INTERVIEW

The people behind the papers – Yuki Naitou and Katsuhiko Hayashi

Specification of primordial germ cells requires a proportion of the cells in the posterior of the epiblast to reacquire pluripotency. A new paper in Development describes how OVOL2 is involved in regulating the balance between mesodermal fate and germ cell fate during gastrulation. We caught up with the first author, Yuki Naitou, and corresponding author, Katsuhiko Hayashi (Osaka University), to find out more about the paper and their future research.

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

KH: I gained my MSc from Meiji University (1996) and then had an assistant professor position in Tokyo University of Science, where I got my PhD (2004). After this period, I worked with Professor Yasuhisa Matsui in Osaka Women’s and Children’s Hospital, and then joined to the lab of Professor Azim Surani, a pioneer of genome imprinting and epigenetics, at the Gurdon Institute, Cambridge (2005-2009). After returning to Japan, I worked with Professor Mitinori Saitou, who is a world-leading scientist in germ cell biology, as an associate professor in Kyoto University (2009-2014). After this period, I had a full professor position in Kyushu University (2014-2021) and recently moved to Osaka University (2021-present). Throughout my career, I have been interested in germ cell development, particularly the mechanisms that underlie differentiation of early germ cell population, such as primordial germ cells and primary oocytes. In collaboration with Azim and Mitinori, we developed a culture system that reconstitutes germ cell development using mouse pluripotent stem cells. With this particularly useful system, we address gene regulatory networks involved in primordial germ cell and oocyte differentiation.

Yuki, how did you come to work in Katsuhiko’s lab and what drives your research today?

YN: I was interested in pluripotent stem cells and regenerative medicine at the beginning of my undergraduate programme. While studying life science and medicine at Kyushu University, I wanted to experience various research fields and in the third year of my undergraduate programme, I had an opportunity to join Katsuhiko’s lab. The interesting research projects in Katsuhiko’s lab made me realise that experiments and discussions are so exciting and fascinating. Therefore, I decided to join the lab as a master’s course student. Fortunately, I could start this interesting project to understand the mechanism of PGC specification. Then, I continued the research in the lab as a PhD student.

Before your work, what was known about the molecular mechanism of primordial germ cell (PGC) specification during gastrulation?

KH & YN: It was known that three transcription factors, BLIMP1/PRDM1, PRDM14 and TFAP2C, are essential for PGC specification. T (brachyury), a well-known mesodermal factor, was isolated upstream of these transcription factors, which brings us to the fundamental question to be solved in this study. Given that T (brachyury) activates both PGC specification and nascent mesoderm differentiation, the question was: how is the balance between germ cells and mesodermal cells determined? We noted previous reports showing that the Drosophila gene ovo plays a role in germ cell development and that Ovol2-mutant mice have a reduced number of PGCs. Furthermore, in other cell types it has been reported that Ovol2 counteracts epithelial-mesenchymal transition. Collecting these findings, we designed this study to understand the role of Ovol2 in PGC specification.

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

KH & YN: The key result of this paper is the functional difference between two splice variants: Ovol2a and Ovol2b. Based on the results of chromatin immunoprecipitation sequencing and luciferase reporter analyses, Ovol2a, a splice variant encoding a repressor domain, directly represses the EMT-related gene Zeb1, resulting in re-acquisition of pluripotency in PGCs. On the other hand, Ovol2b, another splice variant missing a repressor domain, directly upregulates the PGC-associated gene Blimp1/Prdm1. The synergistic effect of these splice variants safeguards a certain number of germ cells in PGC specification.

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Ovol2+/−  Ovol2−/−

The EMT-related gene Hmga2 is upregulated in Blimp1-mVenus (BV) positive cells (dotted line) of Ovol2−/− embryos, whereas BV-positive PGCs (dotted line) of Ovol2+/− embryos show a negligible level of HMG A2 expression.

Why do you think that knockout of Zeb1, but not of other EMT-related genes, was sufficient to rescue PGC-like cell differentiation in the absence of Ovol genes?
KH & YN: As other studies demonstrated, the roles of EMT factors, including Zeb1, Snail and Twist1/2, are cell-context dependent. We think that Zeb1 has a unique role in the epiblast during gastrulation. As a general concept in EMT, it is thought that the transition during gastrulation is a gradual process with multiple intermediate states in the epiblast. There could be a point of no return for restoration of expression of PGC- and pluripotency-associated genes. It is possible that Zeb1, but not other EMT-related genes, has a crucial role at this point by counteracting Ovol2 function. Relevant findings showed that the Zeb1/2-mir200 axis regulates balance between EMT and MET during iPS C reprogramming (Burk et al., 2008; Wang et al., 2013). As Ovol2 possesses the potential to promote iPS C reprogramming (Maekawa et al., 2011), the balance between EMT and MET in pluripotent cells could be extremely sensitive to the axis between Zeb1 and Ovol2. This point should be investigated further in future.

We realised then that the repression of EMT is crucial for re-acquisition of pluripotency.

What are the differences between OVOL2A and OVOL2B that lead to their distinctive roles in PGC specification?
KH & YN: There are differences in the domain structures and expression patterns between OVOL2A and OVOL2B: OVOL2A has both repressive and transactivation domains, whereas OVOL2B has only a transactivation domain. In addition, the peak of Ovol2a expression is earlier than that of Ovol2b expression. Based on these observations, we speculate that the repression of EMT-related gene expression by OVOL2A precedes the activation of PGC-associated gene expression by OVOL2B. This sequence of regulation is consistent with the transient activation of the mesodermal programme followed by robust expression of PGC-associated genes during PGC specification. As these Ovol2 variants are likely regulated by the same promoter, it would be important to see a mechanism regulating the alternative splicing in PGC precursors.

When doing the research, did you have any particular result or eureka moment that has stuck with you?
YN: The most exciting moment for me was when I found the expression of PGC-associated genes and pluripotent genes restored in Ovol-triple mutant PGC-like cells upon deletion of Zeb1. We realised then that the repression of EMT is crucial for re-acquisition of pluripotency.

And what about the flipside: any moments of frustration or despair?
YN: Although I enjoyed this project, I was frustrated with refinement of the conditions for chromatin immunoprecipitation sequencing. As this experiment includes multiple steps, it was difficult to determine the optimal conditions.

What next for you after this paper?
YN: Through this project, I became interested in the cell-to-cell variation that determines individual cell fate during embryogenesis, especially in germ cell development. Although I am satisfied with this project, I realised that I do not have a clear answer to the heterogeneity in differentiation of PGCs at the most proximal layer of the posterior epiblast, which should be in the same environment. Such cell-to-cell variation can be generalised to various cell contexts in embryogenesis. To address the heterogeneity in PGC differentiation, I am going to investigate details such as relevance to the cell cycle, quantification of cell-cell contacts and bias of signalling molecules by using a live-cell imaging system.

Where will this story take your labs next?
KH: As Yuki mentioned, we cannot explain effect of cell-to-cell variation in germ cell development. This is the case not only in PGC specification but also in primary oocyte differentiation, where oocytes seem to make a critical choice on their cell fate in the apparently same environment. We want to understand determinants, or stochasticity, of such cell-to-cell variation in germ cell development.

Finally, let’s move outside the lab – what do you like to do in your spare time?
YN: I enjoy watching the videos of cute animals. I find solace in YouTube videos of animals.
KH: Playing with my kids and dog, visiting historical places such as old castles, testing Sake, and research!

References