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学位論文題目	Studies on extrinsic effects on the pattern formation via Delta-Notch signaling pathway （Delta-Notch シグナル経路を介したパターン形成への外的要因の影響に関する研究）

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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, Yoshihara Masaharu describes the mechanism of how the Delta-Notch signaling pathway leads to the differentiation of progenitor cells into biliary epithelial cells and how it is affected by extrinsic factors. The summary is as follows:

（目的　Purpose）

The Delta-Notch signaling pathway plays a pivotal role in the pattern formation of many organs at histological levels. However, little is known about how pattern formation is affected by extrinsic conditions. The author attempted to uncover the mechanism by which the Delta-Notch signaling pathway yields various patterns, specifically focusing on the spatially restricted biliary formation around the portal vein in the liver.

（対象と方法　Materials and Methods）

In this dissertation, the author took mathematical, bioinformatical, and experimental approaches. He utilized a previously proposed mathematical model (Lateral inhibition with Mutual Inactivation model: LIMI model) in a 20 x 20 cell field. While the original LIMI model is a differential equation model that incorporated many parameters, the author traced the development of Notch signal-dependent pattern formation by calculating different equation form of the LIMI model and analyzed the effect of the existence of a cell (an imaginary portal mesenchymal cell) with an extremely high level of Delta ligands expression ("disturbance") on the whole simulated field using C++-based computer simulation. The author systematically examines whether the production rates of Delta ligand or Notch receptors could affect the pattern formation that is driven by such disturbance. Next, the author used bioinformatical analysis of previously reported two human fetal liver single-cell RNA sequencing (scRNA-seq) datasets at Carnegie Stage (CS) 20 and 23 to examine whether mathematical prediction on the disturbance-driven pattern formation was observed in vivo. Finally, the author used transgenic reporter mouse lines: Notch1-Gal4VP16 (Smith et al., Genesis, 2012), UAS-Cre-T2A-miRFP670 (the author's study), and R26GRR (Hasegawa et al., Exp Anim, 2013) to visualize the past Notch 1 signal at a cellular level.

（結果　Results）

The author confirmed that the pattern formation via the Delta-Notch signaling pathway is dependent on the production rate of Delta ligands and Notch receptors. A series of simulations showed that spatially restricted patterns where Notch signal was enriched in cells neighboring the "disturbing" cell tended to be achieved when other cells had low Delta ligands production rates. Previous scRNA-seq analyses showed that JAGGED1 mRNA expression was high in the cluster with high ACTA2 mRNA expression, which constituted only 6.63% and 4.96% of the CS20 and 23 datasets, respectively. Finally, the author established a new transgenic reporter mouse (UAS-Cre-T2A-miRFP670) and visualized past Notch signals in a Gal4VP16-dependent manner through the action of endogenous Delta ligands and Notch receptors in vivo.

（考察　Discussion）

The author's mathematical analysis suggested that the intrinsic biochemical property of the Delta-Notch signaling pathway could yield several spatial patterns that are affected by extrinsic conditions such as the presence of "disturbing" cells (JAGGED1-expressing portal mesenchymal cells) and low Delta ligands production rates in the progenitor cells. This conclusion was supported by the bioinformatical analysis that JAGGED1 mRNA was expressed in a limited population of ACTA2-expressing portal mesenchymal cells in the liver, whereas the progenitor cells had low JAGGAED1 production rates. The author's new transgenic reporter mouse enabled visualization of the past Notch signal by combining it with two previously established transgenic mouse lines. The author observed that the Notch signal was confined to the progenitor cells around JAGGED1-expressing portal mesenchymal cells in vivo. Since Notch2 is the main Notch receptor responsible for biliary development and is one of the susceptible genes in Alagille syndrome, it would be crucial to establish Notch2-Gal4VP16 transgenic mice. Nevertheless, the author successfully shed light on how Delta-Notch signaling acts, emphasizing extrinsic conditions such as the presence of "disturbing" cells and JAGGED1 production rate in the progenitor cells.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

The author examines how extrinsic conditions affect pattern formation in the Delta-Notch signaling pathway, which plays a crucial role in biliary formation around the portal vein. The author successfully combined mathematical models, bioinformatical analysis, and in vivo mouse analysis to investigate the mechanism that spatially restricts Delta-Notch signaling. The author's study exemplifies the use of a bi-disciplinary approach and may potentially impact liver regeneration.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on December 21, 2022. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

（結論　Conclusion）

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.