cells. Nat. Rev. Gastroenterol. Hepatol. *16*, 19–34. https://doi.org/10.1038/s41575-018-0081-y.

3. Worthington, J.J., Reimann, F., and Gribble, F.M. (2018). Enteroendocrine cells-sensory sentinels of the intestinal environment and orchestrators of mucosal immunity. Mucosal Immunol. *11*, 3–20. https://doi.org/10.1038/mi.2017.73.

4. Beumer, J., Puschhof, J., Bauzá-Martinez, J., Martínez-Silgado, A., Elmentaite, R., James, K.R., Ross, A., Hendriks, D., Artegiani, B., Busslinger, G.A., et al. (2020). High-Resolution mRNA and Secretome Atlas of Human Enteroendocrine Cells. Cell *181*, 1291–1306.e19. https://doi.org/10.1016/j.cell.2020.04.036.

5. Beucher, A., Gjernes, E., Collin, C., Courtney, M., Meunier, A., Collombat, P., and Gradwohl, G. (2012). The homeodomain-containing transcription factors Arx and Pax4 control enteroendocrine subtype specification in mice. PLoS One *7*, e36449. https://doi.org/10.1371/journal.pone.0036449.

6. Mellitzer, G., Beucher, A., Lobstein, V., Michel, P., Robine, S., Kedinger, M., and Gradwohl, G. (2010). Loss of enteroendocrine cells in mice alters lipid absorption and glucose homeostasis and impairs postnatal survival. J. Clin. Invest. *120*, 1708–1721. https://doi.org/10.1172/JCI40794.

7. Naya, F.J., Huang, H.P., Qiu, Y., Mutoh, H., DeMayo, F.J., Leiter, A.B., and Tsai, M.J. (1997). Diabetes, defective pancreatic morphogenesis, and abnormal enteroendocrine differentiation in BETA2/neuroD-deficient mice. Genes Dev. *11*, 2323–2334. https://doi.org/10.1101/gad.11.18.2323.

8. Gehart, H., van Es, J.H., Hamer, K., Beumer, J., Kretzschmar, K., Dekkers, J.F., Rios, A., and Clevers, H. (2019). Identification of Enteroendocrine Regulators by Real-Time Single-Cell Differentiation Mapping. Cell *176*, 1158–1173.e16. https://doi.org/10.1016/j.cell.2018.12.029.

9. Lin, L., DeMartino, J., Wang, D., van Son, G.J.F., van der Linden, R., Begthel, H., Korving, J., Andersson-Rolf, A., van den Brink, S., Lopez-Iglesias, C., et al. (2023). Unbiased transcription factor CRISPR screen identifies ZNF800 as master repressor of enteroendocrine differentiation. Science *382*, 451–458. https://doi.org/10.1126/science.adi2246.

10. Sueda, R., Imayoshi, I., Harima, Y., and Kageyama, R. (2019). High Hes1 expression and resultant Ascl1 suppression regulate quiescent vs. active neural stem cells in the adult mouse brain. Genes Dev. *33*, 511–523. https://doi.org/10.1101/gad.323196.118.

Unraveling the function of FGF signaling in human hypoblast specialization

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Dattani et al.[1] developed a method for inducing hypoblast-like cells from human naive pluripotent stem cells. They elucidated the requirement for FGF signaling in human hypoblast specialization at a specific time window, which was previously controversial.

During the blastocyst stage of mammalian embryos, three cell lineages are formed: epiblast, trophoblast, and primitive endoderm (PrE, also known as hypoblast in humans). The PrE will give rise to yolk sac upon implantation and can further contribute to the gut after gastrulation. Unlike in mice, the gene regulatory network and signaling requirements for hypoblast specification and yolk sac development are poorly understood in humans. PrE stem cells (PrESCs), which recapitulate the molecular and functional features of PrE cells, have been established in mice.[2] Despite identification of several conserved key transcription factors that regulate PrE specialization, such as the upstream GATA6, which subsequently activates SOX17, GATA4, and SOX7, the signaling requirements for hypoblast specialization appear distinct between humans and mice. Additionally, stem cell analogs to PrESCs have not been established for humans.

FGF signaling is known as one of the most important upstream regulators of mouse PrE specialization.[3] For example, FGF/ERK signaling is critical for maintaining GATA6-positive mouse PrE cells, and inhibition of FGF/MEK signaling in mouse embryos suppresses PrE fate.[4] Whether the FGF signaling is required for human hypoblast specialization remains controversial.[5] In this issue of *Cell Stem Cell*, Dattani et al.[1] addressed this issue. They developed a method for inducing the differentiation of human naive pluripotent stem cells (nPSCs, which represent the pre-implantation epiblast) to hypoblast-like cells and revealed the FGF signaling requirement in human hypoblast specialization.

Human nPSCs retain the ability to differentiate into both the trophoblast and the hypoblast. In their current paper, Dattani et al. demonstrated that a combination of the MEK/ERK inhibitor PD0325901 and Activin/Nodal inhibitor A83-01, referred to as the PA condition, can drive nPSCs to differentiate into either the trophoblast or the hypoblast, depending on exposure duration. This same condition was previously used by the same and other labs to generate human

blastocyst-like structures, known as blastoids,[6,7] which contain cells resembling all blastocyst lineages. Comparative single-cell RNA-sequencing (scRNA-seq) analyses with reference human embryo datasets revealed that nPSCs cultured in PXGL medium and exposed to PA for 24 h, followed by 48 h in N2B27 medium (PA-N condition), exhibited a shift in cell fate toward the nascent hypoblast. In contrast, nPSCs continously exposed to PA for 72 h differentiated toward the trophoblast. Thus, PA-N confers a window of emerging hypoblast *in vitro*.

Interestingly, scRNA-seq analysis revealed that despite the presence of PD0325901 during hypoblast differentiation, nPSCs and hypoblast cells highly expressed FGF ligands (FGF2 and FGF4) and their receptors, respectively. To investigate the requirement for FGF signaling in human hypoblast differentiation from nPSCs, the authors treated nPSCs with an FGF receptor inhibitor (PD173074, PD17) at different time points within the 48 h window following PA treatment. Suppressing FGF signaling with PD17 immediately after PA treatment (PA-PD17) greatly prevented hypoblast emergence, while delayed PD17 treatment (PA-N-PD17) had no effect. Moreover, during human blastoid formation, PA-PD17 treatment resulted in almost no hypoblast cells forming. Treating early day 5 human blastocysts with PD17 exhibited reduced, but not entirely absent, hypoblast differentiation, suggesting remaining requirements for other signaling pathways. Additionally, the addition of FGF2 after PA treatment, along with suppressing epiblast and trophoblast differentiation using A83-01 and XAV939, respectively (FA83X condition), further enhanced hypoblast differentiation efficiency from nPSCs. In conclusion, using nPSC-based *in vitro* models and human blastocysts, Dattani et al. demonstrated that nascent hypoblast specialization from the epiblast requires a transient FGF signaling activation.

This study not only provided a method for obtaining nascent hypoblast-like cells *in vitro* but also systematically investigated the dependence of FGF signaling on early human hypoblast specialization. Recently, several other studies have reported successful generation of human hypoblast-like cells. Wei et al. and Okubu et al. independently developed systems for inducing human hypoblast-like cells from nPSCs, both of which also included FGF ligands.[8,9] Wei et al. developed an FTW condition to induce extraembryonic endoderm cells in mice, monkeys, and humans by co-activating FGF, TGF-β/Smad, and WNT/β-catenin signaling pathways. Okubu et al. established a seven-factor condition to induce human hypoblast-like cells from nPSCs, which showed high transcriptome correlation with the hypoblast produced by GATA6 and *in vivo*. Several other signaling pathways, such as BMP, Activin/NODAL, TGF, WNT, PDGF, and retinoic acid, were suggested to play a role in hypoblast specification or *in vitro* maintenance. It is important to determine whether these signaling pathways play a role in the specialization or the maintenance of the hypoblast. Weatherbee et al. dissected the dynamic and potential roles of some well-known signaling pathways (NODAL, BMP, and NOTCH) in the spatiotemporal patterning of human embryos from pre- to post-implantation, highlighting their similarities and differences between humans and mice.[10] They suggested a potential role for NODAL and BMP signaling pathways in hypoblast specialization and revealed their essential role in the maintenance of anterior hypoblast after implantation.

Despite the advances made by Dattani et al. and others, essential signaling pathways regulating hypoblast specification and self-renewal remain to be further elucidated before we can derive bona fide human hypoblast stem cells analogous to their blastocyst counterparts.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Dattani, A., Corujo-Simon, E., Radley, A., Heydari, T., Taheriabkenar, Y., Carlisle, F., Lin, S., Liddle, C., Mill, J., Zandstra, P.W., et al. (2024). Naive pluripotent stem cell-based models capture FGF-dependent human hypoblast lineage specification. Cell Stem Cell *37*, 1058–1071.e5. https://doi.org/10.1016/j.stem.2024.05.003.

2. Ohinata, Y., Endo, T.A., Sugishita, H., Watanabe, T., Iizuka, Y., Kawamoto, Y., Saraya, A., Kumon, M., Koseki, Y., Kondo, T., et al. (2022). Establishment of mouse stem cells that can recapitulate the developmental potential of primitive endoderm. Science *375*, 574–578. https://doi.org/10.1126/science.aay3325.

3. Yamanaka, Y., Lanner, F., and Rossant, J. (2010). FGF signal-dependent segregation of primitive endoderm and epiblast in the mouse blastocyst. Development *137*, 715–724. https://doi.org/10.1242/dev.043471.

4. Nichols, J., Silva, J., Roode, M., and Smith, A. (2009). Suppression of Erk signalling promotes ground state pluripotency in the mouse embryo. Development *136*, 3215–3222. https://doi.org/10.1242/dev.038893.

5. Roode, M., Blair, K., Snell, P., Elder, K., Marchant, S., Smith, A., and Nichols, J. (2012). Human hypoblast formation is not dependent on FGF signalling. Dev. Biol. *361*, 358–363. https://doi.org/10.1016/j.ydbio.2011.10.030.

6. Yanagida, A., Spindlow, D., Nichols, J., Dattani, A., Smith, A., and Guo, G. (2021). Naive stem cell blastocyst model captures human embryo lineage segregation. Cell Stem Cell *28*, 1016–1022.e4. https://doi.org/10.1016/j.stem.2021.04.031.

7. Yu, L., Wei, Y., Duan, J., Schmitz, D.A., Sakurai, M., Wang, L., Wang, K., Zhao, S., Hon, G.C., and Wu, J. (2021). Blastocyst-like structures generated from human pluripotent stem cells. Nature *591*, 620–626. https://doi.org/10.1038/s41586-021-03356-y.

8. Wei, Y., Zhang, E., Yu, L., Ci, B., Sakurai, M., Guo, L., Zhang, X., Lin, S., Takii, S., Liu, L., et al. (2023). Dissecting embryonic and extraembryonic lineage crosstalk with stem cell co-culture. Cell *186*, 5859–5875.e24. https://doi.org/10.1016/j.cell.2023.11.008.

9. Okubo, T., Rivron, N., Kabata, M., Masaki, H., Kishimoto, K., Semi, K., Nakajima-Koyama, M., Kunitomi, H., Kaswandy, B., Sato, H., et al. (2024). Hypoblast from human pluripotent stem cells regulates epiblast development. Nature *626*, 357–366. https://doi.org/10.1038/s41586-023-06871-2.

10. Weatherbee, B.A.T., Weberling, A., Gantner, C.W., Iwamoto-Stohl, L.K., Barnikel, Z., Barrie, A., Campbell, A., Cunningham, P., Drezet, C., Efstathiou, P., et al. (2024). Distinct pathways drive anterior hypoblast specification in the implanting human embryo. Nat. Cell Biol. *26*, 353–365. https://doi.org/10.1038/s41556-024-01367-1.