Chemicals turn human embryonic stem cells towards beta cells

Yu-Ping Yang & Chris Wright

Small-molecule library screening identifies simple imitators of the cellular signaling events that normally guide formation of the pancreas and its insulin-secreting beta cells, further enabling detailed analysis in vitro, or eventual diabetes therapies via large-scale differentiation of human stem cells.

There is an implicit potential of bringing a cellular replacement therapy for diabetes closer to reality. Using a high-content, high-throughput chemical library screen, Chen et al. identify a small-molecule inducer that acts on human embryonic stem cells (hESCs) to promote their effective conversion to a cell type that may be a key staging point along the road toward their final maturation into glucose-responsive, insulin-producing beta cells. The study also highlights the idea that recapitulating and dissecting differentiation programs in vitro will improve our knowledge of the molecular-genetic mechanisms that regulate differentiation and maintenance of many human cell and tissue types.

The endoderm germ layer, from which the pancreas emerges (Fig. 1a), forms in response to instructive signals received in the early embryo. The primitive gut-respiratory tube becomes organ-regionalized according to specific locations, and differentiates the various cell types and structures needed for each organ. Despite much study, our understanding of endoderm specialization is still superficial, but the general principle has arisen that combinations of intercellular signaling molecules have highly selective tissue-inducing effects. In addition to proteins such as fibroblast growth factor (FGF) and Hedgehog, simple chemicals such as retinoic acid can also play critical roles. Studies in various vertebrate model organisms

Yu-Ping Yang and Chris Wright are in the Department of Cell and Developmental Biology and the Vanderbilt University Program in Developmental Biology, Vanderbilt University School of Medicine, Nashville, Tennessee, USA. e-mail: chris.wright@vanderbilt.edu

Figure 1 Chemical-induced differentiation in vitro as a mimic of normal organogenesis. (a) The development of pancreas and its beta cells from endoderm in the mouse embryo (a process highly similar in humans) is shown above a hypothetical series of cell transitions caused in vivo by various intercellular signals (S1, S2 and so on). Each stage has specific combinations of transcription factors (T1, T2 and so on), which also act as cell-autonomous regulators of differentiation steps, their production being activated in response to the types of signals. Small-molecule inducers are indicated (CMixX, CMixY, CMixZ) by ‘step-around’ direct transitions, which ‘avoid’ intermediate stages. Certain chemicals (CMixY) might not instruct formation of proper pro-beta cells, but a close mimic lacking a critical transcription factor (T10 here). This possibility highlights the need to detect when small molecules direct formation of almost-normal cells with a flawed potential for forward progress, maybe never being able to make it to mature beta cells. Note that signals, transcription factors and chemical inducers sometimes appear in multiple transition steps, which suggests context-dependent functions. This diagram omits signal transduction intermediates, such as protein kinases. ICM, inner cell mass. (b) Directed differentiation via restrictive channeling. Chemical inhibitors (I1, I2 and so on) channel cells into a selected differentiation program by working at several choice points to prevent access to additional fate pathways. (c) Instruction of replication of a pro-beta cell by a small-molecule mixture (CMixZ) produces a large pool of cells, which are convertible by other compounds to finally mature beta cells (note congruence with the final step in a above).
have led to significant insights into the genetic programs regulating production of the pancreas buds and subsequent events that produce its islets of Langerhans, where the beta cells reside.2

Reproducing pancreas or beta-cell formation in vitro with a cell therapy goal in mind is being studied intensively. Recently, Baetge and colleagues3,4 showed that a sophisticated series of cocktails (signaling molecules and chemical compounds), their compositions guided largely by in vivo studies on endoderm development, can convert hESCs into cells akin to endocrine cells of the pancreatic islets. With differentiation entirely in vitro, however, endocrine cell formation was incomplete and inefficient; there were few beta cells and they were poorly glucose-responsive. But, pluripotent hESCs have the inherent capacity to become mature beta cells. Partly differentiated hESC-derived ‘pre-pancreatic’ cell clusters, when implanted into mice (in an environment of unknown signaling complexity), moved beautifully through endocrine differentiation, with the end result that the implants produced enough working beta cells to control blood glucose even when the mouse’s pancreatic beta cells were deliberately destroyed.4

The therapy problem could thus be technical: how to produce enough real beta cells in vitro for subsequent transplantation into diabetic patients. The results of Chen et al.1 are most relevant to the first step—producing large numbers of multipotent cells that can develop into all pancreatic cell types, either in vitro or after implantation-maturation in mice. The protein kinase C (PKC) activator indolactam V (ILV) was identified from screening 5,000 compounds for their ability to greatly increase the number of Pdx1-expressing cells. Pdx1 is expressed in the presumptive pancreas and adjacent tissues, and is then maintained in mature beta cells, making it a great entry point for dissecting the development of the posterior foregut endoderm. Absence of Pdx1 results in massive failure of pancreas formation in mouse and humans.

Chen et al.1 report that ILV affects a choice-point where hESC-derived endodermal progenitors must select between foregut/pancreas or another fate. Using conditions that efficiently turn hESCs into definitive endoderm, they showed that while the initial definitive endoderm had ~5% Pdx1-expressing cells, ILV caused an ~fivefold increase, with evidence that, rather than promoting proliferation of the initial 5%, ILV increased the flux of definitive endoderm toward Pdx1 positivity. ILV raised Pdx1-expressing cell numbers synergistically with FGF10, a known positive effecter on pancreas development.

How does ILV work? It may stimulate PKC, and results with PKC agonists and antagonists strengthen this hypothesis. Interestingly, PKC antagonists alone reduced Pdx1-expressing cell numbers far below the starting 5%, which suggests that PKC signaling both induces and maintains this state. A differentiation-instructive influence and PKC-FGF connection is not unprecedented; it has been reported, for example, in neural induction5 and in the developing chicken limb bud6. Nonetheless, how PKC induces specific molecular signaling is unknown. The presence of Pdx1-expressing cells (and various different presumptive endodermal types) in the starting definitive endoderm suggests that this tissue, perhaps associated with being produced in vitro, consists of meta-stable cells, ‘teetering’ on the brink of differentiation, and that ‘stochastic effects’ or cell culture conditions result in a small amount of bleed-through regional specialization and/or differentiation. ILV can clearly enhance these effects, but the degree of instructiveness and selectivity requires further study.

Additional open questions remain, some surely under study already, including determining the molecular function of the other hits from the compound screen. The sets of markers, such as transcription factors and cell surface proteins that accurately define each differentiation stage are improving, but we are still homing in on diagnostic signatures for the most important states. Defining such critical intermediates in detail will help to assure that we are on our way to making exactly the correct cell types in vitro (Fig. 1a).

It is also possible that inhibitor chemicals could be useful for directing differentiation by preventing cells from moving into undesired pathways (Fig. 1b). How to detect regionalized PKC-stimulatory effects during normal pancreas formation, and their interaction with other signaling processes within and between developing tissues, will be interesting to pursue.

With respect to hESC-derived therapies, it will be important to ensure uniform response from the cell population, to purify relevant intermediate populations and to control proliferation (Fig. 1c). Compounds that act as inducers might be the most desirable for growing progenitors suitable for maturation in vitro (possibly in novel implant sites), to leverage the self-assembling abilities of cells grown in communities. More fancifully, compounds that act as tissue-targeted drugs could theoretically be used to stimulate regeneration of a patient’s remaining beta cells, or to elicit the remarkable acinar-to-beta cell transformation7 reported recently. Although the mechanism of ILV action was determined in the current study, in the future, it is debatable as to whether or not such knowledge for other chemicals would in fact be necessary. In the meantime, chemical inducers can potentially provide an end-run around safety issues associated with hESC contact with animal-derived products. In addition, inexpensive, clean-acting chemicals might be easy to apply and remove, allowing recipe-based incubator growth of large quantities of perfect replacement cells.