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Researcher Spotlight

Harnessing spatial biology to uncover the cellular landscape of colorectal cancer

Taking a multi-omics approach towards cancer research can reveal a wealth of valuable information regarding the unique behavior, expression, and morphology of distinct cell populations during carcinogenesis and disease progression. The ability to spatially resolve cells and their transcriptome using tools like *in situ* hybridization (ISH), multiplex immunohistochemistry (IHC), and *in situ* sequencing (ISS), has brought great value to cancer research; enabling researchers to characterize the intricate genomic and architectural landscape of tumors with varying heterogeneity. One such researcher conducting this pioneering work is Dr. Eoghan Mulholland (Junior Research Fellow, Wellcome Centre for Human Genetics, Somerville College, University of Oxford), whose exciting research into the spatial biology of colorectal cancer helped his team to secure ACD's European Spatial Biology Research Grant. Here, Dr. Mullholland discusses his research, and how his project has been bolstered by RNAscope™ ISH technology.

Can you tell us about the main focus of your research?

My current research is focused on stem cell dynamics in colorectal cancer and trying to understand how the epithelium and cancer cells, communicate with immune cells and stromal cells, and vice versa. It's an interrogation of cell compartments, how they communicate and—if a cancer expresses certain mutations—how that conversation changes. I am very interested in a range of different stem cell types, mainly regenerative and classic Lgr5+ cells, and the factors influencing their maintenance and evolution.

What has your preliminary research shown?

We have evidence that the YAP/TAZ signaling pathways converge to push epithelial cells down a stem cell lineage, and we are currently exploring what is driving/resulting from this phenomenon. My current project centers around matching scRNAseq with multiplex IHC images from samples of the same genotype, and then using RNAscope ISH technology to highlight the expression of YAP/TAZ signaling markers and unpack what cell phenotypes are driving stemness via YAP/TAZ.

Can you tell us more about the YAP/TAZ signaling pathways, and how this is pertinent to your research?

As I mentioned previously, we've been focused upon cancer stem cells and their plasticity. Usually in colorectal cancer, you have a standard healthy stem cell which is marked by Lgr5 and spatially, these are always in the very bottom of crypt-like structures, which you find in these types of tissues. As the cancer starts to evolve and proliferate, we see expansion of these stem cells, and they don't stay Lgr5+ – they change. We've termed these regenerative stem cells – they don't respond to treatments in the same way, and we've found that various environmental factors can influence this shift in stem cell phenotype.

One of those things was the extracellular matrix (ECM) and what type of ECM those stem cells are being exposed to, how stiff that matrix is, and how much it's causing cellular tension. We find that one of the markers of this type of tension and stiffness is the YAP/TAZ pathway, which is a major pathway that trickles into a lot of different ones. Its main function seems to be regeneration. It comes up a lot in tissue healing, and is highly expressed in colon

tissues which have colitis. We have highlighted it as one of the main pathways causing the shift in stem cell phenotype to regenerative.

How does RNAscope technology fit into your research?

In our mission to understand and phenotype different immune, stromal, and epithelial cells, it's key for us to determine the transcriptome of the different cell phenotypes. Additionally, we need to spatially understand where in the tumors, or tissue, those different cells are located. Currently in our lab, we use a host of different techniques: multiplex fluorescent imaging of proteins to identify different cell types, large-scale RNAseq from tumors, plus single cell RNAseq. That's just one side of the story though – we know that RNA is in the tumor, but where is it? With the ability to spatially resolve this with RNAscope, we can unpack what each cell is doing and why it needs to express those certain RNAs.

A lot of the recent work in our lab has centered around phenotyping different types of epithelial, stem and cancer cells and now we are able to use RNAscope fluorescent multiplex assays to look at two distinct RNA targets at once – we wouldn't have been able to do that without technology like this.

How has the ability to multiplex helped in your research?

I've been using multiplex RNAscope for several years now. I started off with the brown ISH then moved into the co-ISH stain. Thanks to the grant though, I have been able to explore the use of IHC with RNAscope for the first time, so there's certainly been an evolution in my research. Using ACD's IHC products in conjunction with the RNAscope kits has been fantastic, because up until that point, I had to multiplex proteins on one slide, while conducting multiple RNAs on another slide, but they were never together. Previously, I could determine broad regions in which cells were active/inactive, but now it's definitive.

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How does your work feed into the wider field of cancer research and where do you see it going?

Being able to spatially resolve cells in tissue and use tools like RNAscope to identify and phenotype cells will have a lot of clinical relevance in the future for the pathological analysis of tissues. If there was a whole load of discovery work filtered down into one or two markers in certain types of cancer, which were able to give an indication of how a patient would respond or not to certain treatments, that's where I see this going. If we can understand the dynamics of the cancer through the different compartments, through things like RNAseq right through to spatially resolving that RNA on the slide, that's where the real power of this can be seen going from bench to bedside.