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INTERVIEW

The people behind the papers – Beth Firulli and Anthony Firulli

HAND2 is an important regulator of cardiac morphogenesis and is expressed throughout the heart. A new paper in Development dissects the gene regulatory networks downstream of HAND2 in the endocardium. To find out more about this research, we caught up with co-first author Beth Firulli and corresponding author Anthony (Tony) Firulli, Professor at Indiana Medical School. Co-first author Rajani George has left the Firulli lab and was not available for our interview.

Tony Firulli (L) and Beth Firulli (R).

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

AF: My lab has been focused on the functions of the Twist family of basic helix loop helix (bHLH) proteins for well over 25 years now. We want to understand how these combinatorial transcription factors choose the right dimer complex, bind to the right E- or D-box and facilitate the gene regulatory networks that they modulate. Factors like HAND2 are broadly expressed yet do very specific things within a variety of tissues. The question we have been striving to answer is: How does this family of bHLH proteins do this?

How did the team come together for this project, and what drives your research today?

BF: Tony and I were graduate students together at Roswell Park Cancer Center. We got married and worked separately until Tony became an Assistant Professor and, at the same time, my PI decided to pursue other career options. I was interested in what Tony was working on as I had heard a lot about it and had been involved in discussions on the best ways to approach the questions being asked. Things just fell into place.

AF: In 2017, I was looking for a postdoctoral fellow. While talking to two close colleagues, they suggested I reach out to a former PhD student, Rajani George, who was working in industry but wanted to get back into an academic setting. Rajani joined the lab soon after and is co-first author on our paper.

BF & AF: What drives our research is the question: How does this work? How can a broadly expressed transcription factor that needs a dimer partner do so many specific things when its DNA binding sequence is encountered every 256 base pairs in the genome?

What drives our research is the question: How does this work?

What was known about the role of HAND2 in cardiac morphogenesis prior to your work?

BF & AF: Our understanding of HAND2 has grown quite a bit since 1995 when the first papers on HAND2 (dHAND) were published. HAND2 is interesting as it is expressed in all the cell lineages that compose the heart (myocardium, endocardium, epicardium and the cardiac neural crest). The Srivastava lab performed an elegant study showing HAND2 loss-of-function using a panel of cardiac-relevant Cre driver lines and nicely demonstrated important roles within the myocardium and second heart field (Tsuchihashi et al., 2011). Nathan VanDusen, who was a graduate student in my lab, smartly saw that endocardium was a major source of HAND2 cardiac expression so using endothelial/endocardial Cre drivers, we uncovered the role of endocardial HAND2 is septation and cardiac morphogenesis (VanDusen et al., 2014). This study takes Nathan's observations a step further and identifies HAND2-dependent gene regulatory networks within the endocardium.

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

BF & AF: Well, we knew that loss of HAND2 within the endocardium is embryonic lethal and causes tricuspid atresia or double inlet left ventricle due to defects in septum formation and that HAND2 lies downstream of NOTCH signalling (VanDusen et al., 2014). In this study, we used single cell transcriptomics, combined with HAND2 DNA occupancy data, to identify a number of putative HAND2-dependent genes that exhibit downregulation and HAND2 DNA binding. By testing these HAND2 binding domains for enhancer activity within the endocardium/endothelium,

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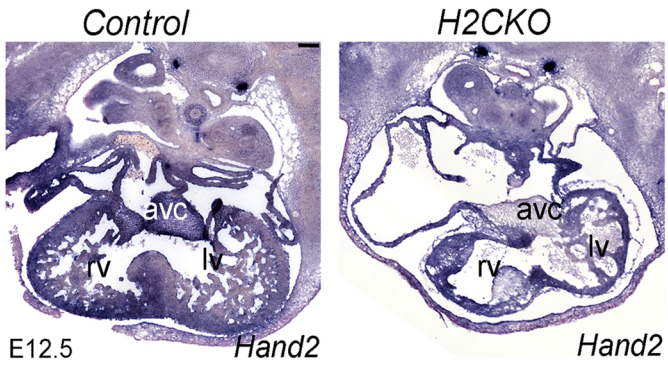

***Hand2* expression in E12.5 *Hand2*$^{fx/fx}$ control (left) and *Nfatc1*^{Cre}*Hand2*$^{fx/fx}$ *H2CKO* (right).** avc, atrioventricular canal; lv, left ventricle; rv, right ventricle.

we identified three novel endocardial enhancers, two of which were located in the gene for the shear-stress master regulator *Klf2* and recapitulate its endogenous expression. Using genome-editing, deletion of the putative enhancer −50 kb upstream of the *Klf* transcriptional start site revealed that ventricular endocardial expression of *Klf2* was reduced.

You identify two endocardial cell populations in your analysis that respond somewhat differently to *Hand2* ablation. How does the identity of these populations differ?

BF & AF: This is a question we wished we knew the answer to, but even with power of single cell transcriptomic technology we cannot be sure whether the cells are heterogeneously mixed within the heart or whether they segregate to specific regions (septum, ventricles, and later fated to be coronaries, and lymphatics). The difference could also reflect a maturation of endocardium. Support for this idea can be seen in our 2014 study where we looked at LYVE1 expression. At E10.5, LYVE1 expression is comparable between controls and *Hand2* knockout mouse embryos. When we looked at E13.5 in control embryos, LYVE1 expression is restricted to the forming lymphatics, whereas in *Hand2* knockout embryos, LYVE1 expression is maintained within the ventricular endocardium. To really answer your question, we plan to employ spatial transcriptomic analysis to maintain the positional identity of these cells. We feel this will allow us to see more, speculate less, and perhaps design new experiments that will confirm the true nature of these two endocardial cell populations.

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

BF: Yes, when we first saw the *Klf2* enhancer construct expression, revealing that these HAND2-DNA binding conserved non-coding sequences recapitulated *Klf2* endogenous expression patterns and validating that this approach can be used effectively.

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

BF: Despair was never an emotion encountered in this study; however, the technical nature of performing the key experiments and the, at times over our heads, bioinformatic analysis was a bit scary. Luckily for us, our collaborators Ram and Doug made these learning curves as shallow as possible.

Beth, what is next for you after this paper?

BF: I hope a lot. I'm hoping that following up on this study will yield another paper in a year or four! We have an interesting set of data that we have nearly finished collecting and analysing on a gene replacement mouse model. We knocked the coding region of HAND2 into the *Hand1* locus such that the regulatory sequences are all *Hand1* sequences, but the two exons and single intron are *Hand2*. The idea was to functionally test the redundancy of these two highly related proteins. Our findings are pretty interesting, especially outside the heart.

Tony, where will this story take your lab next?

AF: Our next steps will be continuing to search for genes that show significant regulation in *Hand2* loss-of function models. We'll look for HAND2 DNA occupancy within conserved non-coding regions of these genes to identify the HAND2-dependent endocardial enhancers that will directly build a HAND2-dependent transcriptional network. In parallel, we'll generate spatial transcriptomic data to reveal whether there are specific regions within the developing endocardium where HAND2 gene regulatory networks are of greater importance.

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

AF & BF: Well, we have a house full of dogs, backyard full of chickens, and when the stars align a few successful beehives. We also like to garden and get our hands dirty.

AF: I have rediscovered my love of cycling and try to get out on the road as much as time, traffic and weather permits. If I cannot get out, I jump on my peloton every morning so I can keep up outside.

BF: I enjoy sewing and construct my own garments and quilts.

References

George, R. M., Firulli, B. A., Podicheti, R., Rusch, D. B., Mannion, B. J., Pennacchio, L. A., Osterwalder, M. and Firulli, A. B. (2023). Single cell evaluation of endocardial Hand2 gene regulatory networks reveals HAND2-dependent pathways that impact cardiac morphogenesis. *Development* **150**, dev201341. doi:10.1242/dev.201341

Tsuchihashi, T., Maeda, J., Shin, C. H., Ivey, K. N., Black, B. L., Olson, E. N., Yamagishi, H. and Srivastava, D. (2011). Hand2 function in second heart field progenitors is essential for cardiogenesis. *Dev. Biol.* **351**, 62-69. doi:10.1016/j.ydbio.2010.12.023

VanDusen, N. J., Casanovas, J., Vincentz, J. W., Firulli, B. A., Osterwalder, M., Lopez-Rios, J., Zeller, R., Zhou, B., Grego-Bessa, J., De La Pompa, J. L. et al. (2014). Hand2 is an essential regulator for two Notch-dependent functions within the embryonic endocardium. *Cell Rep.* **9**, 2071-2083. doi:10.1016/j.celrep.2014.11.021