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Editorial

Integrative biology studies in pluripotent stem cells

Embryonic stem cells (ESCs) have become a paradigm in stem cell research and developmental biology as they are capable of generating all three germ layers and can therefore be used to model development and the ontogeny of genetic diseases. Moreover, ESCs are fascinating due to their ability to self-organize and generate embryonic structures (Shahbazi et al., 2019). Unearthing the molecular mechanisms that underlie these capacities addresses fundamental questions in the fields of stem cell and developmental biology, with strong implications for regenerative medicine.

Mouse ESCs were established in the early 1980s (Evans and Kaufman, 1981; Martin, 1981) and enabled a revolution in developmental biology. The derivation of human ESCs (Thomson, 1998) and reprogramming human somatic cells into induced pluripotent stem cells (Takahashi and Yamanaka, 2006) opened the perspective of applying pluripotent stem cell technology towards regenerative medicine. Advances in deep and single cell sequencing, proteomics and bioinformatics are now helping to reveal the molecular regulation of stem cell fate. To obtain an in-depth understanding of the factors and mechanisms underpinning ESC pluripotency, differentiation and self-organization abilities, essential to harness their full potential, calls for an integrative approach. In this special issue, we bring together studies into pluripotent stem cells at the level of the epigenome, coding and non-coding transcriptome, regulome and proteome, together with modelling approaches. These studies provide new insights into the factors that regulate lineage acquisition and self-organization of pluripotent stem cells.

Development of the early embryo and differentiation of ESCs are dynamic processes that proceed through multiple transient cell states (Weinberger et al., 2016)(Yang 2019, de Los Angeles 2015). Yet, these transitions are mainly studied at the bulk level, averaging out rare populations that emerge transiently during development. To resolve these transient cell types, Patrick Cahan and colleagues have subjected mouse ESCs differentiating in embryoid bodies to droplet-based single-cell capture and RNA sequencing (Spangler et al., 2018). Embryoid body differentiation recapitulates representative events of gastrulation, including formation of mesendodermal progenitors in a primitive streak-like structure (ten Berge et al., 2008). The authors performed a thorough analysis of the emerging progenitors and predicted the cluster dynamics to identify the most likely origin of each cell during the differentiation trajectories. By comparison to embryo development and supported by a marker-driven inspection of gene patterns they went a step ahead and identified biologically relevant novel transcription factors and signaling pathways contributing to embryoid body differentiation.

In a complementary approach, Henning Kempf and colleagues applied a carefully controlled system to differentiate human ESCs to mesendodermal progenitors and studied the underlying dynamics using mathematical modeling (Gaspari et al., 2018). Assumptions for the model were derived from medium refreshment and conditioned medium experiments, mass spectrometry analysis of secreted factors, and knock-down experiments, and the model was validated using time-course data of differentiating cells. The model required paracrine factors produced by differentiated as well as by undifferentiated cells to recapitulate the experimentally observed differentiation kinetics. The experimental data suggested that the Nodal antagonist Lefty1, produced by undifferentiated cells, and the BMP antagonist Cer1, produced by differentiating cells, provided critical negative feedback to the differentiation process. Moreover, the model required induction of a paracrine activating factor to drive transition through the primitive streak-like stage. However, this factor could not be identified in mass spec analysis of the secretome of the differentiating cultures and may therefore not be secreted in the medium.

Interestingly, these findings dovetail with the single cell transcriptome data from Cahan and colleagues (Spangler et al., 2018) as they showed BMP repression and lack of signal activation in the pluripotent cluster combined with Cer1 induction in the mesendodermal cluster. Moreover, the mesendodermal cluster showed activation of the Hedgehog and Notch pathways, which may point at the nature of the missing activating factor. The hedgehog pathway is active in late streak-stage anterior mesendoderm but, due to their hydrophobic nature, hedgehog ligands are poorly secreted in the medium. Likewise, induction of the transmembrane protein Notch will likely not be detected in the secretome. The role of the Notch and Hedgehog pathways in mesendodermal differentiation therefore warrants further investigation.

Over the past decade, hundreds of genes, mostly transcription factors, have been implicated in the processes of self-renewal and differentiation. These processes are often associated with changes in signaling pathways that also govern the intracellular distribution of transcription factors independent of transcriptional changes (Betschinger et al., 2013; Zhou et al., 2017). To systematically characterize protein shuttling in early developmental transitions Hendrik Marks and colleagues, in this special issue, performed a mass spectrometry analysis of the subcellular proteome during the transition from ground state preimplantation blastocyst-like cells towards post-implantation epiblast-like cells (van Mierlo et al., 2018). These data, combined with functional assays, supported a model that centered on stage-specific distinct metabolic programs, which include upregulation of free radical buffering by the glutathione pathway specific for ground-state pluripotent stem cells.

While transcription factors, signaling pathways and chromatin regulators relevant for stem cell decisions have received most of the attention (Weinberger et al., 2016), understanding the involvement of RNA based mechanisms in cell fate choices is in comparison still in its

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infancy (Guallar and Wang, 2014). The importance of these modes of regulation now recognizes roles of long noncoding RNAs (lncRNA) in developmental patterning. Importantly, the same lncRNAs that govern ESCs to become committed to embryonic lineage are often impaired in the final stages of our lives, in particular upon the onset of neurodegeneration (Chung et al., 2018; Modic et al., 2019; Shelkovnikova et al., 2018). Two papers in this special issues have contributed to achieve a quantitative understanding of the lncRNA signatures in hESC-derived motor neurons (Biscarini et al., 2018) and generated a streamlined differentiation protocol to study cranial motor neurons (MN) differentiated from pluripotent stem cells (De Santis et al., 2018). The efficient derivation of MNs is achieved by combinatorial activity of Phox2a, Ngn2 and Isl1 transcription factors that at high efficiency (~90%) and reproducibly convert ESCs towards cranial MNs in absence of patterning molecules and without the need of viral infection. Empowered with MN differentiation protocols, Bozzoni and colleagues have characterized the lncRNAs transcriptome of mouse MNs and compared the trajectories of lncRNA expression patterns between differentiating mouse and human MNs. While sequence conservation of lncRNAs between human and mouse MNs was limited, synteny conservation and conserved expression dynamics for selected lncRNAs between the species was demonstrated. Furthermore, the authors made use of MNs derived of ALS-mouse models to identify three conserved lncRNAs that are affected in FUS ALS model through a loss-of-function mechanism.

Finally, Gennadi Glinsky and Tahsin Stefan Barakat further addressed human-specific features of pluripotency. They performed a comprehensive evolutionary analysis of the functional enhancer landscape in human embryonic stem cells to show that a subset of human enhancers undergo rapid evolution, not only between human and mouse but also between human and the other great apes. This enhancer subset may therefore be a crucial driver of human evolution (Glinsky and Barakat, 2019). Given the differences in human and mouse embryogenesis that are becoming apparent (Shahbazi and Zernicka-Goetz, 2018), these enhancers may play important roles in human-specific features of pluripotency.

References

Betschinger, J., Nichols, J., Dietmann, S., Corrin, P.D., Paddison, P.J., Smith, A., 2013. Exit from pluripotency is gated by intracellular redistribution of the bHLH transcription factor Tfe3. Cell 153, 335–347.

Biscarini, S., Capauto, D., Peruzzi, G., Lu, L., Colantoni, A., Santini, T., Shneider, N.A., Caffarelli, E., Laneve, P., Bozzoni, I., 2018. Characterization of the lncRNA transcriptome in mESC-derived motor neurons: Implications for FUS-ALS. Stem Cell Research. https://doi.org/10.1016/j.scr.2018.01.037.

Chung, C.-Y., Berson, A., Kennerdell, J.R., Sartoris, A., Unger, T., Porta, S., Kim, H.-J., Smith, E.R., Shilatifard, A., Van Deerlin, V., Lee, V.M.-Y., Chen-Plotkin, A., Bonini, N.M., 2018. Aberrant activation of non-coding RNA targets of transcriptional elongation complexes contributes to TDP-43 toxicity. Nat. Commun. 9, 4406.

De Santis, R., Garone, M.G., Pagani, F., de Turris, V., Di Angelantonio, S., Rosa, A., 2018. Direct conversion of human pluripotent stem cells into cranial motor neurons using a piggyBac vector. Stem Cell Res 29, 189–196.

Evans, M.J., Kaufman, M.H., 1981. Establishment in culture of pluripotential cells from mouse embryos. Nature 292, 154–156.

Gaspari, E., Franke, A., Robles-Diaz, D., Zweigerdt, R., Roeder, I., Zerjatke, T., Kempf, H., 2018. Paracrine mechanisms in early differentiation of human pluripotent stem cells: Insights from a mathematical model. Stem Cell Res 32, 1–7.

Glinsky, G., Barakat, T.S., 2019. The evolution of Great Apes has shaped the functional enhancers' landscape in human embryonic stem cells. Stem Cell Res 37, 101456.

Guallar, D., Wang, J., 2014. RNA-binding proteins in pluripotency, differentiation, and reprogramming. Front. Biol. 9, 389–409.

Martin, G.R., 1981. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Dev. Biol. 78, 7634–7638.

Modic, M., Grosch, M., Rot, G., Schirge, S., Lepko, T., Yamazaki, T., Lee, F.C.Y., Rusha, E., Shaposhnikov, D., Palo, M., Merl-Pham, J., Cacchiarelli, D., Rogelj, B., Hauck, S.M., von Mering, C., Meissner, A., Lickert, H., Hirose, T., Ule, J., Drukker, M., 2019. Cross-Regulation between TDP-43 and Paraspeckles Promotes Pluripotency-Differentiation Transition. Mol. Cell 74, 951–965 e13.

Shahbazi, M.N., Siggia, E.D., Zernicka-Goetz, M., 2019. Self-organization of stem cells into embryos: A window on early mammalian development. Science 364, 948–951.

Shahbazi, M.N., Zernicka-Goetz, M., 2018. Deconstructing and reconstructing the mouse and human early embryo. Nat. Cell Biol. 20, 878–887.

Shelkovnikova, T.A., Kukharsky, M.S., An, H., Dimasi, P., Alexeeva, S., Shabir, O., Heath, P.R., Buchman, V.L., 2018. Protective paraspeckle hyper-assembly downstream of TDP-43 loss of function in amyotrophic lateral sclerosis. Mol. Neurodegener. 13, 30.

Spangler, A., Su, E.Y., Craft, A.M., Cahan, P., 2018. A single cell transcriptional portrait of embryoid body differentiation and comparison to progenitors of the developing embryo. Stem Cell Res 31, 201–215.

Takahashi, K., Yamanaka, S., 2006. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Cell 126, 663–676.

ten Berge, D., Koole, W., Fuerer, C., Fish, M., Eroglu, E., Nusse, R., 2008. Wnt Signaling Mediates Self-Organization and Axis Formation in Embryoid Bodies. Cell Stem Cell 3, 508–518.

Thomson, J.A., 1998. Embryonic Stem Cell Lines Derived from Human Blastocysts. Science 282, 1145–1147.

van Mierlo, G., Wester, R.A., Marks, H., 2018. Quantitative subcellular proteomics using SILAC reveals enhanced metabolic buffering in the pluripotent ground state. Stem Cell Res 33, 135–145.

Weinberger, L., Ayyash, M., Novershtern, N., Hanna, J.H., 2016. Dynamic stem cell states: naive to primed pluripotency in rodents and humans. Nat. Rev. Mol. Cell Biol. 17, 155–169.

Zhou, X., Chadarevian, J.P., Ruiz, B., Ying, Q.-L., 2017. Cytoplasmic and Nuclear TAZ Exert Distinct Functions in Regulating Primed Pluripotency. Stem Cell Reports 9, 732–741.

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