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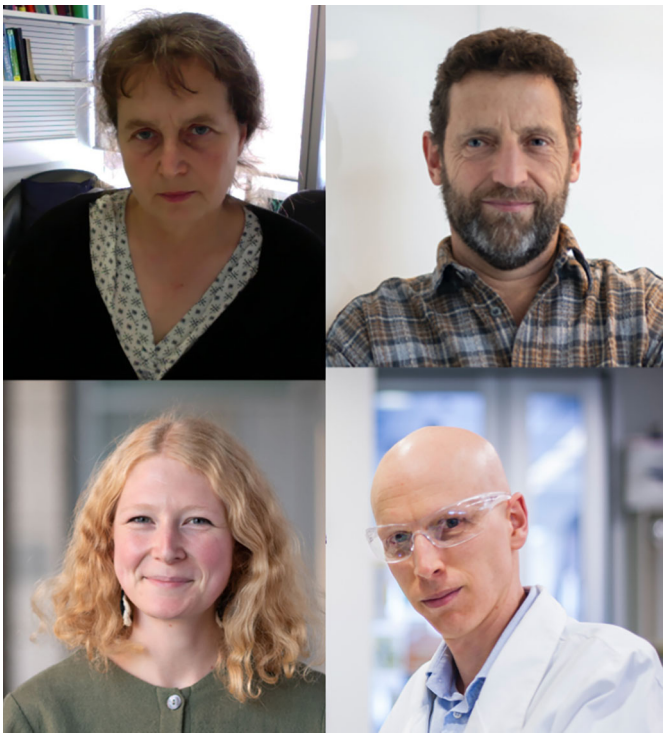
INTERVIEW

The people behind the papers – Zoe Grant, Tim Thomas, Anne Voss and Leigh Coultas

The histone acetyltransferase HBO1 (KAT1) is required for histone H3 lysine 14 acetylation, which is crucial for embryonic development. A new paper in Development reveals that, in the vascular system, HBO1 is required in endothelial cells for sprouting angiogenesis regulation. To hear more about the story, we caught up with first author Zoe Grant and senior authors Professor Anne Voss, Associate Professor Tim Thomas and Leigh Coultas, Business Development Manager, from the Walter and Eliza Hall Institute of Medical Research (WEHI), Australia.



Anne Voss (top left), Tim Thomas (top right), Zoe Grant (bottom left) and Leigh Coultas (bottom right).

Anne and Tim, what is your scientific background and what does your lab currently work on?

AV & TT: We started out as developmental biologists. In the early 1990s, at the Max Planck Institute for Biophysical Chemistry in Göttingen in the group of Professor Peter Gruss, we conducted a gene-trap screen for proteins that are important in embryonic development, in particular proteins regulating the balance between proliferation and differentiation. One of our hits, QKF (querkopf), had sequence similarity to the recently identified histone acetyltransferase called MOZ (monocytic leukaemia zinc finger protein). MOZ was found to be the subject of recurrent chromosomal translocations causing a particularly aggressive form of acute myeloid leukaemia. We showed that QKF had histone acetyltransferase activity, was strongly expressed in the developing cerebral cortex and was essential for brain development. This sparked our interest in proteins similar to QKF and MOZ: histone acetyltransferase binding to the origin of replication complex protein 1 (HBO1), males absent on the first (MOF) and HIV-1 Tat interactive protein, 60 kDa (TIP60). As this MYST family of proteins clearly have important roles in normal development, particularly stem cells, we decided to study them in depth. We generated conventional and conditional mutant mouse strains for all five family members, which allowed us to study their function in mouse embryonic development. TIP60, MOZ, QKF, HBO1 and MOF have since been renamed twice, and are now called lysine acetyltransferases 5, 6a, 6b, 7 and 8 (KAT5, KAT6A, KAT6B, KAT7 and KAT8), respectively.

Zoe, how did you come to work in Leigh's lab and what drives your research today?

ZG: I began working in Leigh's lab for my one-year Honours research year after my Bachelor of Biomedicine degree. The lab studied regulation of endothelial cell apoptosis in the context of developing angiogenic blood vessels with the goal of understanding how this might be disrupted in disease contexts, such as cancer or ischaemic disease. I was initially drawn to the lab because I loved developmental biology and I loved the idea of understanding how developmental processes are co-opted in disease. I also loved mitochondrial biology and apoptosis. I ended up working on this project about the histone acetyltransferase HBO1 and its role in endothelial fate specification. At first, I was a little unsure about the project – I hadn't enjoyed genetics and epigenetics during my undergraduate degree, but very quickly I fell in love with epigenetics, chromatin and thinking about it in the context of dynamic, angiogenic vessel networks. I decided to stay in the lab for my PhD and continue working on this project. Eventually, I also ended up working on a separate project about apoptosis, but it was the HBO1 project that completely shaped and inspired my science career direction. When looking for a postdoc lab, I wanted to go somewhere I could dive deeper into the mechanisms that regulate chromatin organisation to control gene expression and I hope to continue working in this area after my postdoc.

Before your study, what was known about HBO1?

LC, AV, ZG & TT: Our earlier work showed that HBO1 was required for acetylation specifically of histone H3 on lysine 14 (H3K14ac) and the activation of important developmental

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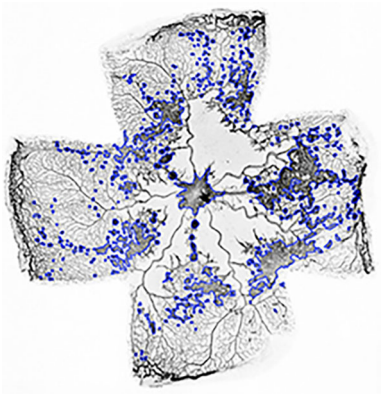

Collagen IV staining of P17 control retinas. Neovascular lesions are outlined in blue.

patterning genes, such as *Tbx1*, *Gata4* and *Nkx2-5* in the developing cardiovascular system and *Otx2* in the developing forebrain. In cultured human and mouse cells, we observed that HBO1 was not essential for cell division. We found that lack of HBO1 during mouse embryogenesis caused developmental arrest at embryonic day 8.5, a failure of vascular maturation, failed allantois outgrowth and failure of chorio-allantois fusion. This sparked an interest in examining the role of HBO1 in blood vessel development further and led to the collaboration with Leigh, an expert in blood vessel development, and the co-supervision of Zoe on this project.

Can you give us the key results of the paper in a paragraph?

LC, AV, ZG & TT: We studied the role of HBO1 in specifying the identities of endothelial cells involved in angiogenic vessel growth. We generated mice in which *Hbo1* was specifically deleted from the endothelium. We found that this led to reduced vessel outgrowth and vessel complexity during both developmental angiogenesis and in a model of pathological vessel growth. Using single-cell RNA sequencing (scRNA-seq) and immunofluorescence staining, we found that the cell type most affected by HBO1 loss was the migratory endothelial tip cells. Although they were more abundant in *Hbo1*-deleted retinas, their ability to undergo the directed migration needed for vessel network expansion was impaired. HBO1 is necessary for acetylation of H3K14 and we found that H3K14ac was widely distributed across the endothelial genome, but enriched at genes that are highly expressed during angiogenesis.

How do you think HBO1 regulates tip cell behaviour during angiogenesis?

LC, AV, ZG & TT: Fundamentally, we think HBO is necessary for organising chromatin in a way that allows the activation of gene expression programmes necessary for efficient sprouting behaviour by endothelial cells. In the absence of HBO1, we found an increased number of tip cells at the sprouting front. Tip cell specification is a dynamic process and HBO1 may prevent endothelial cells from adopting this identity and/or promote their exit from it. We also found that tip cells lacking HBO1 did not migrate normally. This may have been a consequence of the overabundance of tip cells interfering with the collective cell behaviour needed for directed migration and efficient sprouting angiogenesis, but we also found evidence that tip cells need HBO1 to express genes necessary for migratory behaviour.

When you were carrying out the research, did you have any particular result or 'eureka' moment that has stuck with you?

ZG: The moment where things really came together was towards the end of this work. I had been working with the *Hbo1*-deleted retinas for years and always assumed that the reduced angiogenesis phenotype was due to fewer tip cells. It wasn't until I was going over the scRNA-seq data analysed by Dr Peter Hickey that I saw that, in fact, the number of tip cells was increased in the absence of HBO1 and it totally shifted the way I thought about the phenotype. I then analysed the number of cells expressing ESM1, a marker of tip cell identity. This confirmed that there were more tip cells in *Hbo1*-deleted retinas and that an overabundance of this cell type, together with their migration defect, could also delay angiogenesis.

And what about the flipside: any particular challenges or moments of frustration?

ZG: There were certainly many challenges with this project! Working with a rare cell population like postnatal retinal endothelial cells was the major limiting factor and over the years I tried multiple ways of isolating endothelial-specific RNA for sequencing. The advancements in scRNA-seq were timely for this project and helped me finally overcome the challenge of obtaining transcriptome data from these cells. This project taught me so much about patience and perseverance in the lab!

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What next for you after this paper?

ZG: I moved from Melbourne to San Francisco in October 2020 (in the middle of the pandemic!) to start my postdoc with Dr Benoit Bruneau at the Gladstone Institute. My project here focuses on the regulation of gene expression in the developing heart. Someday, I would love to return to Australia to start my own lab studying gene regulation in cardiovascular development and disease.

What is next for the Voss and Thomas groups?

AV & TT: Having investigated the roles of the MYST family histone acetyltransferases in development, as well as identifying and confirming their histone lysine acetylation targets *in vivo*, we have now become interested in their roles in disease.

Our interest in the role of the MYST family of chromatin regulators in cancer is a natural progression, because genes regulating key developmental processes are frequently mutated in cancer. Based on the observation that both *KAT6A* and *KAT6B* are the target of recurrent chromosomal translocations causing acute myeloid leukaemia, we have investigated the roles of MOZ (KAT6A) and QKF (KAT6B) in haematopoiesis and haematological cancers. The roles of KAT6A and KAT6B in the context of cancer appears to be facilitatory. We showed that KAT6A is required for cells to avoid cellular senescence and explored whether it would be a suitable target for drug development. We showed that inhibiting KAT6A arrested lymphoma growth *in vivo*. This has led to a collaboration with a large pharmaceutical company, and MYST inhibitors are currently in Phase I clinical trials for the treatment of cancer. In additional work, we have also assessed the role of HBO1 in haematopoiesis and as a target for drug development. We found, in collaboration with others, that inhibition of HBO1 arrests the growth of human acute myeloid leukaemia cells.

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A second focus is developmental disorders that arise from heterozygous loss of *KAT6A* or *KAT6B*: Arboleda–Tham syndrome (ARTHS) and Say–Barber–Biesecker–Young–Simpson (SBBYS) or genitopatellar syndrome, respectively. We are currently exploring whether our heterozygous loss-of-function mice and transgenic gain-of-function mice can be utilised to model these disorders.

Finally, let's move outside the lab – what do you like to do in your spare time?

LC: I enjoy gardening, making chocolate creations and bike rides with my son.

AV & TT: We enjoy spending time with our son Jan and dog Fletcher in the Victorian Alps hiking, as well as fishing and sailing in Victorian coastal waters. We are both also engaged in wildlife management and conservation.

ZG: I love quiet activities like reading fiction and poetry, and cooking new recipes, but also love seeing live music and having a beer with friends. I'm currently exploring San Francisco one brewery at a time! I also love to get outside for hiking or backpacking when I can. I'm having a wonderful time exploring some of California's mountains and forests!

Reference

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