Clinical Dermatology

UCD CHARLES INSTITUTE SEMINAR SERIES

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The cancer connection in epidermolysis bullosa

*Attendees at UCD's Charles Institute Seminar Series recently heard a presentation by **Dr Andrew South** of Thomas Jefferson University in the US on understanding the molecular basis of fibrosis and aggressive cancer*

The Charles Institute, Ireland's national dermatology research and education centre, hosts a range of guest speakers who cover a variety of topics ranging from skin cancer to psoriasis, among others. The series, which is sponsored by RELIFE (part of the A.Menarini group), is designed to provide expert advice from a range of distinguished national and international experts in their respective fields and is chaired by Prof Desmond Tobin, Full Professor of Dermatological Science at UCD School of Medicine and Director of the Charles Institute of Dermatology. The seminars are broadcast to attendees with a special interest in dermatology and cutaneous science in other locations, who access the talks remotely via an audio-visual link.

Attendees heard a presentation from Dr Andrew South, Associate Professor at the Department of Dermatology and Cutaneous Biology at Thomas Jefferson University, Philadelphia, US, and author of more than 100 peer-reviewed articles on the subjects of genetic skin disease and squamous cell carcinoma (SCC).

Dr South explained that recessive dystrophic epidermolysis bullosa (RDEB) is a devastating skin-blistering disease that is caused by mutations in the gene encoding type VII collagen, and is characterised by epidermal fragility, trauma-induced blistering, and long-term wounds that are difficult to heal. Fibrosis, he explained, develops rapidly in cases of RDEB skin and contributes to both chronic wounds, which develop following cycles of repetitive wound and scar formation, and SCC. This is the single most common cause of death in this patient cohort, he added.

Model

The molecular pathways that are disrupted in a broad spectrum of fibrotic disease are also disrupted in RDEB, and SCCs arising in RDEB are to date molecularly indistinct from other subtypes of sporadic SCC. This makes RDEB a model for gaining a better understanding of the molecular basis of both fibrosis and aggressive, rapidly-developing cancer.

"One of the reasons we are interested in RDEB is because cutaneous squamous cell carcinoma (CSCC) is the biggest cause of mortality in this patient group," said Dr South. "The important thing to note is that mortality tracks incidence, leading to five-year survival of less than 5 per cent — in fact generally, it's pretty much zero." Dr South presented an overview of published studies and told the seminar that SCC arises in a variety of tissues that form a barrier between a person and the outside world, and are characterised by varying five-year survival rates.

"In sporadic SCC, the five-year survival rate among the general population is pretty good," said Dr South. "However, RDEB is quite aggressive and early-onset and tends to arise on bony prominences. Patients get multiple primary SCCs and there tends to be a focus on the hands, knees and feet in terms of where the tumours arise," said Dr South, who also presented a number of case studies to the attendees. "That got us to asking why that is the case, and it is particularly interesting, given that epidermolysis bullosa (EB) is a skin fragility disease of varying severities but really, it is only mutations in collagen 7 (C7) in dystrophic EB that lead to this huge incidence of SCC. There are data to suggest that SCC can arise in other forms of EB, but the prevalence is not anywhere near that of RDEB, so we are really interested in understanding why this is."

Some key points, he explained, include that EB tumours only arise in the skin and that heterozygous carriers, or patients with dominant dystrophic EB, do not have an increased risk of SCC, so it is not "a classical tumour suppressor," he said. Type 7 collagen is the largest gene in the collagen family and is expressed by both keratinocytes and fibroblasts, and forms supramolecular structures called anchoring fibrils, which originate and terminate in the basement membrane, he explained. This basement membrane is the interface between the keratinocytes and the anchoring fibrils.

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Signalling role

"For the longest time, people have thought of C7 as providing just that structural anchoring between the epidermal and the dermal layer, but work over the past 10 years has identified a signalling role for C7," said Dr South, adding that tumour-cell genetics do not explain either the early onset or aggressive nature of RDEB SCC and in fact, tumour-cell genetics mirror sporadic SCC. "We use this as an argument that RDEB is a very important model of inevitable and aggressive tumours," he said.

Dr Andrew South

Dr South outlined published studies by him and his colleagues which showed that there was no difference between EB keratinocytes and non-EB keratinocytes, however there was a major difference in tumour and normal states. His research also identified therapeutic targets, including polo-like kinase 1 inhibitors.

In terms of how this research could affect patient outcomes, Dr South described "hitting the jackpot" when studying fibroblasts: "We identified huge differences between normal fibroblasts and EB-associated SCC fibroblasts — ie, those were primary cells in culture isolated from tumours. The normal fibroblasts in this assay were very distinct, and interestingly, the EB fibroblasts in normal skin from patients who hadn't been diagnosed with cancer were indistinguishable from the UV-induced SCC fibroblasts, suggesting that there was something pre-cancerous about an EB or RDEB fibroblast," he explained. TGF-beta signaling was also greatly increased, he added.

Dressings

Because tumours arise in wounds, he also outlined another study, which examined whether 'used' wound-dressings can be studied genetically to identify pre-cancerous keratinocytes. "We wondered, if you are taking off these dressings, are you removing keratinocytes and other material at the same time?" said Dr South. "We isolated a lot of debris and cells, and in some instances, we thought we could see squamous keratinocytes in these preparations, but unfortunately, we were unable to get any keratinocytes to adhere and proliferate and culture."

However, he and his colleagues were able to identify a lot of non-adherent cells from the dressings, and that prompted further study of these cells. "Essentially, we were able to isolate up to 100 million cells from a wound dressing and certain characteristics of the processing and the size of the wound influenced the number of cells, as well as how long the dressing had been on the wound. But that also meant that it took longer to process them, which means that the viability tails-off," said Dr South.

"We think this could be a method to potentially profile wounds and perhaps identify some differences between an acute and a chronic wound… we have a longitudinal study going on that was disrupted by Covid, but even now we have some pretty interesting data showing that different types of wounds are associated with different percentages of certain immune cells. We are looking forward to resuming that study and following patients for long periods of time."

He summarised by telling the seminar that RDEB SCC is characterised by APOBEC-driven somatic mutation and activation of TGF-beta in the tumour microenvironment.

Dr South told the attendees: "We are looking at what is actually the driver of this TGF beta phenotype within the microenvironment and identifying whether perhaps C7 plays a role in correctly sorting and secreting large proteins. We have some data to suggest that other large proteins are involved… this leads to inherent organelle stress within the fibroblasts, which ultimately could be tumour-promoting. We have a lot of work to do to find out the exact mechanisms, but we have made a start in identifying C7 as a potential scaffold," he said.

Wounded skin

During an interactive Q&A session, Prof Tobin touched on the content of the presentation that addressed the clinical experience, and the unusual nature of so many primary SCCs appearing on the same patient. "These all seem to be associated with bony areas on the body where there is mechanical stress," said Prof Tobin. "Is it true to say then that SCCs can only emerge in RDEB skin if there is a prior blistering event, or can there be a kind of de novo breakout of SCC in so-called intact skin? I note that you sometimes use so-called 'normal' RDEB cells in some of your experiments, but is the clinical observation of the tumour always depending on a previous blistering event?"

Dr South replied: "That's a very good question — in those body maps [used in research] with the focus of the tumours on the hands, knees or shins, for example, the first study to report that sort of data came from Prof John McGrath at St John's Institute in London, and the same type of thing was seen," he said. "In the histogram I showed, there was a patient as young as seven years who was an RDEB patient diagnosed with SCC. That's very young, so I hesitate to say that an SCC cannot arise in intact skin, but yes, the vast majority arise in wounded, damaged, scarred skin."

RELIFE has had no input into the content of this article or series of seminars