Focusing on eye disease

DR JOANNE MATSUBARA

Dr Joanne Matsubara aims to elucidate the underlying pathology of the early stages of age-related macular degeneration in order to halt the progression towards vision loss. Here, she discusses some of the novel treatment strategies that she and collaborators are developing towards this goal

Is there a link between Amyloid-beta (Aß) peptides, age-related macular degeneration (AMD) and Alzheimer's disease (AD)?

AMD and AD are both age-related neurodegenerative diseases: one of the eye and one of the brain. Aß peptides play a role in both, but the specific mechanisms involved in the brain or eye are not completely understood. Aß 1-42 is the neurotoxic peptide associated with AD, while Aß 1-40, the more common species is also present in ocular drusen. Both forms trigger the innate immune response and appear to initiate inflammasome activation in several cell types, including microglia and retinal pigment epithelial (RPE) cells.

AD and AMD have different genetic predisposition profiles, with at least one gene, apolipoprotein E (APOE, E4 allele) appearing to have opposite risks. The two diseases do not usually co-exist in the same group of patients, also suggesting separate pathogenetic mechanisms. Further studies are needed to fully elucidate the links between AD and AMD, but at present, it is apparent that Aß deposits are implicated in the pathogenesis of both. Understanding the role of Aß in the eye may help support novel treatment strategies in AD, and vice versa.

What was the purpose of using gene set enrichment analysis (GSEA) for your AMD studies?

GSEA is a powerful computational method that allowed us to characterise gene expression changes across all known genes in the human genome and compare results with other published studies by accessing differential gene expression data in the public domain. We discovered that RPE cells respond to stimulation by drusen components such as Aß and advanced glycation endproducts (AGEs), by increasing activity in cellular pathways associated with proteasome degradation, caspase signalling, toll-like receptor signalling and interferon gamma. Identification of these networks in RPE gives insight into the cellular mechanisms affected by drusen components. For example, increased activity of proteosome degradation networks suggests that drusen may exacerbate the immunoproteosome response to cellular stress in RPE.

Toll-like receptor signalling networks were also identified by GSEA analysis and implicate the nuclear factor kappa-b (NfKb) pathway, an important pathway for modulation of growth factors such as VEGF which is known to promote the wet form of AMD, and inflammasome-specific cytokines (Il1ß and IL18). This new knowledge suggests two pathways for suppressing changes in the RPE associated with the early stages of AMD: Nfkb activity and TLR signalling. Our job now is to validate their roles in AMD and to identify key players (such as specific receptors, signalling molecules or complement proteins) as candidate targets for new treatments to stop local, chronic retinal inflammation in AMD.

Could you elaborate on the micro electro mechanical systems (MEMS) device you are involved with?

Dr Mu Chiao's group and I are developing a MEMS device for magnetically controlled drug delivery to the eye that eliminates the need for intravitreal injections of drugs for retinal diseases such as AMD and diabetic retinopathy. Intravitreal injections – currently the standard of care – have associated side effects, including retinal detachment, haemorrhage and endophthalmitis. The MEMS device is placed outside of the eye, on the sclera, and will store enough therapeutic drug to last up to six months or longer, thus eliminating the need of monthly intravitreal needles.

Finally, are you collaborating with other laboratories in the course of your investigations?

Dr Max Cynader's group and I are developing a method to suppress early retinal inflammatory events in AMD by using a novel gene treatment triggered by pro-inflammatory cytokine release. This study focuses on enzymes called proteases that shape the cellular environment in the retina. Abnormal activation of these proteases can cause inflammation by releasing harmful molecules such as tumour necrosis factor (TNF).

Our team is developing a way to 'reprogramme' the proteases in the retina so that they release therapeutic factors instead of harmful factors. The goal of this novel and first-in-class strategy is to have retinal cells release engineered therapeutic factors at the site of inflammation, in timely manner, only when the inflammatory processes have reached a certain critical threshold. Our proposed 'smart' therapeutic strategy will be positioned in retinal cells in advance of disease, and function to reduce the negative off-target effects frequently associated with current drug therapies for eye disease.

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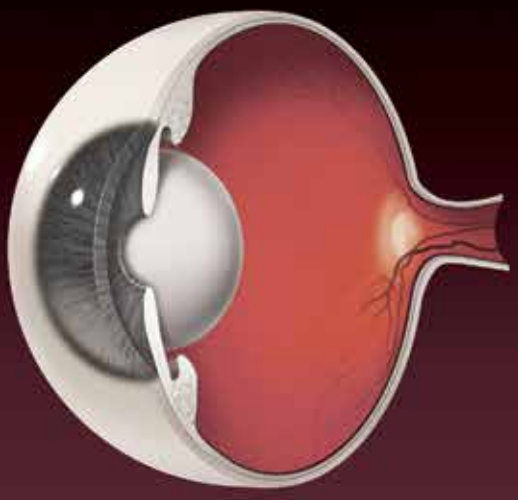

Vision of the future

Researchers at the Department of Ophthalmology and Visual Sciences, **University of British Columbia**, Vancouver, are focusing their efforts on the early molecular triggers that cause age-related macular degeneration, with strong emphasis on preventative treatment

AGE-RELATED MACULAR DEGENERATION

(AMD) is a common eye condition amongst those over the age of 50. It is the leading cause for vision loss in the elderly and involves the gradual deterioration of part of the eye called the macular – the most sensitive part of the retina responsible for providing sharp, detailed, central vision. Those affected lose their ability to detect fine detail and consequently lose the ability to recognise faces or accomplish tasks that rely heavily on complete vision such as driving or reading; patients do, however, retain blurred peripheral vision and are not completely blind. Risk factors associated with the disease include genetics, age, smoking and family history. Despite its prevalence and debilitating symptoms, AMD is relatively poorly understood – there are few effective treatments and the underlying mechanisms have yet to be fully elucidated.

STEPS FORWARD

Dr Joanne Matsubara, Professor and Director of the Research (Basic Sciences), Department of Ophthalmology and Visual Sciences, University of British Columbia, is currently working with collaborators both in Canada and the USA. The main goal of her research is to better understand the biological differences between the healthy ageing eye and the AMD eye. Understanding these differences will help to identify the underlying pathologies of the AMD eye such that they can be exploited therapeutically. This work currently focuses on dry AMD, which comprises around 90 per cent of cases.

Dry AMD represents the earliest stages of the disease, before vision loss occurs, but can progress into geographic atrophy, which is more severe. Furthermore, dry AMD often precedes another form of the disease, wet AMD, in which blood vessels grow and leak into retinal tissue, leading to rapid vision loss. Through understanding the early stages before vision loss, treatments can be devised to prevent the retinal damage associated with dry AMD and before loss of vision occurs.

DRUSEN

AMD is characterised by the development of deposits in the retina called drusen. Drusen can occur naturally during ageing, and sometimes develop without detrimental effect. As AMD progresses from early stages, the number and size of drusen increase, which is thought to contribute to the deterioration of two retinal cell types: photoreceptors and retinal pigment epithelial cells (RPE) that support and maintain photoreceptors. The deterioration of these cells is the underlying reason for vision loss in AMD – if photoreceptors die then vision deteriorates permanently.

Matsubara's research looks for ways to keep RPE and photoreceptor cells healthy, thus preventing the visual loss associated with advanced AMD: "One straightforward approach is to stop drusen forming. However, we cannot do this just yet as we do not understand how, or why, drusen develop in the back of the eye. Therefore, we are not at the stage of understanding how to prevent drusen formation," she explains. Instead, Matsubara focuses her efforts on the effects drusen have on their surrounding environment, investigating how the physiology and behaviour of nearby retinal cells change in response to drusen development.

By fully characterising the pathways involved, novel therapeutic strategies can be devised to prevent drusen from having a detrimental effect on the surrounding cells. If this results in the prevention of photoreceptor death then permanent vision loss could be avoided. Drusen are heterogeneous and consist of a variety of modified proteins, peptides and lipids. One important component is amyloid-beta (Aß), well known for its role in the pathogenesis of Alzheimer's disease (AD).

MATSUBARA LAB

OBJECTIVES

To understand the normal cellular processes in the retina underlying 'healthy' ageing and compare these to the cellular processes in the retina with age-related macular degeneration (AMD), using multiple experimental approaches. One major objective is to elucidate specific cellular pathways that promote chronic retinal inflammation associated with the earliest stages of AMD. Another objective is to identify mechanisms and develop novel treatments to suppress complement activation and MAC formation in the AMD eye. The hope is to develop innovative, novel strategies for suppressing pro-inflammatory cytokines and promoting retinal homeostasis that will protect against vision loss in AMD.

KEY COLLABORATORS

University of British Columbia, Canada: **Dr Max Cynader** and colleagues; **Dr Farzin Fooroghian** and colleagues; **Dr Mu Chiao** and colleagues; **Dr Patrick McGeer**

Simon Fraser University, Canada: **Dr Marinko Sarunic**

Schepens Eye Research Institute, USA: **Dr Andrius Kazlauskas**

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Matsubara's laboratory has found that drusen influence changes in the expression of genes affiliated with damage-associated molecular pattern molecules (DAMPs) that are known to be involved in the initiation and perpetuation of the non-infectious immune response. The researchers have also shown that drusen cause cellular stress in RPE cells. This cellular stress triggers the activation of the inflammasome, a component of the innate immune response. In AMD this can cause the RPE to release long lasting pro-inflammatory cytokines that damage the RPE cells and other surrounding cell types, including photoreceptors and cells in the choriocapillaris vasculature.

COMPLEMENT SYSTEM AND Aβ

Retinal inflammation and the complement immune system have been shown to play an important role in AMD pathogenesis, supported by observations of complement factor H (CFH) mutations that are associated with AMD progression; several groups have found CFH polymorphisms that are linked with AMD. CFH inhibits the alternative pathway of the complement system and mutations in CFH promote chronic inflammation and the accumulation of membrane attack complex (MAC) in the retina. MAC is harmful to cells, causing them to breakdown and die. These processes also cause excessive recruitment of immune cells, macrophages and microglia, into affected retinal tissues. These recruited cells release cytokines and reactive oxygen species that further damage retinal tissue.

Aβ is a candidate trigger of the complement system. Matsubara has so far shown its involvement in retinal inflammation, altered RPE gene expression and changes in cytokine and interferon signalling pathways. It activates the complement immune system and exacerbates the associated MAC accumulation. In collaboration with Dr Patrick McGeer, Matsubara is currently using animal models to develop drugs that suppress cell damage due to this aberrant activation of the complement cascade.

Further to this, through the use of cell cultures and animal models, Matsubara and her group have shown that Aβ activity triggers an increase in the expression of components of the inflammasome and pro-inflammatory chemicals that are thought to exacerbate retinal cell dysfunction. This includes interleukin (IL)-1ß, IL-8, IL-18, caspase-1 and nod-like receptor protein 3 (NLRP3). The exact consequences of this upregulation are currently hypothesised and form a focus of future study. However, intriguing early results suggest that the effects may be context dependent, with different consequences depending on the type and stage of AMD. This illustrates the need for further investigation into Aβ's downstream effects.

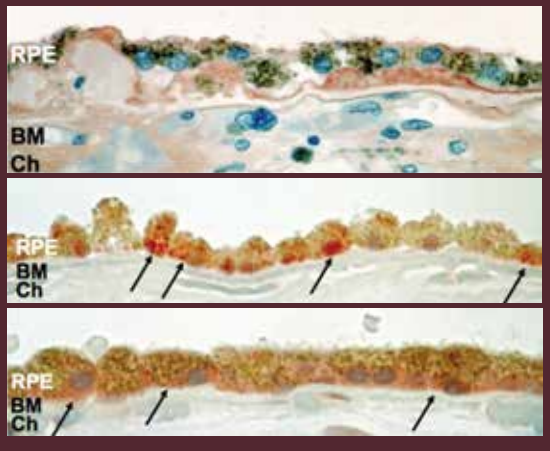
Importantly, this discovery could lead to the development of treatments that suppress the upregulation of these genes and the detrimental effects of drusen might be ameliorated: "In particular, methods to inhibit the NfKb pathways and inflammasome activation look promising in our preliminary studies," elaborates Matsubara.

Moreover, in partnership with retinal specialists at the Eye Care Centre, UBC (Dr Farzin Fooroghian and colleagues), Matsubara has investigated the role of CFH and how it affects, drusen load, choroidal function and systemic cytokines in AMD patients. They found that patients with thinner choroids had a greater occurrence of drusen independent of genotype. Also they found that dry AMD patients with the at-risk variant of the CFH gene (CC) have higher systemic expression of IL6, IL1ß, IL18 and TNFα than patients with the other CFH variants (CT and/or TT), suggesting that systemic cytokine levels may contribute to chronic retinal inflammation and disease progression in dry AMD patients.

COLLABORATIONS AND WIDER SCOPE

Future work is geared towards the study of key cellular pathways and the development of drugs that will interfere with these pathways and thus successfully treat and prevent symptoms in AMD.

Beyond her core AMD research, Matsubara is engaged in a wide range of collaborative studies ranging from the development of novel MEMS-based drug delivery methods (Dr Mu Chiao and colleagues) to the study of the pathogenesis of other retinal diseases such as proliferative vitreoretinopathy (PVR) (Dr Kazlauskas, Schepens Eye Research Institute) and *in vivo* bioimaging of Aβ molecular species in the eye (Dr Sarunic, Simon Fraser University).



These micrograph images are human eye sections through the RPE (RPE), Bruch's Membrane (BM) and choroid (Ch) in the outer retina of postmortem eyes with AMD. Drusen in outer retina (Dr).

The RPE cells shows several unique chemicals (Shown in red- with arrows for example) that indicate abnormal physiology and link to AMD progression.