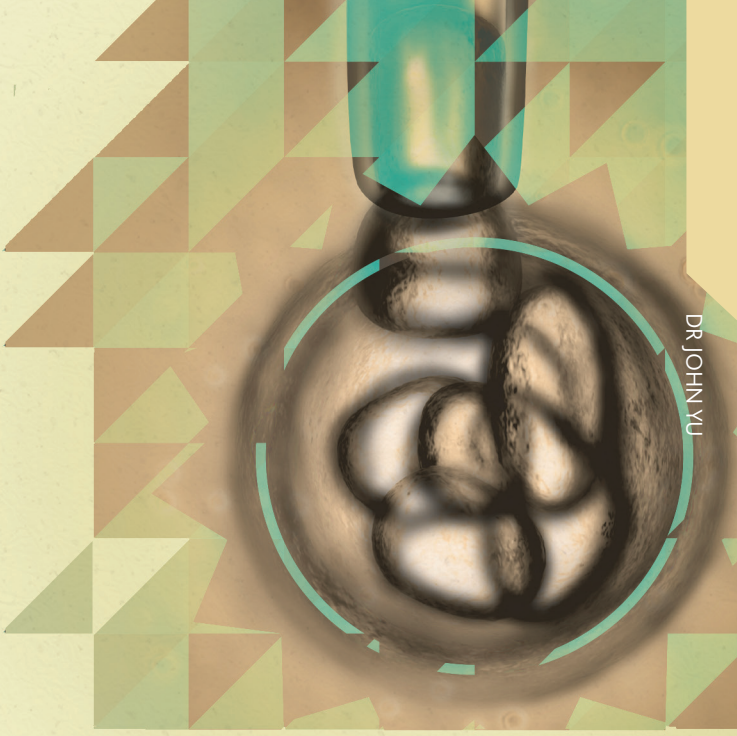
Markers and regulators

Dr John Yu discusses his research on diagnostic tools and therapeutic strategies for cancer



DR JOHN YU

Could you introduce the Institute of Stem Cell and Translational Cancer Research (ISCTCR) and describe your role as Director?

The ISCTCR was established in December 2013 and is located in the Chang Gung Memorial Hospital (CGMH), Linkou. This new Institute is led by myself and Dr Alice Yu, both known for outstanding achievements in stem cell biology and translational cancer research, respectively. The ISCTCR hosts a team of investigators with complementary expertise in a wide range of research areas.

The Institute employs innovative technology platforms such as glycomics, glycoproteomics, phosphoproteomics, high-sensitivity liquid chromatography, mass spectrometry and state-of-the-art tumour immunology to facilitate interdisciplinary and collaborative research for the identification of unique markers and regulators for stem cells and cancer. The ISCTCR aims to develop new diagnostic tools and novel therapeutic strategies for cancer and we hope to contribute to the future development of biomedical research and industry in Taiwan.

What research activities are currently underway at the ISCTCR?

We are investigating the role and downstream regulators of the new markers discovered from human embryonic stem cells (hESCs), breast cancer and cancer stem cells (CSCs). We are also exploring the interrelationship between stem cells and cancers and developing new strategies to target CSCs for cancer diagnosis and therapeutics. Importantly, we are investigating new cancer immunotherapeutic strategies by targeting specific cancer-associated glycans, which function as immune checkpoints.

You are interested in sight-threatening diseases that are caused by progressive neuronal apoptosis, leading to loss of vision. Can you outline the current knowledge regarding this process?

It is known that various retinopathies and age-related ophthalmologic disorders lead to a progressive loss of vision. At present, there is no cure for these age-related eye diseases and blindness. Current developments in the pipeline for new age-related retinopathies are focusing on the use and administration of Avastin and its related derivatives. Both Avastin and the Food and Drug Administration (FDA)-approved Lucentis® (ranibizumab injection), share the same mode of action by inhibiting vascular endothelial growth factor (VEGF). To date, there is no therapeutic agent which can treat such disorders by preventing the loss (death) of the neuronal cells in the eye (ganglion and retinal pigment epithelial cells).

What are the key goals of your research into sight-threatening diseases?

We believe that if we can target and prevent the ultimate death of neuronal cells in the retina, we will be able to retain function in photoreceptors and prevent vision loss. We are planning to use *Puf-A* (or its derivatives) to prevent the retinal degeneration that is associated with various sight-threatening disorders. *Puf-A* prevents apoptosis by blocking the action of caspases, the enzymes involved in cell death pathways. Specifically, we plan to locally inject a FDA-approved adeno-associated virus type 2 (AAV2), encoding *Puf-A* into the sub-retinal or intra-vitreal space in the eye, infecting retinal pigmented epithelial and ganglion cells with high efficiency. We will use this approach to show that *Puf-A* protects retinal cells in retinitis pigmentosa animal models. We will deliver *Puf-A* into the damaged eye and monitor integration, cell survival, and treatment efficacy. This will enable us to determine whether the gene integrates into the correct layers of the retina, makes the corrections and acquires the preventive characteristics of neighbouring cells.

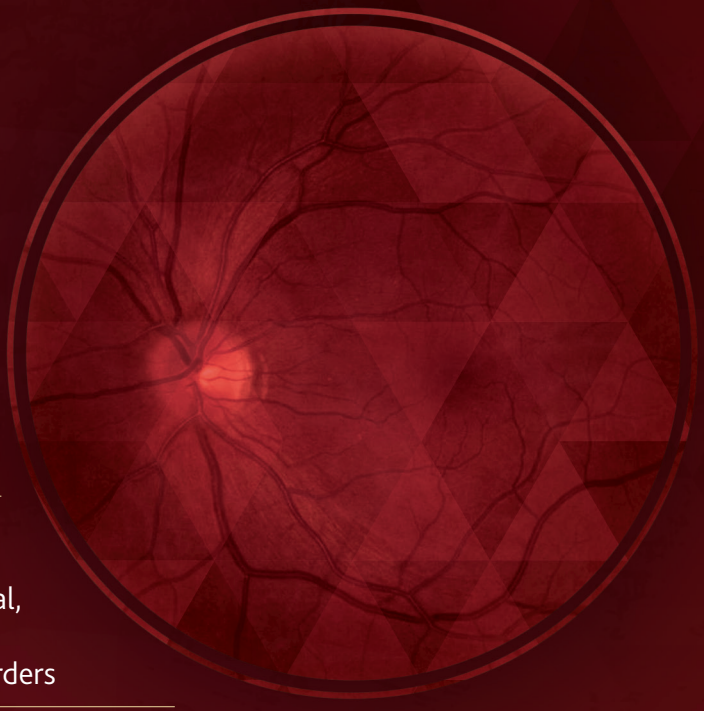
Why are zebrafish an effective model system for the study of human disorders?

Zebrafish have become a favourite vertebrate model for genetic and developmental studies due to their small body size, rapid development, frequent reproductive cycles (one to two weeks), short maturation period (three months), large-scale genetic screening and easy maintenance. In addition, zebrafish mutations are usually faithful phenocopies of many human disorders.

Looking ahead, nearly $4.5 billion of the global $7 billion ophthalmic pharmaceutical market will be subject to US patent expiration over the next few years. Was this a particular stimulus for the project?

Major revenue generators such as Alcon's Patanol, Allergan's Alphagan and Restasis, and the entire Merck Cosopt/Trusopt franchise are likely to face competition. At the same time, the pipelines of the industry leaders contain few, if any, new compounds for major age-related retinopathies in development. This 'perfect storm' of expiring patents, combined with weak pipelines, presents a unique opportunity to develop novel therapies and gain a dominant position in ophthalmology.

Combating **retinopathies**



A team of researchers based at the **Institute of Stem Cell and Translational Cancer Research** in the Chang Gung Memorial Hospital, Taiwan, is working to develop preventive and therapeutic strategies for ophthalmologic disorders

IN A WORLD with an ageing population, a growing number of people are affected by sight-related problems. Many eye diseases are characterised by the gradual decline of retinal ganglion and pigmented epithelial cells, leading to the subsequent loss of photoreceptors in the retina. These sight-threatening diseases include retinitis pigmentosa, glaucoma, age-related macular degeneration (AMD), diabetic retinopathy, and Leber's hereditary optic neuropathy. The ultimate outcome is blindness regardless of the pathogenesis involved.

Unfortunately, there is no cure for retinal neurodegenerative eye diseases. At present, there are more than 1.75 million people in the US with AMD, while an additional 7 million display earlier stages of the disease. By the year 2020, there will be over 79.6 million people in the world with open-angle and angle-closure glaucoma. The overall prevalence of diabetic retinopathy among people with diabetes (approximately 93 million) is 34.6 per cent and there are about 28 million patients with vision-threatening diabetic retinopathy worldwide.

When treating age-related retinopathies the main priority of ophthalmologists is to preserve the patient's eyesight. If the death of retinal cells can be stopped, vision loss can be prevented. Many drugs currently being developed for various age-related retinopathies block retinal angiogenesis, the pathological process through which new blood vessels form from pre-existing vessels. Currently, such an approach of anti-angiogenesis is beneficial for the treatment of patients with 'wet form' AMD, characterised by the growth of abnormal blood vessels. However, the majority of AMD patients (over 85 per cent) have 'dry form' AMD, where excessive angiogenesis does not seem to play a role. This blockage treatment requires at least monthly intraocular injections of drugs, causing discomfort and an excessive burden on the part of patients. Hence, there is an urgent need to pinpoint new drugs and approaches that target back-of-the-eye diseases.

FAR-REACHING FINDINGS

Dr John Yu, Director of the Institute of Stem Cell and Translational Cancer Research (ISCTCR), Chang Gung Memorial Hospital, Taiwan, is leading cutting-edge research on innovative therapeutic and preventive strategies for the protection of the neural retina. With an overarching focus on stem cell biology and regenerative medicine, the Institute hosts projects on the cellular and genomic mechanisms of differentiation and plasticity, the reprogramming of stem cells, the derivation of human embryonic stem cells from novel technology development and somatic cell nucleus replacement. In order to fulfil its research goals, ISCTCR's Stem Cell Programme draws upon international scientific expertise, facilitating dialogue and knowledge transfer between academic institutions and biotechnology companies around the world.

One of Yu's key findings to date is the identification of the novel human gene *Puf-A*. Crucially, this gene displays unique anti-apoptotic activity. As apoptosis is the process whereby programmed cell death occurs in multicellular organisms, Yu and his team were able to demonstrate that over-expression of *Puf-A* in retinal cells could suppress cell death in these cells. This is important because retinal cell death is a central event that leads to retinal neuro-degeneration, vascular dysfunction and eventually irreversible blindness.

Puf-A is primarily expressed in both retinal ganglion and pigmented epithelial cells in zebrafish, mice and humans. Interestingly, using their zebrafish model Yu and his team detected apoptotic cells in significant numbers in zebrafish eyes after the knockdown of *Puf-A*, strongly implying the gene has a significant role in eye development. The researchers are confident establishing approaches that prevent apoptosis in retinal diseases will lead to the development of innovative drugs which, unlike existing drugs, will attack other parts of the disease process.

EXPERIMENTAL ADVANCES

As the onset of many sight-threatening diseases is caused by progressive neuronal apoptosis, Yu and his team believe intraocular delivery of the *Puf-A* gene or its derivatives could prove to be excellent future therapies for neurodegenerative diseases. To explore this exciting possibility, the ISCTCR researchers conducted *in vitro* experiments whereby staurosporine – an alkaloid isolated from the bacterium *Streptomyces staurosporeus* – was used to induce cell apoptosis. Promisingly, the findings showed that the constitutive overexpression of *Puf-A* could reduce staurosporine-induced cell death.

These initial experiments have also been conducted on two animal models using lentiviral constructs containing *Puf-A in vivo*. For example, using the rat model developed by Dr JT Chen at Tri-Service General Hospital, Taiwan, the researchers found that the overexpression

THE INVOLVEMENT OF A NOVEL *PUF-A* GENE PREDICTED FROM EVOLUTIONARY ANALYSIS IN THE DEVELOPMENT OF EYES AND PRIMORDIAL GERM-CELLS

OBJECTIVES

To develop gene-based therapies for retinal diseases.

KEY COLLABORATORS

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DR JOHN YU (MD, PHD) is Distinguished Chair Professor and Director, Institute of Stem Cell and Translational Cancer Research (ISCTCR), Chang Gung Memorial Hospital, LinKou, Taiwan. He is also Distinguished Visiting Research Fellow in Academia Sinica, Taiwan, and was the Director for the Institute of Cellular & Organismic Biology, Academia Sinica (2002-09). Yu is also the founding President for the Taiwan Society for Stem Cell Research. He has been elected to serve in ISCTCR's various committees and task force and is the advisor for Stem Cell Biology at Kumamoto University, Japan. Yu was also the Director of Experimental Haematology (1998-2002) at Scripps Research Institute, La Jolla, USA. He has received an Established Investigatorship Award from the American Heart Association, and several special awards from universities in Taiwan.

The Institute of Stem Cell and Translational Cancer Research's Stem Cell Programme draws upon international scientific expertise, facilitating dialogue and knowledge transfer between academic institutions and biotechnology companies around the world

of *Puf-A* provided neuroprotection against *N*-methyl-D-aspartate (NMDA)-induced cell apoptosis in the retinas of rats. Furthermore, Dr SP Huang from the Tzu Chi General Hospital, Taiwan, utilised another animal model to validate Yu's *in vivo* data using lentivirus: "It was found that intra-vitreal injection of lentivirus expressing rat *Puf-A* in the optic nerve crush model could significantly protect the loss of retinal ganglion cells (RGCs)," explains Yu. "These results indicate that overexpression of *Puf-A* is neuroprotective in the rat model of optic nerve crush, as demonstrated both structurally by RGC density and functionally by flash visual-evoked potentials (FVEP)."

GENERATING GENE THERAPIES

Building on their results, Yu and his team are developing gene-based therapies for retinal diseases. One key gene therapy they are researching involves using adeno-associated virus (AAV), a small virus that infects humans and some animals and is an attractive option as it is not known to cause disease and only induces a very mild immune response. Gene-therapy vectors using AAV can infect dividing and quiescent cells. These viruses can also exist in an extrachromosomal state without integrating into the genome of the host cell.

Several studies have been conducted to test the safety of AAV-targeted gene transfer in mice, primates and, more recently, in humans. The evidence shows that the expression of transgene in AAV intraocular gene therapy can last for many years in animal models, and no retinal toxicity has been found after the viral transgene expression: "These studies were primarily to test the safety and efficacy of viral gene therapy and the trials have already demonstrated acceptable immunologic changes with no serious adverse effects and also evidence of efficacy," Yu discloses. "Recently, it has become a feasible approach for the treatment of inherited retinal degeneration in human patients." In addition, the prolonged expression of transgene in AAV gene therapy may reduce the frequency of intraocular administration of such drugs to patients.

FUTURE DIRECTIONS

The overarching goal of Yu's current research on retinal diseases is to create leading-edge technology for new strategies that prevent blindness. This will be achieved through a more robust understanding of how *Puf-A* and its derivatives induce anti-apoptotic activities and how they might serve as targets for establishing new treatments that prevent blindness. With age-related retinopathies becoming increasingly prevalent, the research at Yu's Institute is vital and is a harbinger of hope for those who suffer from retinal diseases.

For now, the researchers are concentrating on the scientific assessment of the mechanisms of *Puf-A* activities and the product evaluation of AAV-based treatment. They are also aiming to advance 'proof of concept' in animal models and make advances towards translating their research into clinical practice. Looking to the future, the researchers believe that there is enormous market potential for developing new drug targets for retinopathies. They are confident that biotechnology companies will show interest in their research, and get involved in subsequent clinical trials and Investigational New Drug application.