Firstly, could you outline the principal objective of your work?

Angiogenesis is regarded as a major cause of ocular fibrotic scarring, with severe pathological implications leading to irreversible blindness. Our objective was to identify novel molecular targets in the cornea by focusing on pathological induction of angiogenesis and fibrosis.

How did you become interested in this subject?

While investigating corneal injury repair with Elizabeth Fini in Boston, cancer researcher Marsha Moses invited me to learn a new corneal micropocket assay that was developed in the lab of the late Dr Judah Folkman at Harvard. This research has facilitated the discovery of many novel tumour-derived angiogenic molecules and illustrated the activity of angiogenic inhibitors in a defined system.

Inspired by Folkman’s innovation, I was captivated by angiogenesis as a field because it integrated scientists from diverse disciplines. Serendipity took me to the lab of Craig Crews at Yale, who had trained with Stuart Schreiber, the brilliant chemical biologist who pioneered the use of natural products as chemical probes to study protein function.

Could you discuss the new hypothesis that you tested?

Folkman had shown that disparate human diseases shared the common underlying pathological process of excessive blood vessel growth and were also responsive to anti-angiogenic therapies. I deliberated on this while becoming fascinated with ancient medical knowledge in traditional Chinese medicine and Ayurveda, where I found lurking evidence that certain human diseases had been described as having a major angiogenesis mechanism. It seemed logical that a systematic scientific process grouping common uses of medicinal plants might reflect similar ancient medical understanding of angiogenic principles. My explorations of the ayurvedic medicinal plant Withania somnifera, using the most modern techniques, provided proof of this concept.

What did you find regarding the potential of Withania somnifera to inhibit angiogenesis?

At the biotech company EntreMed, Inc, I led a team investigating links between angiogenic and inflammatory pathways, especially the key inflammatory transcription factor nuclear factor-kappa B (NF-κB). In that study, we found that very low doses of the Withania somnifera extract containing withaferin A maintained anti-angiogenic activity in several assays and that NF-κB inhibition occurred only at higher concentrations of the extract. This was confirmed using purified withaferin A, which demonstrated potent activity by inducing endothelial cell growth arrest. Our findings supported Folkman’s concept that anti-angiogenic therapy targeting the tumour vasculature, not the tumour cell, obviates the need for high doses to be effective.

What strategy did you adopt to identify the molecular targets of corneal antiangiogenesis?

My postdoc, Yasuno Yokota, together with Kyung Bo Kim, a former colleague from Yale, helped us synthesise a chemical probe by coupling withaferin A to an affinity reagent. When we applied the probe in vascular endothelial cells and isolated soluble tetrameric vimentin as the intracellular binding target of withaferin A, it was a moment of true excitement, because until that time intermediate filaments were considered undruggable targets. With the withaferin A affinity probe, we then demonstrated that soluble tetrameric vimentin overexpression in corneal fibroblasts undergoing transition into myofibroblasts were also targeted by withaferin A. This led us to reveal withaferin A’s potent anti-fibrotic activity in subsequent mouse studies.

What is particularly significant about the project?

The most significant aspect of discovering vimentin as the target of withaferin A was the illumination of the ligand binding site: this protein domain has been preserved from diverse species over almost 400 million years. Equally amazing was our revelation that the other three intermediate filament family members – desmin, glial fibrillary acidic protein (GFAP) and peripherin – also share this ligand binding site (determined in collaboration with Adel Hamza and Chang-Guo Zhan). We showed that withaferin A bound to filament proteins in their soluble tetrameric forms. When withaferin A was delivered to mice in our alkali injury model, we found co-ordinate reduction in the expression of vimentin and desmin in the injured fibrotic cornea, and co-targeting of vimentin and GFAP expression in retinal Müller glia. Since desmin and GFAP, respectively, are also known to be overexpressed in fibrotic conditions that affect the heart/muscle and nervous system tissues, we believe the significance of our findings goes well beyond angiogenesis or diseases of the eye.

How did your team discover that vimentin deficiency changes the fibrotic response to corneal alkali injury?

We compared the response of normal animals with vimentin-deficient mice after corneal injury and started noticing recovery of corneal clarity in vimentin-deficient mice at later stages post injury.

Much to our excitement, we found that withaferin A delivery in the corneal injury model produced not only an anti-fibrotic effect similar to that of genetic deficiency of vimentin but also recovery of protein markers that revealed restoration of corneal homeostasis.

Serendipitous drug discovery

In search of a treatment for corneal blindness, Professor Royce Mohan and collaborators have unravelled the intricate workings of a potent plant extract used in ancient medicine for its restorative properties.
PLANT CONSTITUENTS IN remedies used by ancient societies and indigenous tribes in their traditional healing practices are progressively being validated as delivering beneficial outcomes. For example, Australian lemon grass has recently been found to have analgesic effects, much like aspirin, and has been used for centuries by aboriginal people for pain relief. The active ingredient in aspirin, acetylsalicylic acid, is derived from the salicin compound found in willow bark, which was known by the ancient Greeks to ease pain: it took more than 2,000 years before salicin and its properties were scientifically validated. Transforming the active ingredient into a form acceptable for consumption took many more years.

AYURVEDIC PLANTS

The Solanaceae are plant species in the nightshade family, and have long been associated with toxic effects. They have also for several centuries been used in magic and healing rituals by many cultures. The family includes belladonna, mandrake, tobacco, henbane, datura and also everyday food plants such as tomatoes, potatoes, aubergines, physalis and capsicum peppers. The leaves, fruits and seeds of a member of the family, Withania somnifera, also known as Ashwagandha or Indian ginseng, have been widely used as a tonic in India for more than 2,000 years and today it is farmed as a medicinal crop. Withania somnifera caught the attention of Professor Royce Mohan, the John A and Florence Mattern Solomon Endowed Chair in Vision Biology and Eye Diseases at the University of Connecticut Health Center, some years ago while he was exploring angiogenesis in cancer and in his spare time, ancient medicinal remedies: “Withania somnifera is a well-described medicinal plant in Ayurveda, known for its ancient medicinal use in the treatment of conditions such as endometriosis and arthritis,” he explains. “I found that many closely-related members of the Solanaceae family, such as Physalis philadelphica – tomatillo – have been incorporated into plant-based medicines by tribal cultures in South and Central America for ailments that were similarly angiogenic-driven within an inflammatory context.”

TARGETING CORNEAL BLINDNESS

After cataracts, corneal blindness is the second leading cause of blindness in the world and it is highly prevalent in developing countries. The condition is brought about indirectly by infections and diseases that affect the cornea, as angiogenesis, the natural fibrotic scarring and revascularisation process employed by the body to heal ocular trauma, effectively render the cornea opaque. Corneal blindness is currently irreversible.

Seeking to unravel the mechanisms that govern tissue repair after eye injury, Mohan and his senior postdoc, Paola Bargagna-Mohan, while at the University of Kentucky, sought new molecular targets to inhibit angiogenic damage to the cornea. They used an unconventional approach to this challenge. Mohan had deduced that the beneficial results demonstrated by Ayurvedic remedies containing parts of the Withania...
MOLECULAR TARGETS OF CORNEAL ANTIANGIOGENESIS

OBJECTIVES

To exploit angiogenic and nonangiogenic models of wound healing to define the ubiquitin proteasome pathway (UPP)-driven healing mechanism(s) of the cornea and validate newly identified molecular targets for anti-angiogenesis. For these investigations, withaferin A will serve both as a pharmacological agent and a cell permeable probe of its binding-protein target’s function.

Specifically, the study will: 1) characterise the drug’s inhibitory mode of action on targeting the angiogenic activation of the UPP, 2) investigate key components of the UPP as mediators of the drugs activity, and 3) investigate withaferin-protein target-deficient mouse models to validate the requirement for drug-protein interaction in the corneal anti-angiogenic mechanism of withaferin.

KEY COLLABORATORS

Paola Bargagna-Mohan, University of Connecticut Health Center

Kyung Bo Kim, University of Kentucky

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Ayurveda-inspired drug target discovery in vascular and nervous systems.

Molecular model of withaferin A bound to tetrameric vimentin.

healing without opacity: “It was surprising to us that there were such close parallels between genetic absence of vimentin and its pharmacological downregulation by withaferin A,” muses Mohan.

OPENING UP NEW OPPORTUNITIES

Mohan has recently obtained a patent for the use of therapeutic withanolide compounds in inhibiting fibrosis and identifying molecular targets for anti-fibrotic drug development. Identifying a possible new therapeutic from an ancient knowledge base is naturally just the first step – refining its means of administration and ensuring that its effects on the eye are solely beneficial will require further effort in requisite drug development steps and clinical trials. Mohan is excited that the outcomes are positive and is cautiously optimistic by the prospect: “I believe the impact could be huge,” he underlines. “A treatment for corneal blindness would fulfill the unmet medical need of millions of people in impoverished nations.” In the short term, the project will therefore continue to investigate the safety of withaferin A and seek to improve the means of its delivery to the eye, and Mohan is equally excited about other recently submitted applications for associated patents that will expand the possible applications of withaferin A, by targeting intermediate filaments other than vimentin.

Meanwhile, Mohan has also become interested in a condition known as trachomatous triachiasis, where infection by the Chlamydia trachomatis bacterium results in in-turned eyelashes which scrape against the cornea, causing painful fibrosis which then can result in permanent blindness. The bacterium is endemic in many impoverished African, Indian, South East Asian and South American countries, where the standards of sanitation and living conditions are inadequate. Triachiasis is currently treated via surgery, an option which is often limited in these poorer countries. Withaferin A may have the potential to become a treatment option for triachiasis but it will require the engagement of other research groups. The researchers are keen to explore this area of study and are hopeful an opportunity for collaboration will soon present itself.