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INTERVIEW

The people behind the papers – Marina Ramiro-Pareta and Ofelia Martinez-Estrada

Wilms tumor 1 (WT1) is a transcription factor known to be expressed in the epicardium and required for heart development, but the role of WT1 outside of the epicardium is less clear. In a new paper in Development, Marina Ramiro-Pareta and colleagues generate an inducible, tissue-specific loss-of-function mouse model to investigate the role of WT1 in coronary endothelial cells (ECs). We caught up with first author Marina Ramiro-Pareta and corresponding author Ofelia Martinez-Estrada (Principal Investigator at the Institute of Biomedicine in Barcelona, Spain) to learn more about their research.

Ofelia Martinez-Estrada (L) and Marina Ramiro-Pareta (R)

Ofelia, can you give us your scientific biography and the questions your lab is trying to answer?

OM-E: After my PhD and postdoc focused on cell biology, I moved to Nick Hastie's lab in Edinburgh to work on WT1 biology. Since then, I have been interested in studying the function of this fascinating protein in heart development and particularly in epicardium development. Those years as a postdoc in Nick's lab had a huge impact on me and were the seed of the science we are doing now at the University of Barcelona. During my first years as a group leader, we remained interested in understanding the molecular mechanism underlying WT1 functions in epicardium development, but more recently we have been working on other cell types and organs. The identification of the role of WT1 in cell plasticity during development and regeneration is one of the main topics of my lab at the moment.

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

MR-P: Surprisingly, I started as a masters student in Ofelia's lab to study possible functions of WT1 in the nervous system, which proved to be really challenging for me as a novice scientist at that time. After many challenges with that project, during my PhD I switched to the cardiology field, where I grew passionate about angiogenic processes, and especially the development and origins of the coronary arteries.

What was known about WT1 before your work?

OM-E & MR-P: Since the initial characterization of the germ line *Wt1KO* mouse model, cardiovascular biologists have known that *Wt1* is an important gene required for heart development. However, most of the cardiovascular defects observed in *Wt1KO* mouse models with defects in heart development have mainly been attributed to WT1 functions in the epicardium and epicardial-derived cells. During the last decade, several groups have reported WT1 expression in coronary ECs, both during development and following myocardial infarction, but the information about WT1 function in these cells was very limited. During Marina's thesis, we decided to generate a new *Wt1* knockout in ECs and characterise its function during coronary blood vessel formation.

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

OM-E & MR-P: In this study, we generated and characterised an inducible, EC-specific *Wt1KO* mouse model. Deletion of *Wt1* in coronary ECs during coronary plexus formation leads to defective coronary blood vessel and myocardium development. RNA-sequencing analysis of differentially expressed genes (DEGs) in coronary ECs isolated from control and mutant mice identified a set of DEGs that are modulated over the course of coronary EC development and, more importantly, many of them regulate blood vessel formation. In summary, the data presented in this article demonstrates that the expression of WT1 in coronary ECs is required for heart development and is highly relevant in the regulation of the transcriptomic signature of embryonic coronary ECs.

Were you surprised that loss of WT1 in ECs also affects myocardium development?

OM-E: To be honest, I wasn't surprised! These mutants have defects in the development of coronary blood vessels and it is logical that the myocardium should be also affected. What has surprised me is the fact that we have not observed embryonic lethality, which is the case in conventional or epicardial *Wt1KO* mouse models.

MR-P: For me, the shock was discovering the extent of the effect on the myocardium, which for a long time had only been attributed to WT1 functions in the epicardium and epicardial-derived cells.

How do you think WT1 contributes to endothelial cell maturation and differentiation?

OM-E & MR-P: In this study, we performed a transcriptomic analysis of coronary ECs isolated from control and *Wt1KO* mice,

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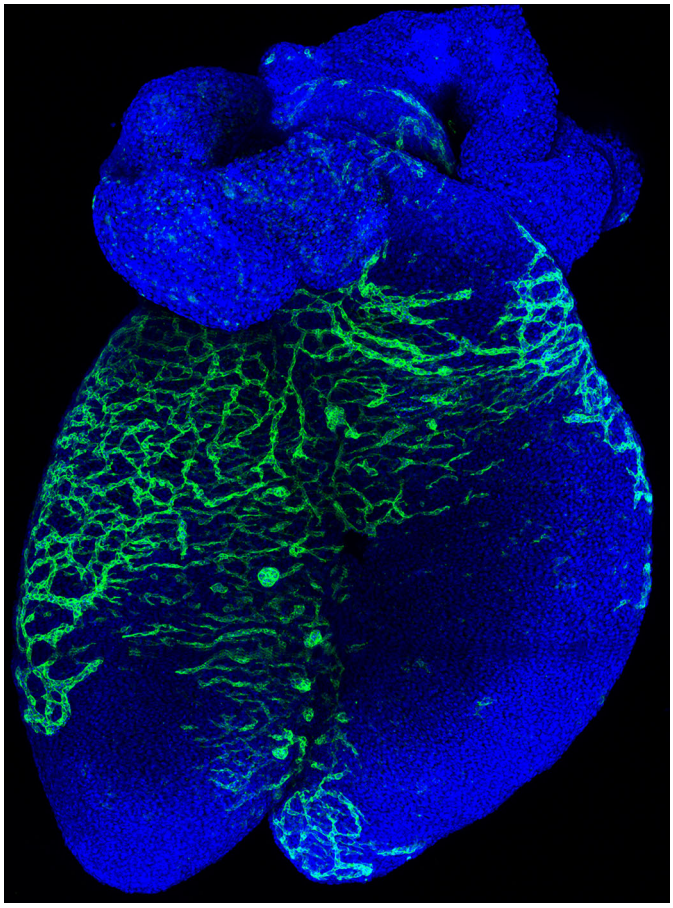

Endothelial deletion of *Wt1* disrupts coronary angiogenesis and myocardium development. Ventral whole-mount images of a *Wt1KO*$^{\Delta EC}$ mouse heart at E13.5 developmental stage, showing a defective coronary plexus in green (expression of GFP) and DAPI (nuclei, blue).

which allowed us to determine how the deletion of WT1 in these cells modified their transcriptomic signature. Interestingly, many of these genes are dynamically expressed during coronary EC development. Gene ontology (GO) analysis has also revealed several very interesting cellular processes. Despite this, at this moment we don't have an answer to your question. We have some candidates in mind, but this is one of the topics that we need to investigate.

When doing the research, did you have any particular result or eureka moment that has stuck with you?

MR-P: As a part of my PhD thesis, I performed some initial experiments testing WT1 functions in angiogenesis using a 'friendlier' system: the retina. When I saw effects after endothelial deletion of Wt1 in that tissue, I was convinced that WT1 might indeed play a key function during angiogenesis of the coronary plexus.

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

MR-P: As the Covid-19 pandemic struck, we were in the middle of performing most of our *in vivo* experiments. As our animal facilities and laboratories were forced to close for at least 3-4 months, we had to reschedule most of the experiments and generate new animals because others grew too old. I felt powerless working remotely!

Marina, what is next for you after this paper?

MR-P: To be completely honest, I don't really know! I feel like taking a break before searching for postdoc positions. I would like to continue in the cardiac/angiogenesis field, but I would also have a hard time leaving Barcelona to do so. So, I need a little time to make up my mind.

Ofelia, where will this story take your lab next?

OM-E: These results form part of Marina's thesis in which she has also studied the effect of WT1 deletion during heart repair. At the moment, we are finishing experiments in which we are studying whether the deletion of WT1 in ECs following myocardial infarction also modulates the formation of new blood vessels. In the near future, I would like to understand how WT1 is regulating these processes. It is extremely challenging because our experiments are performed in embryonic hearts and, usually, the population of cells that we are interested to work with is not the most abundant.

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

OM-E: Barcelona is a fascinating city and it is very well located; it's close to beautiful beaches and mountains, which I love to explore with my family. I like reading as well – I enjoy novels but also books written by scientists. I am Cuban and, as a Cuban, I like celebrations. In the lab, we have many moments of frustration, so every grant or paper is celebrated with mojitos!

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MR-P: I love practising all kinds of sports to set my mind free from my everyday problems: yoga, boulder climbing (my next challenge is to try rock climbing), cycling, skiing, etc. Also, as I listen to a lot of music while in the lab, I have to also do some field research by going to as many live music events as I can (afford)! But there's nothing I love more than drinking a cold beer while eating 'bravas' with my friends on a terrace while sunbathing.

Reference

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