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Screening-Based Chemical Approaches to Unravel Stem Cell Biology

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Cell-permeable compounds provide a convenient and efficient approach to manipulate biological processes. A number of compounds controlling stem cell self-renewal, survival, differentiation, and reprogramming have been identified through high-throughput/content screens. Using these powerful chemical tools, strategies have been developed to direct human pluripotent stem cell (hPSC) differentiation to functional cells. Recently, hPSC-derived cells and organoids are used to model human diseases, which can be adapted to a high-throughput/content platform for chemical screens. The identified compounds provide novel tools for decoding the signaling pathways regulating disease progression and candidates for facilitating future drug discovery. Moreover, humanized mouse models carrying hPSC-derived cells enable an innovative system to evaluate the long-term *in vivo* efficacy of drug candidates on human cells. In summary, screening-based chemical approaches not only expedite strategy development of controlling stem cell fates, but also provide powerful tools for dissecting the molecular mechanisms regulating disease progression.

Chemical Approaches to Maintain Stem Cell Self-Renewal

Embryonic stem cells (ESCs), the *ex vivo* equivalent of the epiblast lineage of the blastocyst, were originally derived using medium containing serum with a feeder layer of mitotically inactivated fibroblasts. Later, leukemia inhibitory factor (LIF), and bone morphogenetic protein 4 (BMP4)-SMAD signals, which are associated with feeder layer cells, were found to be essential for maintaining mouse ESC (mESC) self-renewal (Niwa et al., 1998; Ying et al., 2003). From a high-content screen, we identified a new 3,4-dihydropyrimido(4,5-d)pyrimidine analog, pluripotin/SC1, which could maintain mESCs in the undifferentiated, pluripotent state under chemically defined conditions in the absence of feeder cells, serum, LIF, and BMP4 (Chen et al., 2006). Mechanistic studies revealed that SC1 directly binds and inhibits two target proteins involved in the differentiation-inducing signaling, extracellular signal-regulated kinases (ERK) and Ras GTPase-activating protein. This study provided a conceptual advance that mESC self-renewal can be achieved through inhibiting the differentiation-inducing signals, which highlights the power of chemical approaches in dissecting the complex biology of stem cells. Later, Ying et al. (2008) reported 2i medium, using CHIR99021, a glycogen synthase kinase-3 (GSK-3) inhibitor, and PD0325901, a MEK1/2 inhibitor, to maintain mESC self-renewal.

Chemical approaches were also used to facilitate hESC self-renewal. Watanabe et al. (2007) found Y-27632, a selective Rho-associated kinase (ROCK) inhibitor, which promotes the survival of dissociated single-cell hESCs without the loss of pluripotency. Consistently, Xu et al. (2010) identified thiazovivin from a high-content chemical screen, which increases hESC single-cell survival with enhanced self-renewal. Interestingly, affinity chromatography experiments revealed that thiazovivin enhances E-cadherin stability and cell-cell interactions through the inhibition of ROCK.

Synthetic Small Molecules Facilitate Achieving "Stemness"

In last two decades, a major breakthrough in the stem cell field is cellular reprogramming, in which lineage-committed cells overcome their intrinsic lineage-restriction upon exposure to a specific set of signals, and reverse back to the multipotent or even pluripotent stage. In 2004, we performed a high-throughput screen using C2C12 myoblasts and identified reversine, a 2,6-disubstituted purine, which reverses lineage-committed myoblasts back to the multipotent stage. The reversine-treated myoblasts can efficiently differentiate into osteoblasts and adipocytes only upon exposure to the appropriate differentiation conditions (Chen et al., 2004) (Table 1). Takahashi and Yamanaka (2006) reported the groundbreaking work to reprogram fibroblasts to the pluripotent stage by using overexpression of *Oct4*, *Sox2*, *Klf4*, and *c-Myc*. Subsequently, many chemical screens were carried out to identify the small molecules to replace the transcriptional factors in a stepwise manner. Using a phenotypic screen with *Oct4* promoter-driven GFP expression, Shi et al. (2008) identified BIX-01294 and BayK8644, which enable reprogramming of *Oct4/Klf4*-transduced mouse embryonic fibroblasts. In a focused screen, Huangfu et al. (2008a) found that valproic acid (VPA), a histone deacetylase (HDAC) inhibitor, improves reprogramming efficiency and induces reprogramming in the presence of *c-Myc*. Moreover, VPA enables the reprogramming of primary human fibroblasts with only two factors, *Oct4* and *Sox2* (Huangfu et al., 2008b). Through another chemical screen, kenpaullone was identified to enable the reprogramming of mouse embryonic fibroblasts in the absence of *Klf4* (Lyssiotis et al., 2009). Ichida et al. (2009) identified RepSox, which replaces *Sox2* during reprogramming by inhibiting

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The major research interest in the Chen Laboratory at Weill Cornell focuses on studying the role of genetic factors and environmental factors on pancreatic β cells in type 1 and 2 diabetes. Dr. Chen has published more than 30 papers in peer-reviewed high impact journals, such as *Nature Medicine*, *Cell Stem Cell*, *Nature Chemical Biology*, etc. She has received many awards including New York Stem Cell Foundation Robertson Investigator, American Diabetes Association (ADA) Junior Faculty Award, ADA Innovative Award, NIH Director's New Innovator Award, American Association for Cancer Research Career Development Award, and ISSCR Dr. Susan Lim Award for Outstanding Young Investigator, etc.

transforming growth factor β (TGF-β) signaling. Li et al. (2011b) reported that the combination of VPA, tranylcypromine, CHIR99021, and 616452, was sufficient to induce reprogramming with a single transcription factor, *Oct4*. The treatment of EPZ004777, an inhibitor of the histone H3K79 methyltransferase DOT1-like, significantly increases the yield of induced pluripotent stem cell (iPSC) colonies, and could be substituted for *Klf4* and *c-Myc* (Onder et al., 2012). Finally, Hou et al. (2013) reported a combination of seven compounds identified from a chemical screen, including VPA, tranylcypromine, CHIR99021, 616452, forskolin, 3-deazaneplanocin, and PD0325901, which induces mouse somatic cell reprogramming to PSCs in the absence of transgene overexpression.

In addition to cellular reprogramming, chemical screens were used to identify the compounds that facilitate the conversion between prime and naive pluripotent status. By screening compounds that activate the *OCT4* distal enhancer, Yang et al. (2017) established a minimal condition consisting of human LIF, CHIR99021, (S)-(+)-dimethindene maleate, and minocycline hydrochloride. This minimal condition supports the conversion and long-term maintenance of extended PSCs, which are capable of chimerizing both embryonic and extraembryonic tissues.

Directed hPSC Differentiation Using Chemical Approaches

Human pluripotent stem cells (hPSCs) provide unlimited starting material to generate differentiated cells that can be used to build a functional organ. Essential to this pursuit is an efficient way to differentiate hPSCs into specific types of mature cells. The rational-based chemical approach has been widely used to manipulate the key pathways involved in PSC differentiation. For example, CHIR99021, which activates the Wnt pathway, is commonly used to promote hPSC differentiation (Davidson et al., 2012). Chambers and coworkers found that the combination of SB431542, a TGF-β inhibitor, and, dorsomorphin, a BMP inhibitor, efficiently induces hESC differentiation toward the neural lineage (Chambers et al., 2009; Kim et al., 2010). Li et al. (2011a) reported that the combination of CHIR99021, SB431542, and compound E can efficiently convert hESCs into homogeneous primitive neuroepithelium. Fattahi et al. (2016) found that CHIR99021 promotes the differentiation to neural crest, and that retinoic acid facilitates differentiation to enteric neural crest lineages. In addition, retinoic acid, LDN193189 (a BMP pathway inhibitor), KAAD-cyclopamine (a sonic hedgehog pathway inhibitor) are used to promote pancreatic induction from hESCs (D'Amour et al., 2006). Although chemical agonists and antagonists provide powerful tools for hPSC differentiation, chemical agonists of several key development pathways, such as BMP, TGF-β, epidermal growth factor (EGF), and fibroblast growth factor (FGF), are still missing. Using a high-throughput screening platform based on C2C12 cells containing *Id2-Luc* reporter, we discovered PD407824, a checkpoint kinase 1 inhibitor that increases the sensitivity of cells to sub-threshold BMP4. We showed the utility of PD407824 in the directed differentiation of hESCs toward mesoderm or cytotrophoblast stem cells (Feng et al., 2016).

Although rational-based chemical approaches have provided useful tools to induce hPSC differentiation, the molecular mechanism controlling organogenesis is not fully understood. Screening-based chemical approaches can also be applied to identify the small molecules directing stem cell differentiation (Figure 1). More importantly, the identified small molecules function as tools to discover the pathways controlling cell fate decision. For example,

Table 1. Summary of Compounds Identified from Stem Cell-Based Screens

Compound	Starting Population	Screening Readout	Biological Process Affected	Mechanism of Action	References
Cellular Reprogramming					
Reversine	C2C12 cells	alkaline phosphatase as an osteoblast marker	reprogramming from lineage-committed myoblasts to multipotent stage	a dual inhibitor of MEK1 and nonmuscle myosin II heavy chain	Chen et al. (2004)
BIX-01294	Oct4, Klf4-transduced mouse embryonic fibroblasts (MEFs)	ESC colony and staining with alkaline phosphatase as a pluripotency marker	reprogramming of Oct4/Klf4-transduced mouse embryonic fibroblasts	a potential G9a histone methyltransferase inhibitor	Shi et al. (2008)
BayK8644				a potential L-channel calcium agonist	
Valproic acid	Oct4-, Sox2-, Klf4-, and c-Myc-transduced MEFs	the percentage of Oct4-GFP$^+$ cells	improve reprogramming efficiency of mouse and human fibroblasts	a potential histone deacetylase inhibitor	Huangfu et al. (2008a, 2008b)
Kenpaullone	Oct4-, Sox2-, and c-Myc-transduced MEFs	Nanog-Luc activity	induce reprogramming in the absence of Klf4	a potential GSK-3β inhibitor	Lyssiotis et al. (2009)
RepSox	Oct4-, Klf4-, and c-Myc-transduced MEFs	the number of Oct4-GFP + colonies	induce reprogramming in the absence of Sox2	inhibit TGF-β signaling	Ichida et al. (2009)
Forskolin	Sox2-, Klf4-, and c-Myc-transduced MEFs	the number of Oct4-GFP + colonies	induce reprogramming in the absence of Oct4	a potential cAMP activator	Hou et al. (2013)
(S)-(+)-Dimethindene maleate	primed H9 hESCs	activate the OCT4 distal enhancer	support the conversion and maintenance of dome-shaped hPSCs from primed hPSCs	a potential M$_2$ muscarinic receptor antagonist	Yang et al. (2017)
minocycline hydrochloride				a potential metalloproteinase inhibitor	
Directed Differentiation					
PD407824	C2C12 cells	Id2-Luc reporter	synergize with BMP4 to direct hPSC differentiation toward mesoderm or cytotrophoblast stem cells	inhibit checkpoint kinase 1	Feng et al. (2016)
Atauprimide	mESCs	immunostaining using SOX17 antibody	prime mouse and human ESC differentiation	interact with NME2 and inhibit nuclear localization	Zhu et al. (2009)
IDE1	mESC	Sox17 promoter-driven tdTomato	induce nearly 80% of ESCs to form definitive endoderm	activate TGF-β signaling	Borowiak et al. (2009)
IDE2					
Indolactam V	hESC-derived endodermal cells	immunostaining using PDX1 antibody	induce differentiation toward pancreatic progenitors	activate PKC	Chen et al. (2009)

(Continued on next page)

Table 1. Continued

| Compound	Starting Population	Screening Readout	Biological Process Affected	Mechanism of Action	References
H1152	hESC-derived pancreatic progenitors	immunostaining using insulin antibody	increase the generation of insulin-secreting cells	inhibit ROCKII	Ghazizadeh et al. (2017)
CI-994	hPSC-derived immature hepatocytes	albumin-Venus reporter	promote hPSC differentiation toward hepatocyte-like cells	inhibit histone deacetylase	Li et al. (2018)
SU5402 CHIR99021 DAPT	hPSC-derived neuroectoderm	immunostaining using PAX6 and β3-tubulin antibodies	promote hPSC differentiation toward neurons	a potential VEGFR inhibitor / a potential GSK-3β inhibitor / a potential Notch inhibitor	Chambers et al. (2012)
Selamectin	mESC	immunostaining using tyrosine hydroxylase antibody	transform mPSCs into neurons	a potential GABA$_A$ agonist	Sun et al. (2013)
Phenanthroline	hESC-derived lens placode	*SIX1 H2B::GFP* reporter	promote differentiation from nonneural ectoderm to cranial placode	a potential metalloprotease inhibitor	Tchieu et al. (2017)
KY02111	monkey ESCs	αMHC promoter-driven EGFP	PSC differentiation toward cardiomyocytes	NA	Minami et al. (2012)
Sodium nitroprusside	mESC-derived mesodermal cells	CCS:lacz reporter	promote the generation of cardiac Purkinje fiber-like cells	increase intracellular cAMP	Tsai et al. (2015)
Stem Cell-Based Disease Models					
SKF-86466	wild-type and familial dysautonomia iPSC-derived neural crest cells	alamarBlue to detect cell survival qRT-PCR to detect *IKBKAP* expression	rescue IKAP protein expression and the disease-specific loss of autonomic neuronal marker expression	a potential α$_2$-adrenoceptor antagonist	Lee et al. (2012)
Kenpaullone	wild-type and SOD1G93A HB9::GFP mESC-derived motor neuron	imaging-based assay to count GFP$^+$ cell number	prolong the healthy survival both mouse and human motor neurons	a potential dual inhibitor of GSK-3 and HGK kinases	Yang et al. (2013)
Alsterpaullone	fibroblasts from parental SMA carriers	immunostaining using SMN antibody	increase cellular SMN	a potential GSK-3 inhibitor	Makhortova et al. (2011)
E34	a co-culture of mESC-derived motor neurons, astrocytes, and interferon-γ- and lipopolysaccharide-activated microglial cells	the total neurite length of all neurites of the motor neurons	prevent the neurons from cytokine and lipopolysaccharide-induced degeneration	inhibit nitric oxide synthase	Hoing et al. (2012)

Table 1. *Continued*

Compound	Starting Population	Screening Readout	Biological Process Affected	Mechanism of Action	References
Pepstatin A	*EDNRB*-null mutant hESC-derived enteric nervous system precursors	cell migration assay	rescue loss of *EDNRB*-induced cell migration defects	inhibit BACE2	Fattahi et al. (2016)
Avermectins	TS21 iPSC-derived cortical neurons	the activity of lactate dehydrogenase in medium	increase the relative production of short Aβ peptides at the expense of longer, potentially more toxic peptides	NA	Brownjohn et al. (2017)
Gardiac glycosides	familial hypercholesterolemia iPSC-derived hepatocyte-like cells	ELISA-based assay to detect apolipoprotein B (apoB) production	reduce the production of apoB	potential inhibitors of the membrane sodium-potassium (NA^+-K^+) pump	Cayo et al. (2017)
T5524	*CDKAL1*$^{-/-}$ hESC-derived pancreatic β-like cells	staining with PI and insulin antibody	rescues loss of *CDKAL1* induced increased sensitivity to glucolipotoxcity	inhibit the FOS/JUN pathway	Zeng et al. (2016)
Galunisertib	*GLIS3*$^{-/-}$ hESC-derived pancreatic β-like cells	staining with PI and insulin antibody	rescues loss of *CDKAL1* induced pancreatic β cell death	inhibit TGF-β pathway	Amin et al. (2018)
Emricasan	hNPCs	caspase-3/7 activity assay	block ZIKV-induced hNPC death	a pan-caspase inhibitor	Xu et al. (2016)
Niclosamide			inhibits ZIKV replication	NA	
Hippeastrine hydrobromide	hNPCs	staining with ZIKV antibody	eliminate ZIKV infection	NA	Zhou et al. (2017)
Geneticin	familial adenomatous polyposis-iPSC-derived colonic organoids	staining with CDX2 and Ki67 antibodies	rescue early stop codon caused APC protein decrease and increased cell proliferation	NA	Crespo et al. (2017)

NA, not applicable.
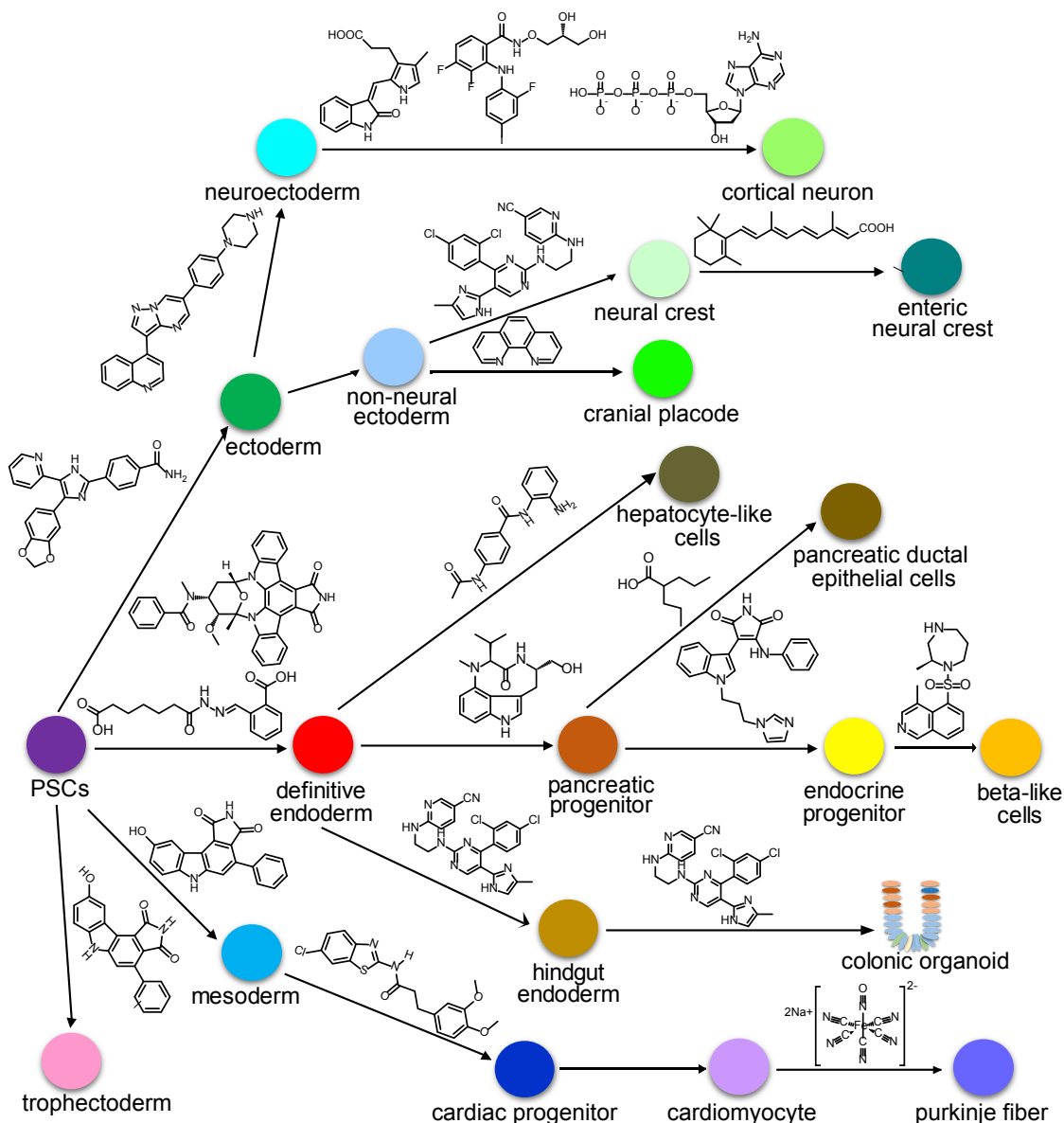
Figure 1. Summary of Small Molecules Regulating Pluripotent Stem Cell Differentiation

Desbordes et al. (2008) reported a high-throughput screen for the identification of compounds regulating self-renewal and differentiation in hESCs. By monitoring SOX17 expression, Zhu et al. (2009) screened 20,000 compounds and identified stauprimide, which promotes efficient induction of SOX17 in both mouse and human ESCs synergistically with low concentrations of activin A. Using *Sox17* promoter-driven tdTomato as a reporter, Borowiak et al. (2009) screened 4,000 compounds and found two small molecules, inducer of definitive endoderm 1 (IDE1) and IDE2, that induce endoderm differentiation in both mESCs and hESCs. In addition, we performed a high-con-

tent chemical screen using hESC-derived definitive endoderm and identified (−)-indolactam V, a protein kinase C (PKC) activator, which promotes the generation of pancreatic progenitors (Chen et al., 2009). Now, a PKC activator has become a standard component of most pancreatic β cell differentiation protocols (Maehr et al., 2009; Pagliuca et al., 2014; Rezania et al., 2014; Vegas et al., 2016). Recently, we screened 2,000 compounds and found that H1152, a ROCKII inhibitor, increases the generation of insulin-secreting cells from hPSCs and improves β cell maturation (Ghazizadeh et al., 2017). Using an albumin-Venus reporter line, Li et al. (2018) identified, CI-994, an HDAC

inhibitor that promotes hPSC differentiation toward hepatocyte-like cells.

Both high-throughput and focused chemical screens were used to develop compounds or compound combinations directing hPSC differentiation to ectodermal lineages. Using a focused screen, Chambers et al. (2012) developed a combination of five chemical pathway inhibitors, LDN193189 and SB431542, SU5402, CHIR99021, and DAPT, which yields hPSC-derived neurons at >75% efficiency. Sun et al. (2013) reported a high-content screen, which revealed that selamectin, an anti-helminthic therapeutic compound, promotes neural differentiation of PSCs. A focused screen by combinatorial application of six pathway inhibitors revealed that XAV939 promotes anterior central nervous system identity; SU5402 and PD0325901 accelerate hPSC differentiation toward neuroectodermal lineages; and the highly transient anterior neuroectodermal precursor state is driven toward post-mitotic cortical fates in the presence of DAPT and SU5402/PD0325901 (Qi et al., 2017). Tchieu et al. (2017) screened the LOPAC library (Sigma) and identified phenanthroline, a metal chelator that can act as a metalloprotease inhibitor, enhances cranial placode differentiation.

Finally, high-throughput or high-content screens also facilitate discovery of compounds inducing mesodermal differentiation. A cell-based screen led to the discovery of KY02111, which promotes hPSC differentiation to cardiomyocytes (Minami et al., 2012). We carried out a high-throughput screen using CCS:lacz and Contactin2:egfp reporter ESC lines, and discovered sodium nitroprusside efficiently promotes the generation of cardiac Purkinje fiber-like cells (Tsai et al., 2015).

hPSC-Derived Cells for Disease Modeling and Drug Screening

Patient-specific hPSCs represent a novel system for modeling human genetic diseases, which can be adapted to a high-throughput/content manner to screen for gene/mutation-specific drug candidates. The identified drug candidates will also facilitate dissecting entangled mechanisms under specific pathological settings. For example, Lee et al. (2012) reported a high-content screen using neural crest precursors derived from iPSCs that were generated from individuals with familial dysautonomia, a rare, fatal genetic disorder affecting neural crest lineages. One hit compound, SKF-86466, rescues IKAP protein expression and the disease-specific loss of autonomic neuronal marker expression. By applying a small-molecule survival screen using motor neurons from both wild-type and mutant $SOD1$ (a gene associated with amyotrophic lateral sclerosis) mESCs, Yang et al. (2013) discovered that kenpaullone, a dual inhibitor of GSK-3 and HGK (hematopoietic progenitor kinase) kinases, prolongs the healthy survival both

mouse and human motor neurons. Spinal muscular atrophy results from mutations that lead to low levels of the ubiquitously expressed protein, survival of motor neuron (SMN). Makhortova et al. (2011) executed an image-based screen and discovered compounds targeting the receptor tyrosine kinase-phosphatidylinositol 3-kinase-AKT-GSK-3 signaling cascade, which increase cellular SMN. A neuroprotective screen was carried out by adding interferon-γ- and lipopolysaccharide-activated microglial cells into the co-culture of mESC-derived motor neurons and astrocytes. This screen identified a number of neuroprotective small molecules that prevented the neurons from degeneration through diverse mechanisms, including inhibition of nitric oxide production by microglia, stimulation of the nuclear factor erythroid 2-related factor 2 pathway in microglia and astrocytes, and direct protection of neurons from cell death (Hoing et al., 2012). By modeling Hirschsprung disease-related migration defects using $EDNRB$-null hESC-derived mutant enteric nervous system precursors, we identified pepstatin A as a candidate therapeutic target for the treatment of Hirschsprung disease (Fattahi et al., 2016). Amyloid precursor protein (APP) processing and the accumulation of APP-derived amyloid β peptides are key processes in Alzheimer's disease. Brownjohn et al. (2017) designed a phenotypic chemical screen to identify modulators of APP processing in trisomy 21/Down syndrome neurons, a complex genetic model of Alzheimer's disease. Avermectins, which are commonly used anthelmintics, were found to increase the relative production of short Aβ peptides at the expense of longer, potentially more toxic peptides. Finally, using hepatocyte-like cells generated from homozygous familial hypercholesterolemia iPSCs, Cayo et al. (2017) identified cardiac glycosides that can potentially be repurposed to lower serum low-density lipoprotein cholesterol.

In addition to modeling monogenic diseases, hPSC-derived cells can also be used to study the complicated diseases caused by a combination of genetic susceptibility and environmental factors. Genome-wide association studies (GWAS) have generated a large database of disease-associated SNPs and loci. One major challenge is to construct a robust system to systematically evaluate the role of these SNPs/loci using disease-relevant cells. Using isogenic hESC-derived pancreatic β-like cells, we found that mutations in $CDKAL1$, $KCNQ1$, and $KCNJ11$, diabetes-associated genes identified from GWAS, led to impaired glucose secretion. $CDKAL1$ mutant insulin$^+$ cells were also hypersensitive to glucolipotoxicity. A high-content chemical screen identified T5524 that rescues $CDKAL1$-specific defects *in vitro* and *in vivo* by inhibiting the FOS/JUN pathway (Zeng et al., 2016). Using these isogenic mutant hESCs, we found an unexpected interaction of two diabetes-associated genes, $CDKAL1$ and *metallothionein* 1E *(MT1E)*. Forced
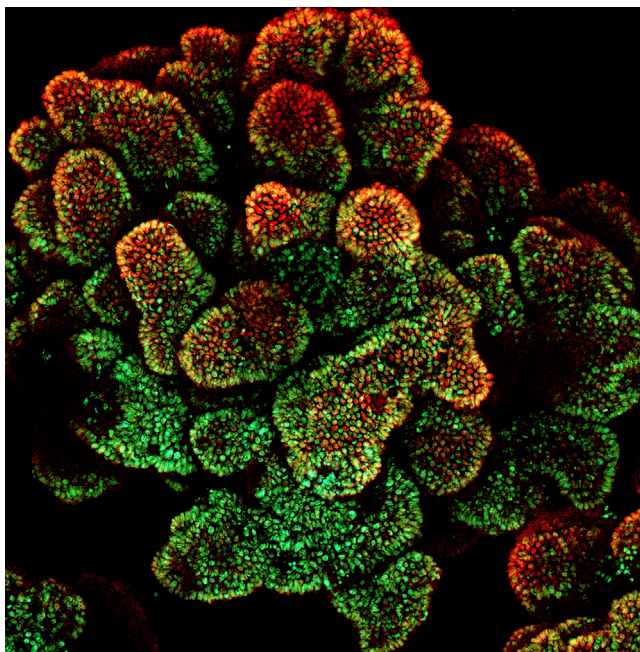
Figure 2. Projection Image of Colonic Organoids Derived from FAP-iPSCs
Red, CDX2; green, CCND1.

MT1E expression rescues both hypersensitivity of *CDKAL1* mutant cells to glycolipotoxicity and pancreatic β cell dysfunction (Guo et al., 2017). We also discovered the haploinsufficiency and dosage-sensitive requirements for *GATA6* and *GATA4*, two diabetes-associated genes, in the formation of both definitive endoderm and pancreatic progenitor cells (Shi et al., 2017). Finally, we studied *GLIS3*, one of the only two genes associated with type 1, type 2, and neonatal diabetes. Using a "minimal component" protocol to generate mono-hormonal pancreatic β-like cells, we discovered that $GLIS3^{-/-}$ hESCs show impaired differentiation with significant death of β-like cells. Furthermore, we performed a high-content chemical screen and identified a drug candidate targeting the TGF-β pathway, which rescues mutant *GLIS3*-associated β cell death both *in vitro* and *in vivo* (Amin et al., 2018). These examples highlight the power of isogenic hPSCs to evaluate GWAS-identified genetic factors and to identify novel gene/mutation-specific drug candidates for precision medicine.

Finally, hPSC-derived cells also facilitate the drug discovery and evaluation for infectious diseases. A focused screen using hiPSC-CMs infected with a luciferase-expressing coxsackievirus B3 strain was used to evaluate anti-viral compounds including interferon β1, ribavirin, pyrrolidine dithiocarbamate, and fluoxetine (Sharma et al., 2014). A recent large outbreak first observed in Brazil brought Zika

virus (ZIKV) to the forefront of public health attention. ZIKV infection is associated with an increased incidence of congenital defects, most prominently microcephaly (Mlakar et al., 2016; Rasmussen et al., 2016). In infected adults, ZIKV is associated with serious neurological complications such as meningoencephalitis and myelitis (Cao-Lormeau et al., 2016; Carteaux et al., 2016; Mecharles et al., 2016; Parra et al., 2016). The World Health Organization and the NIH have compelled the scientific community to find solutions to the ZIKV threat. Xu et al. (2016) used hPSC-derived human cortical neural progenitors (hNPCs) to screen for anti-ZIKV drugs, and identified emricasan, a pan-caspase inhibitor, which protects hNPCs; cyclin-dependent kinases inhibit ZIKV replication; and niclosamide inhibits ZIKV replication. Concurrently, we performed a high-content screen and identified a novel anti-ZIKV compound, hippeastrine hydrobromide. Strikingly, hippeastrine hydrobromide suppresses viral propagation when administered to adult mice with active ZIKV infection, highlighting its therapeutic potential (Zhou et al., 2017).

hPSC-Derived 3D Organoids for Disease Modeling and Drug Screening

In last decade, significant progresses has been made in 3D organoids. Strategies have been reported to differentiate hPSCs to 3D self-organizing organoids representing intestinal (Spence et al., 2011), gastric (McCracken et al., 2014), brain (Lancaster et al., 2013), liver (Takebe et al., 2013), lung (Dye et al., 2015), pancreas (Huang et al., 2015), and esophageal (Trisno et al., 2018) "mini-organs." Concurrently, we (Crespo et al., 2017; Munera et al., 2017) reported strategies to efficiently generate colonic organoids (COs) from hESCs. We made COs from the iPSCs derived from familial adenomatous polyposis (FAP) patients, which show enhanced WNT activity and increased epithelial cell proliferation (Figure 2). A focused screen identified geneticin targets abnormal WNT activity and thereby corrects the deregulated proliferation specific to FAP COs (Crespo et al., 2017). In addition to hPSC-based organoids, organoids derived from primary tumor tissues were also used for drug screens. Broutier et al. (2017) developed a platform to propagate primary liver cancer (PLC) organoids. Screening using PLC-derived organoids identified an ERK inhibitor, SCH772984, as a potential therapeutic agent for PLC. Finally, we evaluated the anti-ZIKV compounds identified from a chemical screen and showed that hippeastrine hydrobromide rescues ZIKV-induced growth and differentiation defects in human fetal-like forebrain organoids, which highlights the value of human forebrain organoids in long-term evaluation of drug candidates in infectious diseases (Zhou et al., 2017).

Humanized Animal Models for Long-Term *In Vivo* Drug Evaluation

Animal models have proven to be a powerful tool in drug discovery. However, due to the species differences, some animal models fail to precisely predict the clinical outcome, which is one of the major reasons of the high failure rate of clinical trials. There is a strong need to develop more predictive *in vivo* models to evaluate the efficacy, pharmacokinetic profile, and toxicological properties of leading compounds. Humanized mice carrying hPSC-derived cells will provide a powerful tool to evaluate the long-term *in vivo* efficacy of drug candidates on human cells. In our recent studies, we created humanized mice carrying hESC-derived pancreatic β-like cells. The mouse pancreatic β cells were destroyed by the administration of streptozotocin. Thus, the humanized mice depend on the transplanted human pancreatic β-like cells to maintain glucose homeostasis. We found that the mice transplanted with *CDKAL1*$^{-/-}$ hESC-derived cells show impaired glucose secretion, coinciding with defective glucose homeostasis. The humanized mice were applied to evaluate the drug candidate identified from a high-content screen. We found that T5224 treatment significantly increases the level of insulin secretion and restores the capacity of the humanized mice to maintain glucose homeostasis (Zeng et al., 2016). In another study using humanized mice transplanted with *GLIS3*$^{-/-}$ hESC-derived pancreatic β-like cells, we found that galunisertib rescues cell death induced by loss of *GLIS3 in vivo* (Amin et al., 2018). Using avatar mice harboring hepatocyte-like cells generated from homozygous familial hypercholesterolemia iPSCs, Cayo et al. (2017) showed that cardiac glycosides reduce the production of apolipoprotein B in the serum of humanized mice. Finally, Verissimo et al. (2016) used xenotransplanted mice carrying RAS mutant organoids to confirm the growth arrest effect of pan-HER/MEK combination therapy.

Conclusion and Prospective

High-throughput/content chemical screening is an emerging, critical approach to identify the small molecules controlling stem cell self-renewal, differentiation, and reprogramming. Using the small molecules identified from focused or unbiased screens, significant progress has been made to derive functional cells or organoids from hPSCs. However, several issues remain to be addressed to apply chemical approaches for broader applications. First, a better chemical toolkit to manipulate developmental pathways, such as chemical agonists of BMP, TGF-β, EGF, and FGF signals, are required. Second, additional chemical screens are needed to identify small molecules inducing differentiation to some poorly characterized cell types, such as cells in the cardiac conduction system, neuroendo-

crine cells, etc. Third, maturation is still a major concern for most directed differentiation protocols. With the appropriate experimental design, chemical screens will expedite the development of strategies promoting cell maturation.

hPSC-derived cells or organoids are widely used to study the role of genetic and environmental factors in human disease progression, which could provide a source of cells for large-scale drug discovery. Indeed, the chemical screens using hESC/iPSC-derived cells or organoids have identified candidates that help future drug development. In the meanwhile, these identified small molecules facilitate dissecting the molecular mechanism under pathological conditions. Most of current screens using hPSC-derived cells are phenotypic-based screens. Additional work needs to be done to further improve the throughput of these types of screens. Moreover, hPSC-derived 3D organoids show large variation due to their complicated structure. Appropriate assays need to be designed to adapt the organoid-based screen to a high-throughput/content manner. Finally, initial attempts of humanized mice have proven the power of this innovative model to evaluate long-term drug efficacy on human cells *in vivo*. Improved humanized mouse models, which contain multiple human organs, and/or the human immune system, will facilitate establishing more predictive *in vivo* models for drug discovery.

In summary, screening-based chemical approaches have been proven to be a powerful tool for controlling stem cell fate decisions, as well as decoding complicated disease progression. With the discovery of novel hPSC differentiation strategies, disease models, and humanized mouse models, the high-throughput/content chemical screens will significantly contribute to the discovery of a novel "toolkit" for regenerative medicine and precision therapy.

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