional prediction tools.

Site-directed mutagenesis was performed to generate GUCY2D mutants and their functional analyses were validated through Sanger sequencing. A wild type and mutant plasmid were transiently transfected into HEK293 cells and confirmed by immunocytochemistry and western blotting. ELISA analysis of the wildtype and mutant transfected proteins revealed a twofold lower expression of cGMP in the mutant than wildtype vectors.

Table 1: Represents the missense mutations identified in the GUCY2D gene.

S.No	No of Patients	Exon	c.DNA change	Amino acid change	Zygosity	MOI	Reported/ Not Reported	ACMG	Reported functional studies
1	6	2	c.T524G	p.LI75R	HOM	AR	R	VUS	NA
2	3	2	c.G169A	p.G57R	HOM	AR	R	VUS	NA
3	2	15	c.G2927A	p.R976H	HOM	AR	R	LP	NA
4	1	2	c.G582C	p.W194C	HOM	AR	NR	VUS	NA
5	1	10	c.G2043T	p.K681H	HOM	AR	NR	LP	NA
6	1	17	c.C3118G	p.R1040G	HOM	AR	R	VUS	NA
7	1	11	c.G2197A	p.A733T	HOM	AR	R	LP	NA
8	1	12	c.C2302T	p.R768W	HOM	AR	R	P	Available
9	1	10	c.T1970G	p.L657R	HOM	AR	NR	LP	NA
10	1	15	c.T2918A	p.V973E	HOM	AR	NR	LP	NA

Hom: Homozygous; AR: Autosomal Recessive; R: Reported; NR: Not Reported; VUS: Variant of Uncertain Significance; LP: Likely Pathogenic; P: Pathogenic; NA - Not Available

Conclusion

The current study examined the effects of GUCY2D missense mutations on RetGC-1 activity in a HEK293 in-vitro model. SDM analyses revealed the mutation in the kinase homology domain (c.T1970G) of the GUCY2D gene significantly reduced enzymatic activity compared to the wild type. In addition to comprehending the consequences of GUCY2D missense mutations, this model also provides an in-depth insight into the consequences of photoreceptor damage..

Analysis of Microstructural changes in an X-linked juvenile retinoschisis patient harboring RS1 G668A mutation by en-face OCT imaging

Investigators : Dr. P. Sundaresan
Dr. Rupa Anjanamurthy
Project Fellow : Susmita Chowdhury

Funding Agency : Lady Tata Memorial Trust – Senior Research Fellowship

Introduction

Juvenile X-linked retinoschisis (JXLR, OMIM 312700) is an inherited retinal degenerative disease, primarily affecting males at the first decade of their life in an X-linked recessive manner. It is associated with early vision impairment and characterised by the foveal schisis resulting from inner retinal layer splitting, thus represents an archetypal spoke-wheel appearance and a negative electroretinogram (ERG) because of reduced b-wave amplitude.

RS1 (chromosome Xp22.2) gene is reported as the candidate gene for JXLR pathogenesis, comprising six exons. It encodes an adhesive 24kDa retinoschisin protein secreted from photoreceptors and bipolar cells. Retinoschisin is reported to involve in cellular adhesion and cell–cell interactions, thus maintains the structural and functional integrity of the retina. Dysfunction in Retinoschisin protein leads to the accumulation of retinoschisin within the inner retina and the development of cystic-like spaces primarily in the inner nuclear and outer plexiform layers of the retina.

Hence, mutations in retinoschisin protein might reduce the adhesion across retinal layers resulting in schisis formation or the accumulation of dysfunctional protein within the inner retina which can develop cystic-like spaces primarily in the inner nuclear and outer plexiform layers of the retina. So far, more than 300 unique variants of the RS1 gene have been reported, which are documented in the Leiden Open Variation Database (https://databases. lovd.nl/shared/genes/RS1; Leiden Open Variation Database, LOVD v. 3.0 Build 26c). However, a very few studies from India have reported RS1 mutations associated with X-linked retinoschisis till now.

Spectral-domain optical coherence tomography (SD-OCT) images are reminiscent of histopathology of the retina. Retinal imaging through OCT has been in use for various ocular disorders including retinoschisis. With the advent of OCT angiography (OCTA) and en-face OCT, the ultra-structure of retinal layers can be studied more precisely. Hitherto, few studies demonstrated the en-face OCT imaging for retinoschisis patients. Therefore, present study

Table 2: Clinical, En-face OCT angiography findings and RS1 mutation details of the proband.

	Age at onset (years) / Sex	BCVA		Macular & Peripheral retinal changes		En face OCT imaging findings		ERG Findings	Identified RS1 mutation	In silico Prediction
		OD	OS	OD	OS	OU	OU			
Proband	7/ M	6/24	6/24	Foveal schisis	Foveal schisis	Characteristic spoke like foveal and parafoveal schitic lesions in the GCL, IPL, INL and OPL	b/a ratio < 1 in both eyes	Foveal schisis	Exon 6: c.G668A [p.Cys223Tyr] Hemizygous	Deleterious

M-Male, BCVA-Best corrected Visual acuity, OD-Right eye, OS-Left eye, OU- Both eyes, GCL-Ganglion cell layer, IPL-Inner plexiform layer, INL-inner nuclear layer, OPL-Outer plexiform layer. In silico analysis was done using SIFT, PolyPhen2 and Mutation Taster tools

was communally aimed to understand the precise in-depth details of retinal layers changes using en-face OCT imaging and ascertaining the causative RS1 mutation in a JXLR patient from south India.

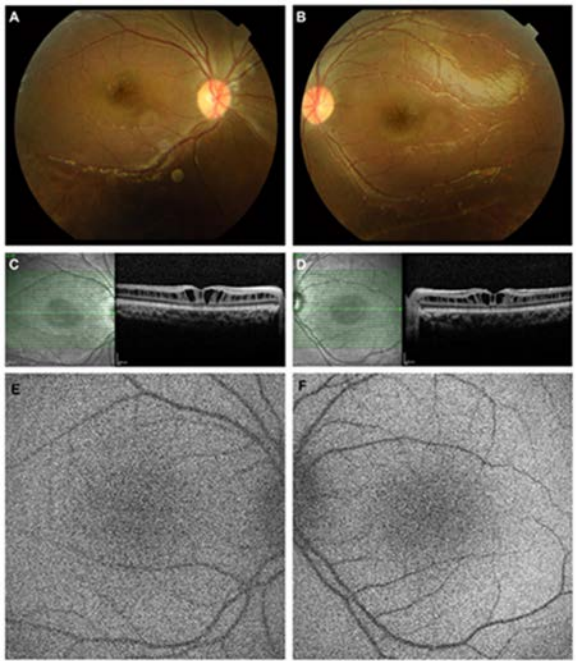


Fig 1. A and B: Fundus photo of both the eyes of the proband showing cart-wheel appearance at the macula due to foveal schisis. C and D: Optical coherence tomography (OCT) images of both the eyes showing foveal schisis. OCT B scan was taken passing through the center of the fovea and 8.9 mm in length. E and F: Confocal scanning laser ophthalmoscopy (CSLO) blue light fundus auto fluorescence (FAF) image of both the eyes showing reduced FAF signal in the macular area, a finding similar to the eyes without any macular pathology.

Results

Herein, the study deal with a 7-year-old male child from non-consanguineous parents presented with JXLR phenotype on a comprehensive ophthalmic assessment by OCT en-face imaging, fundoscopy, and electroretinography (ERG) (Figure 1& 2). RS1 mutational screening for proband and his parents were performed through bidirectional Sanger sequencing followed by in silico analysis (Table 2).

On clinical evaluation for both eyes (OU), the patient's fundus showed a cart-wheel appearance at the macula without peripheral retinoschisis. Horizontal OCT B-scan showed schisis in different retinal layers. En-face OCT imaging revealed characteristic schitic lesions exclusively in the ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL) and outer plexiform layer (OPL). Selective reduction in the b wave was also seen. RS1 screening of proband detected a hemizygous pathogenic mutation (c.G668A; p.Cys223Tyr) in exon-6. Segregation analysis demonstrated heterozygosity for the mother and normal genotype for the father, corroborating X-linked inheritance.

Conclusion

The study describes the collective findings of en-face OCT of a JXLR patient harboring Cyst223Tyr mutation from south India for the first time. Cysteine residue 223 is vital for RS1 octamerisation and cellular adhesion, thus maintaining the retinal structure integrity. The report adds to Indian RS1 mutations spectra and aid in carrier determination for genetic counseling. This study casts further insights into the importance of en-face imaging to understand schisis microstructure for JXLR.

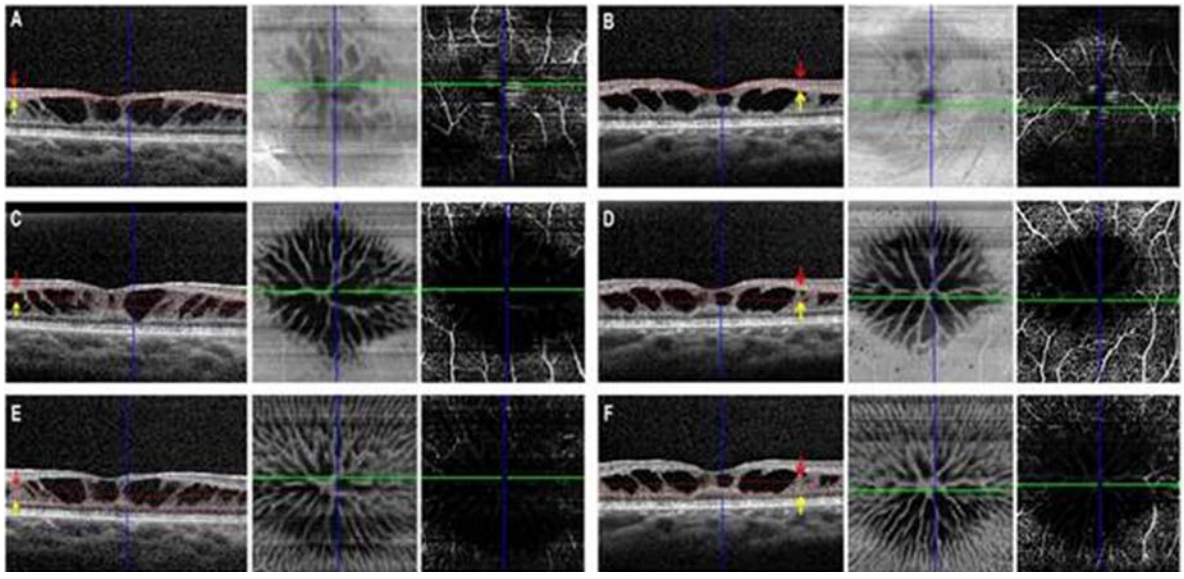


Fig 2. An en-face OCT image. A and B: retinal nerve fiber layer (RNFL) of the RE and the LE respectively; C and D: GCL of the RE and the LE respectively; E and F: IPL - OPL complex of the RE and the LE respectively. The left panel of each image represents the OCT B scan. Please note the two dashed red lines (also indicated by the red and yellow arrows) indicate the layers of the retina where the segmentation was done manually. The middle panel of the images represents the en-face structural slab. No definite schitic pattern in the RNFL layers was observed. In the GCL, a spoke-like pattern in the foveal region was observed. In the IPL-OPL layers, there is a spoke-like pattern in the foveal as well as the perifoveal regions. The right panel of the images represents the OCTA flow signal.

GENETICS OF OCULAR TUMORS

Ocular tumors in India are mostly presented at very late stages and hence their treatment remains challenging. The research spans around three important aspects of the ocular tumors that include early diagnosis, improved prognosis and better therapeutics. Genetic and epigenetic approaches are used to develop diagnostic tests that are efficient and cost-effective. Understanding the pathological processes help in improved prognostication and categorising the patients based on their clinical features. Application of high end methods to dissect out the tumorigenesis process is useful to develop newer and better therapeutics for ocular tumors including retinoblastoma and lymphoma.

Genetic testing of Retinoblastoma

Investigators : Dr. A. Vanniarajan, Dr. Usha Kim, Prof. VR. Muthukkaruppan
Genetic Analyst : K. Murugan
Funding Agency : Aravind Eye Care System, Madurai

Introduction

Retinoblastoma has a diverse spectrum of mutations that requires multiple methods such as Sanger sequencing, Multiplex ligation dependent probe amplification, Real time PCR and Next generation sequencing. Use of the suitable methods makes the process faster and cost effective. Prenatal genetic testing is now possible for retinoblastoma that supports the affected families.

Results

RB1 genetic testing was carried out in 58 patients including 36 unilateral and 22 bilateral RB during this year. Of the 58 patients, 12 were older patients who were visiting the clinic for follow-up and willing to know their genetic status. Germline mutations identified in 22 patients indicated the increased risk of RB in the next generation. Somatic mutations identified in 4 tumors and absence of the mutations in the blood samples of other patients implied the low risk of RB in the future generation.

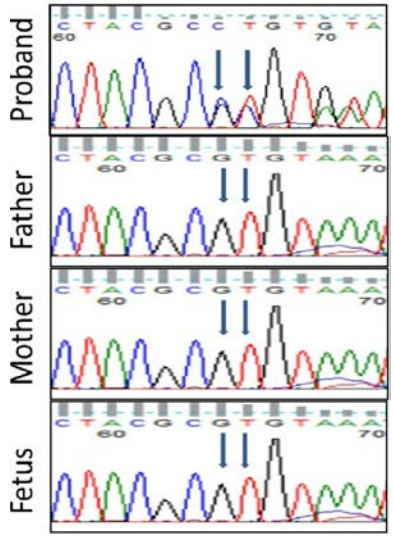


*Fig 1. Frameshift mutation c.1861_1862insCC p.R621Pfs*3 in Exon 19 identified in the proband but absent in parents and fetus samples*

During this year, prenatal genetic testing was performed in a family with known familial history of RB. The proband was identified with a frameshift mutation in exon 19, but the parents did not have the mutation (Fig.1). When the mother conceived her second child, amniotic fluid was collected and fetal DNA was isolated. Sample was received at AMRF for RB genetic analysis after testing for maternal cell contamination. Fetal DNA did not have the mutation that gave the relief for the family. The pregnancy was continued and the mother delivered a healthy child.

Conclusion

Prenatal genetic testing of retinoblastoma provides comfort for the families with positive history. Even in the case of the child identified with mutation, early intervention with appropriate treatment soon after birth can save the child from loss of vision or globe.

RB1 transcript analysis detects novel splicing aberration in retinoblastoma

Investigators : Dr. A. Vanniarajan, Dr. Usha Kim, Prof. VR. Muthukkaruppan
Project Fellow : K. Jeyaprakash
Funding Agency : Science and Engineering Research Board; Department of Health Research

Introduction

RB1 gene testing is crucial for genetic counselling and clinical management of RB. Conventional RB1 gene analysis identifies alterations in exonic and near-exonic regions but not in intronic regions. To uncover such imperceptible changes in introns, RB1 transcript was analysed in two RB tumors with no RB1 coding alterations.

Results

Transcript analysis identified RB1 splicing anomalies in two tumors (RBRT38T and RBRT99T). In RBRT38T, two amplicons with shorter sizes were noted on agarose gel electrophoresis. Sequencing analysis showed skipping of exons 21 and 22, along with skipping of exon 22 alone (Fig. 1A), causing in-frame deletions. In another tumor (RBRT99T), an intense and slightly larger amplicon was noted with an insertion of 52 bp from intron 9 (IVS9+532_+584), leading to out-of-frame and premature termination at codon 321. This variant was predicted to cause cryptic exon activation (Fig. 1B).

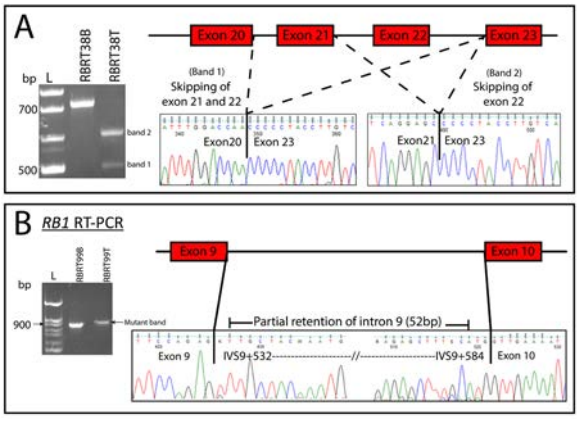


Figure 1: RB1 splicing anomalies in RB tumors: (A) RBRT38T and (B) RBRT99T

Conclusion

RB1 transcript analysis should be utilized in tumors with no detecatable RB1 coding mutations.

Genomic characterisation of kinome related genes in retinoblastoma

Investigators : Dr. A Vanniarajan, Dr. Usha Kim, Dr. R Shanthi, Prof. VR Muthukkaruppan
Research Fellow : K Jeyaprakash
Funding : Aravind Eye Foundation, USA

Introduction

Retinoblastoma (RB) is an aggressive intraocular pediatric cancer and chemotherapy is the standard of care in most RB. Unfortunately, some patients do not respond to treatment and become drug resistant. Newer targets need to be identified for their treatment. Kinases are excellent class of drug targets for cancer treatment. This study was designed to investigate alterations of kinase genes using targeted exome sequencing.

Results

This study included 16 RB tumors that were grouped into responsive (chemoreduced) (n=7) and nonresponsive (chemoresistant) (n=9) tumors and analysed for alterations in kinase genes.

NGS analysis identified three pathogenic somatic mutations affecting two genes (ERBB4 and EGFR) in three nonresponsive tumors. Copy number analysis revealed more CNVs in nonresponsive tumors compared to responsive tumors (p value). Interestingly, amplification of receptor tyrosine kinase (RTK) genes was seen in 6 (66.6%) nonresponsive

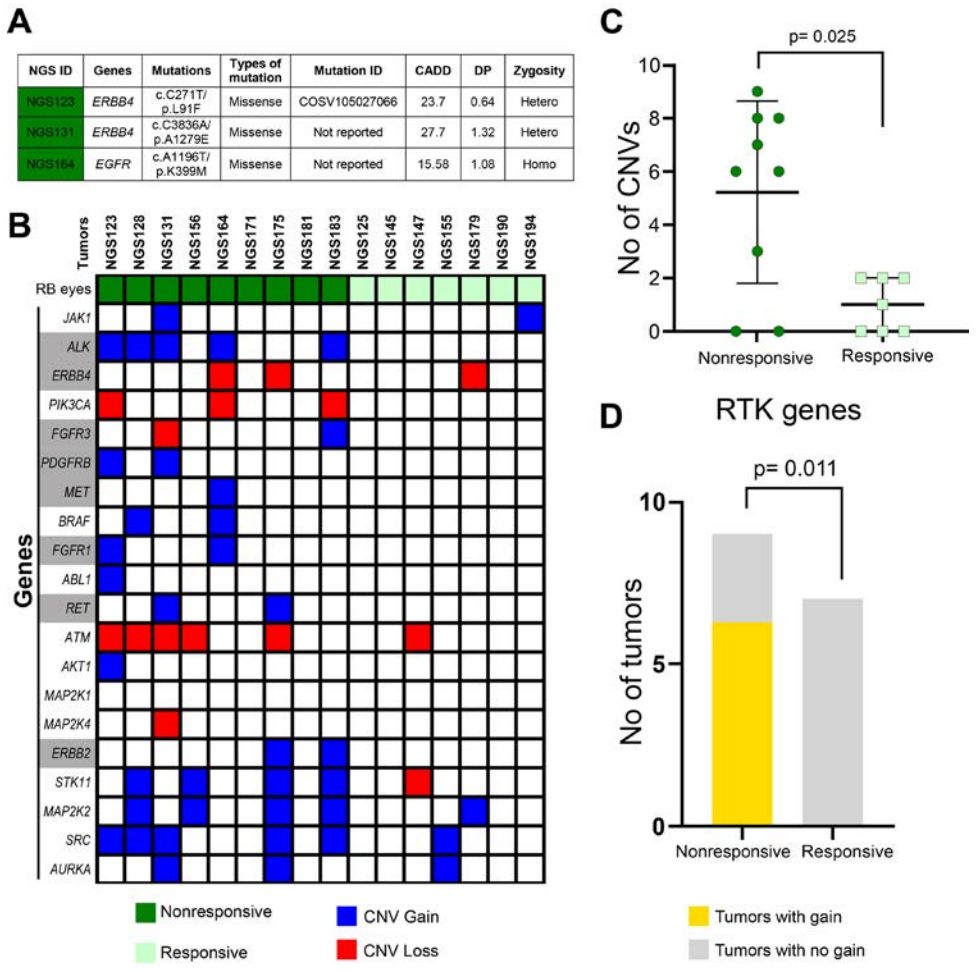


Figure 1: Genetic alterations in nonresponsive vs responsive RB tumors (A) The pathogenic mutations identified in ERBB4 and EGFR (B) CNV gain/loss of individual genes, RTK genes are highlighted in gray boxes. (C) Increased number of CNVs in nonresponsive tumors (D) Gain of RTK genes in nonresponsive tumors.

tumors but not in responsive tumors. Among RTK genes, ALK gain was significantly observed in 5 nonresponsive tumors (Figure 1 A-D).

Conclusion

The nonresponsive tumors showed higher gain of RTK genes especially ALK, which can serve as potential drug target. Further expression study of RTKs may open up new therapeutic options.

Molecular characterisation of tumor progression in retinoblastoma

Investigators : Dr. A. Vanniarajan, Dr. Usha Kim, Prof. VR. Muthukkaruppan
Project Fellows : T. Shanthini
Funding Agency : DST-INSPIRE (Fellowship)

Introduction

MicroRNAs (miRNA) are involved in development and progression of many cancers. MiRNAs can either act as tumor suppressors or oncogenes targeting key cancer pathways. In this study, analysis of miRNAs and their target genes are carried out to understand their underlying role in retinoblastoma progression.

Results

MiRnome and transcriptome sequencing data identified 246 significant differentially expressed miRNAs (DEmiRNAs) and 80 differentially expressed genes (DEGs) respectively. The integration analysis showed the inverse correlation of 108 upregulated miRNAs targeting 17 genes of down regulation and 138 downregulated miRNAs targeting 63 genes of upregulation. Functional enrichment of 8 genes showed altered pathways including cell cycle, cell

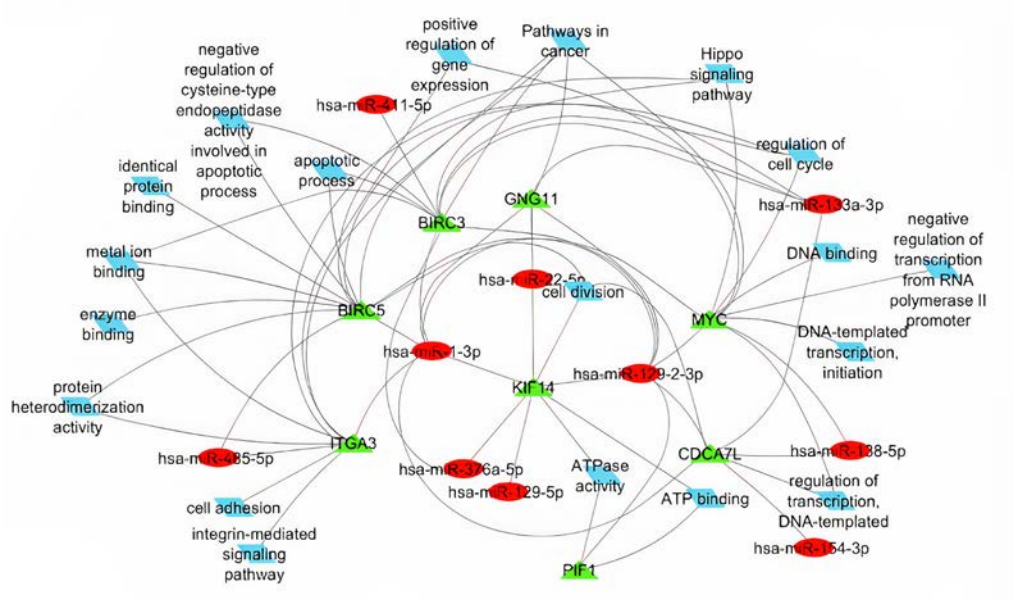


Fig.1: Network analysis using Cytoscape showing the integration of DEmiRNAs and DEGs.

adhesion, and apoptotic process. KIF14 involved in cell division and ATPase activity was regulated through 5 miRNAs: hsa-miR-22-5p, hsa-miR-1-3p, hsa-miR-376a-5p, hsa-miR-129-5p, hsa-miR-129-2-3p. Similarly, other seven upregulated genes were involved in the key cancer pathways as shown in Fig.1

Conclusion

The role of key microRNAs that regulate the major pathways involved in cell growth and division was elucidated. Functional validation of these microRNAs would confirm their role in tumor progression of RB.

Identification and Validation of Dysregulated Pathways in Retinoblastoma

Investigators : Dr. A. Vanniarajan, Dr. Usha Kim, Prof. VR. Muthukkaruppan

Project Fellows : K. Saraswathi, R.Sethu Nagarajan

Funding Agency : SERB

Introduction

Gene expression studies have paved the way for deducing the pathways involved in cancer. The present study is aimed at investigating the genes and pathways that are dysregulated in RB tumors using a focused transcriptomic approach and validating them at protein level.

Results

In the previous years, the expression analysis of cancer related genes indicated the dysregulation of CDC20, MCM2, MK167, PGF, WEE1 and COX5A in RB tumors. Western blot analysis was performed to validate the data at protein level using RB tumors (n=3) and control neural retina (n=3) samples (Fig.1). The genes with transcriptional upregulation were confirmed at protein levels as well.

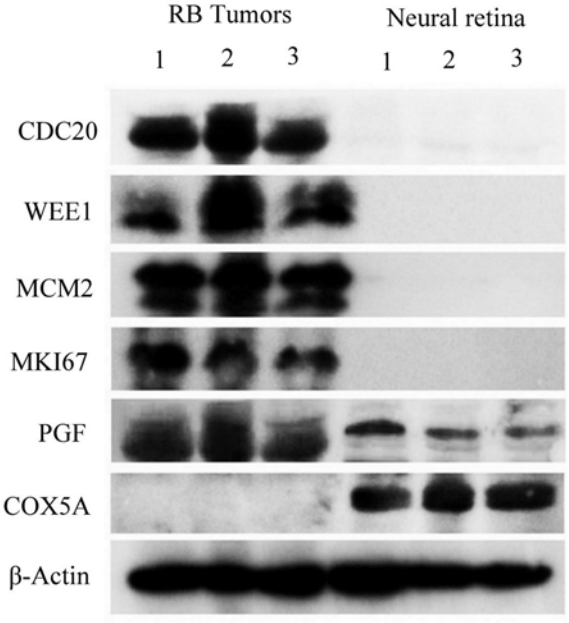


Figure 1: Differentially expressed proteins in retinoblastoma tumors compared to neural retina controls

Genes with down regulation did not show any expression of protein. Except for PGF, all the proteins showed no expression in non-RB tissues. COX5A was totally absent in tumors and highly present in normal tissues.

Conclusion

Identification of transcriptional alterations of CDC20, MCM2, MK167, PGF, WEE1 and COX5A and its validation at the protein level confirms the role of these genes in RB tumorigenesis and hence could be used as potential molecular targets

Translational Genomics of Ocular Cancers

Investigators : Dr. A.Vanniarajan, Dr. Usha Kim, Dr. R. Shanthi, Dr. D. Bharanidharan, Prof. VR. Muthukkaruppan
Project fellow : K. Saraswathi
Funding Agency : Aravind Eye Foundation; Lady Tata Memorial Trust (Fellowship)

Introduction

Orbital neoplasms may arise primarily from the orbit or secondarily from an adjacent source. Ocular Adnexal Lymphoma is the most frequent orbital neoplasm found in 8–10% of all extranodal lymphomas. This study is aimed at reviewing the clinical characteristics and their effect on survival and outcome in ocular adnexal lymphoma patients of Aravind Eye Hospital, Madurai presented during 2012 to 2019. Correlation analysis was performed using cox regression analysis and the survival data were analysed using the Kaplan-Meier plot.

Results

During the 8 year period, one hundred and twelve patients diagnosed with ocular adnexal lymphoma were included in the study. The median age of presentation was 57 years (range, 32 to 81 years). The majority of patients were of B-cell origin (98%) and 2% of patients had T cell lymphoma. With a median follow-up of 34 months, complete remission was observed in 53% of patients and disease recurrence was observed in 20% of the patients at the last follow-up. Disease dissemination was observed in 23% of patients with 56 months as a median overall survival. The median progression free survival was 24 months. The occurrence of relapse was associated with secondary lymphoma (p=0.003, $\chi 2$ test). The 3, 5-year overall survival and disease specific survival for ocular adnexal lymphoma was 88%, 88% and 92%, 88%, respectively (Fig.1). Disease specific survival was found to be better in patients under 55 years of age (p= 0.0102) among the ocular adnexal lymphoma patients.

Conclusion

The prognosis of ocular adnexal lymphoma was majorly influenced by age at presentation. A long-term follow-up is warranted for understanding the association of these risk factors in survival outcomes of ocular adnexal lymphoma patients.

Targeted Modulation of E2F3 and KIF14 pathway in Retinoblastoma refractory to existing chemotherapeutic drugs

Investigators : Dr. A. Vanniarajan, Dr. Usha Kim, Prof. K. Dharmalingam
Project Fellow : R. Sethu Nagarajan

Introduction

Conventional chemotherapeutic regimen has several disadvantages and can affect untargeted cells as well. Towards the molecular therapy, earlier analysis from the lab showed frequent copy number alterations of cell cycle modulators E2F3 and KIF14. Targeting these altered genes could be a competent treatment approach in retinoblastoma treatment.

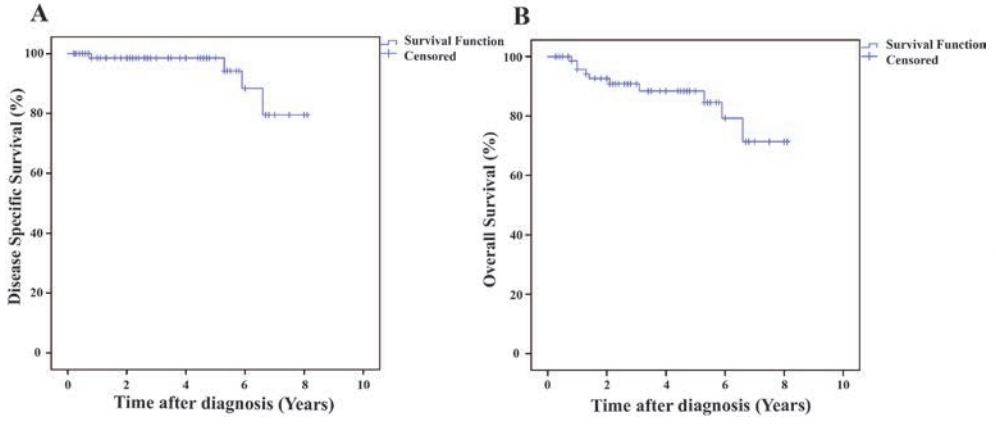


Figure 1: (A) Disease-Specific Survival and (B) Overall Survival in ocular adnexal lymphoma patients

Results

The cells with overexpression of E2F3 and KIF14 were treated with small molecule inhibitors HLM006474, Ispinesib and their inhibition was studied at transcript and protein level. Drug treated cells showed an increased number of TUNEL positivity compared to the controls indicating the apoptotic cell death. Growth kinetics assay showed the reduced cell proliferation and increased doubling time in the drug treated cells (Fig.1a & 1b).

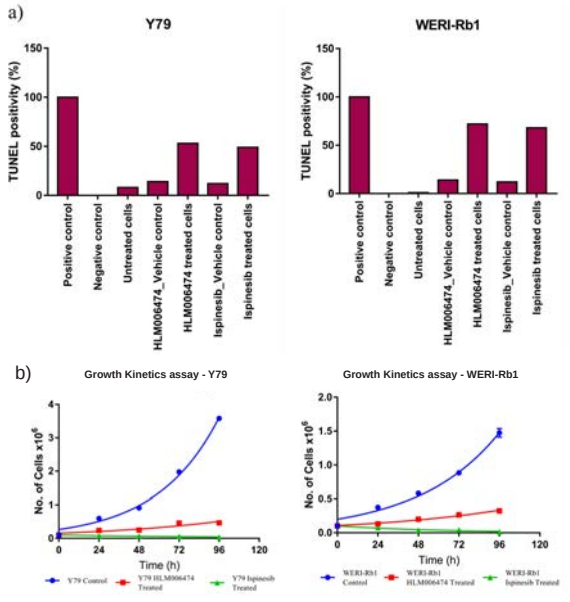


Figure 1: a) Increased apoptotic cell death in drug treated cells as shown by TUNEL positivity and (b) Decreased cell proliferation as shown by growth kinetic assay

Conclusion

The functional analysis confirmed the effects of small molecule inhibitors on E2F3 and KIF14 and hence could be used as a therapeutic option for the tumors that are refractory to the conventional chemotherapy.

Evaluation of GD2 synthase as a prognostic biomarker in retinoblastoma

Investigators : Dr. A. Vanniarajan, Dr. Shanthi, Dr. Usha Kim

Project Fellow : R. Sethu Nagarajan

Funding : VISTA

Introduction

Pathological risk factors such as poor differentiation pattern and tumor cell dissemination are considered as poor prognostic indicators. Tumor cell dissemination into the central nervous system (CNS) is generally fatal. To evaluate the CNS involvement, cytology of cerebrospinal fluid (CSF) is performed. An effective biomarker that can indicate the invasion will be ideal. In this study, the role of GD2 synthase as a biomarker in tumor dissemination of retinoblastoma was investigated.

Results

Previous year, primers for GD2 synthase were optimised and found increased expression in few RB tumors and cell lines. Further, isolation of RNA from the CSF sample was optimised with the classical Trisolution method. Standards were made with the known copies ranging from 1010 to 100 of U87MG cell line amplicons. Absolute quantities of CSF cDNA samples (n=9) were calculated after RT-qPCR along with standards. The samples with high pathological risk factors like optic nerve head invasion, pT4 tumor staging had higher copies of GD2 synthase compared to patients with low risk factors (Fig.1a &1b).

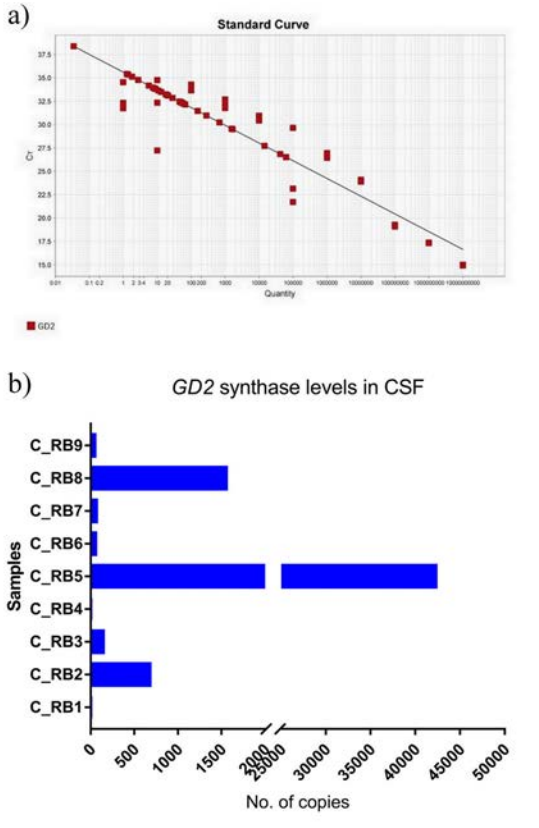


Figure 1: a) Standard curve made with known concentration of GD2 synthase amplicons (b) Increased levels of GD2 synthase in CSF samples of retinoblastoma patient

Conclusion

Absolute quantification of GD2 synthase in CSF samples showed a high number of copies in the patient with high risk factors. Further validation in a large cohort of patient samples is needed for utilising GD2 synthase as a prognostic biomarker.

IMMUNOLOGY AND STEM CELL BIOLOGY

Understanding the basic biology of adult ocular stem cells – location, characterisation, molecular regulation, their role in maintenance of tissue homeostasis, changes in their content and functional properties with ageing and in diseased condition are essential to develop better stem cell based therapies for several ocular conditions/diseases. Recent reports from this laboratory have identified the miRNAs regulating limbal epithelial stem cells, age-related reduction in the trabecular meshwork stem cell content and a drastic reduction in glaucomatous condition, loss of lens epithelial stem cells in cataractous lens and identified the location of stem cells in retinal pigment epithelium. The current focus is to understand the molecular regulation of the adult tissue resident stem cells present in human trabecular meshwork, lens epithelium and retinal pigment epithelium both by intrinsic and extrinsic factors.

Characterisation of adult human lens epithelial stem cells in the maintenance of tissue homeostasis throughout life and their functional status in cataractous lens

Investigator : Dr. Madhu Shekhar
Co-Investigators : Dr. Gowri Priya Chidambaranathan
Prof. VR. Muthukkaruppan,
Dr. Haripriya Aravind
Research Scholars: P. Saranya, M. Lakshmi Priya
Funding agency : Science and Engineering Research Board

Introduction including Background

The crystalline lens is entirely derived from a single cell type –the anterior lens epithelial cells. The epithelial cells are known to differentiate into lens fibres throughout life. The location of stem cells in the lens epithelium remains controversial. Hence, the study aims to identify and characterise the lens epithelial stem cells, their role in maintaining tissue homeostasis and in the development of age related cataract. The four zones in the human anterior lens epithelium were demarcated based on the expression of specific markers (Cx-43 and crystallins). SOX-2^+ but Cx-43^- adult lens epithelial stem cells were confined to the central zone of human anterior lens epithelium. Further, the absence of SOX-2^+ cells in the cataractous lens indicated a probable association with cataract development. In continuation, studies were carried out in this year to analyse the functional changes in the lens epithelial stem cells upon aging and in cataractous donor tissues.

Age related changes of the adult stem cells in human anterior lens epithelium

The excised globes (after the removal of cornea for transplantation) having normal lens from three different age groups <30 years, 30-60 years, >60 years were obtained from Eye Banks of Aravind Eye Care System (Madurai and Tirunelveli). The whole mount of human anterior lens epithelium was immunostained for stem cell marker SOX-2, and a differentiated cell marker Cx-43. The sequential confocal images were acquired from one end to the other end of the wholemount and the quantification of cells in a ROI of 200 x 200 μm^2 from five fields in

the central zone (1.66±0.1mm) was analysed using ImageJ software. Stem cells were defined as SOX-2^{+} CX-43^{-} cells and progenitor cells as SOX-2^{+} Cx-43^{+} cells.

The percentage of stem cells with SOX2^{+} Cx-43^{-} expression was observed to be 2.2±1.3% (n=5) in younger donors (<30 years), 2.3±1.1% (n=5) in 30-60 years and 1.3% in above 60 years (n=1) (Table below). The percentage of progenitor cells with expression of SOX2^{+} Cx-43^{+} was also identified to be 6.7±3.9 in younger age (<30 years), 9.9±5.3 in middle age (30-60 years) and 3.2% in older age group (>60 years). Thus, upon aging, there is no reduction in adult human lens epithelial stem cells.

S.No	Age (Years)	Stem Cells (Sox-2^{+} Cx- 43^{-})	Progentior Cells (Sox-2^{+} Cx-43^{+})
> 30 Years			
1	21	3.69	10.99
2	23	1.05	4.09
3	27	2.53	8.22
4	28	3.05	8.95
5	28	0.79	1.32
Mean ±SD		2.2±1.3	6.7±3.9
30-60 Years			
1	32	3.54	17.35
2	36	3.28	15.04
3	39	0.90	3.56
4	46	3.02	7.94
5	56	1.5	8.98
Mean ±SD		2.2±1.1	9.9±5.3
> 60 Years			
1	63	1.34	3.15

Table:Lens Epithelial Stem Cell Content in three different age groups

Expression of SOX-2 and Cx-43 markers at mRNA level

The whole mount of human anterior lens epithelium (n=3) was dissected into three zones (Central+Germinative 2mm, transitional zone 4mm, equatorial zone 8mm). The cells were subjected to RNA isolation followed by cDNA conversion and real-time semiquantitative RT PCR analysis for the expression of GAPDH, SOX-2 and Cx-43 genes. Compared to the house keeping gene GAPDH, the expression of SOX-2 and Cx-43 revealed that there is no significance between the three zones.

Label retaining assay (LRCs)

The adult SCs with slow cycling property retains label like 5′-bromo-2′deoxyuridine (BrdU), a thymidine analog, for an extended period and thus called the LRCs. The LRC property of anterior lens epithelial cells was evaluated using explant cultures from central+germinative (2mm), transitional (4mm) and equatorial zones (8mm). Cultures were pulse labeled followed by a 21-day chase to identify the LRCs (Fig.1). Double immunostaining of explant cultured lens epithelial cells revealed that the expression of BrdU and SOX-2 in 0-30 yrs: central zone 21.8±12.7%, transitional zone - 6.56±2.4%, equatorial zone - 18.9±24.6% and in 30-60yrs: central zone 12.9±3.7%, transitional zone - 12±3.9%, equatorial zone - 13.7±5.1%. In cataract tissues, the label retaining SOX-2^{+} cells were restricted and reduced in the central zone (1.72±0.86%) compared to normal lens.

Characterisation of human lens spheres

Explant cultured primary lens epithelial cells (1×10^3 cells) from the different zones were analysed for their sphere forming ability (n = 3). After seven days of culture, the cells from the central+germinative

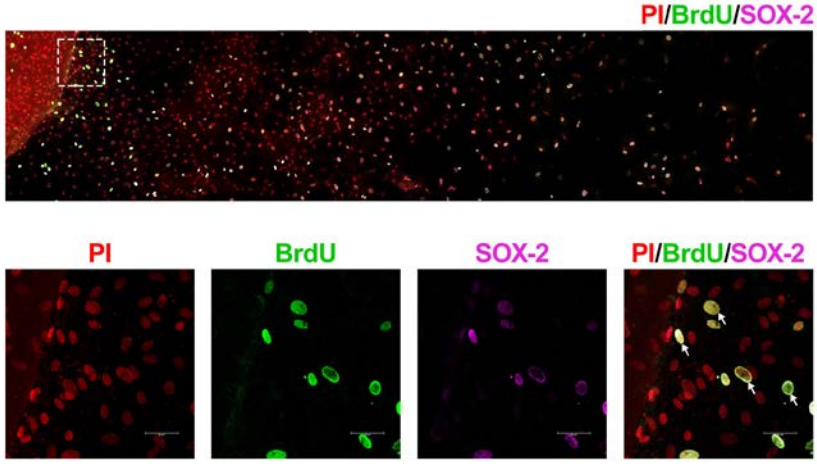


Figure 1: Representative confocal images of the cultured human lens epithelium from central + germinative zones, pulse labeled for BrdU followed by 21 days chase. The label retaining/slow cycling cells were identified by the expression of BrdU (green) along with the expression of the stem cell marker SOX-2 (Violet). The nuclei were counterstained with propidium Iodide (red).

Spheres formed/10^3 cells (spheres >40 µm)						
Age (years)	Central + Germinative Zone		Transitional Zone		Equatorial Zone	
	% of spheres	size (µm)	% of spheres	size (µm)	% of spheres	size (µm)
41	4.9	63.91	0	0	0	0
22	1.4	62.14	0.5	55.8	1.6	51.4
20	4.6	56.27	1.4	45.26	0.7	46.17
Mean ± SD	3.6 ± 1.1	60.8 ± 19.5	0.6 ± 0.4	50.5 ± 9.2	0.8 ± 0.5	48.8 ± 10.2

Sphere forming ability of human anterior lens epithelium (three zones)

zone were identified to have higher sphere forming ability - 3.6±1.12% (60.77±19.5µm) and larger spheres compared to other zones; transitional zone: 0.63±0.41% and equatorial zone: 0.76±0.46%.

Conclusion

- The mean percentage of lens epithelial stem cells ($SOX\text{-}2^+Cx\text{-}43^-$ cells) in the central zone remained the same with ageing.
- There was no significant difference in the expression of SOX-2 and Cx-43 at mRNA level between the three zones in the normal lens epithelium.
- Cells from the central+germinative zones had higher sphere forming ability (3.6±1.12%) compared to transitional (0.63±0.41%) and equatorial (0.76±0.46%) zones.
- The absence of SOX-2 positive cells in native tissue and the reduction in the percentage of LRCs in cultured lens epithelial cells in cataractous lens indicated a probable association of these stem cells with the cataract development.

Role of trabecular meshwork stem cell-derived extracellular vesicular miRNAs in human trabecular meshwork regeneration

Investigator : Dr. Gowri Priya Chidambaranathan
Co-Investigators : Dr. S. R. Krishnadas, Prof. VR. Muthukkaruppan, Prof. K. Dharmalingam
Research Scholar : R. Iswarya
Funding agency : Sun Pharma

Introduction including Background

Trabecular meshwork (TM), a tiny porous tissue located in the irido-corneal angle of the eye, is responsible for intraocular pressure (IOP) homeostasis. In glaucomatous condition, a drastic reduction in TM cells and oxidative stress that leads to altered extracellular matrix, have been associated with increased IOP. Previous studies from this laboratory identified the transplantation of cultured TM Stem cells (TMSC) in a cell loss human organ culture of anterior segment model to reduce the IOP. A few other reports in animal models also demonstrated the ability of TMSCs to regenerate TM. However, the main obstacle in cell based therapy is the maintenance of stemness in culture. The current study aims to evaluate the proliferative and anti-oxidant potential of TMSC derived exosomes on TM cell survival as an attempt towards cell free therapy for glaucoma. In this study, the exosomes from TM and TMSC conditioned medium were isolated by ultracentrifugation followed by characterisation using western blotting, nanoparticle tracking analysis (NTA) and transmission electron microscope (TEM). To evaluate the functional efficacy- in vitro scratch wound and anti-oxidant assays were carried out.

Results

Isolation and characterisation of exosomes

The isolated TM and TMSC exosomes expressed the exosomal markers syntenin, neuropilin and emilin (Figure below). NTA analysis revealed the size of TM (150.7±20.03nm) and TMSC (137.7±11.9nm) exosomes to be within the exosomal size range of 30-200nm. TEM images revealed the exosomes to be poly-dispersive and spherical in nature (Fig.1).

Wound healing and anti-oxidant efficacy of exosomes

In vitro wound healing assay results indicated that the TMSC exosomes significantly increased the wound closure rate (96.47±2.95%) compared to control (media - 75.80±4.62%) and TM exosomes (79.34±9.26%) at 48 hours (Fig.2).

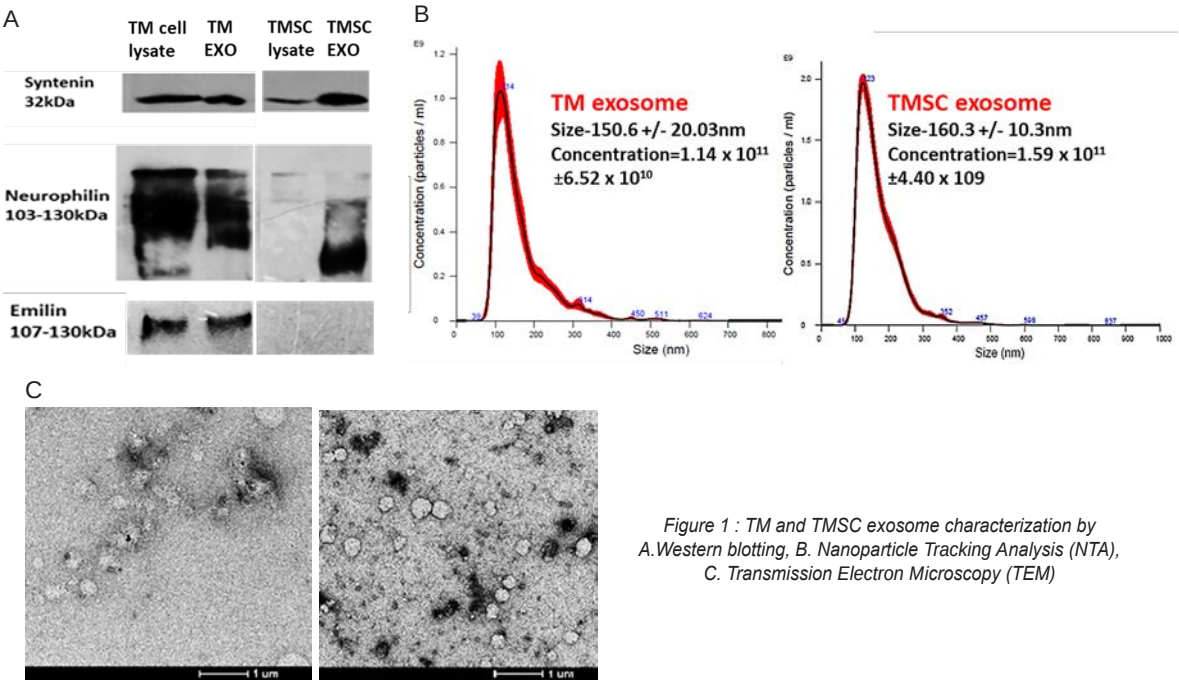
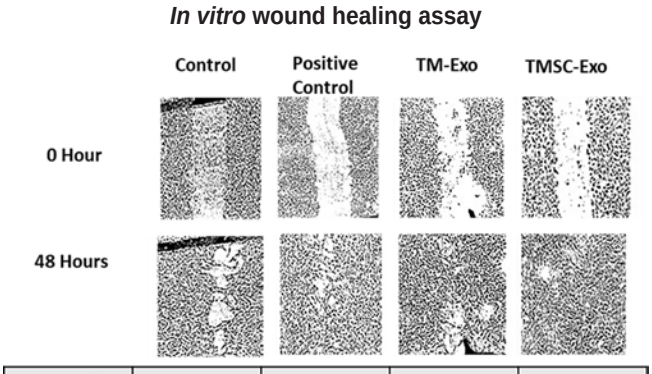


Figure 1 : TM and TMSC exosome characterization by A.Western blotting, B. Nanoparticle Tracking Analysis (NTA), C. Transmission Electron Microscopy (TEM)



Wound closure%	75.8±4.6	94.9±3.5	79.3±9.2	96.4±2.9

Figure 2 : Inverted phase contrast microscopic images of wounded TM cells with different treatment (top panel - 0 hours, bottom panel - 48 hours) - converted and analayzed using image J sofwere.

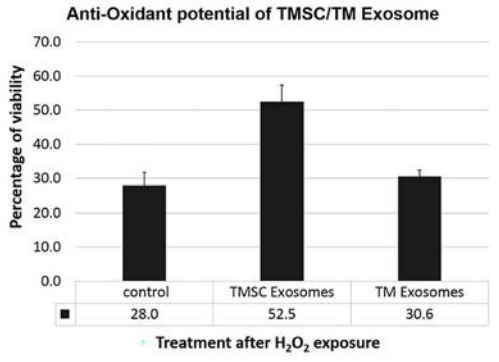


Figure 3: Viability of TM cells under oxidative stress, TMSC - exosomes enhanced cell viability compared to TM cells treated with TM exosomes and control

MTT assay revealed that upon oxidative stress the TM cell viability was reduced to 28% (100 µM H_2O_2) which increased to 52.5% with TMSC exosome treatment, while with TM exosomes it was only 30.6% (Fig.3).

Conclusion

The TMSC exosomes enhanced wound healing efficacy and anti-oxidant potential of TM cells indicating the possibility of developing a TMSC exosome based therapy for patients with primary open angle glaucoma.

Molecular regulators associated with the maintenance of human trabecular meshwork stem cells in relation to their reduction in ageing and glaucoma

Investigator : Dr. Gowri Priya Chidambaranathan
Co-Investigators : Prof. VR. Muthukkaruppan
Dr. S. R. Krishnadas
Dr. D. Bharanidharan
Research Scholar : R. Nerethika
Funding Agency : Science and Engineering Research Board

Previous reports from this laboratory confirmed the presence of adult tissue resident stem cells to be located in the anterior non-filtering (NF) region of human trabecular meshwork (TM). An age-related reduction in the total TM cells was identified to be significantly correlated with the TMSC loss. This reduction in both TM cells and TMSCs was drastically higher in donor tissues with known history of glaucoma. This study was carried out to identify the molecular regulators - the miRNAs that are associated with the stemness by comparing the cells from filtering (F) and NF regions of TM.

Immunostaining for stem cell marker confirmed the separation of filtering and non-filtering regions of human TM.

In order to elucidate the molecular regulation of the TM stem cells, the filtering (F) and NF region of human TM were dissected (n=3 donor tissues) and separation was confirmed by haematoxylin-eosin staining of paraffin sections of the tissues after dissection as well as by immunostaining for the universal stem cell marker - ABCG2 and neural crest stem cell marker - p75. Confocal microscopic analysis revealed higher expression of ABCG2 and p75 in the NF region compared to the F region (Fig.1).

MiRNA profiling of the filtering and non-filtering TM

RNA from the F and NF regions of TM were isolated and quality was checked by (i) Qubit - high sensitivity RNA assay as well as miRNA assay and (iii) Agilent Bioanalyser 2100 – pico chip. The RNA concentration was 4.2 to 19.7 ng/µl; miRNA – 1.08 to 8.54 ng/µl with a RIN value between 2.3 to 2.6. MiRNA profiling was carried out on Nanostring nCounter SPRINT.

Principal component analysis of the miRNA data from all three donors (Fig.2) indicated distinct clustering of miRNAs between the two regions of TM in the 14 and 62 year-old donor. In the 69 year old donor the difference was minimal.

This was also reflected in the heat map analysis (Fig.3). Analysis of the miRNAs identified that all the 11 miRNAs – hsa-miR -143-3p, miR-182, miR-26a-5p, miR-204-5p, miR-22-3p, let-7a-5p, miR-148a-3p, miR-181a-5p, miR-191-5p, let-7i-5p and miR-27b-3p, were reported to be expressed commonly in native human TM, limbus and ciliary body by Drewry et al.,

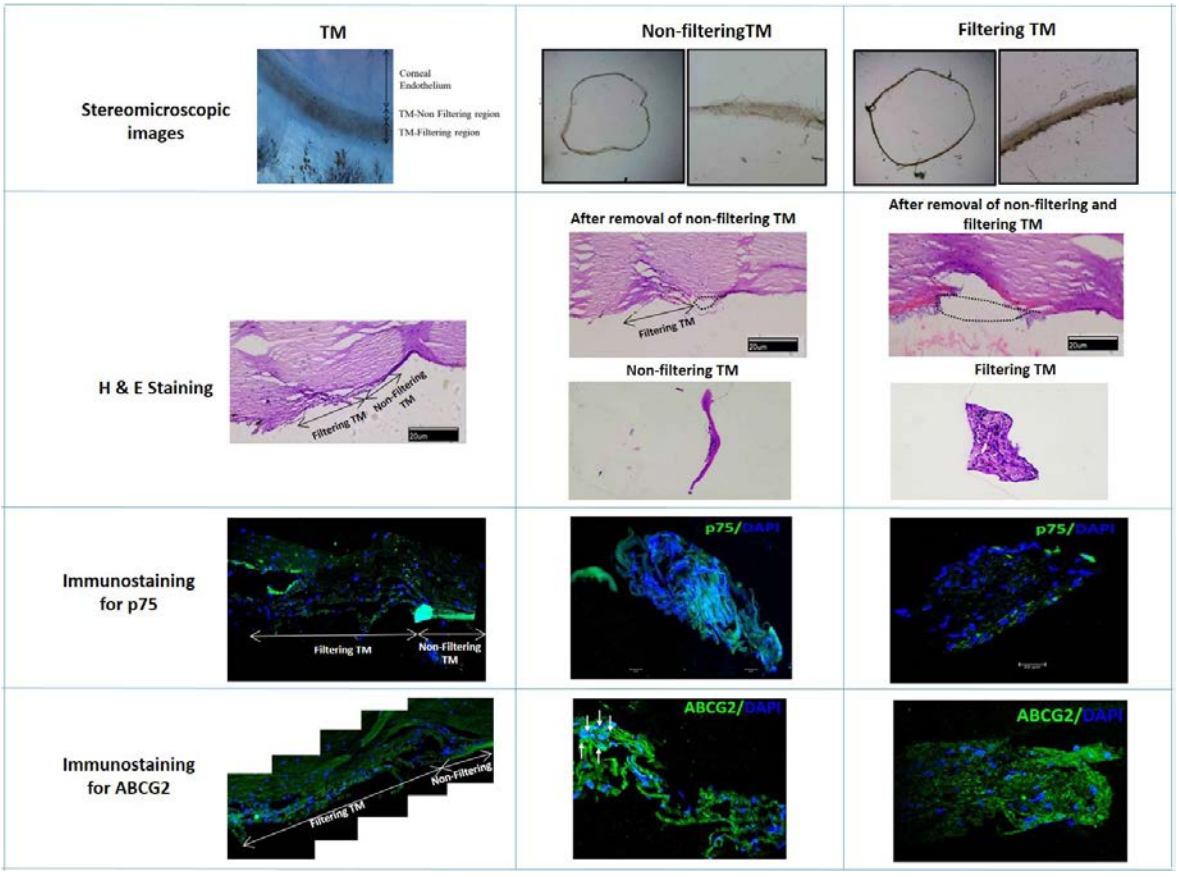


Figure 1 : Separation of filtering and non-filtering regions of human trabecular meshwork. Immunostaining of the dissected filtering and non-filtering TM sections from donor tissues indicated the expression of p75 and higher expression of ABCG2 in the nonfiltering TM, confirming the proper dissection.

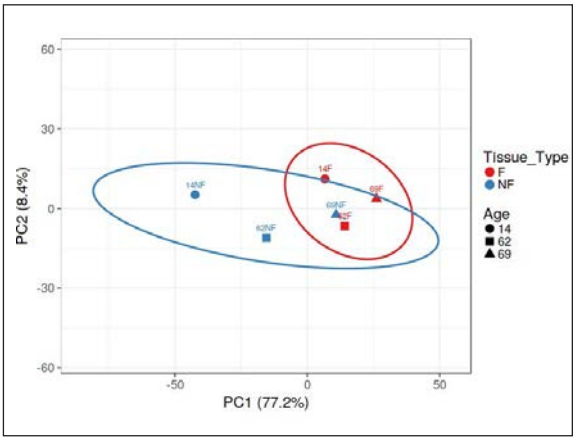


Figure 2: Principal component analysis

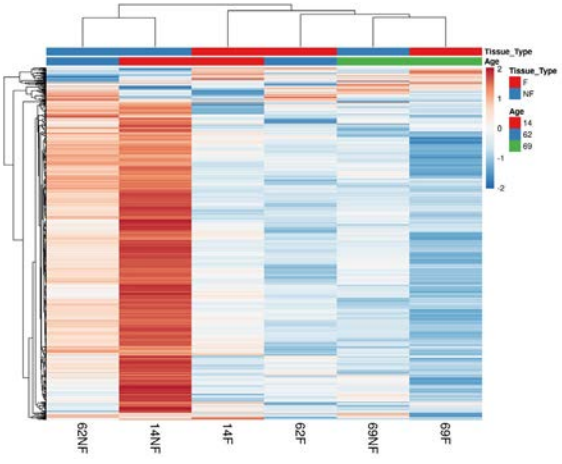


Figure 3: Heat map of the expression of miRNAs expressed in the filtering and non-filtering regions of the human trabecular meshwork from three donors. The expression of these miRNAs is illustrated in this heat map as the mean normalized mumber of reads, with blue representing relatively low mumber of reads and red as relatively high mumber of reads.

IOVS (2016), were also expressed in both the filtering and non-filtering regions of TM. In addition, miRNAs that were highly expressed in TM – miR-10b-5p, miR-21-5p, miR-30a-5p, miR-125b-5p, let-7f-5p, miR-92a-3p were also expressed in both the regions of TM, confirming the validity of the data generated.

MiRNAs differentially expressed in non-filtering TM

Analysis of the differentially expressed miRNAs by nSolver identified the following miRNAs to have a significant fold change:

(i) Upregulated in non-filtering region with fold change >= 1.2: miR-585-3p, miR-1246, miR-184, miR-664b-3p, miR-1283, miR-499a-3p, miR-346, miR-601, miR-494-3p, miR-1827 (14 years); 302d-3p (62 years); 126-3p (69 years)

(ii) Downregulated in non-filtering region with fold change <= -1.2: let-7i-5p, miR-145-5p, miR-4454, let-7c-5p, miR-99a-5p (14 years); let-7i-5p (62 years); miR-1-3p, miR-133a-3p, miR-143-3p, miR-145-5p (69 years)

Further studies are being carried out to (i) elucidate the role of these miRNAs and their targets in relation to TM stem cells, which are known to be located in this region and (ii) to understand the age-related changes in the expression of these miRNAs.

Conclusion

MiRNA profiling of the filtering and non-filtering regions of human trabecular meshwork from three donors identified miRNAs that were differentially expressed significantly in the non-filtering TM indicating a probable role with the trabecular meshwork stem cells, which are known to be located in this region.

Identification, characterisation and maintenance of stem cells in adult human Retinal Pigment Epithelium

Investigators : Dr. Gowri Priya Chidambaranathan
Co-Investigators : Prof. VR. Muthukkaruppan
Dr. K. NareshBabu
Dr. R. Kim
Research Scholar : A.Waseema

Introduction including background

Retinal Pigment Epithelium (RPE) is a pigmented monolayer beneath the photoreceptors of the human eye. It is important for the functioning of photoreceptors and its degeneration leads to age related macular degeneration, a leading cause of blindness in the elderly population. Salero et al., 2012 have demonstrated the presence of stem cells (10.6%) in cultured RPE cells (RPE-SC) based on its self-renewal and ability to differentiate into multiple cell types. But no report is available on the stem cells in native RPE and their role in tissue homeostasis. Previous studies from this laboratory identified the presence of cells with high proliferative potential in the peripheral region of human RPE (Waseema et al., IERG 2021). This study aims to analyse the changes in the functional characteristics of stem cells in the three regions of human RPE with ageing.

Results

Changes in sphere forming ability with ageing

The presence of 1.7% of cells with sphere forming ability in total human RPE was established in the previous report from this laboratory. Further, when the three regions of RPE were analysed (Fig.1),

1.53% of sphere forming cells were identified only in the peripheral RPE. In order to analyse the age-related changes, cells isolated from the entire or different regions of RPE (Figure below) from donors aged 30-60 and >60 years were subjected to sphere formation.

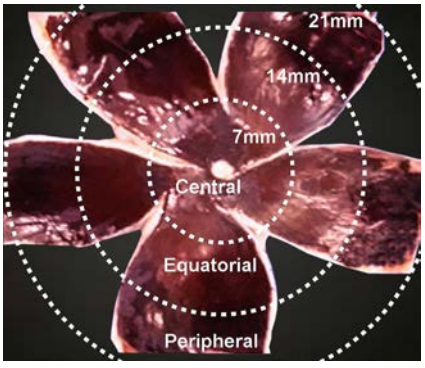


Figure 1: Demarcation of three regions of RPE (i.e) three equidistant concentric circles centered on the optic nerve head

Upon ageing, the cells with sphere forming ability were identified to be completely lost (Fig.2).

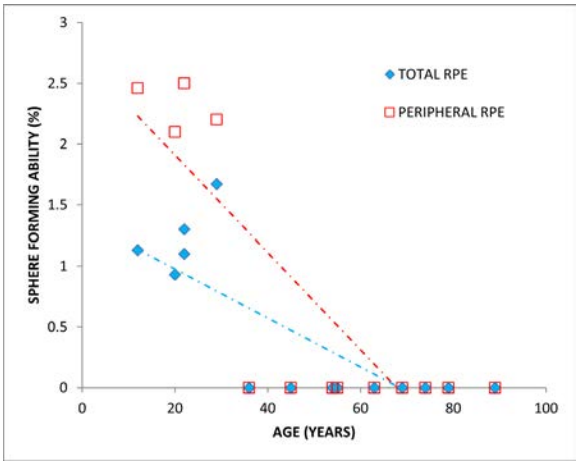


Figure 2: Correlation plot between age and sphere forming ability of RPESCs. Each dot in the plot represents the percentage of cells exhibiting the functional characteristics.

Changes in clone forming ability with ageing

Cells isolated from total and regional RPE of donors aged 30-60 and >60 years were cultured single cell per well in adherent conditions for 28 days. The percentage of cells with clone forming ability reduced from 3.54±1.58% in the middle aged donors to 0.2±0.45% in the older donors (Fig.3). A similar trend was also identified in the peripheral region, reducing from 11.62±2.25% of clone forming cells in the middle aged donors to 2.41±1.51% in the older donors.

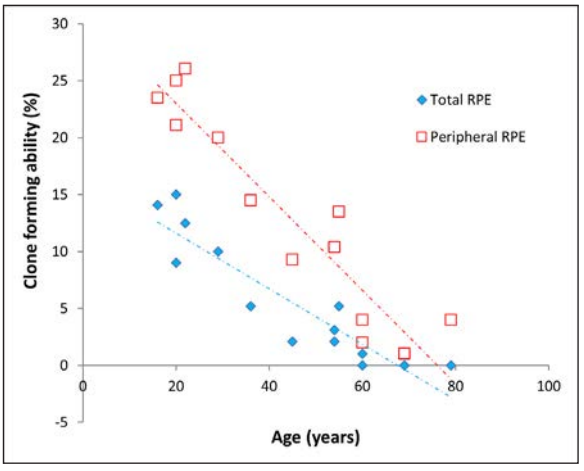


Figure 3: Correlation plot between age and clone forming ability of RPESCs. Each dot in the plot represents the percentage of cells exhibiting the functional characteristics represents the percentage of cells exhibiting the functional characteristics.

However, no such cells were observed in the central and equatorial regions of RPE.

Changes in label retaining property with ageing

RPE cells (total and different regions) from donors aged 30-60 and >60 years were pulsed with 10 μM BrdU for 2 days after adherence and cultured for 16 days without BrdU. The label retaining property of these cells revealed a negative correlation ($P<0.05$) with ageing (Fig.4). The small percentage of LRCs previously observed in the younger age group (< 30 years) in the equatorial region was lost with ageing (Table).

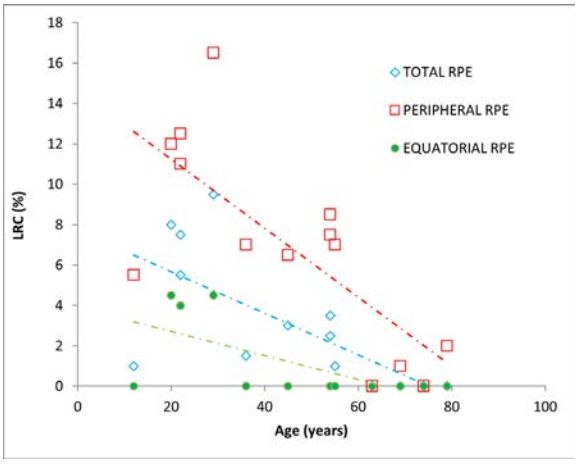


Figure 4: Correlation plot between age and label retaining ability of RPESCs. Each dot in the plot represents the percentage of cells exhibiting the functional characteristics.

Functional characteristics	Age	Total RPE	Peripheral RPE	Equatorial RPE	Central characteristics
Sphere formation	30-60 years	-	-	-	-
	60 years	-	-	-	-
Clone formation	30-60 years	3.54 ±1.58	11.62± 2.25	-	-
	60 years	0.2± 0.45	2.4 ±1.51	-	-
LRC analysis	30-60 years	2.3 ±1.04	7.3 ±0.76	-	-
	60 years	-	1.5± 0.71	-	-

Table : Changes in functional characteristics of stem cells with ageing

Conclusion

A reduction in the functional characteristics of RPESCs was observed with ageing. Additional studies are required to elucidate whether this reduction is associated with AMD.

PROTEOMICS

The ocular diseases that are focused on in the Proteomics department are diabetic retinopathy, fungal keratitis, keratoconus, and pterygium, all of which contribute a lion's share towards vision loss in the Indian population. The major techniques that are employed to study disease mechanisms and develop therapeutic interventions are proteomics, transcriptomics and state-of-art cell molecular biology techniques. The proteomics facility is equipped with two mass spectrometers to perform gel-based and non-gel based proteome analysis from ocular tissues, tear, blood and cells. Further, to study the role of nano-sized vesicles called extracellular vesicles in various ocular diseases, the proteomics department is equipped with ultracentrifuge and NanoParticle Tracking Analysis NS300 instruments. The outcomes of the basic research are translated as diagnostic or therapeutic strategies to improve disease management.

Role of Extracellular Vesicles in the pathogenesis of diabetic retinopathy Sub-project 1

Title: Deciphering the proteome signature of plasma extracellular vesicles from proliferative diabetic retinopathy patients

Investigator details: Dr. Daipayan Banerjee,
Prof. K. Dharmalingam,
Dr. K. Naresh Babu, Dr. R. Kim,
Dr. Sagnik Sen,
Dr. Prithviraj Udaya

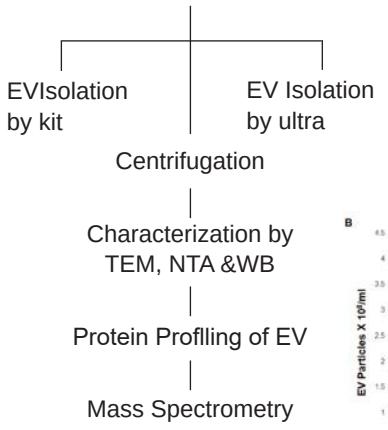
Research Scholar : Aadhithiya T. Gr
Funding : Sun Pharmaceuticals

Introduction including background

Diabetic Retinopathy (DR) is a serious ocular diabetic complication and is the leading cause of vision impairment in the working-age population globally. This progressive, microvascular disease is characterised by leaky abnormal retinal blood vessel formation/growth and hemorrhagic blood vessels. Clinically, DR is divided into two stages: i) non-proliferative DR (NPDR), which is characterised by alteration in vascular permeability, microaneurysms (alteration in the retinal blood vessel) and macular edema in an advanced stage. ii) Proliferative DR (PDR), which causes significant vision loss and is characterised by neovascularization, vitreous haemorrhage, and retinal detachment. Aberrant angiogenesis is the primary cause of vision loss. The anti-VEGF treatment routinely used for DR is expensive and not effective in about 40-50% of patients. Further, no prognostic marker is available to predict if a diabetic patient will progress to develop DR or if the milder stage of NPDR will progress to PDR. Thus, with the increasing disease burden, there is an urgent need to identify disease signatures and development of alternate treatment routes for efficient disease management.

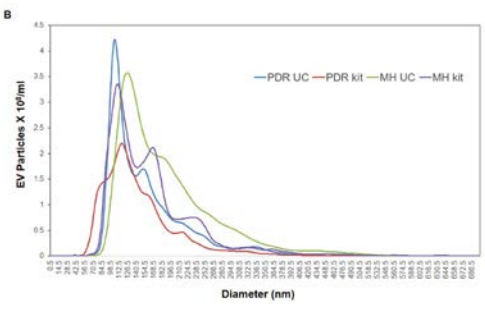
Extracellular vesicles (EV) are lipid-encased nano-carriers that are released by all eukaryotes and prokaryotes in health and diseased condition. The EV cargo consists of nucleic acids, proteins, lipids, and

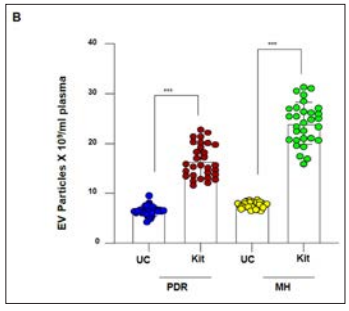
A

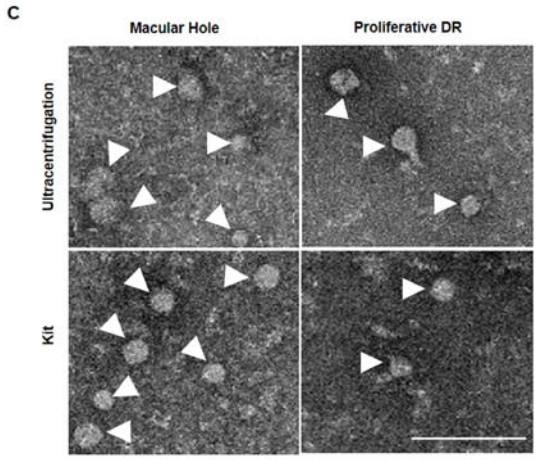


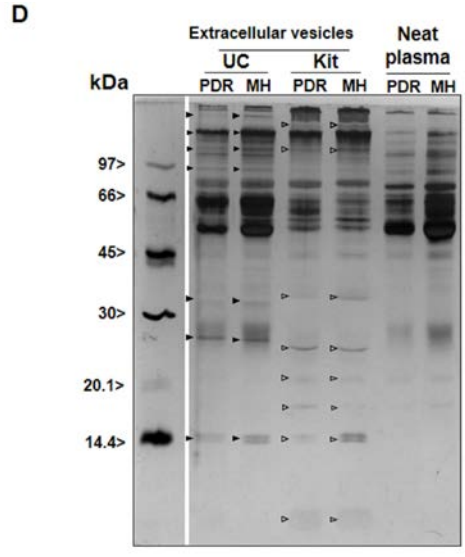
B

	PDR		MH	
in (nm)	UC	Kit	UC	Kit
Mean	163.1 ± 11.9	147.5 ± 22.1	189.5 ± 10.7	164 ± 17.8
Mode	111.9 ± 12.5	113.8 ± 21.2	127.9 ± 14.8	117.4 ± 21.3
D10	101.2 ± 6.3	94.7 ± 16.9	114 ± 6.8	101.8 ± 8.8
D50	138.5 ± 10.1	132 ± 19.2	166 ± 11.4	145.2 ± 17.1
D90	258 ± 30.5	221.5 ± 38.2	294.4 ± 21.1	251.9 ± 32.7









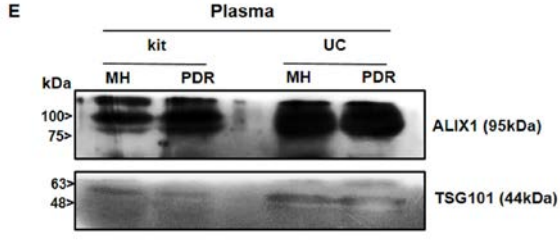


Figure 1. Plasma EV isolation and characterization from PDR and MH patients.

A. Experimental outline

*B. Nanoparticle Tracking Analysis (NTA): Table shows vesicle mean, mode, D10, D50 and D90; histogram represents the concentration of EVs (from NS300) corresponding to the size of EVs in the sample. Graph shows concentration of EVs per ml of plasma. Data presented are mean ± s.d.; two-tailed unpaired t test; *p < 0.05 indicates significance compared to respective groups; n=30 independent readings from NanoSight NS300 (2 samples, 15 readings per sample).*

C. Transmission Electron Microscopy images for the Extracellular Vesicles (EVs) isolated by ultracentrifugation and kit method from plasma of Proliferative Diabetic Retinopathy (PDR) and Macular Hole (MH) patients, the arrowheads denote the EVs in the samples. Representative images of n=5 fields are presented. Scale bar = 100 µm.

D. SDS-PAGE silver-stained image shows the difference in the proteome profile of EVs between neat plasma and EVs and the difference between two isolation methods.

E. Immunoblots for EV markers ALIX1 and TSG101

metabolites and EVs are mediators of intracellular communication in several key physiological processes like gene transcription, translation, cell migration, proliferation, immune response and angiogenesis to name a few. Tumor angiogenesis is needed for the tumour growth and metastasis. Endothelial cells are critical in microvessel sprouting and angiogenesis. Several studies have revealed pivotal roles of EVs in tumor biology, where under hypoxic conditions, tumor cells secrete functional EVs that induce endothelial cell sprouting, neovascularization and aid in tumor metastasis. Angiogenesis is a key process in DR pathology, however, the role of EVs in DR pathogenesis is not well understood. Recent studies have revealed the role of EV in DR. IgG-laden plasma EVs activate the classical complement pathway and contribute to microvascular damage in DR. Further, EVs secreted by retinal astroglia cells (RACs) under oxidative stress promote the proliferation and migration of endothelial cells.

The objective of this study was to identify the plasma protein cargo of EVs from proliferative diabetic retinopathy (PDR) patients by isolating EVs by two different methods and using a shotgun mass spectrometry-based proteomic approach. The research team also identified the biochemical pathways enriched specifically in DR patient EVs using computational tools.

Results

Extracellular vesicle isolation and characterisation

Plasma was separated from blood collected from DR patients and patients with MH (as control) and EVs were isolated from pooled plasma using two different methods: The ultracentrifugation method and the commercially available ExoQuick ULTRA kit (System Biosciences). EVs were characterised by Transmission Electron Microscopy (TEM), Nanoparticle Tracking Analysis (NTA) and immunoblotting using exosome specific markers. A bottom-up proteomics approach was conducted and plasma EV proteins were detected using a nano-LC Orbitrap mass spectrometer (MS). Raw data from MS were searched against the human database to identify EV's protein cargo and proteins were analysed using the online bioinformatics platform DAVID to associate with biological pathways. The overall workflow is illustrated in Figure 1A.

Plasma EVs isolated by the two methods were subjected to NTA (NanoSight NS300, Malvern Panalytical) for size determination and particle count. Figure 1B and Table within shows the size distribution and the concentration of the EVs per ml of plasma based on NTA analysis. Analysis of the

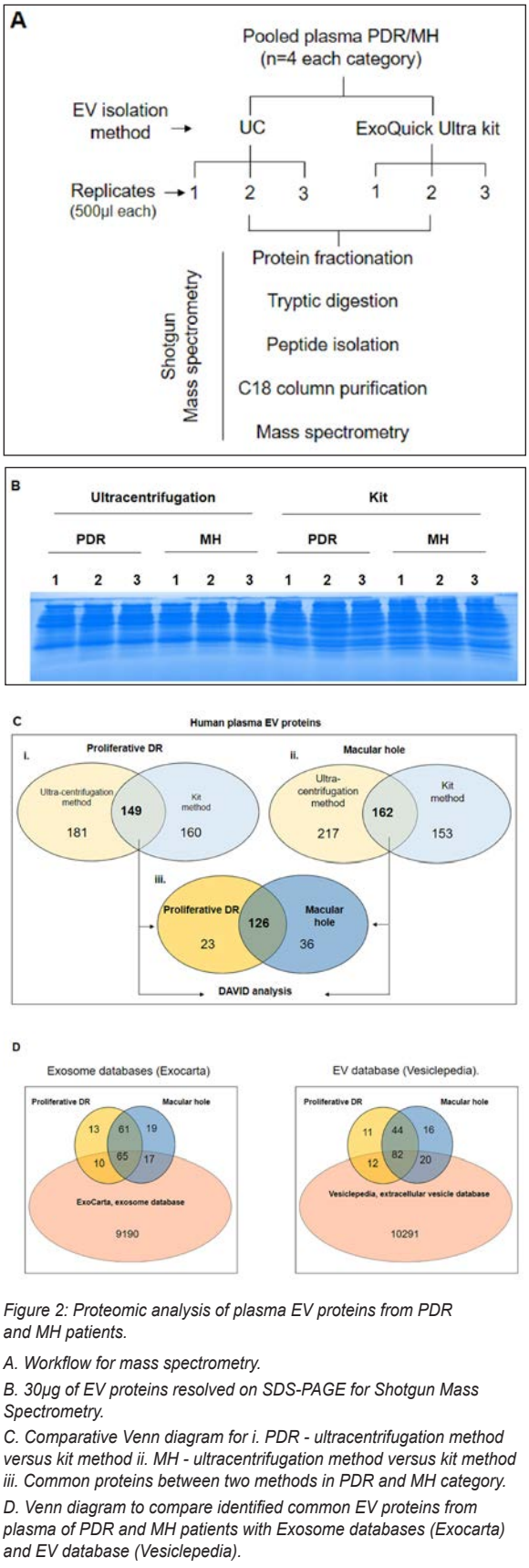


Figure 2: Proteomic analysis of plasma EV proteins from PDR and MH patients.

A. Workflow for mass spectrometry.

B. 30µg of EV proteins resolved on SDS-PAGE for Shotgun Mass Spectrometry.

C. Comparative Venn diagram for i. PDR - ultracentrifugation method versus kit method ii. MH - ultracentrifugation method versus kit method iii. Common proteins between two methods in PDR and MH category.

D. Venn diagram to compare identified common EV proteins from plasma of PDR and MH patients with Exosome databases (Exocarta) and EV database (Vesiclepedia).

plasma EVs by transmission electron microscopy revealed different shaped vesicles as shown in Figure 1C. The enrichment of EVs from plasma was confirmed by immunoblot analysis of EV protein using two EV markers, ALIX and TSG101 (Figure 1E). The representative protein profile of plasma EVs separated on a 1D PAGE followed by silver staining is shown in Figure 1D. The EV protein profile was distinctly different from the neat plasma profile (as indicated by arrows) and differences in banding pattern were observed in EV profile depending on the isolation method (indicated by black arrows). Further, differences in EV protein profile were noted in plasma EVs from PDR patients compared to plasma EVs from MH patients. Thus, differences in protein profiling indicate differences in the proteomic landscape in plasma EVs from PDR compared to MH and also depending on the EV isolation technique.

Proteome profiling of extracellular vesicles

An equal amount of plasma from 4 patients in each disease category (PDR or MH) was pooled and 0.5ml of pooled plasma was used for each EV isolation method with 3 experimental replicates in each isolation category. Each of the isolated EVs was subjected to shotgun analysis for in-gel tryptic digestion and peptide extraction followed by peptides desalting cleanup using C18 columns and were analysed in a nanoLC-Orbitrap mass spectrometer (Figure 2A and 2B). Raw data when searched against the human proteome database identified non-redundant 330 and 379 total plasma EV proteins in the PDR and MH categories respectively using the ultracentrifugation EV isolation technique. A total of 309 PDR and 315 MH EV proteins were identified from the EVs prepared by the ExoQuick Ultra kit. The two independent EV isolation methods revealed 149 common EV proteins from plasma EVs of PDR patients and 162 common proteins from plasma EVs of patients with MH and were taken for functional analysis using a bioinformatics tool (Figure 2C). The proteins identified in the plasma EVs were then compared with those reported in the Exocarta exosome database and Vesiclepedia extracellular vesicles database. Figure 2D shows the comparison of the plasma EV proteins with the total proteins reported in the database.

Discussion

Based on the EV-based biomarker studies in cancer biology, the research team postulate that evaluating the plasma EV protein cargo in DR patients can be an excellent starting point for biomarker identification and exploring novel therapeutic targets in the angiogenic ocular disease DR. PDR is a more advanced stage of diabetic retinopathy which causes significant vision loss and is characterised by neovascularization, vitreous haemorrhage, and retinal detachment. Rarely have the protein signatures in plasma EVs obtained from PDR patients been investigated. This is the first study to explore the plasma EV proteome profile from PDR patients in comparison to another non-angiogenic ocular complication idiopathic macular hole. Some of the proteins that are uniquely found or are enriched in plasma EVs from PDR patients are involved in tumour progression, anti-oxidant activity, glucose homeostasis/insulin resistance, inflammasome activation and cell-cell adhesion. Future studies will entail validating plasma EV proteins for individual patients in a large cohort of patient population in each disease category. These proteins can be investigated at length for potential biomarker identification in progressive DR disease states and their role in DR pathogenesis.

Overall, through this study AMRF have identified the plasma EV protein cargo from patients with an angiogenic ocular disease PDR in comparison to a non-angiogenic ocular condition of idiopathic macular hole is identified. This study is a stepping stone towards evaluating the role of EVs in progressive ocular angiogenic disease, specifically in the context of the key protein components.

Sub-project 2
A comparative proteomic analysis of plasma and vitreous humor derived small Extracellular Vesicles (SEVs) from Proliferative Diabetic Retinopathy (PDR) patients

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Dr. K.Naresh Babu, Dr. R.Kim,
Research Scholar : Aadhithiya T. Gr
Funding : Sun Pharmaceuticals

Background

EVs can be broadly classified into small and large EVs based on their size and biogenesis pathway. In this study, the research team compared the proteome landscape of the plasma-derived small EVs (SEVs) to the vitreous humor-derived SEVs from patients with PDR. The objective is to identify the VH SEVs and plasma SEVs, understand their contribution to DR pathology and shortlist circulatory SEV proteins that may be used for biomarker analysis.

Results

Small Extracellular vesicle isolation and characterisation

Plasma was separated from blood collected from PDR patients. Vitreous humor was collected from the same patients undergoing vitrectomy surgery. Large EVs were segregated by passing the pooled plasma samples and the pooled vitreous samples through a .22µm filter followed by centrifugation at 30,000g. From the supernatant, SEVs were isolated by ultracentrifugation at 1,20,000g. The SEVs were characterised by Transmission Electron Microscopy (TEM) and Nanoparticle Tracking Analysis (NTA). The plasma SEVs and VH SEVs were subjected to mass spectrometry to identify the protein content. The proteins from plasma SEVs and VH SEVs were compared to find the common and enriched proteins in each biological fluid test. The overall workflow is shown in Figure 3a. Interestingly, the NTA particle concentration and size distribution show a significant difference between the plasma SEVs and VH SEVs (Figure 3b and 3c). Transmission Electron Microscopy (TEM) reveals the shape of the plasma SEVs and VH SEVs from PDR patients (Figure 3d). Using shotgun mass-spectrometry, a total of 147 proteins were identified in VH SEVs and 125 proteins were identified in plasma SEVs. These protein groups were compared and 68 proteins were identified as common proteins, 57 unique proteins in plasma SEVs and 79 unique proteins in VH SEVs (Figure 3e).

Fig: 3a

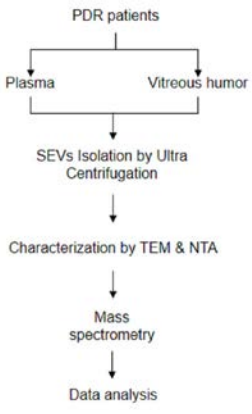


Fig: 3b

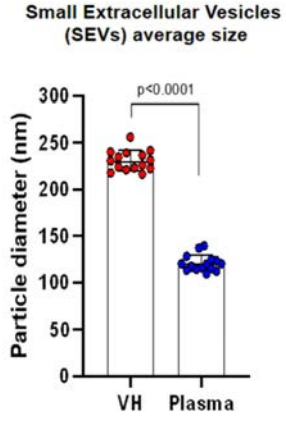


Fig: 3c

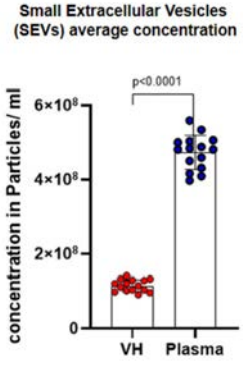


Fig: 3d

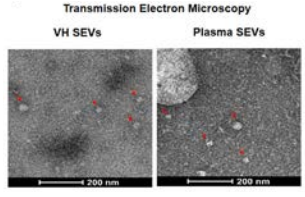


Fig: 3e

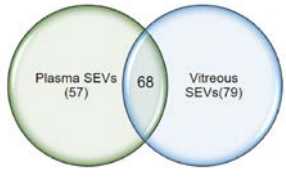


Figure 3: Comparison of small EVs from plasma and vitreous humor of PDR patients.

A. Experimental outline

B and C. Small EV size and concentration comparison using Nanoparticle Tracking Analysis (NTA)

D. Transmission Electron Microscopy images for the Small Extracellular Vesicles from plasma and vitreous humor.

E. Venn diagram to compare identified common and unique EV proteins from plasma and VH from PDR patients.

Conclusion

In this study, SEVs from the pooled plasma is identified and the pooled VH samples from the PDR patients by ultracentrifugation method. The isolated SEVs were characterised by NTA and TEM analysis. The SEVs cargo proteins were identified by mass spectrometry and the team has identified the proteins that are unique and common to the two biological fluids are short listed. Using both MaxQuant-LFQ Analyst and Proteome Discoverer, amongst the common proteins identified in the SEVs, the proteins that are enriched in plasma SEVs are Complement component 3 and the acute phase protein alpha2-macroglobulin. The proteins that are enriched in VH SEVs are retinal homeostatic protein zinc-alpha2-glycoprotein and the acute phase response protein SERPINA3. The proteins that are identified uniquely in plasma SEVs are the regulator of classical pathway of complement activation C4b Binding Protein (α-chain), the Complement C1q subcomponent subunit C and subunit A which is a component of C1 complex and the cell adhesion protein Fibronectin. The proteins that are identified uniquely in VH SEVs are the antioxidant protein Peroxiredoxin 2, the photoreceptor secreted retinol transport protein Retinol Binding Protein 3 (RBP3), and the acute phase protein Serpin B3, which is upregulated in serval cancers.

This study has identified several small EV proteins in the plasma and VH of PDR patients that are potentially interesting both as a putative biomarker and further mechanistic studies to decipher their role in DR pathogenesis.

Project 2
Deciphering predictive and preventative methods in the progression of pterygium using multi-omics approaches.

Investigator details: Dr. Daipayan Banerjee
Research Scholar : Mathan Loganathan
Funding : SERB-Startup Research Grant, 2years.

Introduction including background

Pterygium is a highly prevalent, progressive conjunctival eye disease that has a significant impact on quality of life and causes vision impairment, mostly affecting people with low socioeconomic status working outdoors. It is characterised by wing-shaped conjunctival fibrovascular overgrowth typically originating from the nasal side and migrating towards the cornea. In southern India, the incidence rate of pterygium is 25.2 per 100 person-years and contributes to ~4% of the corneal blindness burden. UV exposure is one of the critical causative factors of the disease, however, the disease etiology remains obscure. Despite the high prevalence rate of 12%, no pharmaceutical intervention prevents pterygium progression, and surgical removal is the only treatment option. Further, no prognostic tools to predict disease progression/recurrence exist. Rationale: Dysregulated epigenetic modifications play a critical role in disease pathogenesis and are used as biomarker/interventional therapies. It is established that chronic sunlight exposure causes alteration in skin epigenetics and is a causative factor in skin cancer. Pterygium is an ocular surface disease that is constantly exposed to sunlight, however, the role of epigenetic changes and how it regulates gene expression in pterygium subtypes is vastly unexplored.

In this study we will Decipher the role of epigenetic modifications in regulating gene expression in pterygium progression and recurrence and Evaluate pharmacological inhibitors and exosomes as adjuvant therapy to prevent pterygium progression.

Understanding the mechanism of action of a novel chemical cross-linker designed to treat keratoconus

Investigators : Dr. O. G. Ramprasad, Prof. K. Dharmalingam and Dr. Venkatesh Prajna
Research fellow : Adhithya Subramanian
Clinical fellow : Dr. Pooja Deepak Andhare
Funding : ICMR

Introduction including background

Keratoconus (KC) is one of the major bilateral corneal ectasias affecting the young population in their second and third decades of life. The mild form of keratoconus, characterised by slight increase in the curvature of cornea is prevalent among 1.4% of the Indian population while moderate to severe keratoconus cases are seen in 0.21% to 0.035% of the population. The severe form of keratoconus needs corneal transplantation. The severity of the disease is classified on the basis of radius of curvature of anterior segment of the cornea. Though the disease affects both the eyes, the presentation and severity at any point of time is unilateral. The disease is characterised by a decrease in the mechanical strength of the collagen fibrils in the cornea and significant loss of extracellular matrix, leading to the thinning of the cornea followed by the formation of cone shaped central cornea. In addition to that, decreased collagen cross-linking activity leads to keratoconus. Consequently, defective vision develops in the form of severe astigmatism.

The collaborators at the University of Liverpool, UK have developed a novel, PBS soluble, eye-drop based chemical cross-linker, consisting of EDCI/NHS [1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide] mediated chemistry and a suberic acid spacer that has the potential to be developed as an alternative form of treatment for mild to moderate keratoconus. It can cause corneal cross-linking without removing the corneal epithelium, or the use of UV-A irradiation, therefore avoiding the pain associated with the conventional crosslinking treatment of keratoconus and the risk of infection. Aravind Medical Research Foundation and Aurolab have established the proof-of concept in human corneas, wherein treatment of the diseased keratoconus cornea for 15 minutes at 37°C with the novel chemical cross-linker is able to increase the stiffness of the weak keratoconus cornea by cross-linking collagen molecules. The cross-linker also does not cause cytotoxicity or morphological changes to the corneal cell layers. The details about the investigation are available in the previous progress reports as well as in the publication (Haneef et al., 2021). The mechanism of action of the cross-linker in stiffening the cornea is not clearly understood. By unravelling the mechanism of action of the novel chemical cross-linker solution, this present study will aid in the further development and or refinement of optimal formulation of the cross-linker for further pre-clinical trials.

Results

In the results reported here, the research team analysed the effect of cross-linker on the modulation

of transcripts in crosslinked vs non-crosslinked Keratoconus cornea (epithelium and stroma) and its comparison to normal cadaver cornea. The team also studied the effect of cross-linker on the enzyme activity of matrix modulating enzymes and collagen fibril assembly and ECM modulation by the cross-linker. The difference in the arrangement of epithelial cells in the corneal epithelium and the stromal cells in the corneal stroma necessitated the separate analysis of differential gene expression and the activity of matrix modulating enzymes in these layers

Transcriptome profiling from corneal layers after crosslinker treatment

Total RNA from epithelium and stroma of the cadaver cornea and keratoconus cornea was isolated after separating the layers. The keratoconus cornea was treated with the cross-linker, the layers were separated and RNA was isolated. The RNA yields ranged from 36.3 ng/ul to 115 ng/ul for epithelial layers and from 30.6 ng/ul to 109 ng/ul for the stromal layers as measured by a Qubit fluorometer.

Corneal epithelium: The characteristic genes expressed in central corneal epithelium with significant expression included, Keratin 3 (KRT3), Keratin 5 (KRT5), Keratin 12 (KRT12), aldehyde dehydrogenase 3 family member A1 (ALDH3A1), clusterin (CLU) and enolase 1 (ENO1). KRT3 and KRT12 are markers for differentiated corneal epithelial cells and are only expressed in the central corneal region. These genes were expressed in both cadaver and cross-linker treated corneal epithelium.

There were a total number of 1102 differentially expressed genes and out of that, 390 genes were upregulated and 712 genes were downregulated. Select upregulated genes (log Fc>1) and select downregulated genes (log Fc<1) are listed in Tables 1A and 1B respectively.

The effect of the cross-linker was more pronounced by the upregulation of some of the following genes in the cross-linker treated keratoconus corneal epithelium:

COL4A3: basement membrane collagen seen in Bowman's layer, ICAM1 and CDH23: genes involved in enhancing the cell to cell adhesion and contact of the epithelial layer.

The upregulation of these genes makes the epithelial cells in the keratoconus corneal layer more organised and in close contact with each other.

Regarding the downregulated genes, MMP9 was down regulated 4.9 folds meaning the cross-linker is able to decrease the inflammation and the effect of metalloprotease. Collagens COL3A1, COL4A2 and COL5A1 were down regulated implying that the cross-linker was able to inhibit the genes associated with fibrosis.

A real-time PCR validation of some of the transcripts was done. MMP-9 was found at similar levels of expression in both the keratoconus and the cross-linker treated corneal epithelium in comparison

Table 1A: Select upregulated genes in the cross-linker treated keratoconus epithelium

Gene name	Log Fc compared to control	Description
FAM107A	3.91	family with sequence similarity 107 member A
SNORD31	4.11	small nucleolar RNA, C/D box 31
FGF7	3.90	fibroblast growth factor 7
KCNJ13	3.06	potassium channel family protein
COL4A3	2.63	Collagen Type IV Alpha 3 Chain
SERPINA3	2.27	Serpin Family A Member 3, Serine (Or Cysteine) Proteinase Inhibitor
CLEC2B	2.05	C-Type Lectin Domain Family 2 Member B
SMAD3	1.59	SMAD Family Member 3, functions in TGF-β signalling pathway
VEGFA	1.51	Vascular endothelial growth factor, A subtype
ICAM1	1.6	Intercellular adhesion molecule
CDH23	1.89	Cadherin 23, Helps in cell -cell adhesion
KLF10	1.47	Kruppel Like Factor 10, transcriptional repressor, effector of TGF-β signalling
KLF11	1.18	Kruppel Like Factor 11, transcription factor, inhibitor of cell growth

Table 1B: Select downregulated genes in the cross-linker treated keratoconus epithelium

Gene name	Log Fc compared to control	Description
WNT7A	-1.72	Wnt family 7A
MMP9	-4.9	Matrix-metalloprotease 9
SLC17A9	-4.62	solute carrier family 17 member 9
KRT78	-4.65	keratin 78
COL4A1	-4.66	collagen type IV alpha 1 chain
KLK12	-4.75	kallikrein related peptidase 12
COMP	-4.88	cartilage oligomeric matrix protein
KRT13	-4.88	keratin 13
COL4A2	-4.89	collagen type IV alpha 2 chain
COL5A1	-4.93	collagen type V alpha 1 chain
ZNF469	-5.00	zinc finger protein 469
CEACAM5	-5.00	CEA cell adhesion molecule 5

to control (it was downregulated in RNA seq analysis) (Fig. 1). LOX (Lysyl oxidase; the natural cross-linking enzyme), KRT6A (Keratin 6A) and COL1A1 were expressed at lower levels in keratoconus epithelium and at higher levels in cross-linker treated epithelium. In keratoconus condition, the expression of the above three genes is lower as a result of considerable reduction in stiffness of the corneal structure. The cross-linker restores the stiffness and as a result the expression of genes related to the structural changes is higher. LOX gene expression was also higher in the human corneal epithelial cell line treated with the cross-linker solution. KRT3 is a marker of the central corneal epithelium, its expression was found to be higher in keratoconus and cross-linker treated conditions.

Corneal stroma: The matrix modulating genes like MYOC, COMP, GSTM1, SFRP2, ANKRD35 were upregulated in cross-linker treated corneal stroma in comparison to KC stroma. Some of the downregulated genes were CD55, MYO5B, ZNF750 and IL18. Those downregulated genes corresponded to cell adhesion, cell growth and immunomodulatory genes. The DAVID functional annotation analysis classified the differentially regulated genes to ECM modulation, metalloprotease signaling, WNT signaling and TGFβ2 signalling pathways. The cross-linker

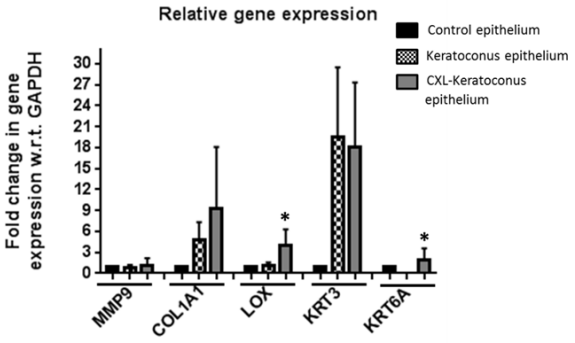


*Figure 1: Real-time PCR validation of select genes from the control cadaver, keratoconus and the cross-linker (CXL) treated keratoconus corneal epithelium. * represents p<0.05 by Wilcoxon's t-Test.*

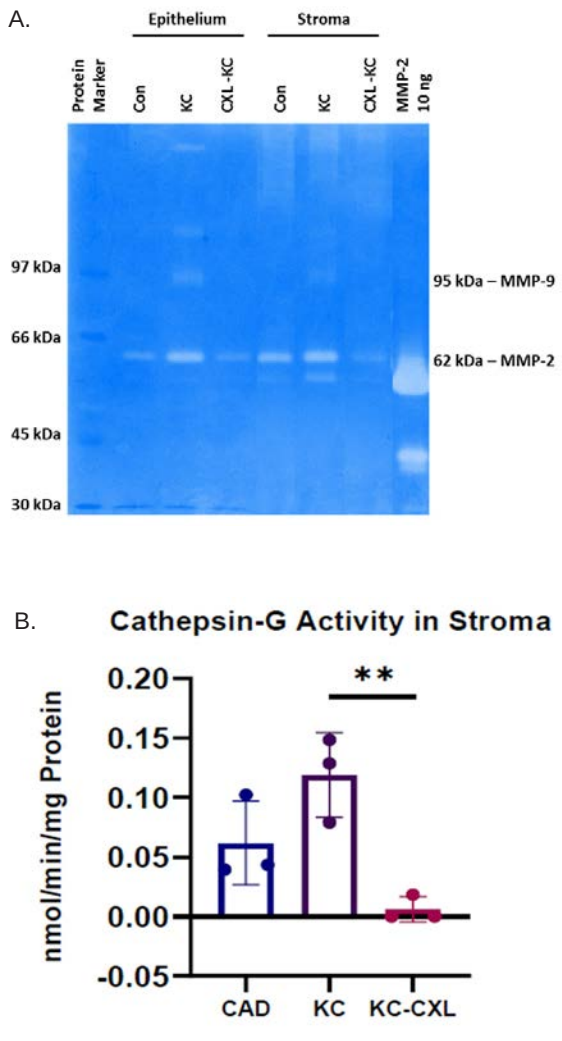


Figure 2: (A) Gelatin zymography of corneal epithelial and stromal layers before and after cross-linker treatment. (B) Cathepsin-G activity measured in stromal layer of various categories of cornea. KC represents keratoconus condition. KC-CXL represents keratoconus cornea treated with cross-linker. ** represents p<0.05 by t-Test between the indicated conditions.

affected the above pathways in the stroma. Further validation of differentially regulated genes by real time PCR has to be carried out.

Effect of cross-linker on the activity of matrix metalloproteases (MMPs) in the corneal layers

Gelatin zymography assay was performed to assess the effect of the cross-linker on the activity of secreted MMP-s 2 and 9, which are gelatinases. The control cadaver cornea, keratoconus cornea and cross-linker treated keratoconus cornea were separated into epithelial and stromal layers. Lysates from these layers before and after cross-linker treatment were used for analysing the gelatin digesting activity of the MMPs. The gelatin containing native PAGE gels exhibit white bands at the molecular weight positions of different MMPs indicating the digestion of gelatin. The strength of the gelatinase activity depends on the thickness of the bands. The results of this experiment are represented in Figure 2A. In the keratoconus corneal epithelium, more of MMP9 activity (95 kDa) was observed. After cross-linker treatment, the activity of MMP9 was attenuated. Similar was the case with MMP2 activity at 62 kDa. The control cadaver epithelium did not have any MMP9 activity. In stroma, MMP2 activity was highly upregulated, which explains the corneal stromal degradation in keratoconus condition. After cross-linker treatment, the MMP2 activity diminished.

Keratoconus corneas have elevated levels of cathepsins-B and -G, which can stimulate oxidative stress and cause matrix degradation. There was increased Cathepsin G. activity in corneal stroma which diminished after cross-linker treatment (Figure 2B). The cross-linker deactivates the metalloproteases MMPs- 2 and 9 and Cathepsin-G and probably halts the degradation of the matrix.

Modulation of collagen fibre assembly in the corneal stroma by the cross-linker

Transmission electron microscopy (TEM) analysis of corneal stroma from the control cadaver, KC and cross-linker treated KC corneas was done. The control cadaver stroma had the typical orderly orthogonal arrangement and packing of collagen fibres. In KC stroma, the packing was less dense and collagen fibres were disorganised. After the cross-linker treatment, the collagen fibres re-organised to a great extent, restoring the orderliness and arrangement of the fibres (Figure 3A). The collagen fibril density was measured in multiple sections of different samples. The fibril density in KC was almost

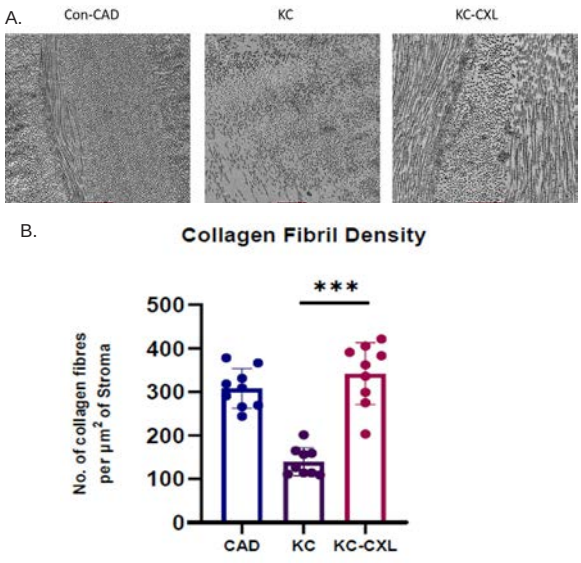


*Figure 3: (A) Collagen fibre assembly imaging in the corneal stroma by TEM analysis in control cadaver (Con-CAD), KC and KC cornea treated with cross-linker (CXL). (B) Measurement of collagen fibril density in the above TEM images. *** represents p<0.005 between KC stroma and KC-CXL stroma*

half of the control samples, cross-linker treatment restored the fibril density to the control samples (Figure 3B).

Conclusions:

The novel chemical cross-linker regulates genes related to ECM, focal adhesion, WNT signalling pathways in the corneal epithelium. In corneal stroma, ECM, metalloprotease, collagen synthesis and TGFβ signalling pathways are affected coupled with the reduction in inflammatory genes. The cross-linker could reduce the inflammatory conditions associated with keratoconus. MMP-2 and 9 analysis by gelatin zymography in the corneal epithelial and stromal layers showed consistent decrease of MMP-2 and MMP-9 activity after crosslinker treatment. Cathepsin G activity is decreased in stroma of keratoconus cornea after CXL treatment. The CXL possibly inhibits the surge in activity of MMP-2, MMP-9 and cathepsin-G, thereby reducing the degradation of matrix in keratoconus cornea. CXL also brings together the disorganised collagen fibres in stroma leading to the increase in stiffness in keratoconus corneas. The novel chemical crosslinker seems to be associated with inhibition of the signalling pathways involving the MMP-2 and MMP-9, leading to corneal stiffening, thereby halting the progression of keratoconus disease.

OCULAR PHARMACOLOGY

The main research focus of the Department of Ocular Pharmacology is to understand the molecular mechanism(s) involved in the pathogenesis and to develop therapeutic targets for the management of glucocorticoid (GC) induced ocular hypertension (OHT)/glaucoma. The funding support from Wellcome-DBT/India Alliance enabled the research team of the department to investigate the role of microRNA in the regulation of GC signaling and to develop miRNA based therapeutics for GC-induced glaucoma.

Role of microRNA in regulating glucocorticoid receptor signaling in steroid-induced ocular hypertension/glaucoma

Investigators Details	:	Dr. S. Senthilkumari , Dr. C. Gowripriya, Dr. D. Bharanidharan, Dr. R. Sharmila
International Collaborator:		Prof. Colin Willoughby, Faculty of Life & Health Sciences, University of Ulster, Northern Ireland, UK
Mentors	:	Prof. VR.Muthukkaruppan, Dr. S.R. Krishnadas, Prof. K. Dharmalingam
Research Associate	:	Dr. R. Haribalaganesh, Yogamaya Prabu
Funding Agency	:	The Wellcome Trust-DBT/ India Alliance (Intermediate Fellowship)

Evaluating the Efficacy if has-miR-483-3p in an ex vivo Model of SI-OHT

Introduction

The aim of the study was to investigate the role of miRNA in the regulation of glucocorticoid receptor (GR) signaling and to develop new miRNA-based therapeutics for the treatment of steroid-induced glaucoma. In order to achieve this, firstly an ex vivo model of SI-OHT was established using HOCAS and assessed the differential GC responsiveness in Indian donor eyes (Haribalaganesh et al., 2021). Followed by this, HTM cells with known GC responsiveness were established to investigate the differential expression of mRNAs and miRNAs between GC-responder and non-responder HTM cells after DEX treatment. Interestingly, this study revealed a distinct gene and miRNA signatures and pathways identified between them using next generation sequencing technology (Kathirvel et al., 2021[transcriptome profiling]; Kathirvel et al.,2023 (un-published) [miRNA profiling]). Further, in order to demonstrate the role of miRNA in SI-OHT/glaucoma, the hsa-miR-483-3p has been chosen from the differentially expressed miRNAs. It was found that the hsa-miR483-3p was found to regulate ECM proteins through smad4/ TGF-β2 signaling, more pronounced in GC-R as compared to GC-NR HTM cells in vitro. Further, this study was extended to demonstrate the efficacy of miR483-3p in an ex vivo model of SI-OHT and to validate the findings of in vitro miRNA functional role using HOCAS.

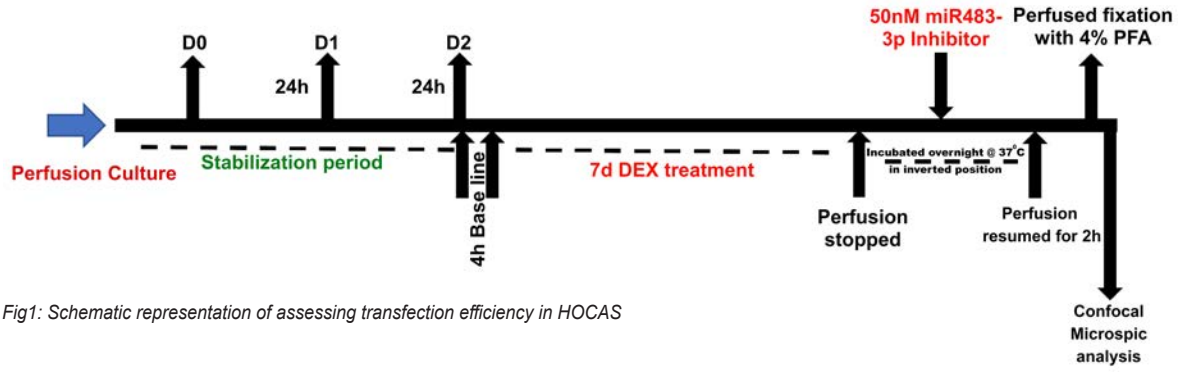


Fig1: Schematic representation of assessing transfection efficiency in HOCAS

Assessment of hsa-483-3p Inhibitor Transfection Efficiency in HOCAS

For assessing the transfection efficiency, HOCAS was established using paired human donor eyes and the GC responsiveness was assessed after DEX treatment for 7d based on IOP change. Then, miR-483-3p inhibitor (50nM) was injected into the anterior segment and the pump was stopped. The petri dish in which the anterior segment mounted was kept in an inverted position overnight. Following the next day, the flow rate was resumed to normal flow rate (2.5µl/min). At the end of 24h post transfection, the anterior segments were perfusion fixed and analysed by confocal microscopy (Fig.1).

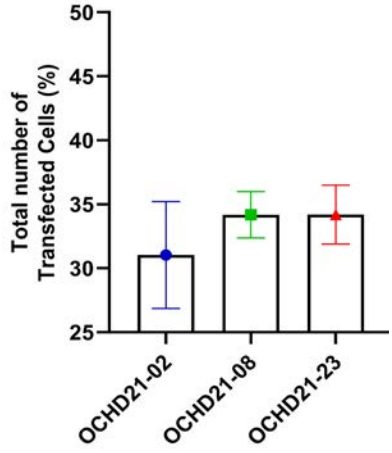


Fig 2: Transfection Efficiency of hsa-483-3p inhibitor in HOCAS Anterior Segment. The percentage of transfection efficiency was found to be 33.1±1.2 %.

Effect of Hsa-483-3p inhibitor on Smad4/TGFβ2 Signaling

After having demonstrated the effect of miR483-3p mimic/inhibitor on the regulation of ECM proteins in GC-R and GC-NR HTM cells, the effect of the same was studied in the DEX-induced OHT ex vivo model. For this, HOCAS was established using paired human donor eyes and the GC responsiveness was assessed after DEX treatment for 7d based on IOP change. Then, miR-483-3p inhibitor (100nM) was injected into the anterior segment and the pump was stopped. The petri dish in which the anterior segments mounted was kept in an inverted position overnight. Following the next day, the flow rate was resumed to normal flow rate (2.5µl/min) and continued for 3 days. IOP was monitored continuously. At the end of the experiment, TM tissues were isolated and the silencing of mRNA target gene (SMAD4) was analysed by western blotting. (Fig.3)

MiR-483-3p down regulates SMAD4 and TGFβ2 Signaling by RT-PCR

In the studies using cultured HTM cells, AMRF found that miR-483-3p targets SMAD4 and the presence of 483-3p inhibitor showed up-regulation of SMAD4 and TGFβ2 expression. The same trend was seen in TM tissues isolated from the ex vivo culture of human anterior segment tissue treated with DEX for 7d with

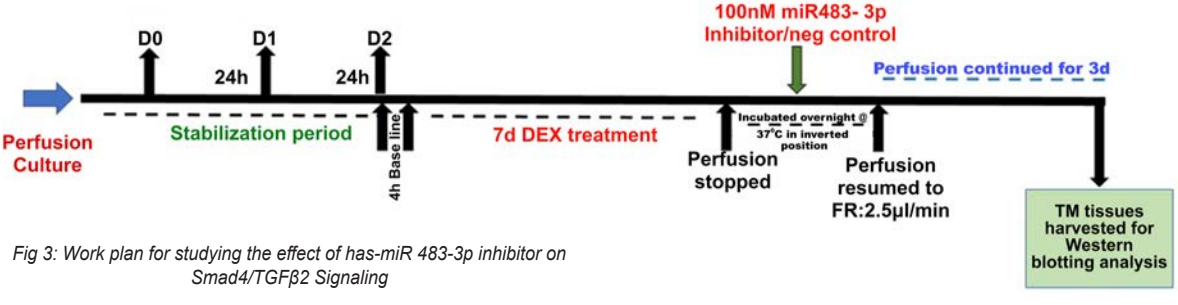


Fig 3: Work plan for studying the effect of has-miR 483-3p inhibitor on Smad4/TGFβ2 Signaling

Table1: Characteristics of Human Donor Eyes Used for the Present Study

Code	Age	Sex	Cause of Death	Time B/W Death & Enucleation (h)	Time B/W Enucleation & Culture (h)	Experiment Eye	HOCAS	Treatment	Transfection
OCHD22-01	43	M	Cardiovascular disease	5.33	51.25	OD	HOCAS	DEX	hsa miR483-3p inhibitor
						OS	HOCAS	ETH	
OCHD22-02	55	M	Accidental fall	13.33	24	OD	HOCAS	DEX	
						OS	HOCAS	ETH	
OCHD22-09	17	F	Railway accident	10.58	76.33	OD	HOCAS	DEX	
						OS	HOCAS	ETH	
OCHD22-10	75	M	Road traffic accident	4.75	25.5	OD	HOCAS	DEX	
						OS	HOCAS	ETH	
OCHD22-13	75	F	Cardiovascular arrest	2.5	65.5	OD	HOCAS	DEX	
						OS	HOCAS	ETH	
OCHD22-14	60	M	Heart disease	7.5	57.5	OD	-	-	
						OS	HOCAS	DEX	
OCHD22-15	27	M	Tablet poison	1.91	7.5	OD	HOCAS	DEX	
						OS	HOCAS	DEX	
OCHD22-26*	60	M	Cardiovascular arrest	2.5	53.91	OD	HOCAS	DEX	
						OS	HOCAS	DEX	
OCHD22-27*	32	M	Road traffic accident	6	69.5	OD	-	-	
						OS	HOCAS	DEX	
OCHD22-28*	75	F	Cardiovascular arrest	2.33	23.66	OD	HOCAS	DEX	
						OS	-	-	

*Note: *-DEX-treated Responder eyes. The mean age of the donor eyes used for the study was found to be 51.9±21.2 years. The mean elapsed time between death and enucleation was found to be 5.7±3.8 h*

and without an inhibitor by RT-PCR analysis.

Total RNA was extracted from TM tissue after ex vivo anterior segment culture by the trizol method and it was quantified using a Nano drop-spectrophotometer. Further, 1µg of total RNA was subjected to cDNA conversion using a verso cDNA synthesis kit. Eventually, real-time PCR was performed through the SYBR Green master mix for the following genes: fibronectin, TGF beta, and smad4. GAPDH was used as an internal control (Fig.4).

Western blot analysis revealed the up-regulation of SMAD4 and Col1A expression in presence of miR-483-3p inhibitor which was reduced by miR-483-3p treatment. This is in agreement with the RT-PCR analysis.

As expected, the presence of miR 483-3p up-regulated the expression of SMAD4 and Col1A in both GC-responder and non-responder eyes whereas the presence of miR-483-3p mimic down-regulated the expression of SMAD4 and Col1A (Fig.5).

In conclusion, DEX treatment elevated IOP through SMAD4/TGFβ2 signaling which resulted in the enhanced production of ECM proteins such as Col1A/fibronectin. The presence of miR483-3p mimic down-regulated SMAD4/TGFβ2 signaling and hence decreased the production of ECM proteins in HTM cells. Therefore, the up-regulation of miR483-3p and subsequent down-regulation of its target Smad4 may serve as a protective mechanism to regulate ECM proteins in HTM cells upon DEX treatment.

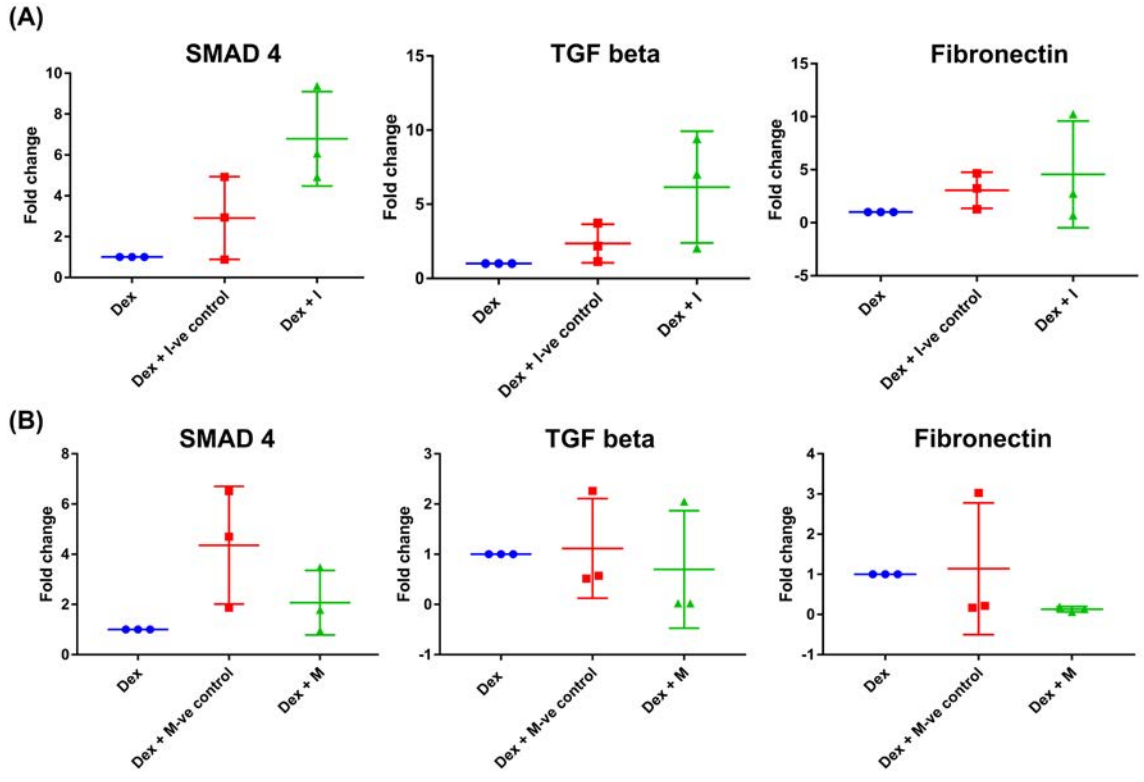


Fig 4: Human Anterior segments were treated with DEX for 7d and then treated with miR-483-3pinhibitor (100nM) (A) and mimic (100nM). (B) The presence of miR-483-3p inhibitor up-regulated the expression of SMAD4, TGFβ2 and fibronectin expression which was reduced by the presence of has-483-3p mimic by RT-PCR analysis.

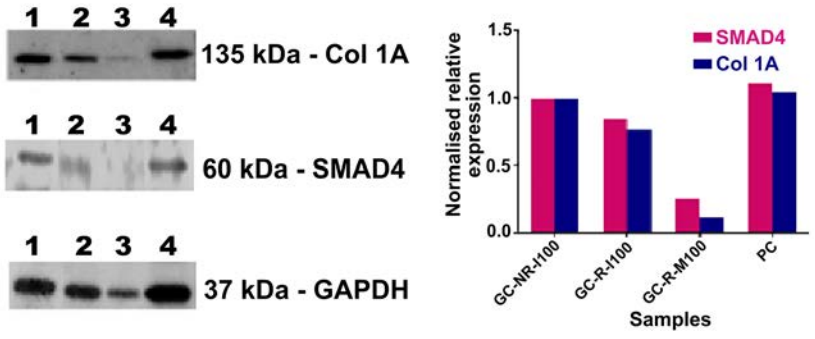


Fig 5: Western blot analysis of SMAD4 and ECM protein-Col1A after transfection of miR 483-3p mimic/ inhibitor in HOCAS. Note: Lane 1 - 22-15-OS (GC-Non-responder eye treated with miR-483-3p Inhibitor); Lane 2 - 22-28-OS (GC-responder eye treated with 483-3p Inhibitor); Lane 3 - 22-28-OD (GC-responder eye treated with miR-483-3p Mimic); Lane 8 – Positive control (cell lysate from GC-Responder eyes treated with miR 483-3p inhibitor). B) Densitometry analysis of the WB analysis of SMAD4 and Col1A in GC-R and GC-NR in presence of miR-483-3p mimic and inhibitor.

Profiling of differentially expressed miRNAs in human trabecular meshwork tissues from steroid responder and non-responder eyes using NanoString technology

NanoString miRNA expression assay was carried out to profile the miRNAs present in TM tissues obtained from GC-R and GC-NR eyes after HOCAS experiments. In order to perform this assay, total RNA from FFPE blocks after HOCAS experiments was isolated using Trizol reagent as per manufacturers' instructions with some modifications. TM tissues from each GC-R (n=4), GC-NR (n=5) and vehicle control (n=2) were subjected to RNA extraction after DEX or 0.1% ETH treatment for 7 days. The miRNA quantity was measured using the Qubit miRNA assay kit (Invitrogen) and the quality was assessed on an Agilent 2100 bio analyser. 100ng RNA was used to measure the expression of 800 pre-selected human miRNAs using the nCounter Human v3 miRNA expression assay kit from NanoString Technologies (Seattle, WA, USA), as per the manufacturer's instructions. After specific sample preparation and

overnight hybridisation, digital readouts of the relative miRNA abundance were obtained, translating to miRNA expression. The assay was outsourced to a commercial service provider in India.

The total number of DEMIRs identified in group #1 and group #2 were 519 and 230 respectively. There were 210 DEMIRs found to be common (Group#3) between Group 1 and 2. The unique DEMIRs identified in GC-responder and non-responder eyes were found to be 309 (Group #4) and 20 (Group #5) respectively (Figure 1).

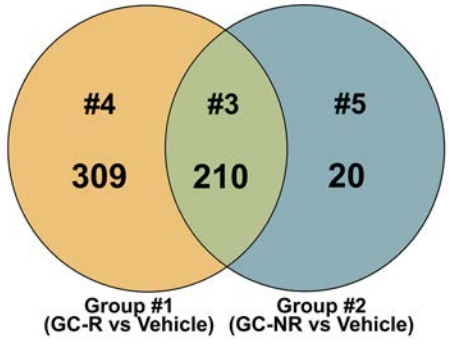


Figure 1. Venn diagram Showing Differentially Expressed MiRNAs Identified in Each Group. Differentially expressed miRNAs (DEMIRs) of three groups from NanoString miRNA Expression data are shown. Only genes with LogFC>2 or <-2 and significant p value <0.05 were included in these groupings. Group#1: DEMIRNAs between ETH and DEX-treated cells of GC-R HTM cells, Group#2 : DEMIRNAs between ETH and DEX-treated cells of GC-NR HTM cells, Group #3: Overlapping DE miRNAs between Group #1 and Group #2; Group #4: uniquely expressed miRNAs in GC-R and Group #5: uniquely expressed DEMIRNAs in GC-NR

Top10 DEMIRs in Group #4

miRNA	R vs Vehicle	
	Log FC	p-Value
hsa-miR-302d-3p	3.39424	0.032526
hsa-miR-143-3p	3.36721	0.033932
hsa-let-7b-5p	3.3307	0.035914
hsa-miR-551b-3p	3.13278	0.048457
hsa-let-7c-5p	3.1158	0.049686
hsa-miR-4488	3.0462	0.055003
hsa-miR-1180-3p	3.03771	0.055683
hsa-miR-3131	3.02696	0.056555
hsa-miR-1247-5p	3.01112	0.057859
hsa-miR-423-5p	2.97598	0.060841

Note: Group #4: uniquely expressed miRNAs in GC-R

The list of DEMIRS identified in group #3-#5 is represented in Table1.

Table1 showing the list of top 10 DEMIRs in Group#3 - #5
Top10 DEMIRs in Group #3

miRNA	Group #1(R vs Vehicle)		Group #2 (NR vs Vehicle)	
	Log FC	p-Value	Log FC	p-Value
hsa-miR-1246	6.26295	8.14E-05	3.11686	0.129267
hsa-miR-630	6.15388	0.000108	3.54633	0.084365
hsa-miR-3195	4.62092	0.003622	2.77938	0.176116
hsa-miR-1268a	4.5974	0.003797	3.21612	0.117515
hsa-miR-320e	4.40793	0.005512	3.43253	0.09481
hsa-miR-1202	4.35477	0.006106	2.5618	0.212417
hsa-miR-601	4.07146	0.01035	3.18272	0.121372
hsa-miR-124-3p	3.98466	0.012097	3.10269	0.131016
hsa-miR-575	3.92583	0.013424	2.95087	0.150932
hsa-miR-1305	3.85942	0.015076	2.73734	0.182742

Note: Group #3: Overlapping DE miRNAs between Group #1 and Group #2

Top10 DEMIRs in Group #5

miRNA	NR vs Vehicle	
	Log FC	p-Value
hsa-miR-1253	4.76363	0.020463
hsa-miR-494-3p	3.85263	0.060811
hsa-miR-128-3p	3.24096	0.11471
hsa-miR-193b-3p	2.29106	0.264765
hsa-miR-329-3p	2.25619	0.272104
hsa-miR-4485-3p	2.20866	0.282329
hsa-miR-516a-3p+hsa-miR-516b-3p	2.17887	0.28887
hsa-miR-548n	2.16175	0.292675
hsa-miR-3613-3p	2.14183	0.297144
hsa-miR-125a-3p	2.13994	0.29757

Note: Group #5: uniquely expressed DEMIRNAs in GC-NR

To validate the miRNA expression data obtained from Nanostring, 12 miRNAs were chosen for the validation by ddPCR [12 miRNAs (let-7a-5p, miR-9-5p, miR-143-3P, miR-145-5-P, miR-1246, miR-320e, miR-124-3P, miR-494-3P, miR-483-3P, miR-548aa, miR-630, miR-1253 and snoRNA as housekeeping gene)]. This assay was out-sourced to a commercial service provider, Theracues Innovations Pvt. Ltd, Bangalore, India. The ddPCR assay was performed using Biorad Automated DDPCR QX200 platform, according to the manufacturer's instruction and the data was normalised against corrected counts of sno-RNA as well as against the corrected counts of vehicle control samples. Out of 12 miRNAs validated by ddPCR, 9 and 10 miRNAs matched with the expression pattern of NanoString analysis in GC-R and GC-NR eyes respectively (Figure 2).

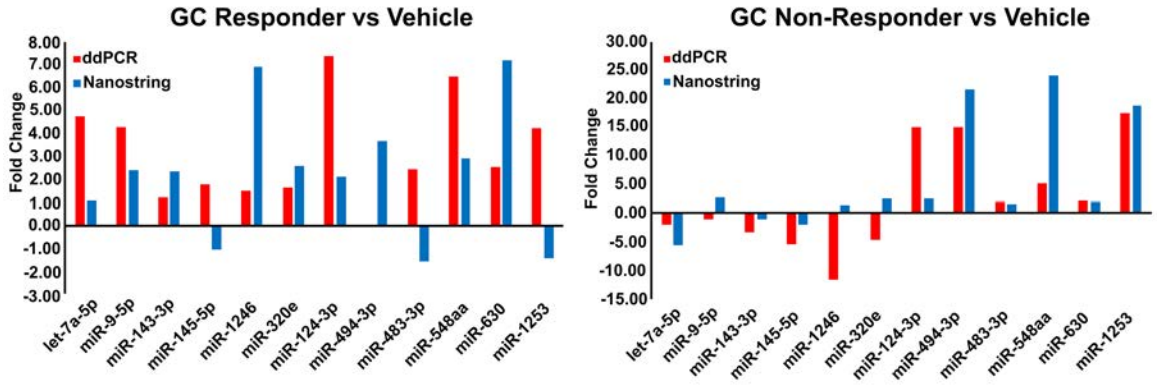


Figure 2 Showing the Comparison Between the expression of selected miRNAs by NanoString and ddPCR analysis

BIOINFORMATICS

The primary goal is to use next-generation sequencing methods and to provide bioinformatics methods to understand the role of the genome, transcriptome and epigenome on eye diseases diagnosis, prognosis and pathogenesis. This includes i) developing data analysis pipelines for whole genome/exome genome data analysis to identify eye-disease-specific pathogenic variants ii) transcriptomic analysis to detect the altered gene expression and transcripts and its role in the disease pathogenesis, iii) ocular microbiome analysis in fungal keratitis.

A computational framework for the identification of eye-disease pathogenic variants from whole Exome/Genome sequencing

Investigator : Dr. D. Bharanidharan
Research Scholar : K. Manojkumar

Background

Genome Sequencing (GS) and Exome Sequencing (ES) have become valuable tools for diagnosing patients with genetic diseases as Next-Generation Sequencing (NGS) technologies have advanced dramatically. These methods find between 60,000 and 100,000 variants per person and most of them are harmless or unrelated to the patient's disease phenotype. Thus, finding pathogenic variants is still a bottleneck due to the lack of high-throughput evaluation for the functional changes, especially for SNVs and InDels which account for 85% of disease-causing (pathogenic) variants. Recent studies showed that disease-specific tools such as PathoPredictor, CardioBoost and DVPred outperformed the pan-disease specific tools such as Exomiser, Phenovar, and VarElect. These tools have applied machine learning models using specific-disease gene panels of pathogenic and benign variants. However, specific-disease models may not prioritise pathogenic variants among several genes in a particular disease or novel genes. Thus, as proof of concept, in this study, AMRF aims to develop a disease-specific model using features of pathogenic variants of a specific disease compared to other diseases.

Results and conclusions

AMRF developed an eyeVarP pipeline, as shown in Figure:1.1 consists of three modules: (I) variant calling and annotation, (II) VarP, and (III) eyeVarP, available at github online link: https://github.com/bharani-lab/WES-pipelines/. AMRF developed the variant calling pipeline (I) based on the previous practices with the performance evaluation of various tools (10), which was updated and re-evaluated (data not shown). Therefore, the user gets annotated VCF automatically using the best performing automated pipeline for variant calling and annotation. Second, AMRF developed a VarP module to filter and predict the pathogenicity of both SNVs and InDels. Briefly, the VarP filters functional variants from annotated VCF using default settings provided in the parameter file. The user can modify all the default settings (Figure 1). A prediction score greater than 30 was

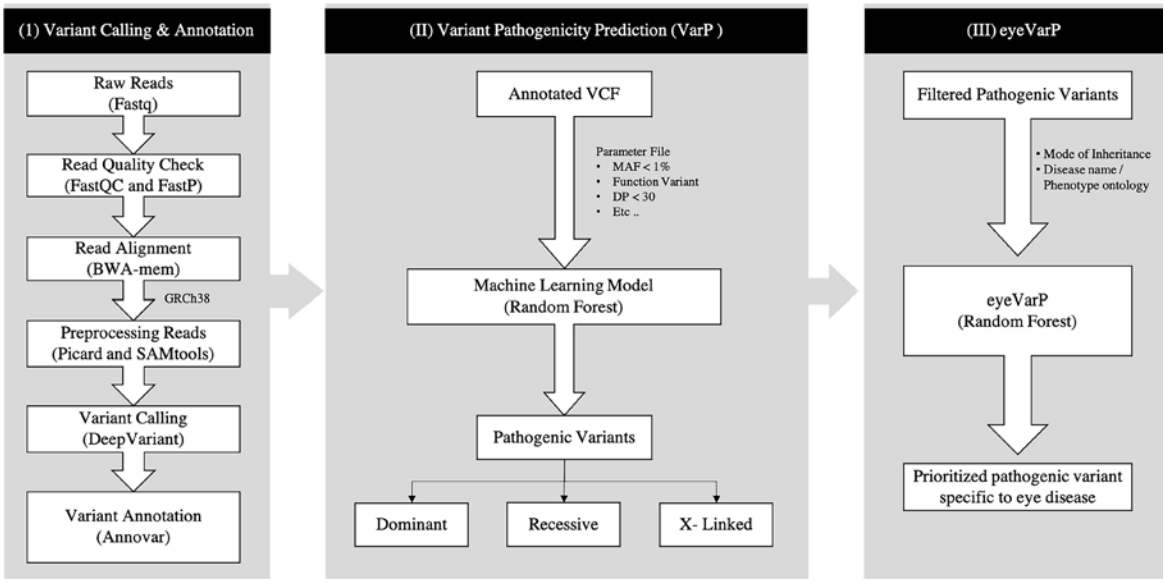


Figure 1.1. The schematic representation of eyeVarP pipeline.

defined as disease-causing variants; based on the comparison of RF model with a known dataset. Eventually, AMRF developed the eyeVarP module as the final step for prioritising the pathogenic variants specific to eye diseases using the Random Forest (RF) model. The eyeVarP takes the users inputs such as mode of inheritance and diseases name, and output of VarP (.txt file) as inputs and outputs a comma-delimited file (.CSV) containing the probability score. The probability score of one is said to be the variant with a high chance of causing eye disease. Likewise, 0.5 suggests that the variant has 50% chance of causing eye diseases. AMRF used 2272 pathogenic variants for eye diseases and 6,693 for other diseases, covering 317 eye-disease-specific genes and 1,243 for other diseases. The variant features selection and Gene Expression Profile scores, along with HPO and GO, significantly improved the model with an accuracy of 85% on the test set. Further, the eyeVarP performance was evaluated by ten-fold cross-validation.

In addition to the ten-fold cross-validation, AMRF evaluated the performance of the eyeVarP with widely used pan-disease-specific tools such as Exomiser (7) and Phenovar (8). The results showed that eyeVarP performance was significantly different from other tools as shown in Figure:1.2 eyeVarP identified 81% of reported pathogenic variants in the top 5 ranks, whereas Exomiser and Phenovar showed less than 75 %. Moreover, almost all the reported pathogenic variants were obtained under the top 10 list with eyeVarP compared to other tools (Figure:1.2). In addition, based on the inheritance pattern, eyeVarP showed better performance over other tools, as shown in Figure:1.2. Further, AMRF tested the eyeVarP to predict new eye-disease specific pathogenic variants in 30 Korean inherited retinal degeneration (IRD) patients who had one reported heterozygous pathogenic variant and 52 unsolved cases of Korean IRD (PRJNA167850) patients. Among the 30 IRD patients, eyeVarP

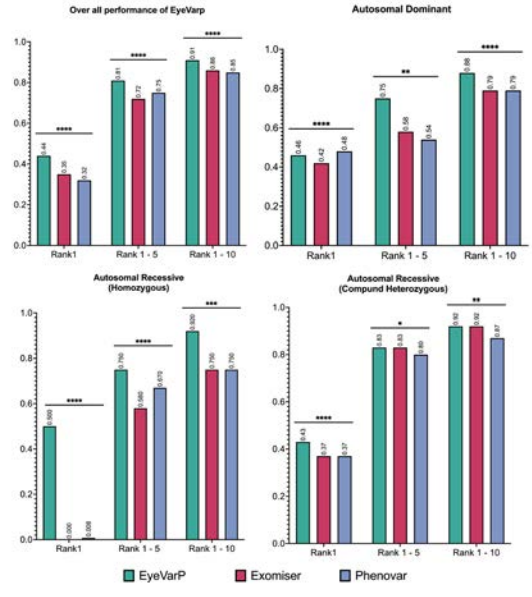


*Figure 1.2. Comparison of eyeVarP performance with other pan-disease-specific tools. The accuracy of reported pathogenic variants was plotted against their rank at various levels predicted by the tools. Comparison of tools for all the reported pathogenic variants (A), autosomal dominant variants (B), autosomal recessive homozygous variants (C), and autosomal recessive compound heterozygous variants (D). **** represents P-value <0.0001, *** represents < 0.0002, ** represents <0.007 and * represents < 0.01*

identified pathogenic variants with AR inheritance in 10 patients and 4 among 52 unsolved cases (data not shown). Thus, the eyeVarP improved the pathogenic variant detection rate to 59.5% (100/168 patients) from 51.2% (86/168) significantly (P-value = 0.00024) in the Korean IRD patients, demonstrating the power of eyeVarP.

Conclusion

AMRF demonstrated that the eyeVarP method developed using pathogenic variants of eye diseases and other diseases performs better in identifying eye disease-specific pathogenic variants over pan-disease-specific methods. Implementing such complete computational procedures would significantly improve the clinical variant interpretation for specific diseases.

Identification and Analysis of Alternative Transcripts in Retinoblastoma Progression.

Investigators : Dr. D. Bharanidharan.
Research Scholar : Mohamed Hameed Aslam A.
Funding : ICMR-SRF

Background

Retinoblastoma (RB) is a rare Pediatric eye cancer initiated by biallelic inactivation of the tumour suppressor gene RB1. MYCN amplification without RB1 inactivation causes a small percentage of retinoblastomas. Beyond RB1 mutations, other events such as genomic instability, copy number variation, and altered gene expression drive retinoblastoma progression. The role of alternative transcripts in the context of retinoblastoma progression has not been studied. Hence, AMRF elucidates the role of alternative transcripts in RB progression.

Results and Conclusions

First, AMRF used available transcriptome data of RB for the meta transcriptome analysis. AMRF developed a comprehensive bioinformatics pipeline for alternative transcript analysis and differential gene expression analysis using meta transcriptome data. Further, AMRF investigated the correlation between differential gene expression (DGE) and differential alternative splicing events (DAS) by network analysis using the results from gene set enrichment analysis (GSEA) of hallmark gene sets from the Molecular Signatures Database (MSigDB). The GSEA showed activation of cell-proliferative pathways such as the G2M checkpoint, the E2F target, and the mitotic spindle, all of which have been linked to drug response and patient survival in many cancers (Figure 2.1 (a)).

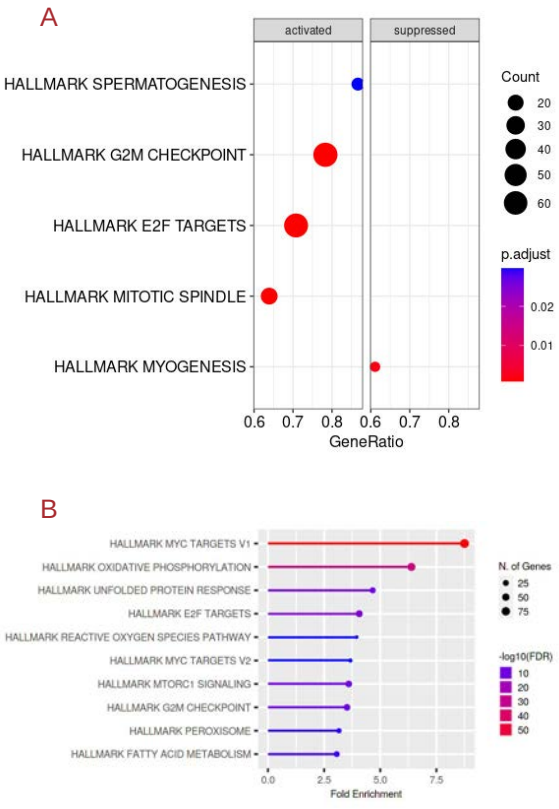


Figure 2.1 (a): Gene-Set Enrichment Analysis (GSEA) of Differential Expression of Genes (DEGs) against the Molecular Signature Database (MsigDB); (b) Gene-Set Enrichment Analysis (GSEA) of Differential Alternative Splicing Events (DAS) against the Molecular Signature Database (MsigDB).

In addition to GSEA, the functional network was constructed with significantly enriched pathways and their genes, identified by GSEA of alternative splicing events (Figure 2.2). All the nodes connected between E2F and G2M have upregulated gene expression, indicating tumour progression. All the nodes connecting MYC_Target_V1 are down-regulated compared to normal tissue, indicating that the pathway was stable and didn't undergo aberrant splicing. Since MYC expression controls cell cycle entry and exit, decreased splicing events may help MYC mRNA and protein levels in the quiescent state. Interestingly, Fatty Acid Metabolism showed mostly down regulated AS. Of these, Enolase had more events. Enolase2 (ENO2) is a glycolysis enzyme dysregulated in cancer that fuels Tumor Growth by giving Energy. However, ENO2's AS role in RB remains elusive. The protein-protein interaction (PPI) network analysis showed that ENO2 interacting with insulin growth factor1 and 2, suggesting the AS of ENO2 may play an important role in RB progression.

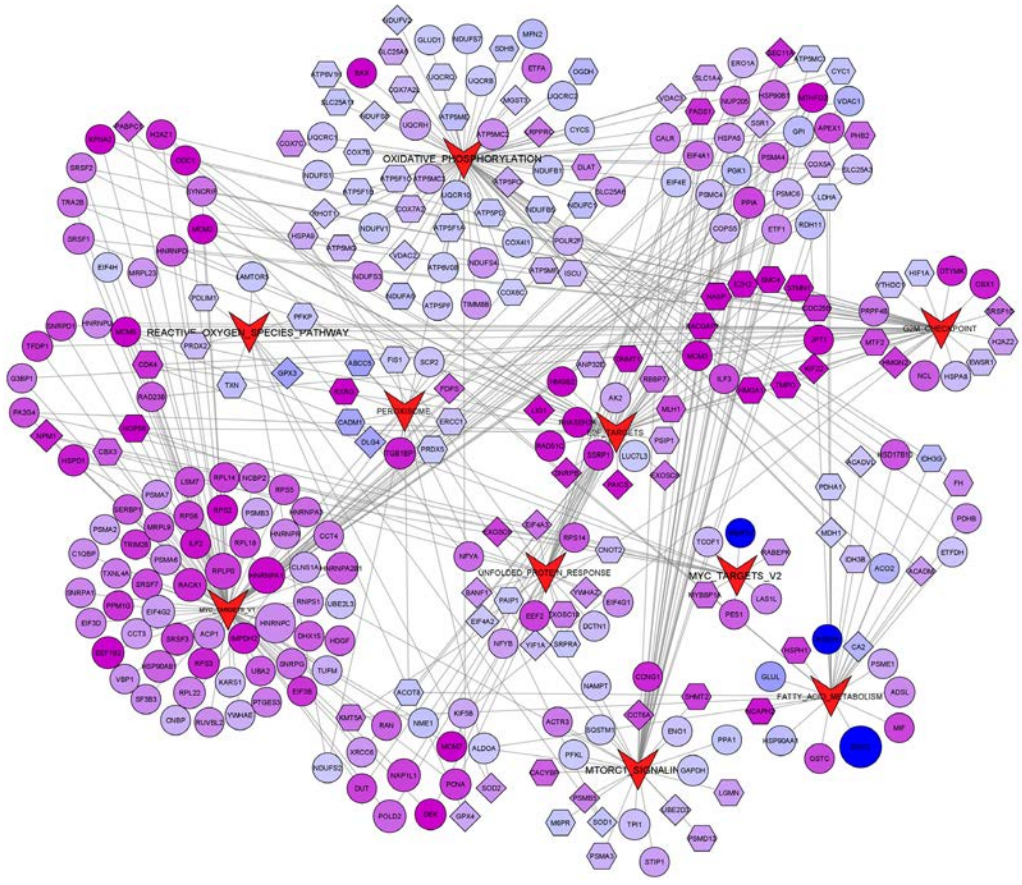


Figure 2.2. The functional network of enriched pathways and genes in RB progression; Among the genes, diamond shape represents the down regulated Alternative Splicing events (DAS), hexagon represent upregulated DAS and circles are non-significant. The degree of red to blue colour represents highly upregulated gene to highly down regulated gene expression. Downward triangles represent pathways.

OCULAR MICROBIOLOGY

The department majorly focuses on the diagnosis and prognosis of ocular infections and understanding their pathogenesis. Molecular methods such as PCR, Real Time PCR, DNA bar coding and Multi locus sequence analysis are used for the identification of ocular pathogens including bacteria and fungus as a part of routine diagnosis. However, new methods or molecular biomarkers such as miRNAs are explored for no growth or unidentified organisms. The department uses genomic and transcriptomic approaches, and invitro cell culture and exvivo model system to understand the pathogenesis of ocular pathogens.

Diagnostic markers for Intra-Ocular Tuberculosis

Investigators : Dr. D. Bharanidharan,
Dr. S. R. Rathinam,
Dr. Lalitha Prajna
Research Scholar : C. Swathi
Funding : DBT, ICMR-SRF

Background

Intraocular tuberculosis (IOTB) presents most commonly as uveitis, an intraocular inflammation that leads to visual loss in patients if undiagnosed early (1-4). However, the differential diagnosis of Intraocular TB is challenging because it is a great mimicker of various uveitis entities. Moreover, the limited size of ocular fluids and the paucibacillary nature of ocular M. tuberculosis infection create additional barriers in the diagnosis. The diagnosis is only presumptive and corroborated by laboratory tests and therapeutic response to Anti-TB treatment (ATT). These leads to undiagnosed TB uveitis carry a risk of high visual morbidity. Moreover, overzealous ATT treatment in the absence of TB infection carries a significant risk of systemic side effects. Therefore, advanced molecular markers in the diagnosis of ocular tuberculosis are required. Thus, for the first time, the research profiled human miRNAs in AH and VH of the IOTB patients using next generation sequencing. The research team also validated selected miRNAs in small set samples through qPCR. Four miRNAs such as miR-423-5p, miR-328-3p, miR-21-5p, and miR-16-5p and three miRNAs were identified as potential markers in AH and VH respectively. However, serum miRNAs' role as biomarkers has not been studied in IOTB. As circulating miRNAs are promising biomarkers for various diseases, the research team investigated whether miRNAs have potential as an IOTB diagnosis.

Results and Conclusions

Metadata analysis of systemic tuberculosis:

To select potential serum miRNAs that are altered in IOTB, first AMRF carried out meta analysis of small RNA sequencing data of systemic TB patients. The

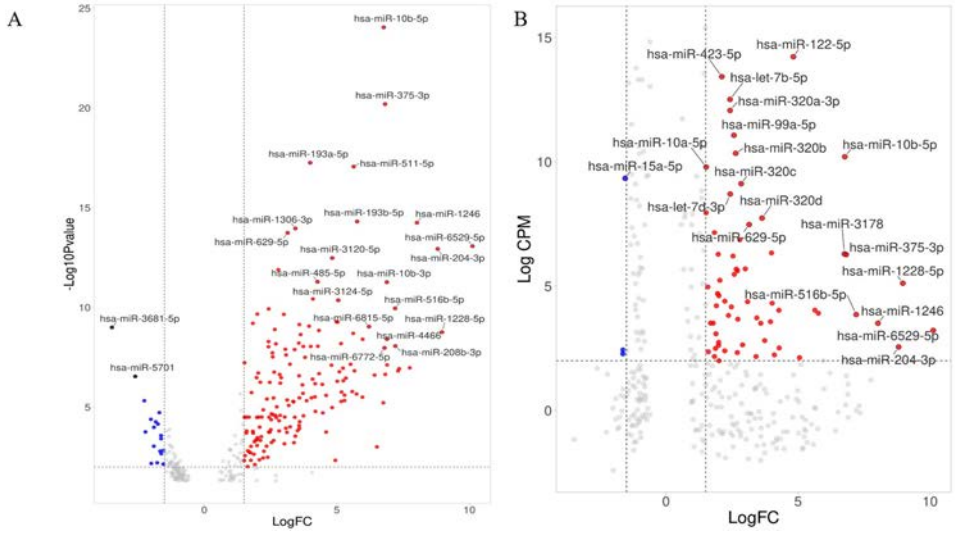


Figure 1.1. The volcano plot shows the differential expression of microRNAs in Active TB samples compared with healthy control samples. Red colour represents upregulated miRNAs and blue colour represents downregulated miRNAs. Top 20 miRNAs were labeled in the plot. The research team considers miRNAs significant based on absolute fold change was >1.5 and adj. p <0.05 and log CPM greater than or equal to 2. (A) X-axis shows Log2 FC, and Y-axis shows the minus Log10 P-value. (B) X-axis shows Log2 FC, and Y-axis shows the log CPM.

team retrieved 13 small RNA sequencing datasets including peripheral blood, serum, and serum exosome samples of TB patients from NCBI-SRA database. Extrapulmonary datasets, patients with other infections like HIV, cells infected with MTB, and ATT-treated LTB cases were excluded from this study. Totally, 131 samples were segregated into healthy controls, LTB, and active TB. Data processing and differential expression analysis were performed using the bioinformatics pipeline.

Five hundred and seventy-five miRNAs were differentially expressed in active TB cases compared to healthy controls. From there, 281 miRNAs were upregulated, and 294 were downregulated. The topmost differentially expressed miRNAs ordered by log2foldchange (FC) are presented in Figure: 1.1.

AMRF also identified seven hundred sixty-six DE miRNAs in active TB compared with LTB samples. Of these, 496 miRNAs were upregulated (those with a positive Log2 FC), and 270 were downregulated (those with a negative Log2 FC). Further, based on their expression profile in active TB compared to controls and LTB, AMRF selected the miRNAs, as shown in Figure 1.2, as TB-specific, active TB, LTB-specific and miRNAs specific to disease progression (data not shown).

Finally, the research team selected ten miRNAs, miR-423-5p, miR-150-5p, miR-21-5p, miR-328-3p,

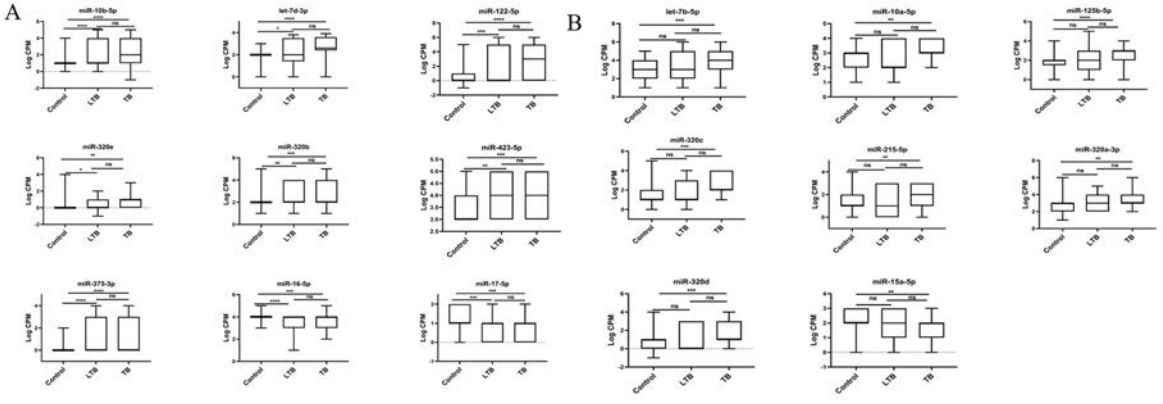


*Figure 1.2. Bar graphs were plotted for both TB-specific and active TB miRNAs in blood. CPM - the mean read counts per million RNA-seq reads and its standard deviation. Data were plotted using the parametric t-test statistical analysis method. *P<0.05, ** P<0.01, ***P<0.001, and ****P<0.0001. (A) The differential expression of miRNAs was identified in both active TB and LTB compared to healthy controls. (B) The differential expression of miRNAs was identified in active TB compared to healthy controls.*

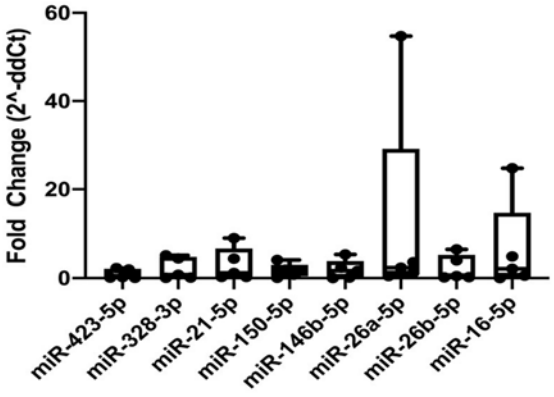


Figure: 1.3. Validation of miRNAs in serum of IOTB samples using qRT-PCR. A. Relative expression of miRNAs in serum of IOTB samples (n=5) compared to Immunosuppressive patients with uveitis (n=5).

miR-155-5p, miR-16-5p, miR-146b-5p, miR-26b-5p, miR-26a-5p, and miR-1-3p based on the systemic TB meta-analysis and their DE expression in ocular fluids for identifying their diagnostic potential in serum of IOTB patients.

AMRF analysed the expression of selected miRNAs in serum of IOTB patients (n=5) patients compared to control samples (n=5) and identified eight DE miRNAs as shown in Figure:1.3. Further validation in large cohort will allow their potential diagnostic role in serum of IOTB patients.

Role of Human corneal miRNAs in the onset and severity of Fungal Keratitis

Investigators : Dr. D. Bharanidharan,
Dr. K. Dharmalingam,
Dr. Venkatesh Prajna,
Dr. Lalitha Prajna
Research Scholar : Shreya Dinesh, Gayathri.M
Funding : ICMR

Background

Fungal keratitis is more prevalent in India, and 65% of infection is due to either *Aspergillus flavus* or *Fusarium solani*. Nearly 40% of the patients are refractory to anti-fungal treatment and lack of newer drugs greatly limits treatment options, necessitates to find the factors that could identify the severity of disease early on. The previous studies have showed dysregulation of miRNA in patients who fail to respond to antifungal treatment, which clearly shown the involvement of miRNA in the regulation of onset and progression of the disease in the case of *Aspergillus* keratitis. The current project aims at exploring the molecular mechanism and the host target gene involved in the miRNA dependent host response to *Fusarium* and *Aspergillus* keratitis.

Results and Conclusion

Totally, 289 corneal tissue samples were collected. Of these, 111 tissue samples from keratitis patient samples who were culture positive for *A.flavus*, *Fusarium* species and *P. aeruginosa* were taken forward for the downstream experiments. Also, ten cadaver normal corneal tissues were used for the

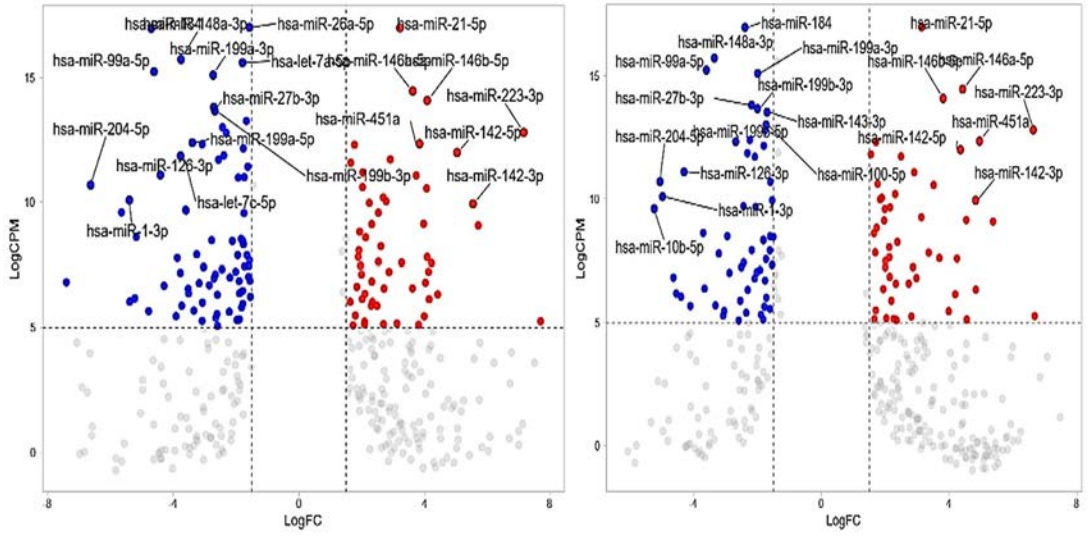


Figure 2.1. Volcano Plot for A: A. flavus poor outcome samples vs Cadaver Cornea B: Fusarium sp. poor outcome samples Cadaver Cornea Red color represents upregulated miRNAs and blue color represents downregulated miRNAs. We consider miRNAs significant based on absolute fold change was >1.5 and adj. log CPM greater than or equal to 4. X-axis shows LogFC, and Y-axis shows the log CPM.

controls. Similarly, 153 swab samples were collected at the patient's first presentation and at the first review (7-10 days) after treatment. 32 samples were sequenced at Biokart Company for small RNA sequencing and 15 samples for mRNA sequencing. The differential expression analysis was performed using an in-house bioinformatics pipeline.

The differentially expressed (DE) miRNA were filtered based on the following criteria, log fold change > ± 2; -Log2P-value >2 and LogCPM>4 from each two-group analysis. Figure 2.1 shows the volcano plot of differentially expressed miRNAs in *A. flavus* and *Fusarium sp*. poor outcome samples compared to Normal cadaver cornea samples.

Target prediction was performed on these selected DE miRNAs. To identify true gene targets, the research team carried out RNA-seq of five *Aspergillus* and *Fusarium* Keratitis patient's tissues and five cadaver controls. The DE of genes was obtained through an in-house pipeline. The DE genes were matched with predicted targets and obtained the final miRNA gene targets. A functional network analysis was carried out with filtered mRNAs. Figure 2.2 shows the functional network of pathways significant to fungal pathogenesis and pathways significant to the host response to pathogen infection. The P13K-Akt signaling pathway is reported to have a significant role in the biological responses of corneal epithelial cells and maintains the tissue homeostasis (Kuangqi Chen., 2022). AMRF could identify Phagosome maturation as a top-hit pathway from the analysis. It has been already studied that cytokines can enhance the antifungal activity of neutrophils and macrophages. MAPK signaling has been reported earlier to be involved in pathogenesis of fungal keratitis via p38 MAPK pathway. Based on the miRNA's potential role in fungal keratitis pathogenesis, AMRF prioritised the list of miRNAs with prognostic value. Also, AMRF selected fungal species-specific miRNAs, which require further validation in a large cohort. Real-time PCR on small subset of samples identified hsa-miR-184, hsa-miR-149-5p, hsa-miR-223-3p, hsa-miR-21-5p and hsa-miR-451a that plays important role in *Aspergillus* keratitis pathogenesis and disease severity.

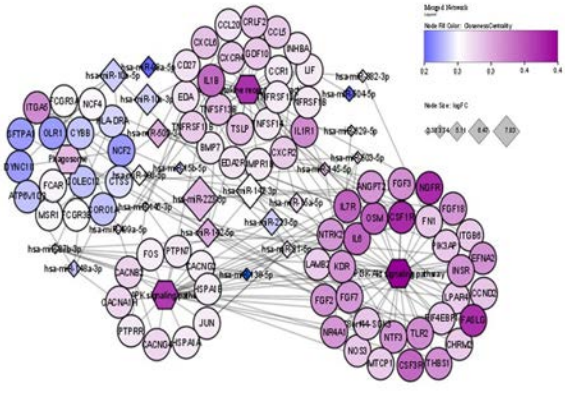


Figure 2.2. miRNA-target Functional Network of Pathways involved in Fungal keratitis

The functional network of enriched pathways and genes Fungal keratitis pathogenesis. The diamond shape represents the miRNAs with larger ones having higher fold change and their target genes are shown in circles. The hexagon represents the significant pathways.

Altogether, the research team has identified several potential miRNAs that involve in fungal keratitis pathogenesis, disease progression, and severity. These miRNAs will serve as potential biomarkers and requires further validation in a large cohort of samples.

Expression profiling of human corneal miRNAs and their role in *Pseudomonas aeruginosa* keratitis

Investigators : Dr. D. Bharanidharan,
Dr. Venkatesh Prajna,
Dr. Lalitha Prajna
Research Scholar : R. Praveenkumar
Funding : DBT-BET

Pseudomonas aeruginosa keratitis is regarded as one of the rapidly progressing and painful infection which could result in subsequent corneal melting and vision loss if not treated properly (Shrestha et al., 2021). Currently, PA keratitis is treated with topical administration of antibiotics. However, an unresolved infection may lead to vision loss or poor visual acuity eventually requiring a surgical intervention. The reasons for such poor outcomes upon antibiotic treatment are many, broadly categorised as pathogen associated or host associated factors. The pathogen associated factors are bacterial virulence, development of antibiotic resistance and biofilm formation. The host associated factors responsible for worsening of bacterial ulcer are the inflammatory pathways and epigenetic factors such as miRNAs. Recent reports on altered miRNA expression in human corneal diseases suggest their regulatory role in pathogenesis (Derrick et al., 2017). Particularly, miR-155-5p (Yang et al., 2014) and miR-183/96/182 (Muraleedharan et al., 2016) have been shown to play an important role in *P. aeruginosa* keratitis and may provide potential targets. Even though the role of few individual miRNAs has been reported in *Pseudomonas aeruginosa* keratitis, a comprehensive expression profiling and dysregulated miRNAs that

are associated with good outcome is not studied. Furthermore, their role in mitigating the infection is not studied.

Results and Conclusions

Expression profiling of miRNAs was carried out from the *Pseudomonas aeruginosa* infected poor outcome corneas (n=3), cadaver corneas (n=3) and corneal swabs from healed patients (n=4) by miRNAseq. The top highly expressed miRNAs in poor outcome cornea were miR-26a-5p, miR-184, miR-148a-3p, miR-99a-5p, miR-199a-3p, let-7f-5p and let-7a-5p, whereas, miR-21-5p was highly expressed in poor outcome cornea (Figure 3.1). miR-223-3p, let-7a-5p, miR-205-5p, miR-18, miR-165-5p were found to be highly expressed in corneal swabs from healed patients (Table 1b). AMRF performed differential expression analysis of the expressed miRNA using the edgeR platform with filtering criteria FDR adjusted p-value < 0.05 and Fold change > 1.5 in the online tool called DEapp. The differential expression of miRNAs was visualised in a Volcano plot (not shown). As shown in the Table 1a, 223-3p, miR-23-3p, miR-3614-5p, miR-21-5p and miR-155-5p were upregulated and miR-485-3p, miR-10b-5p, miR-514a-3p, miR-135a-5p and miR-126-3p were downregulated from the top 10 DE list.

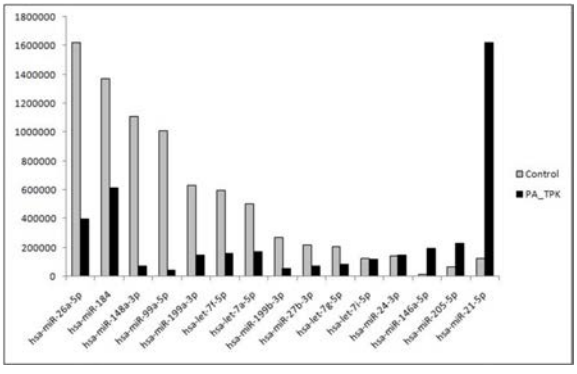


Figure 3.1: Highly expressed miRNAs in poor outcome cornea (black) and controls (grey) plotted against their raw counts.

MicroRNAs in cancer	53	5.15E-13	4.95E-11
Cellular senescence	34	2.09E-11	1.34E-09
Pathways in cancer	69	5.67E-11	2.72E-09
Pancreatic cancer	23	8.27E-11	3.17E-09
FoxO signaling pathway	30	1.12E-10	3.59E-09
EGFR tyrosine kinase inhibitor resistance	23	1.91E-10	5.23E-09
Prostate cancer	25	4.17E-10	1.00E-08
Hepatitis B	32	1.22E-09	2.60E-08
Hepatocellular carcinoma	32	3.08E-09	5.91E-08
Signaling pathways regulating pluripotency of stem cells	29	4.61E-09	8.05E-08
PI3K-Akt signaling pathway	49	7.99E-09	1.28E-07
p53 signaling pathway	20	1.16E-08	1.71E-07
Renal cell carcinoma	19	2.72E-08	3.73E-07
TGF-beta signaling pathway	22	3.70E-08	4.74E-07

Table 2: List of enriched KEGG pathways for the predicted targets of selected differentially expressed miRNAs

From the differential expression analysis, 102 miRNAs were selected based on their abundance, significance and fold change. The gene targets for the selected miRNAs were predicted using miRWalk tool. Gene ontology analysis was performed for the predicted targets and significantly enriched KEGG pathways were found (Table 2). Among the

S No	miRNAs	logFC	logCPM
1	hsa-miR-10b-5p	-7.98	10.69
2	hsa-miR-135a-5p	-4.87	7.86
3	hsa-miR-4485-3p	-5.15	7.03
4	hsa-miR-509-3-5p	-5.96	6.59
5	hsa-miR-126-3p	-5.71	12.14
6	hsa-miR-223-3p	5.48	10.82
7	hsa-miR-21-3p	4.84	8.85
8	hsa-miR-514a-3p	-4.73	9.58
9	hsa-miR-3614-5p	5.24	3.1
10	hsa-miR-1-3p	-6.91	11.18

S No	miRNA	Log(CPM)
1	hsa-miR-223-3p	5.414226
2	hsa-let-7a-5p	4.916928
3	hsa-miR-205-5p	4.866616
4	hsa-miR-184	4.805991
5	hsa-miR-16-5p	4.789884
6	hsa-miR-191-5p	4.648037
7	hsa-miR-26a-5p	4.511297
8	hsa-miR-92a-3p	4.509696
9	hsa-miR-103a-3p	4.502094
10	hsa-let-7b-5p	4.438273

Table 1: 1a (left): The top 10 differentially expressed miRNA in Pseudomonas aeruginosa keratitis cornea vs Cadaver controls; 1b (right): The highly expressed miRNAs in corneal swabs of Pseudomonas aeruginosa keratitis patients with good outcome compared to cadaver controls.

significantly enriched pathways, FOXO, PI3K-Akt and TGF-β signalling pathway were relevant in ulcer pathology. Foxo pathway is activated during cellular defence against stress and they are essential in cell survival and longevity. PI3K-Akt signalling via GSK-3β regulates epithelial cell morphology and function. TGF-β, is an important factor involved in wound healing which promotes fibroblast differentiation and scarring.

Interestingly, AMRF observed that 2 let-family of miRNA (let7a-5p and let 7b-5p) were upregulated in good outcome patients only. Its noteworthy to look upon this group of miRNAs since recently, a let family miRNA (let-7b-5p) was shown to be secreted in exosomes by lung epithelial cells upon infection by *Pseudomonas aeruginosa*, which affects antibiotic susceptibility and biofilm-forming ability of the bacteria (Koeppen et al., 2021). Thus, AMRF will explore the role of the let-7 family in mitigating the bacterial infection in-vitro and in-vivo models.

Mechanotransduction in the homeostasis of retinal pigmented epithelium: implications in age related macular degeneration

Investigators details : Dr. Siddharth Narendran
Funding : SERB
Research Scholar : S. Karvannan

Introduction including background

Age-related macular degeneration (AMD) refers to progressive degeneration of the macula, the photoreceptor dense central retina which is necessary for fine visual acuity. AMD is classified into atrophic and neovascular forms. Geographic Atrophy (GA), the untreatable advanced form of atrophic AMD is responsible for 20% of all blindness due to AMD and is characterised by degeneration of the retinal pigmented epithelium (RPE). GA is characterised initially by sub-RPE deposits that accumulate between the RPE and the Bruch's membrane (BM). The last three decades of AMD research have been primarily focused on identifying the biochemical components of these sub-RPE deposits and targeting individual components of these deposits which has been the predominant treatment strategy. However, these strategies thus far, have been a futile endeavor as evidenced by multiple clinical trials. Despite being the pathological hallmarks of GA and AMD, the effect of the mechanical changes caused by these deposits on RPE homeostasis have not been studied. Mechanotransduction, a phenomenon governing the fates and functions of biological systems by mechanical forces, has been found to occur in all corners of the biological realm with an extensive and a diverse repertoire of mechanisms. Excitingly, in new preliminary studies, the research team observed the role of mechanotransduction in RPE homeostasis and degeneration in GA. Hence, although inflammation is considered to be the primary process by which RPE cell death occurs in GA, the team hypothesises that while RPE degeneration is perpetuated by inflammatory mediators, it is initiated by mechanical factors.

Results:

Spatiotemporal interrogation of the molecular mechanobiology of the BM using Atomic Force Microscopy:

Stiffness of Macular BM in donor eyes with AMD is significantly higher compared to normal donors' eyes (Figure 1).

Spatiotemporal interrogation of the human RPE resurfacing on BM using Scanning Electron Microscopy:

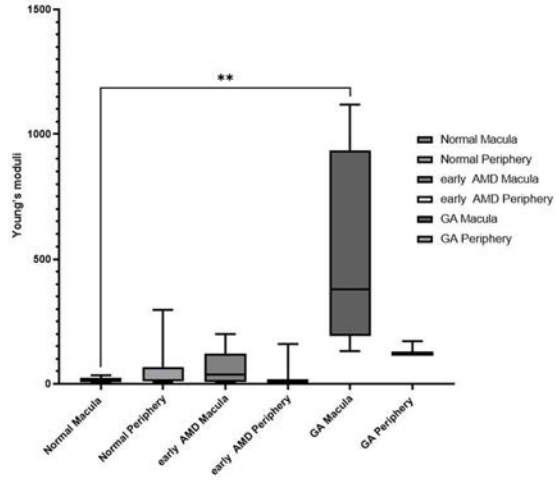


Figure 1: Spatiotemporal profiling of the Young's moduli of BM in normal and in donor eyes with GA using atomic force microscopy.

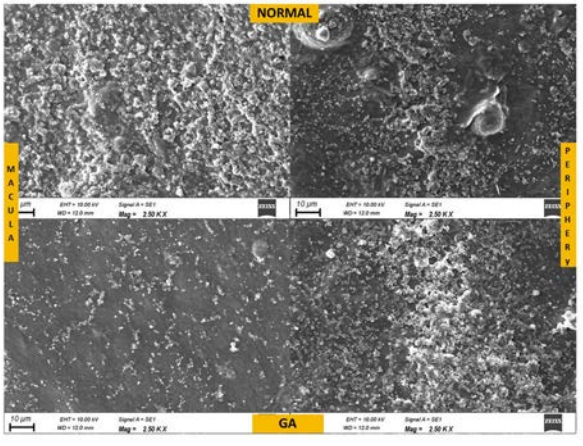


Figure 2: RPE resurfacing of the Bruch's membrane assessed using Scanning electron microscopy.

Resurfacing of human RPE on macular BM was significantly diminished compared to BM of normal donor eyes (Figure 2).

Development and Clinical Evaluation of a CRISPR/Cas12a-based Nucleic Acid Detection Platform for the Diagnosis of Keratomycoses

Investigators details: Dr. Siddharth Narendran,
Dr. Venkatesh Prajna,
Dr. Lalitha Prajna
Funding : Velux Foundation
Research Scholar : D. Hanithraj

Introduction including background

Fungal diseases are estimated to be responsible for more than 1.6 million deaths annually and over 1billion people suffer from fungal infections worldwide. Despite the substantial morbidity and mortality associated with fungal infections, they remain an under estimated and neglected global public health problem. Though pathogenic fungi can infect various organ systems of the human body, fungal infections of the eye are particularly devastating for several reasons. Fungal Keratitis (FK) is the most common ocular fungal infection and even in best-case scenarios, visual rehabilitation and long-term visual outcomes are not optimal. Expedient initiation of treatment drastically improves the clinical outcomes with time to diagnosis being one of the most important risk factors influencing morbidity and mortality in ocular and systemic fungal infections. However, conventional mycological diagnostic modalities require expertise and are often time-consuming. Given the fact that fungal infections disproportionately afflict the rural population in resource-limited settings (RLS), there exists an unmet clinical need for the development of newer diagnostic modalities for rapid and accurate diagnosis of fungal infections. Microbial Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (CRISPR-Cas) adaptive immune systems contain programmable endonucleases with distinctive enzymatic properties that can be leveraged for the detection of microbial nucleic acids.

Recent studies have highlighted the potential of these CRISPR-based nucleic acid detection methods as rapid and highly sensitive diagnostic modalities to detect pathogenic bacteria and viruses. However, the utility of these CRISPR-based diagnostic methods to diagnose fungal infections and their role as a potential diagnostic platform for ophthalmic infections remains to be elucidated. Here, AMRF describes the development of a rapid, ultrasensitive easy-to-implement CRISPR–Cas12a-based tool, and Rapid Identification of Mycoses using CRISPR (RID-MyC), for the detection of fungal nucleic acids. The research team has also validated its method using contrived reference samples and clinical samples from patients with suspected microbial keratitis.

Results

Figure 1 illustrates the workflow of the RID-MyC assay for clinical specimens. The RID-MyC diagnostic platform combines Recombinase Polymerase Amplification (RPA) and CRISPR/Cas12a detection. The analytical sensitivity of the RID-MyC assay was determined and the Limit of Detection (LoD) was 13.8 genomic copies for *Aspergillus flavus*, 16.6 for *Fusarium* solani, 13.9 for *Curvularia lunata*, and 13.3 for Candida albicans. Corneal swabs/scrapings from 75 consecutive patients with presumed microbial keratitis presenting to its tertiary eye care facility were collected. Of the 75 scrapes, 72 (96 %) were positive for fungus by standard mycologic methods of which 55 (73%) were positive by both microscopy and culture and 17 (22.7%) were positive only by microscopy. Between RID-MyC and culture, 56

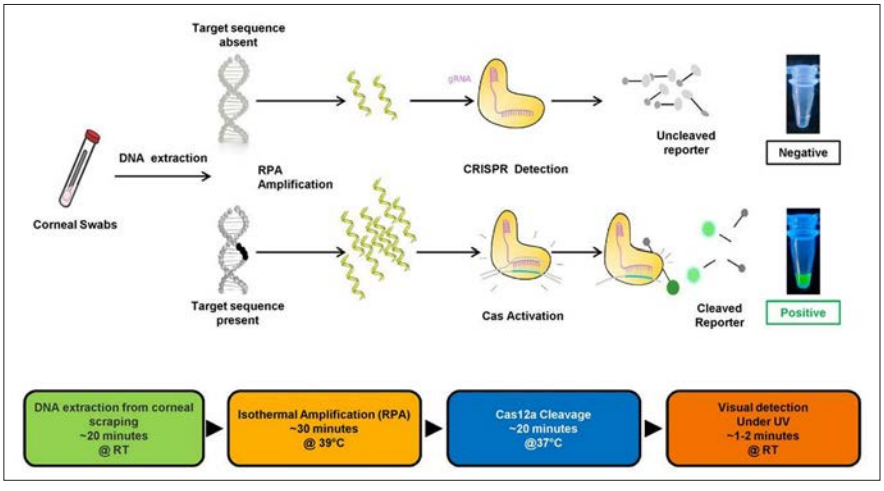


Fig.1: Schematic of RID-MyC assay

Table 1: Performance of PCR, RID-MyC assay and culture in comparison to reference standard in microbial keratitis samples

Results (n = 75)	Reference Standard*		Performance of Assay				
	Positive	Negative	% Sensitivity	% Specificity	% Positive Predictive value	% Negative Predictive value	p- value
PCR			87.5 (77.6 - 94.1)	100 (29.2 - 100)	100	25 (15.3 - 38.1)	
Positive	63	0					0.16 ˜
Negative	9	3					
RID-MyC			94.4 (86.4 - 98.5)	100 (29.2 - 100)	100	42.9 (22.4 - 66)	
Positive	68	0					
Negative	4	3					0.002 †
Culture			76.4 (64.9 - 85.6)	100 (29.2 - 100)	100	15 (10.4 - 21.1)	
Positive	55	0					
Negative	17	3					

**Reference Standard - Includes results of both microscopy and culture; considered positive if either was positive. Values within brackets in performance parameters indicate 95% Confidence Interval.*

˜ - Indicates p-value between PCR and RID-MyC

† - Indicates p-value between RID-MyC and culture

Abbreviations: PCR – Polymerase Chain Reaction, RID-Myc – Rapid Identification of Mycoses using CRISPR, CRISPR – Clustered Regularly Interspaced Short Palindromic Repeats

concordant (52 positives and 4 negatives) and 19 discordant (16 RID-MyC positive but culture negative, 3 RID-MyC negative but culture positive) results were observed. All 16 scrapes were positive for RID-MyC but negative for culture were found to be microscopy-positive for fungus. Of the 55 scrapes positive on fungal culture, 36 harbored *Fusarium*; 8 had *Aspergillus*; *Cylindrocarpon* and *Scedosporium* were identified in 2 cultures each, *Lasiodiplodia*, *Curvularia*, and *Exserohilum* were identified in 1 culture each while 4 culture isolates were not speciated. Among the 4 specimens negative by both RID-MyC and fungal culture, 3 showed bacterial growth (*Pseudomonas* in 2 and *Streptococcus* in 1) and one had no growth. Being a nucleic acid detection modality, the diagnostic performance of the RID-MyC assay was compared to PCR. A sample was considered positive for FK if it demonstrated positive results by culture or microscopy. This definition of positive results was used as the gold standard to calculate and compare the test performances (sensitivity, specificity, PPV, and NPV) between RID-MyC, PCR, and culture (Table 1). The sensitivity, specificity, PPV, and NPV of RID-MyC were 94.4%, 100%, 100 %, and 42.9% respectively. The sensitivity, specificity, PPV, and NPV of PCR were 87.5%, 100%, 100%, and 25% respectively. There was no significant difference between the sensitivities and specificities of PCR and RID-MyC (p=0.16).

Mechanisms modulating endogenous retinal pigmented epithelium regeneration

Investigator : Dr. Siddharth Narendran

Introduction including background:

The RPE is a monolayer of specialised pigmented epithelium strategically poised at the interface between the light-sensitive outer segments of the “image forming” photoreceptors and their source of nutrition, the choriocapillaris. The inconspicuousness of this monolayer contrasts sharply with the multi-functional and indispensable role it plays in maintaining retinal homeostasis. The critical role played by the RPE in ensuring the optimal functioning of the visual system is further highlighted by the number of clinical disorders that take origin from these cells.

Degeneration/dysfunction of the RPE is a seminal event in initiating and maintaining the progressive degeneration of the macula, the photoreceptor dense central retina necessary for fine visual acuity, in several inherited and degenerative diseases of the eye such as age-related macular degeneration (AMD) and Stargardt’s disease (STGD). Despite significant progress in understanding the molecular mechanisms driving RPE degeneration and a several number of therapeutic approaches being suggested including immune modulation and cell replacement therapies to prevent and/or delay

the neurodegenerative process, currently there are no proven treatments for the management of macular degeneration.

Endogenous tissue regeneration has recently received growing interest as a potentially less invasive and cost-effective therapeutic strategy to maintain and reinvigorate cellular function in situ. Conventionally, endogenous regenerative research was limited to tissues with resident stem cells such as skin and the gastrointestinal tract and also to tissues with committed progenitor cells such as the liver and lungs. However, the recently unearthed plasticity of differentiated cells and the replication potential of cells previously believed to be mitotically quiescent, and have kindled a lot of interest in exploring and exploiting the regenerative potential of such cells in chronic diseases such as diabetes mellitus, cardiac disorders and neurodegeneration.

The mature adult RPE cells are generally considered to be terminally differentiated and incapable of replication and regeneration under physiological conditions. However, this dogmatic view on the mitotic quiescence of the adult RPE has been challenged by recent studies elucidating the regenerative potential of the RPE in non-mammalian vertebrates. Additionally, the plasticity and replicative potential of adult human RPE is seen in pathological conditions such as proliferative vitreoretinopathy and recent cell culture studies have also highlighted the plasticity and transdifferentiation potential of the human RPE. Despite these evidences, there is minimal information on the regenerative potential of mammalian RPE. Through exciting new studies, AMRF observed the regenerative potential of the adult mammalian RPE and identified a potential role for inflammation and mechanotransduction in the regulation of RPE regeneration.

Results

Mammalian RPE is capable of recovering structure and function after injury. In a murine model of SINE RNA-induced RPE degeneration, which demonstrated many features reminiscent of AMD, AMRF investigated RPE health at different time points after the injury. Briefly, RPE health was assessed by fundus photography and immunofluorescence staining of zonula occludens-1 (ZO-1) on RPE flat mounts at 7,14 and 28 days after subretinal injection of SINE RNA in 8-12 weeks old C57BL/6J mice. Fundus photographs revealed a decrease in the area of hypopigmentation from Day 7 to day 14 (Fig.1A). RPE degeneration was quantified using two methodologies: The area of degeneration and polymegethism (coefficient of variation of cell size, a prominent feature of RPE cells in AMD) were measured in a semi-automated fashion. Both the area of RPE degeneration (Fig. 1B, 1C) and polymegethism (Fig. 1D) were significantly reduced on Day 28 compared to Day 7, indicating anatomical recovery of the RPE after injury. Next, AMRF investigated if the RPE function was also restored, and for this, AMRF performed RPE phagocytosis assay in mice. Phagocytosis of shed photoreceptor rod outer segments (POS) by the RPE is a vital function of the RPE essential for retinal function. Briefly, RPE flat mounts were prepared from SINE RNA injected mice at 1-hour intervals starting at the onset of light. Ingested POS were measured by immunostaining rhodopsin-positive particles in phalloidin-delineated RPE cells as described previously and compared between the injected eye and the contralateral uninjected eye of the same mice.

Consistent with previous reports, in the uninjected eyes, uptake of POS by the RPE increased after light onset and peaked approximately 2 hours later. In the injected eyes, the number of phagocytosed POS was significantly decreased seven days after SINE RNA injection, while there was significant improvement in the phagocytic function of RPE at 28 days after the insult (data not shown). These exciting preliminary observations strongly suggest that the RPE is capable of undergoing anatomical and functional recovery after injury.

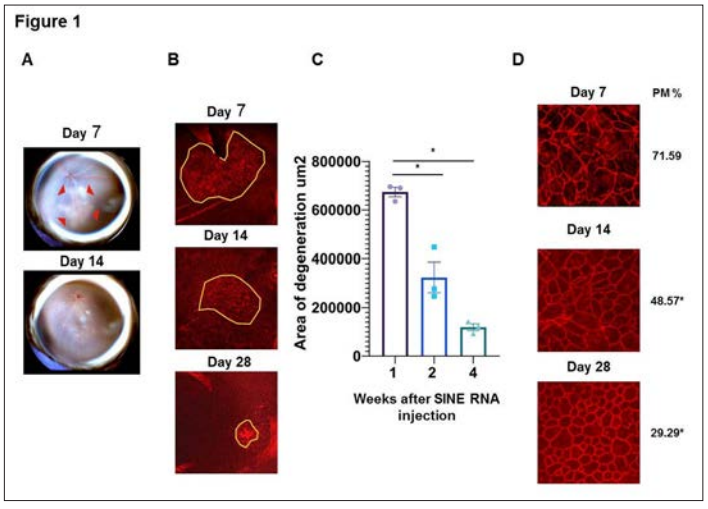


*Fig.1: Adult mouse RPE are capable of restoring structure after injury. RPE health was evaluated at different time points after subretinal injection of SINE RNA (day 0). Fundus photographs(A) show a decrease in the area of hypopigmentation at day 14 compared to day 7 (red arrow heads point to the area of hypopigmentation / degeneration). The area of degeneration (demarcated in yellow) (B&C) and polymegethism (PM) (D) were significantly reduced at day 28 and day 14 compared to day 7 (*P< 0.05).*

CONFERENCES / MEETINGS

National Conference on Biotechnology and OMICS Sciences-2022

Alagappa University, Karaikudi, May 27-28, 2022

Prof. K. Dharmalingam inaugurated the event and delivered the keynote address titled, “Genotype to phenotype: A bridge too far" at the national conference organised by Alagappa University, Karaikudi. Dr. C. Gowri Priya, Dr. A. Vanniarajan, Dr. D. Bharanidharan and Dr. J. Jeya Maheswari were the resource persons of the conference. Four students from AMRF participated and three of them presented their research work.

Invited Talks:

- Dr. J. Jeya Maheswari: *Proteomics in fungal keratitis research: A road map to personalised treatment*
- Dr. A.Vanniarajan: *Omics to precision medicine in eye cancers*

Oral Presentation:

- K. Saraswathi: *Recurrent alterations of NF-kb pathway in Ocular Lymphoma*

Poster Presentation:

- R. Praveenkumar: *Human corneal miRNAs and their role in Pseudomonas aeruginosa keratitis*
- Hanithraj: *Development of an ultrasensitive CRISPR-based diagnostic platform for point-of-care diagnosis of fungal keratitis*

28th ARVO-India–Indian Eye Research Group Meeting (Hybrid)

Hyderabad, September 9-11, 2022

Prof. K. Dharmalingam delivered the presidential talk and chaired the “D. Balasubramanian Oration lecture”. He was elected as the President of IERG for the year 2022 . Dr. C. Gowri Priya and Dr. A. Vanniarajan gave invited talks on their recent research findings. Dr. C. Gowri Priya and Dr. Senthilkumari chaired the scientific sessions.

Invited Talks:

- Dr. C. Gowri Priya: *Biology of adult ocular stem cells and their role in regenerative medicine*
- Dr. A. Vanniarajan: *Epigenetic alterations in retinoblastoma*

Oral Presentation:

- Iswarya Radhakrishnan: *Trabecular meshwork stem cell derived exosomes enhance TM cell survival and proliferation*
- Hanith Raj Deivarajan: *Development and validation of a novel CRISPR/Cas12a-based nucleic acid detection platform for the diagnosis of ocular fungal infections*

Poster Presentations

- Saraswathi Kannan: *Exome sequencing unveiled recurring mutations in nf-κb Pathway in ocular b-cell lymphoma*
- Susmita Chowdhury: *Analysis of microstructural changes in an x-linked juvenile Retinoschisis patient harboring rs1 g668a mutation by en-face Optical coherence tomography imaging*
- Sethu Nagarajan: *Targeted modulation of e2f3 and kif14 in retinoblastoma*
- Shreya Dinesh - *Understanding the role of miRNA in aspergillus flavus keratitis Progression*
- Swathi Chadalawada: *Identification of potential blood miRNAs for diagnosis of intraocular tuberculosis*

Prof. K. Dharmalingam at Alagappa University, Karaikudi

AMRF faculty and research scholars at IERG meeting

- Nerethika Ravichandran: *Separation of filtering and non-filtering region of human Trabecular meshwork*
- P. Saranya: *Adult human anterior lens epithelial stem cells–changes during ageing and in cataract*
- Waseema Arif: *Age-related changes in the functional characteristics of adult stem cells in human retinal pigment epithelium*
- Swagata Ghosh: *In-vitro infection models of fungal and amoebic keratitis reveal host cell-type and pathogen specific features of host inflammatory response*
- Daipayan Banerjee: *Deciphering the proteome signature of plasma extracellular vesicles from proliferative diabetic retinopathy patients*

IInd International Conference cum Workshop on Recent Trends in Structural Bioinformatics and Computer-Aided Drug Design

Alagappa University, Karaikudi, November 2022

A. Mohamed Hameed Aslam and Dr. D. Bharanidharan presented a poster on the topic, *Identification and analysis of alternative transcripts in retinoblastoma progression*.

Genomics India Conference - 2023

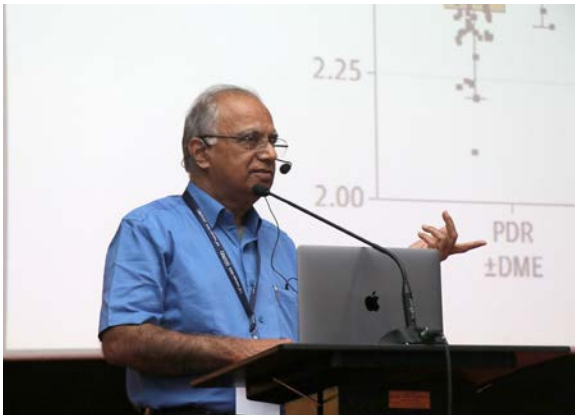
Bangalore, February 2-3, 2023

Dr. P. Sundaresan delivered a talk on *Gene therapy for an inherited eye disorder and Mitochondriopathy* at the conference organised by Genotypic Technology in the NIMHANS Convention Centre, Bangalore.

International Conference on Consortium of Universal Research Erudition (iCURE)

Madurai, February 03-06, 2023

Prof. K. Dharmalingam delivered two invited lectures at the international conference organised by Madurai Kamaraj University, Madurai. The topics were:



Prof. K. Dharmalingam at iCURE meeting at MKU

- *Diabetic retinopathy-Prognostic biomarkers and disease biology*
- *Immune system and the eye*

International Symposium on Mitochondria, Cell Death and Human Diseases

New Delhi, February 18-19, 2023

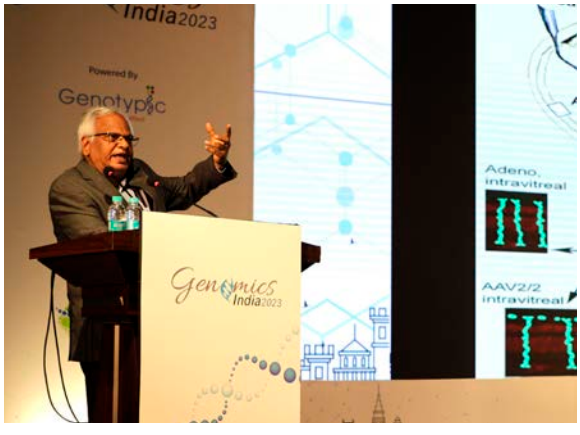
Dr. P. Sundaresan, delivered an invited talk on *Mutation profile and functional studies of neurodegenerative mitochondriopathy-LHON* at the international symposium organised by the School of Life Sciences, Jawaharlal Nehru University, New Delhi.

Workshop on Next Generation Sequencing data analysis

Punjab, March 9-10, 2023

Dr. D. Bharanidharan was a resource person for the two-day workshop held at the Central University of Punjab, Bathinda. He delivered a lecture on *Hunting Disease-Specific Pathogenic Variants from genome/ exome sequencing with a focus on eye diseases* and conducted a hands-on session on *Exome/Genome Data analysis*.

Dr. P. Sundaresan at NIMHANS, Bangalore



Dr. P. Sundaresan at JNU, New Delhi

Dr. D. Bharanidharan at the Central University, Punjab

CONFERENCES CONDUCTED

One Day Training Programme on Tissue Culture Techniques

Madurai, September 3, 2022

The department of Immunology and Stem Cell Biology, conducted a one day training programme on "Tissue Culture Techniques" as a part of the Scientific Social Responsibility activity of an on-going project funded by Science and Engineering Research Board, New Delhi. The programme included invited talks by Prof. VR. Muthukkaruppan, Advisor, AMRF and Dr. Indumathi Mariappan, Research Scientist, LV Prasad Eye Institute, Hyderabad. Laboratory sessions were conducted on the basics of tissue culture. A total of 10 PhD scholars from nearby colleges and universities were selected for the programme.

One-day Seminar on Biomedical Applications of Fundamental Research and Future Potential

Madurai, October 14, 2022

AMRF organised a one-day seminar as a part of October Summit 2022. Twenty-two participants from various colleges in and around Madurai attended. An Open house programme was conducted to introduce the college teachers about the research activities of Aravind Eye Hospital (AEH) and AMRF and also motivated them to encourage their students to take up research.

Listed below are the talks delivered by the clinicians and experts at the event:

- Dr. S. R. Rathinam: *Bench to bedside - Trematode induced granulomatous uveitis*
- Dr. Lalitha Prajna: *Progress in diagnostics for ocular infections at Aravind*
- Dr. Usha Kim: Retinoblastoma management: *Our journey from research to patient care*
- Prof. K. Dharmalingam: *Biomarkers - discovery, development and clinical use*
- Prof. S. Karutha Pandian: *Evaluation of natural compounds for biofilm inhibition*
- Prof. H. Shakila: *Nanobiomaterials - From fundamentals to biomedical applications*
- Prof. K. Balamurugan: *Caenorhabditis elegans, A useful model for biological research*

AMRF–Dartmouth Education and Research Conference

Madurai, November 29-30, 2022

AMRF conducted a two-day Conference in collaboration with Dartmouth College. This conference is a unique activity wherein two institutions share educational and research activities, which would pave way for future collaborative research projects. The conference was organised by Prof. K. Dharmalingam and co-ordinated by Dr. D. Bharanidharan, and Dawn E. Carey, Associate Director, Global Health and Development, The Dartmouth Institute for Health Policy and Clinical Practice, Lebanon, New Hampshire.

Eleven students from Dartmouth College presented research proposals on various eye

Participants of Tissue culture techniques workshop

Participants of Biomedical Applications of Fundamental Research and Future Potential Seminar

AMRF - DARTMOUTH Education and Research Conference

diseases to clinicians, scientists and research scholars. The research work of AMRF was presented as posters by the research scholars. The details of the core research facilities and on-going research projects at AMRF were also presented at the event.

Workshop on Basics of Tissue Culture

Madurai, February 7-11, 2023

A total of 16 participants attended the workshop including two Junior Faculty, 11 Research Scholars and three Postgraduate Students from various colleges and Universities The workshop included a series of lectures by AMRF faculty followed by laboratory sessions to give hands-on training on tissue culture techniques.

21st Research Advisory Board Meeting

March 25, 2023

The faculty members of AMRF presented their works and received feedback. Research scholars presented their findings as posters and interacted with the committee members. The best poster was selected for Prof. VR. Muthukkaruppan Endowment Award.

AWARDS

Best Paper in Clinical Sciences

Hanith Raj Deivarajan, Project Fellow, Department of Microbiology received the Best Paper award in Clinical Sciences for his work titled *Development and validation of a novel CRISPR/CAS12A based Nucleic-Acid detection platform for the diagnosis of ocular fungal infections* at the IERG meeting held on September 9-11, 2022 at LVPEI, Hyderabad.

Recipient of Travel Grant

R. Iswarya, Senior research Fellow, Department of Immunology and Stem Cell biology received a travel grant for her work titled *Trabecular meshwork stem cell derived exosomes enhance TM Cell survival and proliferation* at the IERG meeting held on September 9-11, 2022 at LVPEI, Hyderabad.

21st Research Advisory Board Meeting

Iswarya receiving travel grant award from Prof. Mark Petrash, University of Colorado, USA

Hanithraj D receiving best paper award from Prof. Mark Petrash, University of Colorado, USA

Best Oral Presentation Award

C. Prakash, Senior Research Fellow, Department of Genetics, received the "Best Oral Presentation Award" for his presentation titled *Nuclear gene implications in LHON probands decodes autosomal recessive forms of inheritance* at the 4th International Conference on Genome Biology (ICGB-4) & 54th Annual Aqua-Terr Day (Feb 28 - Mar 1, 2023) at Madurai Kamaraj University, Madurai.

Prof. VR. Muthukkaruppan Endowment award 2022

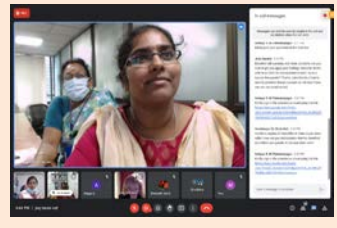
In 2014, students and colleagues of Prof. VR. Muthukkaruppan established an endowment in his name. From this endowment fund, an award is given every year to the best researcher of the institute based on the scientific merit of their research abstracts and presentation. This award carries a certificate and a cash prize.

Hanithraj Deivarajan, Department of Microbiology, won the award for his outstanding research work on *Development of a novel non-invasive nucleic acid extraction-free CRISPR/cas12a based diagnostic approach for fungal keratitis* for the year.

Hanithraj receiving Prof. VR. Muthukkaruppan Endowment award

Ph.D awarded by Alagappa University

Aloysius Abraham
Department of Molecular Genetics
Thesis: Identification of modifier genes involved in Tumorigenesis of Retinoblastoma
Guide : Dr. A. Vanniarajan



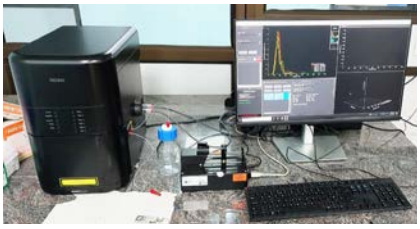
K. Lavanya
Department of Immunology and Stem Cell
Thesis: Micro RNAs Specific to Corneal Epithelial Stem Cells
Guide: Dr. Gowri Priya Chidambaranathan

A. Divya
Department of Proteomics
Thesis: Interaction of Pathogenic Fungi with Human Corneal Epithelial cells
Guide: Prof. K. Dharmalingam

NEW FACILITY

Exosome Innovation Centre

Extracellular vesicles (EVs) or exosomes (small EVs) are lipid-encased nanoparticles that carry information from parent cells in the form of DNA, RNA, protein, lipids and metabolites and can alter the cellular physiology of local cells or distal cells by travelling through peripheral blood. The involvement of EVs in several (patho) physiological processes along with their molecular composition reflecting the parent cell in health and disease state makes EVs an excellent tool for diagnostic biomarkers and understanding their role in disease pathogenesis.



AMRF established the state of art facility to facilitate exosome research in ocular and other related areas. This facility is a part of the translational initiative for the basic research programmes at AMRF. AMRF is expanding the scope of this exosome research facility to be available for any group working in the exciting yet challenging area of exosomes research. The facility includes the infrastructure for carrying out the end-to-end workflow right from isolation to confirmation and to characterisation of exosomes.

The facility encompasses the following major equipment essential for the entire workflow:

1. Tabletop ultracentrifuge for exosomes isolation (Optima Max-XP Table Top Ultra Centrifuge (Beckman Coulter)
2. Nano particle tracking analysis (NS300, Malvern Panalytical)
3. UV-Vis Spectrophotometer (Spectramax 3, Moleculardevices)
4. Mass Spectrometer for proteome profiling OrbitrapVelos Pro Spectrometer (ThermoFisher)
5. 2D PAGE and 2D DIGE

Regional Research Centre

The research at Aravind is always a collaborative work of clinicians and scientists. It is quite successful in Madurai where the hospital and research centre are located in the same vicinity. For expanding the scope of the research across the Aravind Eye Care System, the clinicians should have access to basic research facilities and better ways of communicating with the researchers through video conferencing. In a major initiative, the President of AMRF, Dr. P. Namperumalsamy formulated the idea of strengthening the research at Aravind Eye Hospitals in Tirunelveli, Coimbatore, Pondicherry, and Chennai. Towards this objective, Regional Research Centres of AMRF are being established in these hospitals. These centres will have adequate infrastructural facilities to start research programme in collaboration with AMRF, Madurai.

GUEST LECTURE

Dr. Beth Mills, UKRI Future Leaders Fellow, Translational Healthcare Technologies Group, Centre for Inflammation Research, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK

Topic : "Diagnosis of Infection: Utilising Optical Molecular Smart Probes from the Lung to the Cornea"

12th May

Dr. K. Gokulakrishnan, Assistant Professor, DBT/ Wellcome Trust India Alliance–Intermediate Fellow, Department of Neurochemistry, National Institute of Mental Health and Neuro Sciences (NIMHANS), (Institute of National Importance), Bengaluru

Topic: "Clinical and Molecular significance of Retinol Binding Protein-4 (RBP4) and Adiponectin in obesity-mediated type 2 diabetes".

30th September

Dr. Bini, Proteomics Technical Lead, MRC Toxicology Unit, University of Cambridge, Cambridge, UK

Topic :"An Introduction to Single Cell Proteomics".

21st November

Prof. Michael E. Zegans, MD, Professor, Chief-Ophthalmology, Dartmouth Hitchcock Medical Centre Lebanon, USA.

Topic : "Anti-Fungal effects of Beta-adrenergic antagonists".

1st December

Dr. V. Prabhakaran, Professor, Neurochemistry Laboratory, Department of Neurological Sciences, Christian Medical College and Hospital, Vellore.

Topic: "Peripheral blood genomics and proteomics to understand Neurocysticercosis pathogenesis".

28th December

Dr. Anand Tadas, Regional Application Specialist, Malvern Panalytical, Aimil Ltd, Chennai.

Topic: "NanoSight and its application".

31st March

PUBLICATIONS 2022 - 2023

Divya Arunachalam, Shruthi Mahalakshmi Ramanathan, Athul Menon, Lekshmi Madhav, Gopalakrishna Ramaswamy,Venkatesh Prajna Namperumalsamy, Lalitha Prajna, Dharmalingam Kuppamuthu

- *Expression of immune response genes in human corneal epithelial cells interacting with Aspergillus flavus conidia*

BMC Genomics 2022;23:5

Chitaranjan Mishra, Roopam Duvesh, Susmita Chowdhury, Rupa Anjanamurthy, Naresh Babu Kannan, Kim Ramasamy, Periasamy Sundaresan

- *Analysis of microstructural changes in an X-linked juvenile retinoschisis patient harboring RS1 G668A mutation by en-face optical coherence tomography imaging*

Indian J Ophthalmol Case Rep 2022;2:136-9

Mohan M, Shetty S, Perumalsamy V, Prakash C, Sundaresan P.

- *Clinical and genetic aspects of a child with monilethrix and visual rehabilitation*

Indian J Ophthalmol Case Rep 2022;2:211-3

Umadevi Subramanian , Bharanidharan Devarajan

- *Identification of dysregulated pathways and key genes in human retinal angiogenesis using microarray metadata*

Gene Reports 26 (2022) 101434

Poigaialwar Gowri, Ponraj Sathish, Shanmugam Mahesh Kumar, Periasamy Sundaresan

- *Mutation profile of Neurodegenerative Mitochondriopathy – LHON in Southern India*

Gene 2022 Jan 30 (Epub)

Angeline Julius, Remya Rajan Renuka, Waheeta Hopper, P. Babu Raghu, Sharmila Rajendran, Senthilkumari Srinivasan, Kuppamuthu Dharmalingam, Amer M. Alanazi, Selvaraj Arokiyaraj, and S. Prasath

- *Inhibition of Aldose Reductase by Novel Phytocompounds: A Heuristic Approach to Treating Diabetic Retinopathy*

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Gurudas S, Frudd K, Maheshwari JJ, Revathy YR, Sivaprasad S, Ramanathan SM, Pooleeswaran V, Prevost AT, Karatsai E, Halim S, Chandra S, Nderitu P, Conroy D, Krishnakumar S, Parameswaran S, Dharmalingam K, Ramasamy K, Raman R, Jones C, Eleftheriadis H, Greenwood J, Turowski P.

- *Multicenter Evaluation of Diagnostic Circulating Biomarkers to Detect Sight-Threatening Diabetic Retinopathy.*

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Kandasamy Kathirvel, Ravinarayanan Haribalaganesh, Ramasamy Krishnadas, Veerappan Muthukkaruppan, Colin E.Willoughby, Devarajan Bharanidharan, Srinivasan Senthilkumari

- *A Comparative Genome-wide Transcriptome Analysis of Glucocorticoid Responder and Non-Responder Primary Human Trabecular Meshwork Cells*

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Kandasamy Kathirvel, Lester Karen, Ravinarayanan Haribalaganesh, Ramasamy Krishnadas, Veerappan Muthukkaruppan, Brian Lane, David A.Simpson, Kasia Goljanek-Whysall, Carl Sheridan, Devarajan Bharanidharan, Colin E.Willoughby, Srinivasan Senthilkumari

- *Short and long-term effect of dexamethasone on the transcriptome profile or primary human trabecular meshwork cells in vitro*

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Lavanya Kalaimani, Bharanidharan Devarajan, Venkatesh Prajna Namperumalsamy, Muthukkaruppan Veerappan, Julie T. Daniels, Gowri Priya Chidambaranathan

- *Hsa-miR-143-3p inhibits Wnt-β-catenin and MAPK signaling in human corneal epithelial stem cells*

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Linda Maria Genoveva De Piedade Sequeira, Gowri Poigaialwar , Shashikant Shetty, P Sundaresan, P Vijayalakshmi

- *A new entity of hypomyelination with atrophy of basal ganglia and cerebellum-like syndrome with bilateral developmental cataract*

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Roopam Duvesh, Krishnadas SR, Sundaresan P

- *Genetics of Congenital Glaucoma*

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- *Retinal Pigment Epithelium in Human Donor Eyes Contains Higher Levels of Bisretinoids Including A2E in Periphery than Macula*

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Kumar Jeyaprakash, Kannan Thirumalairaj, Usha Kim, Veerappan Muthukkaruppan, Ayyasamy Vanniarajan

- *RB1 transcript analysis detects novel splicing aberration in retinoblastoma*

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Prakash Chermakani, Periasamy Sundaresan

- *Traboulsi Syndrome: A Rare Eye Disease and its Genetic Association*

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ONGOING RESEARCH PROJECTS

No	Projects	Funded by	Investigators	Research Scholar
PROTEOMICS				
1.	Study on human mycotic keratitis	AMRF & AEH	Dr. N. Venkatesh Prajna Dr. Lalitha Prajna Dr. J. Jeya Maheshwari Dr. K. Dharmalingam Dr. O. G. Ramprasad	A. Arun Alexander
2.	Development of aptamer-based assays for diagnosis of infectious keratitis and absolute quantitation of proteoform markers of diabetic retinopathy	Sun Pharma Sep 2019 – Aug.2022	Dr. J. Jeya Maheshwari Dr. K. Dharmalingam Dr. N. Venkatesh Prajna Dr. R. Kim	Dr. Susan Immanuel
3.	Screening the Kadaladi family with early onset Glaucoma for Myocilin gene mutations	Sun Pharma Sep 2019 – Aug.2022	Dr. K. Dharmalingam Dr. S.R. Krishnadas Dr. Mohideen Abdul Kader Dr. D. Bharanidharan	V. Saravanan
4.	Understanding (Deciphering) the role of extracellular vesicles in the modulation of host immune response in fungal keratitis	Sun Pharma Mar.2021 – Aug.2022	Dr. J. Jeya Maheshwari Dr. K. Dharmalingam Dr. N. Venkatesh Prajna Dr. Lalitha Prajna	Subash KK Hariharan G
5.	Understanding the mechanism of action of a novel chemical cross-linker designed to treat keratoconus	ICMR 2020 – May 2023	Dr.O.G.Ramprasad Prof. K. Dharmalingam Dr. N.Venkatesh Prajna Dr. Naveen Radhakrishnan	G. Adhithya Subramanian
6.	Identification of druggable targets for attenuating the progression of pterygium development	Sun Pharma Sep 2019 – Aug 2022	Dr. Daipayan Banerjee Dr. Vishnu Teja Dr.N. Venkatesh Prajna Dr. K.Dharmalingam	Aadithiya T Gr
7.	Ectosomes and exosomes in ocular diseases: Towards the development of novel diagnosis and reconfigurable therapeutics (Role of Extracellular Vesicles in pathogenesis of Diabetic Retinopathy)	Sun Pharma Mar.2021 – Aug.2022	Dr. Daipayan Banerjee Dr. Sagnik Sen Dr. Naresh Babu Dr. R.Kim Dr. K.Dharmalingam	Aadithiya T Gr
8.	A comparative proteomic analysis of plasma and vitreous humor derived small Extracellular Vesicles (SEVs) from Proliferative Diabetic Retinopathy (PDR) patients.	Sun Pharma March 2023-February 2024	Dr. Daipayan Banerjee Dr. Bhavani S Dr. K. Naresh Babu Dr. R. Kim Prof. K. Dharmalingam	Aadithiya T Gr
9.	Deciphering predictive and preventative methods in the progression of pterygium using multiomics approaches	SERB 1st November 2022-31st October 2024	Dr. Daipayan Banerjee	L. Mathan
10.	Whole genome sequence analysis of selected family members of the Kadaladi family and identification of markers for the early detection of JOAG using tear extracellular vesicles	Sun Pharma 2022-2023	Dr. K. Dharmalingam Dr. P. Sundaresan Dr. D. Bharanidharan Dr. S. R. Krishnadas Dr. Mohideen Abdul Kader	Karthik A

MICROBIOLOGY				
11.	Development and validation of a non-invasive point-of-care diagnostic tool for fungal keratitis	VELUX STIFTUNG 01.08.2021-31.07.2025	Dr.N.Venkatesh Prajna Dr. Lalitha Prajna Dr.K. Dharmalingam Dr.Thulasiraj Ravilla Dr.N. Siddharth	Hanithraj D. Padmapriya S Humera Khathun Vignesh P
12.	Mechanotransduction in retinal pigmented epithelium homeostasis and degeneration: Implications in age-related macular degeneration	SERB 27.12.2021-26.12.2023	Dr. Siddharth Narendran	S. Karvannan
13.	Identification of Dysregulated MicroRNAs In Ocular Fluids as Diagnostic Markers for Intraocular Tuberculosis	ICMR-SRF Jul.2019 – June 2022	Dr. D. Bharanidharan	Swathi Chadalawada
14.	Role of Human Corneal MiRNAs in the onset and severity of Fungal Keratitis	ICMR Nov.2021 Nov.2023	Dr. D. Bharanidharan Dr. K. Dharmalingam Dr. N. Venkatesh Prajna Dr. Lalitha Prajna	Gayathri M
15.	Dysregulated human Corneal miRNAs and their role in disease progression	ICMR-SRF 2022-2025	Dr. D. Bharanidharan	Shreya Dinesh
MOLECULAR GENETICS				
16.	Molecular Characterisation of Leber's Congenital Amaurosis in South Indian Cohort	Lady Tata Memorial Trust 02.01.2021 – 01.01.2026	Dr. P. Sundaresan	A.S. Sriee Viswarubhiny
17.	Investigating the Crosstalk between Nuclear and Mitochondrial Genome in Patients with Leber's Hereditary Optic Neuropathy	ICMR-SRF 01.03.2021 – 28.02.2024	Dr. P. Sundaresan	C. Prakash
18.	Molecular Genetics of Juvenile X-linked Retinoschisis in South Indian Population	Lady Tata Memorial Trust 01.08.2019 – 31.07.2023	Dr.P.Sundaresan	Susmita Chowdhury
19.	Molecular characterisation of tumor progression in retinoblastoma	DST INSPIRE Fellowship 09.06.2017 - 08.06.2022	Dr. A. Vanniarajan	T.Shanthini
20.	Translational Genomics of Ocular Cancers	Aravind Eye Foundation	Dr. Usha Kim Dr. A. Vanniarajan Dr. D. Bharanidharan Dr. R. Shanthi Dr. VR. Muthukkaruppan	K. Jeyaprakash
21.	Molecular characterisation of ocular lymphoma for improved disease prognosis	Lady Tata Memorial Trust 01.08.2020 – 31.07.2025	Dr.A. Vanniarajan	K. Saraswathi

22.	Elucidating the role of cancer stem cells in chemoresistant retinoblastoma and their therapeutic implications	ICMR-SRF 2022-2025	Dr. A. Vanniarajan	R. Sethu Nagarajan
IMMUNOLOGY AND STEM CELL BIOLOGY				
23.	Characterisation of adult human lens epithelial stem cells in the maintenance of tissue homeostasis throughout life and their functional status in cataractous lens	SERB 29.05.2019 – 30.11.2022 Lady Tata Memorial Trust – SRF 01.08.2019 – 31.07.2023	Dr. Madhu Shekhar Dr. Gowri Priya Chidambaranathan Dr. Haripriya Aravind Dr. VR. Muthukkaruppan	P. Saranya
24.	Identification and Characterisation of adult human retinal pigment epithelial stem cells	Part time PhD from 17.03.2022	Dr. Gowri Priya Chidambaranathan	A.Waseema
25.	Adult stem cell derived extracellular vesicular miRNAs for trabecular meshwork regeneration in glaucoma	Sun Pharma UGC-SRF 03.06.2020 – 02.06.2025	Dr. Gowri Priya Chidambaranathan Dr. S. R. Krishnadas Dr. VR. Muthukkaruppan	R. Iswarya
26.	Molecular regulators associated with the maintenance of human trabecular meshwork stem cells in relation to their reduction in ageing and glaucoma	SERB Oct.2021 – Oct.2024	Dr. Gowri Priya Chidambaranathan Dr. D. Bharanidharan Dr. S. R. Krishnadas Dr. VR. Muthukkaruppan	R. Nerethika Sneha Nair
27.	Molecular characterisation of human retinal pigment epithelial stem cells and their role in age related macular degeneration	ICMR Dec 2022-Dec 2025	Dr.C.Gowri Priya Dr.D.Bharanidharan Dr.R.Kim Dr. VR. Muthukkaruppan Dr. Siddharth Narendran	M. Lakshmi Priya
28.	A preliminary study to generate tear producing lacrimal gland organoids from human cadaveric and biopsy samples	Sun Pharma Nov.2021 – Apr.2022	Dr. Anwar Azad P. Dr. Usha Kim Prof. K. Dharmalingam	Gowthami Shankar
OCULAR PHARMACOLOGY				
29.	Role of miRNA in the regulation of Glucocorticoid Receptor (GR) signalling and Development of New therapeutics for Steroid-induced glaucoma	Wellcome-DBT /India Alliance Intermediate Fellowship (2017-30.09.2022)	Dr. S. Senthilkumari Dr. C. Gowri Priya Dr. D. Bharanidharan Dr. R. Sharmila	R. Hari balaganesh K. Kathirvel D.Yogamaya
BIOINFORMATICS				
30.	Expression profiling of human corneal miRNAs and their role in Pseudomonas aeruginosa induced keratitis	DBT-BET 2022-2027	Dr. D. Bharanidharan	R.Praveen kumar
31.	Identification and Analysis of Alternative Transcripts in Retinoblastoma Progression	ICMR-SRF 2022-2025	Dr. D. Bharanidharan	Mohd Hameed Aslam

Much has been done, but much remains to be done… we look to the future with renewed strength to continue the mission of providing quality eye care and hope that some of what we have learned will be useful to other eye care workers around the world.

G. Venkataswamy

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