Commentary

Stem Cell INVESTIGATION

BMP and WNT: the road to cardiomyocytes is paved with precise modulation

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Production of cardiomyocytes *in vitro* is a booming topic in the stem cell field at present. Using pluripotent cells, it is now possible to generate human cardiomyocyte-like cells for high-throughput platforms of analysis or screening. For the field of cardiology, there are immediate applications to drug discovery, including high-throughput screens and toxicity testing. Furthermore, differentiating human cells to cardiomyocytes may shed light on early stages of human fetal development, which would otherwise remain inaccessible for ethical reasons. In the effort to meet these ambitious goals, recently the quest for defined protocols with precise conditions has garnered much attention and been accompanied by significant advancements. However, the issues of high reproducibility and efficient maturation grade remain. One aspect linked to those pitfalls is the lack of comprehensive knowledge in the necessary mechanisms governing the *in vitro* differentiation of human pluripotent cells towards cardiomyocytes. This is precisely the research question that Rao and colleagues address in a recent report (1).

Using human embryonic stem cells as *in vitro* model, Rao *et al.* specifically examined the capacity of modulating BMP and WNT signaling to coax differentiating cells into the cardiac lineage. Based on the empirically optimized protocol that the group previously reported (2), the study shows that a pulse of BMP and WNT activation is necessary at an early commitment stage. Both signals cooperate to decrease mRNA levels of *SOX2*, a marker of pluripotency and regulator of differentiation (3). BMP activation stabilizes a microRNA, *miR-877*, which in turn targets *SOX2* and accelerates its mRNA degradation. In a coordinate role, WNT activation leads to expression of *EOMES*, a marker of mesendoderm specification, which in turn binds *SOX2* promoter and also inhibits *SOX2* expression. At a subsequent stage of maturation, WNT inhibition leads to increased number of differentiated cardiomyocytes. WNT inhibition leads to decreased levels of *MSX1* and *CDX2*, two genes important for fetal patterning and organogenesis (4). In this context, *MSX1* and *CDX2* repress the terminal specification of differentiating cardiac cells. Hence, timely modulation of BMP and WNT is important to remove specific roadblocks from the cardiogenic path of human pluripotent cells.

Rao and colleagues investigated mechanisms of culture conditions previously defined by empirical methods. For each putative mechanism, a combinatorial study of signal concentration led to extensive genome-wide transcriptome analyses. Once a putative pattern was defined, clonal cell lines with inducible transgenes or CRISPR-mediated gene deletion were designed and tested. The resulting body of evidence was convincing and carried information valuable not only for cardiomyocyte-oriented protocols but also for other lineages, such as combinations of BMP/WNT modulation for endodermal or ectodermal differentiation. A notable merit of the study was its addressing BMP and WNT pathways not as single-factor variables, but as interdependent signals with a bi-phasic pattern. The findings therefore point at the *in vitro* differentiation process as integrated complex of signals, rather than sum of individual actors. Moreover, the study unveiled a novel epigenetic factor, *miR-877*, as important contributor for cardiomyocyte specification. Probably the most intriguing picture emerging from this study is that modulation of BMP/WNT pathways is beneficial through the inhibition of potent repressors of the cardiogenic program, rather than the activation of specific inducers.

Notwithstanding the merits of the study, several questions remain open. For example, the role of *MSX1* and *CDX2* in this context needs further exploration. The *MSX1*- and *CDX2*-deleted lines did not demonstrate a straightforward amelioration in cardiogenic potential as expected. Moreover, further studies are required to explain how *MSX1*- and *CDX2*-dependent genes actively or passively repress cardiac induction. Furthermore, a more detailed understanding of the Activin-A and FGF pathways is needed. The cardiogenic protocol used in this study relied on a pulse of Activin-A and FGF signaling. In the initial combinatorial screening of signals, Activin-A and FGF signaling resulted cross-compensating. However, a subsequent experiment showed that pharmacological inhibitors of either of those two pathways abrogated cardiac differentiation.

Finally, a more general question is how these findings can be implemented on human induced pluripotent stem cells (iPSCs). Rao and colleagues utilized embryonic stem cells. However, iPSCs have a number of benefits including their genetic match to an individual patient genotype. Importantly, iPSCs often display biased differentiation efficiency according to the lineage of source cells (5), including the cardiac lineage (6). In this respect, the results presented in this study open the question of whether cardiac propensity of iPSC lines is measurable in inherited responsiveness to BMP and WNT signaling cascades.

This study was able to shed new light on signals and molecular cascades associated with early cardiogenic fate choice using human cells. Hence, these findings are relevant for not only cell differentiation assays, but also human development modeling. An intriguing application of this work is the generation of expandable cardiac cells *in vitro*. Viable and representative cell lines for the heart have long been a limitation for the field. At present, the number of generated cardiomyocytes from this method mainly relies on the number of pluripotent cells gathered at the protocol onset. Refining the knowledge about molecular signals will drive protocols to stabilize *in vitro* cardiac-committed cells, even those that may still proliferate. Such strategy would likely increase reliability and efficiency, and possibly maturation grade, of current *in vitro* cardiomyocyte platforms for pharmacology and tissue modeling.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Comment on: Rao J, Pfeiffer MJ, Frank S, *et al*. Stepwise clearance of repressive roadblocks drives cardiac induction in human ESCs. Cell Stem Cell 2016;18:554-6.

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