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MEETING REVIEW

At new heights – endodermal lineages in development and disease

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ABSTRACT

The endoderm gives rise to diverse tissues and organs that are essential for the homeostasis and metabolism of the organism: the thymus, thyroid, lungs, liver and pancreas, and the functionally diverse domains of the digestive tract. Classically, the endoderm, the 'innermost germ layer', was in the shadow of the ectoderm and mesoderm. However, at a recent Keystone meeting it took center stage, revealing astonishing progress in dissecting the mechanisms underlying the development and malfunction of the endodermal organs. *In vitro* cultures of stem and progenitor cells have become widespread, with remarkable success in differentiating three-dimensional organoids, which – in a new turn for the field – can be used as disease models.

KEY WORDS: Endoderm, Keystone, Development, Disease, Organogenesis, Stem cells, Progenitors

Introduction

The first Keystone conference on Endoderm Lineages in Development and Disease was held in February 2015 at the mountain resort of Keystone in Colorado, USA. The conference was organized by Hans-Willem Snoeck, Lori Sussel (both Columbia University, New York, USA), James Wells and Aaron Zorn (both Cincinnati Children's Hospital, OH, USA), who had assembled an exciting, timely and diverse program. It attracted about 150 researchers from many countries, discussing both historical and new ideas around the endoderm – ranging from germ layer specification, organ induction, expansion and morphogenesis to cell fate choices, functional differentiation and disease. Questions were asked in different species *in vivo*, but the meeting also saw a fair number of studies that are developing models of endoderm organogenesis in culture. As the endoderm gives rise to many diverse organs, which are themselves often topics of specialized conferences, this Keystone conference was unique in covering most areas of endoderm organogenesis. This was reflected by lively 'cross-organ' discussions and remarkably high attendance at poster sessions. Here, we summarize some of the highlights and emerging themes of the meeting.

Endoderm induction and patterning

Cell fate restriction occurs in a sequential fashion. A major early choice is the specification of endoderm and its segregation from mesoderm and ectoderm, for which there are multiple levels of control: chromatin state, priming pioneer transcription factors, other transcription factors and inductive extracellular signals. Determining how these players operate and interact is key to understanding differentiation of organ-specific stem cells, as well as for achieving and controlling reprogramming.

A morphological hallmark of the differentiation process is the transition from large euchromatic nuclei to a more compact nuclear architecture. Examining this process in *C. elegans*, where plasticity becomes significantly restricted with the onset of gastrulation, Susan Mango (Harvard University, Boston, MA, USA) reported a concomitant increase in chromatin organization, indicating that most of the chromatin compacts over time, as lineages are established.

The specific loci defining endoderm specification were studied by Ken Cho's group (University of California, Irvine, USA), who used genome-wide mapping of transcription factor binding in *Xenopus* to address how transcription factors cooperate during mesendoderm induction by Nodal. This work suggests different roles of Nodal mediators Foxh1 and Smad2/3, including Smad2/3-independent preloading of Foxh1 onto the genome, which is often associated with Oct4/Pou5f binding. Subsequently, Smad2/3 binds to a subset of these sites, which are negatively regulated by Oct4/Pou5 binding (Chiu et al., 2014).

At an early stage, the endoderm is regionalized into several organ primordia, a step that includes dynamic signaling between mesenchyme and endoderm. Combining embryonic manipulation in frog embryos with mouse genetics, Aaron Zorn and John Shannon (Cincinnati Children's Hospital, OH, USA) outlined the spatiotemporally tightly controlled sequence of retinoic acid (RA), hedgehog (HH) and Wnt2/2b signaling that coordinates endodermal competence and inductive signals to establish the lungs (Havrilak and Shannon, 2015). In some cases, organs form from multiple primordia, which further complicates the induction process. For example, Kimberly Tremblay (University of Massachusetts, Amherst, USA) showed by fate mapping and laser ablation, that the lateral progenitors form the majority of the liver bud and require vascular endothelial growth factor (VEGF) for survival. By contrast, the medial progenitors contribute to the anterior bud and require fibroblast growth factor (FGF) signaling for specification (Wang et al., 2015b). It is unknown whether the progeny of these distinct progenitor populations produce functionally equivalent hepatic tissue.

The liver and ventral pancreas arise from closely related territories in the embryo and share some induction events (Rodriguez-Seguel et al., 2013). Francesca Spagnoli (Max-Delbrück Center, Berlin, Germany) discussed two TALE transcription factors that serve as a switch between liver and pancreas fate in organ specification. When deleted in mouse embryos, they form enlarged liver domains at the expense of the pancreas, suggesting that they promote pancreas formation by repressing liver fate. Moreover, expression in cultured hepatocytes or by AVV injection in the adult organ demonstrated their potential to reprogram liver cells to a pancreatic identity. Lori Sussel presented another such example, showing that when GATA4 and GATA6 are deleted in the pancreatic bud or the endoderm, the pancreatic bud remains small and acquires the fate of neighboring

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tissues: stomach for the dorsal pancreas and intestine for the ventral pancreas (Xuan et al., 2012). This indicates the importance of GATA4/6 for pancreas specification.

Expansion and differentiation of endoderm organs

Organ formation requires that progenitors differentiate into specialized cell types, but also that an appropriate number of progenitors is maintained to ensure organ expansion. To address this issue in the pancreas, Anne Grapin-Botton and co-workers (University of Copenhagen, Denmark) traced single cells by three dimensional live imaging and clonal analysis. Monitoring symmetric and asymmetric division patterns of single progenitors, they found that stochastic priming of progenitors mediates endocrine cell differentiation and controls the balance between renewal and differentiation (Kim et al., 2015).

Multiple talks addressed chromatin-based mechanisms of regulating differentiation. Allen Wang (University of California, San Diego, USA) showed how pancreas enhancers acquire a poised chromatin state prior to their activation. He proposed that annotation of these poised enhancers in early endodermal intermediates can be predictive of future cell fate, such as pancreas, liver and lung (Wang et al., 2015a). Nisha Patel from the same group described that lysine-specific histone demethylase 1 appears to prime pancreatic progenitors towards an endocrine fate.

In the lungs, the first lineage choice is the segregation between proximal and distal progenitors. Edward Morrisey (University of Pennsylvania, Philadelphia, USA) reported that histone deacetylases 1 and 2 redundantly maintain the proximal domain. At the same stage, Wellington Cardoso (Columbia University Medical Center, New York, USA) showed that activity of the Hippo pathway mediator YAP is needed to initiate a program of proximal progenitor cell specification that ultimately generates the airway epithelium and limits expansion of the distal domain (Mahoney et al., 2014).

At the next step, the cell types are specified within each of these domains. Both Morrisey and Marie-Liesse Asselin-Labat (The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia) showed that EZH2, a component of the PRC2 complex, represses basal cell genes in the proximal airways but promotes the secretory club cell lineage (Galvis et al., 2015; Snitow et al., 2015). Morrisey stressed the general importance of transcriptional repressors in lineage switches and developed another example where deletion of four alleles of *Foxp1/2/4* leads to an upregulation of goblet fate at the expense of club cells and subsequent asthma caused by neuropeptide Y secretion. Meanwhile, Cardoso described a later function for YAP as a crucial factor for club and ciliated cell differentiation.

Daniel Swarr and Michael Herriges (both University of Pennsylvania, Philadelphia, USA) showed that the lncRNAs *Falcor* and *Nanci* are associated with the *Foxa2* and *Nkx2.1* loci, respectively; they control their expression levels, and thereby early endoderm and lung development (Herriges et al., 2014). Indeed, roles for lncRNAs in controlling cell fate were an emerging theme of the meeting, also addressed by Kaveh Daneshvar (Harvard Medical School, Boston, MA, USA) looking at early endoderm differentiation.

Tissue and organ morphogenesis

Organ formation requires not only the differentiation of the right cell types, but also cell movement and reorganization, which was the focus of a dedicated workshop. Live-imaging was an essential component of most presentations. Sonja Nowotschin (Sloan Kettering Institute, New York, USA) described essential functions for the transcription factor SOX17 that enable endodermal cells to undergo mesenchymal-epithelial transition, integrate into the visceral endoderm and segregate from the mesoderm by assembling a basement membrane between both tissues (Viotti et al., 2014).

Examining endoderm development in *Drosophila*, Kyra Campbell (Institut de Biologia Molecular de Barcelona-CSIC, Institut de Recerca Biomèdica de Barcelona, Spain) described how transient expression of the *Gata4/6* homolog *serpent* initiates epithelial-mesenchymal transition in the endoderm by downregulating Crumbs, resulting in a loss of apicobasal polarity and delocalization of E-Cadherin (Campbell et al., 2011). Dynamic E-Cadherin punctae are maintained throughout endoderm migration, and cell tracking shows that it is required for coordinating collective migration of midgut progenitors.

Nandan Nerurkar (Harvard Medical School, Boston, MA, USA) presented work on the formation of the avian gut tube, illustrating the rising interest in tissue mechanics and their interplay with

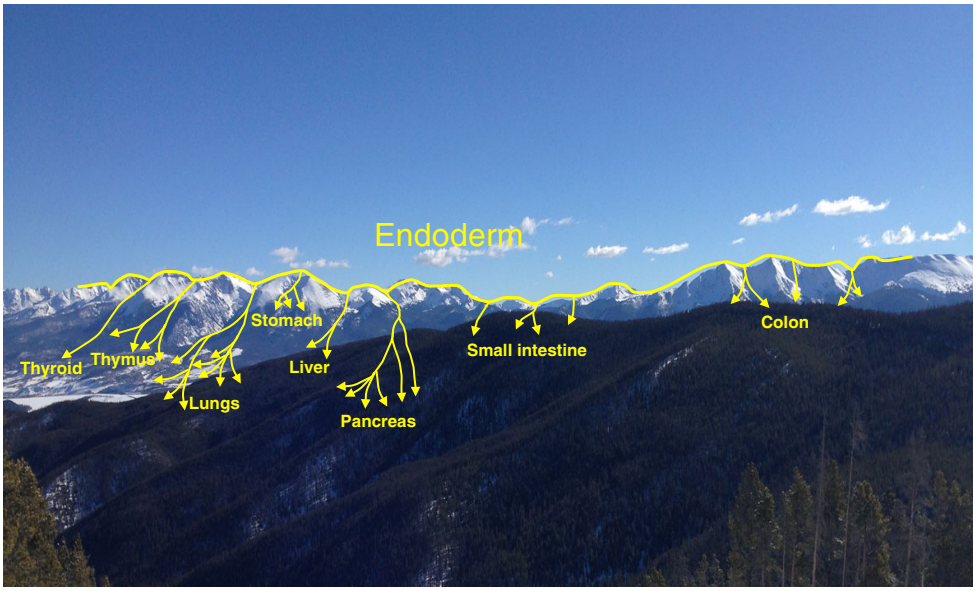

Fig. 1. The endoderm lineages depicted as routes down the mountains surrounding the Keystone resort, where the meeting took place. Image courtesy of Heiko Lickert (Institute of Diabetes and Regeneration, Neuherberg, Germany).

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signaling events. Live-imaging of endodermal cells uncovered an anteroposterior flux of cells leading to posterior compaction and folding of the caudal intestinal portal. Tissue cutting revealed a mechanical tension gradient, which directs endodermal progenitors to the posterior and is controlled by a parallel chemical gradient of FGF8. Tyler Huycke from the same laboratory argued that, at a later developmental stage, the mesenchyme of different gut regions can generate differential mechanical constraints, leading to distinct luminal topologies, including villus formation. Deborah Gumucio (University of Michigan, Ann Arbor, USA) illustrated an alternative model in mice, in which clusters of HH-responsive mesenchymal cells induce overlying epithelial cells to form villi (Walton et al., 2012). Bone morphogenetic protein (BMP) signaling, rather than smooth muscle elements, seem to play a central role in determining cluster pattern in this system. The interplay between signaling and mechanics in this context remains to be determined.

Even though much of the cellular movement is driven by endodermal cells, events in the mesoderm can also play an important part in shaping the endoderm. The different organ progenitors are specified at the midline and then undergo dramatic morphogenetic processes whereby each organ adopts its characteristic position and shape. Elke Ober (University of Copenhagen, Denmark) showed that tight coordination of liver progenitor movement with the migration of the adjacent lateral plate mesoderm is essential for asymmetric liver bud formation in zebrafish.

In addition to single cell migration, reorganization of epithelial sheets is an important driver of organ morphogenesis. How this controls tissue size and shape, as well as cell type differentiation, was highlighted by a number of contributions. Mitsuru Morimoto (RIKEN Center for Developmental Biology, Japan) explored how the size of the mouse trachea is established. Cell shape analyses and 3D reconstruction revealed that epithelial remodeling, including radial intercalation, is involved in late tube enlargement. In the same spirit, Nanette Nascone-Yoder (North Carolina State University, Raleigh, USA) found that, surprisingly, acetylcholinesterase (ACHE) is expressed in the *Xenopus* gut epithelium. Blocking ACHE function revealed a non-canonical role for ACHE in controlling the cell rearrangements that lead to gut elongation and cavitation.

Two presentations illustrated mechanisms that enable cells to leave an epithelial community. In the pancreas, Mostafa Bakhti's work (Institute of Diabetes and Regeneration, Neuherberg, Germany) showed that synaptotagmin 13 (SYT13) controls the delamination of endocrine cells from the branched pancreatic epithelium. Although synaptotagmins are known for their activity in vesicular trafficking, SYT13 appears to control apical basal polarity and spindle orientation in this system. Philip Pauerstein (Stanford University, CA, USA) provided evidence that semaphorin 3a in the mesenchymal periphery of the pancreas attracts neuropilin 2-expressing endocrine cells, enabling islet formation away from the duct from which they originate.

Endoderm differentiation from pluripotent cells

Our understanding of endoderm organ formation has reached the level where information can be used to coax pluripotent stem cells (PSCs) towards specific cell types and tissues. Not all cell types can be generated in a dish and some maturation steps still require engraftment into a host organism. Nevertheless, progress in the past few years has been tremendous. From the initial exposure of PSCs to activin and Wnts, which is generally used to induce endoderm, different culture media have been developed for stepwise differentiation. Such protocols also benefit from the ability to sort specific endoderm populations with increased potential for further differentiation into specific cell types. Gordon Keller (McEwen Centre for Regenerative Medicine, Toronto, Canada) has developed protocols for differentiating hepatocytes (Ogawa et al., 2013), pancreatic β-cells and cholangiocytes. He also showed a new endoderm surface marker that discriminates endodermal populations with liver or pancreatic potential. Along the same lines, Valerie Gouon-Evans (Icahn School of Medicine at Mount Sinai, New York, USA), whose work focuses on generating hepatic cells, also made use of new surface markers to identify a bi-potent progenitor with hepatic and endothelial fate (Goldman et al., 2013, 2014).

Stem cell models can be used to perform experiments that are difficult in whole organisms. For example, the Vallier lab (Sanger Institute, Cambridge, UK) investigated the interplays between cell cycle regulation and cell fate decisions using hPSCs as an *in vitro* model. In this system, endoderm induction occurs in G1, where cyclin D-CDK4/6 controls the phosphorylation of SMAD2 and thereby its ability to promote endoderm differentiation (Pauklin and Vallier, 2013). Furthermore, human PSCs (hPSCs) display a short G1 phase that increases during their differentiation. This change in cell cycle profile could improve the capacity of PSCs to respond to differentiation signals and thus could represent a mechanism determining the activity of extracellular stimuli. However, the presentation by Kat Hadjantonakis (Sloan Kettering Institute, New York, USA) highlighted that some observations in stem cells cannot be extrapolated to an embryonic setting. Using fluorescent reporters for NANOG (pluripotent epiblast) and PDGRFA (primitive endoderm) in the embryo, fluctuations in NANOG or PDGRFA expression were rarely observed, in contrast to previous observations in embryonic stem cell (ESC) cultures. This is possibly due to the timescale of embryonic development, which is too short to observe such oscillations (Xenopoulos et al., 2015).

Organoids: three-dimensional endoderm organs from stem cells

The meeting revealed a surge in our ability to engineer 3D organ models *in vitro*: organoids or spheres are being developed for most endoderm organs either from ESCs or induced PSCs (iPSCs), embryonic progenitors or adult stem cells. The protocols developed often differ between labs and include steps in embryoid bodies or monolayer culture before moving into Matrigel or decellularized matrices. Another important consideration is the long duration of these protocols, particularly using human cells, taking from 15 days up to 7 months.

Hans-Willem Snoeck and Jason Spence (University of Michigan, Ann Arbor, USA) discussed protocols to induce lung fate. After endoderm induction by activin and WNTs, the most anterior foregut endoderm is specified by transient inhibition of BMP, transforming growth factor β (TGFβ) and WNT signaling in mouse (Longmire et al., 2012) and human ESCs (Green et al., 2011). It can then be ventralized by transiently applying BMP, FGF and, in some cases, RA and a WNT activator to promote lung airway epithelium formation (Huang et al., 2015, 2014). Importantly, functional tests revealed that the cells could take up and recycle surfactant. Further development of this and other protocols leads to the formation of 3D structures containing type 1 and type 2 cells (Dye et al., 2015). The Snoeck lab also reported encouraging progress in the formation of saccular-stage alveoli in three dimensions. Alveolospheres containing type 1 and 2 alveolar cells were also derived from adult stem cells by Brigid Hogan's lab (Duke University, Durham, NC, USA).

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Darrell Kotton (Boston University, MA, USA) presented data demonstrating the derivation of functional thyroid follicular cells from mouse embryonic and iPSCs. WNT signaling was dispensible for thyroid lineage specification, and maturation of the resulting endodermal NKX2.1+ thyroid progenitor cells yielded cells that produced multiple thyroid-specific proteins, including the machinery required for thyroid hormone biosynthesis. Important progress was also made towards thyroid regeneration by harnessing the resulting cells to rescue mice with hypothyroidism following radioactive iodine destruction.

James Wells' lab showed how increased FGF and WNT levels, together with inhibition of the BMP pathway and RA activation, promote posterior foregut formation and, eventually, organoids resembling antral stomach (McCracken et al., 2014); fundal fate was obtained by more sustained WNT and FGF signaling. The lab is also making progress in generating colon organoids.

Although the Grapin-Botton lab has generated pancreas organoids from mouse progenitors (Greggio et al., 2013), pancreas organoids from hESCs are still under development. Grapin-Botton's work revealed the importance of heterogeneity in seed clusters to promote organoid formation, which remains to be investigated in other systems.

Tissue maintenance, aging and regeneration: many paths and plenty of plasticity

The homeostasis of endodermal organs is ensured either by stem cells (intestine, stomach) or the expansion of differentiated cells (liver, pancreas). Similarly, upon injury, recovery can be achieved by expansion of differentiated cells, activation of senescent progenitors or reprogramming of cell types.

The plenary lecture by Brigid Hogan reminded us that different types of injury trigger diverse regenerative responses. For example, in the airway, basal cells behave as stem cells in mouse and probably in human. They can give rise to ciliated and club cells. However, upon basal cell ablation, they can be regenerated from club cells. Further down in the airway, the club cells can make type 2 alveolar cells following bleomycin treatment or some types of injury. Type 2 cells give rise to type 1 cells during development and upon some injuries. Partial elimination of type 2 cells leads to expansion of the surviving type 2 cells but upon bleomycin treatment or pneumonectomy, type 2 to type 1 cell conversion is observed. Jason Rock and his student Andrew Lechner (University of California, San Francisco, USA) showed that, during this regeneration, type 2 cells produce CCL2 and attract non-resident CCR2-positive macrophages, which promote regeneration. Hogan also observed that there can be conversions of type 1 cells (marked by HOPX) into type 2 cells upon pneumonectomy.

Other examples of cell type conversion were provided in the *Drosophila* midgut, where normal replenishment is ensured by stem cells. Upon starvation, the gut shrinks to half of its length and many stem cells are eliminated, particularly in central regions. Under such injury, Benjamin Ohlstein (Columbia University, New York, USA) observed a spectacular dedifferentiation of tetraploid enterocytes into diploid stem cells.

In the pancreas and liver, cell type homeostasis occurs by proliferation of differentiated cells. Pancreatic β-cells proliferate extensively in the few weeks after birth, whereas proliferation is strongly reduced in adults. The Lickert lab (Institute of Diabetes and Regeneration, Neuherberg, Germany) showed that this reduced proliferation correlates with upregulation of the gene encoding flattop (cilia and flagella-associated protein 126). Flattop is a basal body protein (Gegg et al., 2014) also expressed in stem cells and enteroendocrine cells of the mouse intestine, where it may act as a sensor of planar polarity.

The thymus is an unusual organ that regresses in the adult, correlating with a progressive decrease of FOXN1 expression. Clare Blackburn (University of Edinburgh, UK) showed that Foxn1 overexpression prevents thymus involution and keeps it in a more juvenile state (Bredenkamp et al., 2014); furthermore, overexpression of FOXN1 in MEFs, which are normally *Foxn1* negative, was sufficient to reprogram the MEFs into functional thymic epithelial cells that could form an organized and functional thymus on transplantation.

Exploiting the potential of converting one cell type into another, Holger Willenbring (University of California, San Francisco, USA) described an AAV capsid targeting liver stellate cells, a cell compartment that is expanded in hepatitis and cirrhosis. This was used to target hepatic transcription factors to stellate cells, leading to reprogramming into hepatocytes. These cells function and proliferate similar to normal hepatocytes. However, residual expression of some stellate cell markers suggests that reprogrammed hepatocytes retain some of their original identity.

The ability to reprogram cell types into others raises questions about the mechanisms that maintain cell identity. Ramesh Shivdasani (Dana-Farber Cancer Institute, Boston, MA, USA) showed that during maturation of intestinal stem cells to enterocytes, only 10% of the genes that change expression also change levels of histone H3K27me3 marks. Upon inactivation of the repressive PRC2 complex component EED, no precocious differentiation occurs and few genes alter expression, mostly activating developmental regulators from other germ layers. Thus, the PRC2 complex seems more important to maintain the silencing of genes repressed much earlier in development, rather than to modulate recent transcriptional programs.

Disease mechanisms: *in vivo* models

Several talks illustrated how cells escape the normal differentiation mechanisms, leading to tumor formation. Lineage tracing, which has enabled characterization of stem cells and their potential in homeostasis, is also crucial to determine the cells of origin for various cancers. As discussed by Trudy Oliver (Huntsman Cancer Institute, Salt Lake City, UT, USA), in the lung, different types of tumors exhibit different molecular changes and presumably also different cells of origin. The group focused on two non-drug-sensitive lung cancers: small cell lung cancer (thought to originate from neuroendocrine cells) and squamous cell cancer. Oliver reported the generation of a new mouse model of squamous cell carcinoma – using overexpression of SOX2 and loss of LKB1 (Mukhopadhyay et al., 2014).

Lineage tracing in the pancreas and intestine also suggests that some cell types are particularly tumor prone, such as a DCLK1 (doublecortin-like kinase)-positive population described by Wang's lab (Columbia University, New York, USA) (Westphalen et al., 2014).

The Stanger lab (University of Pennsylvania, Philadelphia, USA) combined lineage tracing with a genetic model of pancreas adenocarcinoma to show that tumors are polyclonal in this model, with three or four zones originating from different cells. Interestingly, most metastases were polyclonal in the peritoneum, diaphragm and lymph nodes, whereas in the liver and lung only 10% of metastases were polyclonal and formed small lesions, while large lesions were monoclonal, suggesting the dominance of some clones over time.

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Moving away from cancer models, Didier Stainier (Max-Planck Institute for Heart and Lung Research, Bad Nauheim, Germany) presented an unbiased forward genetic approach in mouse to uncover respiratory disease genes and the first mutants from the screen exhibiting, for example, cell type specification defects, pulmonary fibrosis or collapsed lungs.

Development of disease models based on stem cells

In addition to the importance of these *in vivo* disease models, the meeting revealed the adaptation of stem cells as disease models in 2D or organoid conditions. A second development in the stem cell field is the proof of concept that PSCs can be used to decipher the mechanisms of human diseases. In this direction, Danwei Huangfu (Sloan Kettering Institute, New York, USA) used an inducible Crispr/Cas9 system to generate hundreds of hESC lines, bearing homo- and heterozygous inactivation of 13 genes implicated in monogenic forms of diabetes. This constitutes a complementary approach to other studies recapitulating exact patient mutations. Notably, these studies revealed a new haploinsufficient requirement for PDX1 in human. As shown by Matthew Kuhar (Cincinnati Children's Hospital, OH, USA), stem cell models can be applied to contexts where the exact mutation is not yet known, such as intractable diarrhea of infancy – where iPSCs derived from patients had a decreased ability to differentiate into enteroendocrine cells.

James Wells has also modeled infectious disease, specifically *Helicobacter pylori* infection, in gastric organoids (McCracken et al., 2014), while Hans-Wilhelm Snoeck has investigated influenza sensitivity and immune response in airway cells derived from iPSCs from an individual with repeated influenza infection (Ciancanelli et al., 2015). Moreover, these cellular phenotypes could be rescued by IFN exposure. Preliminary data from Jorge Munera (Cincinnati Children's Hospital, OH, USA) suggest that Crohn's disease and ulcerative colitis may be the next models where the interaction between multiple cell types can be modeled with colon organoids.

The influenza model above shows how treatments can be tested in such models. This was also illustrated by Gordon Keller for cholangiocyte spheres [the generation of which was also reported by the Huebert lab (Mayo Clinic, Rochester, NY, USA) (De Assuncao et al., 2015)]. When generated from iPSCs from individuals with cystic fibrosis (CF) with *CFTR* mutations, the CFTR-based ion transport was perturbed and resulted in cyst swelling. This was reverted by drugs used to treat CF. Nicholas Hannan (Sanger Institute, Cambridge, UK) also reported success in reverting cellular phenotypes using CF drugs on patient-derived lung organoids.

Finally, the ability of such stem-cell-based models to be maintained for long periods of time makes them amenable to tumor modeling, as presented by Gustavo Mostoslavsky (Center for Regenerative Medicine, Boston University, MA, USA) showing how different doses of APC influence the cellular and molecular phenotype of intestinal organoids derived from isogenic human iPSCs.

Conclusion

One main theme emerging from this meeting is how closely developmental biology and stem cell research are interconnected. Stem cell biologists use information on organ development to differentiate cell types and trigger their organization into organoids. The converse was also highlighted during the meeting, whereby the large amounts of cells differentiated from ES cells can be used to address developmental issues that would be difficult to elucidate in model organisms. Anticipating that a meeting on endoderm will be re-organized in a few years, we expect to witness a more widespread use of insights from physics and more insights into human developmental biology through exploiting stem cells. Notably, groups now often cross traditional boundaries between developmental and stem cell biology, embryonic development and adult tissue homeostasis or normal and disease contexts, a trend we anticipate will become more widespread in the next few years.

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Competing interests

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