Introduction

The months preceding the NYSCF conference were marked by extraordinary developments in the field of stem cell research. Within the last twelve months progress continued in developing disease-specific stem cell lines; advances were made in creating induced pluripotent stem (iPS) cell lines using technologies compatible with clinical use; and the FDA gave approval for the first clinical trial using cells derived from human embryonic stem cells (hESC) for spinal cord injury. These developments in the laboratory were matched by important activity on the political front. In March 2009, President Barack Obama issued an executive order directing the National Institutes of Health (NIH) to put new rules in place for federal funding of stem cell research; in July 2009, the NIH announced the new rules and procedures for approval; and as of December 2009, the NIH had approved the first 13 stem cell lines for federal research support, and 27 others have been recommended and await approval by the NIH director.

The “Fourth Annual Translational Stem Cell Research Conference: Breaking Ground” opened with the panel session “Road to the Clinic: Funding the Cure” moderated by Lee Rubin (director of Translational Medicine at the Harvard Stem Cell Institute and NYSCF scientific advisor). The panel, composed of leaders from the pharmaceutical, biotechnology, and healthcare industries, focused on translational research and on interactions between academia and the private sector, reflecting NYSCF’s conviction that the development of clinically available therapies will require close cooperation between the two sectors. A second panel session “Stem Cell Policy: Shifting Ground,” moderated by Alice Park, staff health writer for *Time* magazine, focused on recent policy developments, particularly the impact of the recent change in federal policy. Participants included representatives of the NIH and the Food and Drug Administration, as well as an ethicist and a scientist.

The second day of the conference was devoted largely to sessions on specific diseases, including diabetes, cancer and blood diseases, heart and muscle disease, and neurodegeneration and spinal cord injury, followed by a panel on recent progress in programming and reprogramming stem cells. NYSCF was pleased and honored to have Janet Rossant (The Hospital for Sick Children, University of Toronto) give the conference keynote address. Dr. Rossant is a pioneer in understanding early mammalian embryonic development, work that laid the
Breaking ground on translational stem cell research

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Human pre-implantation embryo at the blastocyst stage (6.5 days post-fertilization). Inner cell mass nuclei, labeled blue, express the pluripotency marker Oct3/4. Outer cell nuclei, labeled red, express the trophoblast marker Cdx2. Green-labeled nuclei express the visceral endoderm marker Gata6. Courtesy of Daylon James, Nikica Zaninovic, and Josep Pareja.

fundamental groundwork for the subsequent identification and isolation of hESC. She spoke about regulating pluripotency in embryos and stem cells and about the pathways and transcription factors that specify cell fate in the blastocyst.

Road to the clinic: funding the cure

Lee Rubin opened the session by discussing some of the many promises that stem cells offer. Currently, stem cells are being studied as therapeutic agents in the field of regenerative medicine for the repair of damaged tissue and organs. An emerging view is that cell-based approaches using stem cells derived from individuals with specific diseases would be extremely valuable as approaches to understanding and treating diseases. Examples of such approaches include identifying new targets of conventional therapeutics and discovering new conventional therapeutics that promote muscle and/or neuron development.

David Glass (global head of muscle disease research, Novartis) described the process of sarcopenia (muscle loss) during aging and how satellite cells—stem cells on the surface of muscles—fail to proliferate. Glass’ group used human myoblast stem cells in a screen to show that the failure of the stem cells to proliferate was due to the effects of myostatin and TGF-β family members via the SMAD signaling pathway.1 Glass and colleagues also showed that mitochondrial function decreases with age of the cell, from which they concluded with the open question: can stem cell treatments ameliorate mitochondrial diseases simply by restoring mitochondrial function?

Ira Mellman (vice president of research oncology, Genentech) posed the questions of whether cancer stem cells (CSCs)2 exist and, provocatively, whether an answer really matters. On the basis of the current hypothesis, CSCs are a self-renewing stem cell population required for the initiation and maintenance of tumors. Purportedly, cancer stem cells share most, if not all, the properties of conventional stem cells, meaning that CSCs—like conventional stem cells—may be sensitive to anticancer drugs, and thus CSCs could be good targets of anticancer therapeutics. However, identifying, characterizing, and defining CSCs is difficult: indeed, a first assumption is that CSCs exist and share surface markers associated with stem cells in general. Yet, according to Mellman, experimental data have not born this out. Mellman consequently proposes to reframe the issue on empirical grounds: rather than assume cancer stem cells exit, find and attack “tumor-reinitiating cells” (TRICs) among an existing tumor (not all cells of a tumor have this property). Importantly, TRICs may be a genetically distinct cell population required for tumor growth that possesses distinct features and contains chemo- and radio-resistant cells. However, according to Mellman, whether or not TRICs are bona fide stem cells is a question yet to be answered.

Randall T. Moon (director of the Institute for Stem Cell and Regenerative Medicine, University of Washington) spoke about the role of WNT/β-catenin signaling in the regeneration of cardiac cells. He presented data showing that activation of the WNT/β-catenin pathway in zebrafish, mice, or hESCs contribute to cardiac regeneration in vitro, and that injection of human spinal cord blood into mice activates WNT/β-catenin signaling in mouse cells.4 For example, Moon showed that even if one removes the lower one quarter of a zebrafish heart it will completely regenerate in approximately one month via a WNT/β-catenin-dependent signaling
pathway. He also discussed how knowledge of this signaling pathway could be beneficial to the drug development and biotech industry.

Clifford Woolf (director of the neural plasticity research group at Massachusetts General Hospital) spoke on the use of hESC/iPS to study the genetic heritability of chronic pain susceptibility, which, according to Woolf, is at least 50% genetically determined. By employing a specific method (the Melton method\textsuperscript{5}) of stepwise derivation to produce nociceptors from normal and disease-specific patients, one can screen for analgesic efficiency \textit{in vitro} and determine individuals at risk for developing chronic pain.

Stem cell policy forum: shifting ground

Leading stem cell researchers, ethicists, government representatives, and policy makers examined the public and private sectors in stem cell research given the newly revised federal policies. The discussion centered around President Obama's signing of the Executive Order revising the Bush administration's restrictive funding of stem cell research through the NIH. The conversation also included the development and implementation of the NIH guidelines called for in the Obama administration's Executive Order. The panelists included Susan L. Solomon, (chief executive officer of the New York Stem Cell Foundation), Kevin Eggan (chief scientific officer of NYSCF), Celia Witten (director of the Office of Cellular Tissue and Gene Therapy at the Center for Biologics Evaluation and Research at the FDA), Insoo Hyun (associate professor of bioethics at Case Western Reserve University), and Story Landis (director of the National Institute of Neurological Disorders and Stroke, and director of the Stem Cell Task Force at the NIH).

It was a dynamic panel that engendered lively discussion between the panelists and insightful
audience members. Susan Solomon summarized the recent policy changes at the federal level. In November 2008 a pro-science president was elected, and on March 9, 2009, President Obama signed the Stem Cell Executive Order. On July 7, 2009, the final NIH Guidelines for Human Stem Cell Research were announced, which determined what stem cell research would be eligible for federal funding. And on August 17, 2009, Francis S. Collins, M.D., Ph.D., was officially sworn in as the 16th director of the NIH. Both Eggan and Solomon outlined the limitations of the NIH guidelines and the already existing restrictions of the Dickey Wicker Amendment. They explained that despite the expansion of federal funding opportunities outlined in the new guidelines, many significant restrictions remain in place. These include the derivation of new hESCs lines, somatic cell nuclear transfer, and parthenogenesis, which are still not eligible for federal funding.

Dr. Landis took a more positive view of the revised federal policy and spoke to the opportunities that the expansion would allow. She pointed out that as medical discoveries were made, a constant reexamination of the guidelines would occur at the NIH. Celia Witten outlined the process for approval of biologics at the FDA and felt that existing paradigms for FDA approval were already in existence and would be applied to any products derived from stem cell research. Discussion centered on the possibility of the scientific community forming a partnership with the FDA to make the process as seamless as possible. Finally, Insoo Hyun framed the conversation around the fact that stem cell research would remain controversial and would need to be closely monitored because many Americans view hESCs research with suspicion and as a practice contrary to valuing human life.

Diabetes

Diabetes is a disorder characterized by a loss of β cell mass and/or a loss of β cell–autonomous function, which normally leads to a deficiency of insulin and defective regulation of blood glucose levels. In type 1 diabetes (T1D) loss of β cell mass is caused by an autoimmune assault on β cells; β cell destruction is chronically progressive, occurring over months or years before overt diabetes develops, and continues until, in most instances, nearly all β cells are destroyed. Type 2 diabetes (T2D) is often associated with an increased body mass index (BMI) and insulin resistance that is initially compensated for by increased pancreatic insulin production but eventually results in β cell exhaustion and failure. The possibility of regenerating β cells is therefore relevant for both types of diabetes.

Yuval Dor (The Hebrew University, Israel), the first speaker of the diabetes session, studies the regeneration of β cells in the pancreas. Dor and colleagues have made a number of important findings regarding adult β cells: for example, they have a limited ability to regenerate in the adult organism from preexisting β cells. Of importance clinically, their expansion in number is sensitive to immunosuppressants used after transplantation of heterologous β cells, a finding that is likely relevant to future attempts to transplant stem cell–derived β cells. Dor also presented exciting new data on the molecular signals that stimulate the proliferation of β cells. Upon β cell ablation—in which the majority of β cells are eliminated chemically—mice can recover from the insult by generating new β cells via proliferation of surviving β cells. These data suggest that adult β cells are generated mostly by duplication of preexisting β cells rather than by differentiation of stem cells. Dor and colleagues also showed that proliferation of endogenous β cells is reduced when glucose levels are low; this occurs, for example, after transplantation of glucose-utilizing exogenous β cells. That finding likely explains the ineffectiveness of islet cell transplantation therapy in which loss of grafts is seen one to five years after transplantation.

To further study the role of glucose levels in stimulating β cell replication, Dor and colleagues generated mice with a β cell–specific deletion of glucokinase, the key enzyme responsible for regulating glucose levels; they found that glucokinase–deficient mice develop diabetes and have reduced β cell proliferation and mass. Conversely, pharmacologic activation of glucokinase in vivo increases the rate of β cell proliferation. These data suggest that blood glucose levels, acting via glycolysis in β cells, are a key determinant for β cell proliferation.

Christopher V. Wright (Vanderbilt University, Tennessee) presented data on generation of β cell–like cells from hESCs and on the transcription factors involved in β cell differentiation. He pointed out that thus far mature human β cells have not yet been generated from hESCs in vitro. Instead, the β cell–like cells that
can be generated are defective, produce little insulin, and have limited ability to respond to glucose. Wright showed that such defective/immature cells could be generated in vivo (mice) by a genetically reducing expression of the key transcription factor pancreatic and duodenal homeobox 1 (Pdx-1) in neurogenin 3–expressing (Ngn3+) endocrine progenitors. Conversely, activation of Pdx-1 in vivo reduced the formation of glucagon–producing cells and drove them to a β cell fate. These results show that differentiation of hESCs to mature β cells requires transcription factors such as Pdx-1 to be expressed at amounts comparable to those that occur in vivo.

The final speaker of this session, Peter Arner (Karolinska Institute, Sweden), presented studies on fat cell turnover in humans. Arner and colleagues have previously shown, by taking advantage of the fact that 14C from atomic bomb tests can be detected in genomic DNA of individuals, that only 10% of fat cells are renewed annually. This means that increased body fat mass is due to both increased fat cell number as well as to increased size of individual fat cells. However, there is tremendous variation in adipocyte size of people with comparable body mass index (BMI). Adipose tissue can contain many small adipocytes (hyperplasia) or a comparatively small number of large adipocytes (hypertrophy). Arner presented data on the molecular and cellular causes for this variability. He and colleagues studied fat cell morphology in 764 subjects with BMIs of 18–60 kg/m²; subjects with hypertrophy had a lower rate of adipocyte generation than subjects with hyperplasia. Hypertrophy was also associated with higher serum insulin levels and decreased insulin sensitivity, in agreement with previous studies. Evaluating the mechanisms responsible for adipocyte generation, they showed the importance of follistatin, monomeric TRAP, and the signaling molecules TNF-α, IL-6, and MCP-1 for adipocyte function.

Cancer and blood disease

Hematopoietic stem cells (HSCs), which continuously refresh the vast reservoir of blood cells...
in the body, self renew in the bone marrow and yet retain the capacity for rapid mobilization and differentiation/proliferation in response to insult. The microenvironment (or niche) that dictates the balance between these two conditions is comprised of numerous molecular stimuli that represent promising therapeutic modulators of hematopoiesis normal and pathological conditions. Using a combination of techniques, including real-time ex vivo two-photon microscopy, in vitro hematopoietic cell culture, genetic mouse models, and an immunotherapeutic approach, speakers in the session “Cancer and Blood Disease,” chaired by Dan R. Littman (New York University School of Medicine), explored the spatial, cellular, and molecular influences that govern HSC expansion and self-renewal, and examined mechanisms by which leukemia evades immune surveillance. In the first talk of the session, Linheng Li (the Stowers Institute/University of Kansas) shed light on the molecular signaling apparatus that dictates the balance of HSCs and their derivatives between the endosteal and vascular niches in the bone marrow. Using an innovative live imaging approach to capture the first real-time observations of HSCs in bone marrow, Li’s group tracked the behavior of green fluorescent protein (GFP)–labeled HSCs in vivo and showed that, when transplanted into irradiated mice, HSCs home to the inner bone (endosteal) surface of the marrow. Li also expanded upon this by showing that the localization of HSCs to the endosteal niche is mediated in part by N-cadherin, and that the signaling cascade that promotes self-renewal of HSCs involves the activation of the WNT pathway downstream of PI3Kinase/Akt. Li’s work provides many lines of evidence to support a “niche paradigm” for hematopoietic regulation in which the bone marrow is divided into two zones: the central marrow zone, in which proliferating and differentiating hematopoietic progenitors are found, and an endosteal zone, in which HSCs are normally maintained as relatively quiescent, self-renewing cells until an insult promotes their expansion. The next speaker, Shahin Rafii (the Howard Hughes Institute/Weill Cornell Medical College), focused on the contribution of vascular endothelial cells to maintenance of HSC self-renewal. Rafii’s group established endothelial cell lines (by transducing the primary endothelial cells with the adenovirus E4ORF1 gene) that can be cultured indefinitely in the absence of serum and cytokines. With only kit ligand as a supplement, these endothelial cell cultures generate large numbers of phenotypically and functionally competent HSCs that rescue lethally irradiated mice and engraft, self-renew, and expand in single cell- and serial-transplantation assays. Rafii and colleagues showed that E4ORF1-transduced endothelium mediated its effect via Notch signaling; and they went on to demonstrate an essential role for this pathway in mediating the repopulation/reconstitution of HSCs in the bone marrow in vivo. The E4ORF1/endothelium-based platform functioned similarly for human bone marrow–derived HSCs, maintaining their phenotype and preserving their ability to engraft in immunodeficient mice; they also supported expansion of leukemic and solid tumors in serum-free, cytokine-free conditions. These studies reveal a robust capacity of endothelium to support the expansion of multiple stem and tumor-initiating cells; provide a practical therapeutic avenue for generating transplantable material and/or mobilizing endogenous stem cell pools; and offer novel insights into the role of the vascular niche in fostering hematopoietic and cancer stem cells in vivo.

The last speaker of the session, Irving Weissman (Stanford University), described a clinical study of acute myeloid leukemia and a preclinical immune-based approach to therapy. The approach was based on the premise that certain abnormalities arise within clonal populations of pre-leukemia stem cells, and that these abnormalities allow the stem cells to evade immune surveillance. Weissman and colleagues focused on CD47, demonstrating that this surface molecule protects hematopoietic cells from being engulfed by macrophages—a physiological function that prevents depletion of mobilized HSCs. However, Weissman and colleagues showed that this normal physiological function may be co-opted by subsets of leukemia cells to promote their survival. For example, high CD47 expression in human leukemia is associated with poor prognosis. Weissman and colleagues thus speculated that targeting CD47 cells could serve as a novel therapeutic strategy. Using antibodies targeted to CD47 that could block its capacity to prevent macrophage activation, they showed that the antibodies prevented engraftment of leukemia cells in mouse bone marrow. Further, treating mice that had received...
leukemia grafts with the blocking antibody to CD47 cleared the leukemia. According to Weissman, these results provide a powerful proof of principle for therapeutic approaches based on antibodies. Considering that CD47 is expressed on bladder, ovarian, and medulloblastoma cancer cells as well, this molecule may provide a valuable additional therapeutic target, along with traditional chemotherapeutic approaches, in the effective treatment of multiple cancers.

**Repairing our heart and muscles**

Understanding the basic biological processes governing stem cells during tissue development and aging and applying these to research of new therapeutic modalities were the themes of the session “Repairing Our Heart and Muscles,” chaired by Robert S. Kass (Columbia University).

In the first presentation, Gordon Keller (McEven Centre for Regenerative Medicine, University of Toronto) illustrated how directed differentiation of embryonic stem cells (ESCs) and iPS can be based on the lessons from the early embryonic development, with the challenges of obtaining functional cells in an efficient and reproducible manner. Work with mouse ESCs has shown that signals present in the gastrulating embryo (e.g., BMP, Wnt, and Nodal signaling pathways) can be used to differentiate cells into endodermal and mesodermal populations via *in vitro* formation of a primitive streak-like population. Moving to hESCs, Keller’s group has identified both the human hemangioblast and the human cardiovascular progenitor. Importantly, correct concentrations and timing of growth factor supplementation were key for successful differentiation and had to be adapted for various pluripotent cell lines (e.g., HES2, H1, Y2–1). More recently, markers of different mesodermal populations have been investigated. For example, successful separation between early cardiovascular and hematopoietic populations has been achieved based on the expression of PDGFRα and KDR. Monitoring the emergence of KDR+/PDGFRα− populations
allowed optimization of cardiac differentiation protocols for different cell lines and enabled routine generation of >50% cardiac troponin+ cardiomyocyte populations without cell sorting. The upshot of Keller’s work is that ESC-derived cardiomyocytes can be used for predictive toxicology and drug discovery.

Next, Gordana Vunjak-Novakovic (Columbia University) discussed the multiple levels of functionality of cardiac tissue and how tissue-engineering approaches—in particular the construction of biomaterial scaffolds—could aid in cardiac tissue regeneration. Biomimetic environments developed from combinations of extracellular matrices, biochemical signals, biophysical signals, and various cell types have been used for the culture, maturation, and delivery of stem cells. For example, cardiac tissue patches can be cultured from rat neonatal cardiomyocytes that are seeded on native and synthetic polymer scaffolds. Inclusion of channels into the scaffold structure and dynamic perfusion culture of the constructs allows for high cell viability and homogenous tissue formation. Electrical stimulation can be used to condition the contractile function of constructs in vitro, whereas endothelial cells seeding into the scaffold channels can improve vascularization after implantation. Also, in vivo tests, such as in a rat infarction model of cardiac patches prepared from mesenchymal stem cells, demonstrate specific paracrine effects on tissue regeneration.

To obtain relevant cardiovascular populations, Novakovic’s group studied the culture and differentiation of hESCs in hyaluronic acid and dextran hydrogels; they found that incorporation of growth factors in these scaffolds enabled sustained delivery of the factors sufficient for ESC differentiation. Novakovic’s group also studied delivery of multiple factors (with control over timing and concentration) in microarray bioreactor systems and bioreactor arrays designed for electrical conditioning of the ESC-derived cardiomyocytes; preliminary data indicated positive effects of electrical conditioning on synchronous beating of the cardiomyocytes. The technologies developed in Novakovic’s lab may be used to establish controllable models for in vitro studies, as well as for development of new therapeutic approaches in vivo.
Irina Conboy (University of California, Berkeley) presented studies on the mechanisms controlling stem cell aging and the possible therapeutic targets to reverse this process. Based on the premise that the amount of damage throughout life is constant while the rate of regeneration differs, Conboy hypothesized that older individuals possess stem cells that retain regenerative capacity (“young cells”) but that are functionally repressed by the aging environment. Choosing muscle as an experimental system, Conboy’s group developed techniques to isolate muscle stem cells from individuals, which enabled them to show that the numbers of muscle stem cells are comparable in old and young animals. After injury, in contrast, only muscle stem cells in young animals could proliferate and regenerate muscle tissue. Studying the underlying mechanisms to explain the difference, Conboy and colleagues found downregulation of Notch signaling (responsible for stem cell activation after injury) and upregulation of TGF-β signaling (blocking activation by outcompeting Notch signal) in the old muscle stem cell niche. They also determined that these underlying mechanisms were evolutionarily conserved between mice and humans. On the systemic level, heterochronic parabiosis experiments (i.e., physically connecting the blood circulation systems of individual old and young mice) resulted in rejuvenation of tissue repair in old animals. Similar effects were shown in vitro for human stem cells from muscle of older individuals after it was exposed to serum from younger individuals. These data demonstrate that the transplantation of human glial progenitor cells can (at least in this mouse model) effectively treat disorders of congenital hypomyelination.

The next speaker, Lorenz Studer (Memorial Sloan-Kettering Institute), spoke about recent work using hESCs as a tool to study human neurological disease. hESCs can give rise to neural tissue using various methods, most of which involve using feeder cells or via the formation of embryoid bodies that typically lead to a heterogeneous population of cells. Studer’s group showed that a rapid, defined neural induction protocol from hESCs is very important as an efficient way to generate neural cells for future studies. To do this, they utilized signals involved in neural development, attempting to mimic the signals in the dish. In particular, blocking both BMP and TGF-β signals resulted in rapid induction of neural progenitor cells that, under defined conditions, could become different types of brain and spinal cord neurons. Studer’s talk then shifted gears to a discussion of induced pluripotent stem cell technology and how...
this could be used to model neurological disease. In particular, Studer focused on a disease called familial dysautonomia (FD), a rare but fatal peripheral neuropathy, caused by a mutation in the IKBKAP gene involved in transcriptional elongation. The disease is characterized by the depletion of autonomic and sensory neurons that arise from neural crest cells. To date, the mechanisms of neuron loss in FD are poorly understood owing to the lack of an appropriate model system. In their study, derived iPS lines from FD patients were differentiated into cells of all three germ layers, including peripheral neurons. Gene expression analysis in the purified cell lineages demonstrated tissue-specific mis-splicing of IKBKAP. Interestingly, neural crest precursors from FD patients have been demonstrated to express particularly low amounts of wild-type IKBKAP transcript, suggesting a mechanism for disease specificity. Studer and colleagues characterized the FD pathogenesis further by using cell-based assays to reveal marked defects in neurogenic differentiation and migration behavior. Finally, they used FD-iPS for validating the potency of candidate drugs in reversing aberrant splicing and ameliorating neuronal differentiation and migration. Their study illustrates the promise of iPS technology for gaining new insights into human disease pathogenesis and treatment.

In the final talk of this session Arturo Alvarez-Buylla (University of California, San Francisco) presented work on neural stem cells (NSC) from the adult mouse brain. While NSCs are most prevalent in the embryo, there are two regions of the adult brain that contain NSCs: the subventricular zone (SVZ) in the walls of the lateral ventricles. Previous work from Alvarez-Buylla’s lab showed that these NSCs retain an epithelial apical-basal organization and that they have a fixed position in the epithelium which offers positional information that ultimately determines what types of neurons they will produce. Alvarez-Buylla went on to discuss the role of cilia in neural progenitor cells. He described how conditional ablation of cilia demonstrated the necessity of cilia for stem cell function in the brain. He then discussed how this phenomenon is linked to the sonic hedgehog (SHH) pathway. SHH is a protein that when active leads to an increase in smoothened activity, thus inducing the downstream transcriptional cascade involved in SHH signaling, and NSC proliferation. Alvarez-Buylla’s group showed that tumors acting via SHH require the abolishment of primary cilia to form those tumors. This suggests that primary cilia could serve as a diagnostic tool, as well as bring new insight into the mechanism of tumorigenesis from neural precursor cells.

**Programming and reprogramming**

Reprogramming and the control of pluripotency in ESCs are important areas of study for learning how to use stem cells for in vitro disease modeling and regenerative medicine. The speakers in this session presented studies showing how the pluripotency network of transcription factors is maintained, how it controls epigenetic processes in the cell, and how small molecular modulators of cell signaling can manipulate pluripotency.

Sheng Ding (The Scripps Research Institute) presented work identifying small molecules that may be useful for stem cell biology and regenerative medicine. Ding began by reviewing data showing that a small molecule inhibitor of RasGAP and Erk can keep mouse ESCs in a pluripotent state in the absence of feeder cells and leukemia inhibitory factor (LIF). He then summarized experiments showing that small molecules can replace individual iPS transcription factors for reprogramming to the pluripotent embryonic state and that another molecule can revert a differentiated C2C12 muscle cell line to a multipotent progenitor state. Ding finished by showing that certain small molecules can help to induce the formation of human stem cells with a mouse ESC-like morphology, which may represent a more pluripotent human cell type than the traditional hESC.

Philip Avner (Institut Pasteur, France) presented work demonstrating that key pluripotency transcription factors directly regulate the epigenetic process of X-inactivation, thereby providing a link between the timing of X-inactivation and the development of the mammalian embryo. The expression of Xist RNA is an early event in X-inactivation; it is typically expressed at very low levels in mouse ESCs in which both X chromosomes are active. Avner’s group measured the binding of RNA polymerase II to the Xist promoter and found that Xist is not transcribed in mouse ESCs. They went on to show that the key pluripotency transcription factors Oct4, Sox2, and Nanog bind to intron 1 of...
Ihor Lemischka (Mount Sinai School of Medicine) presented recent work on global analysis of epigenetic and gene expression changes that occur with perturbations in the pluripotency network. In particular, they looked at changes in histone methylation, transcription, mRNA expression, and nuclear protein expression that occur when either Nanog or Esrrb expression is depleted in ESCs. Interestingly, when Nanog was depleted, half of the proteins measured changed with an inverse relationship to their corresponding mRNAs. While the reason for this is unknown, Lemischka’s group also observed this phenomenon when Esrrb was depleted from ESCs. Lemischka then overlaid the Nanog depletion data with computational and experimentally derived data from the literature to demonstrate the long-term goal of defining global interactomes for specific cell types. In the final part of his talk, Lemischka presented work his group has done to set up an in vitro model of Leopard syndrome. He showed that they have made iPS cells from patients with Leopard syndrome and can differentiate these cells into cardiomyocytes that show signs of hypertrophy, which is a characteristic of cardiomyocytes from Leopard syndrome patients.

Justin Ichida (Harvard University and a Druckenmiller postdoctoral fellow, New York Stem Cell Foundation) presented work demonstrating that a small molecule inhibitor of TGF-β signaling can replace both Sox2 and cMyc in the defined-factor reprogramming of mouse fibroblasts. Ichida and colleagues performed a high-throughput chemical screen with a functional, reprogramming readout to find molecules that can replace Sox2 in reprogramming. They found that one molecule, RepSox, could replace Sox2 and cMyc by inhibiting TGF-β signaling. Interestingly, while RepSox acts on the starting fibroblast cells to replace cMyc activity, it does not act on the starting cells to replace Sox2. Instead, RepSox acts by inhibiting TGF-β signaling in trapped, partially reprogrammed intermediate cells that are distinct from other reprogramming intermediates. This inhibition leads to the induction of Nanog, which functionally compensates for Sox2 and induces pluripotency in these cells. Therefore, Ichida and colleagues found that one small molecule can replace two reprogramming factors by different mechanisms and that reprogramming can proceed through different routes and cellular intermediates.

Keynote address

Prior to implantation and overt development of tissues in the embryo during gastrulation, mouse embryos must establish cell lineages that guide successful implantation and development of the embryonic epiblast. Janet Rossant (The Hospital for Sick Children, University of Toronto) ended the conference by shedding light on the signaling mechanisms that guide these first cell fates, which establish
proliferating populations of trophectoderm (TE) and primitive endoderm (PE) cells in the embryo.\textsuperscript{51} While her group and others have previously reported the isolation of self-renewing stem cell lines from each of the three lineages present in the embryo before implantation, much of her talk focused on the role of the FGF and Hippo signaling pathways in guiding these “decisions” \textit{in vivo}.

During compaction and polarization of embryonic blastomeres in the two-day-old mouse embryo, two different environments are established between the future inner cell mass (ICM) inside the embryo and trophectoderm cells outside the embryo.\textsuperscript{52} Her group has shown that the history of an embryonic cell’s exposure to Hippo signaling and signal integration with the FGF/MAPK pathway differentiates inside cells from outside cells, leading to lineage segregation at the blastocyst stage.\textsuperscript{53} They have recently published evidence that the Hippo/Wrt pathway, by regulating TEAD4 expression, may specify TE fate in the embryo.\textsuperscript{54} Hippo is active in inside cells, inhibiting TE specification. Additionally, Rossant reported that while activating the MAPK pathway can maintain but not initiate TE specification, the MAPK pathway is required later to specify PE cell fate. This later “choice” may be a stochastic determination of cells in the ICM encountering different levels of MAPK activation. Over time, these signals are reinforced to commit to a fate in the late blastocyst. Rossant’s group is currently trying to determine the identity of the Hippo signal and whether or not Hippo signaling may also control pluripotency of the inner cells.

While this work was performed with mouse embryos, human embryos may establish these cell types differently. For instance, the conditions used to promote self-renewal of mouse TE are good at promoting hESC self-renewal. However, the human blastocyst TE is invasive rather than proliferative and is likely unresponsive to FGF proliferative signals. As there is a slightly later proliferative phase after implantation of human embryos that may be responsive to FGF signaling, there may be timing differences in response to these signals. Therefore, it will be important to look in the human blastocyst to understand early lineage determinants before we can relate them to corresponding stages in the mouse. It will also be interesting to see if mouse ESC-like cells can be derived from human blastocysts by manipulating these signaling pathways. Together, these results are beginning to explain the cell signaling pathways that allow self-renewing stem cells to be derived from the early embryos.

Conclusions

The New York Stem Cell Foundation’s “Fourth Annual Translational Stem Cell Research Conference: Breaking Ground” brought together leading researchers from the United States and abroad to share results and advance innovative ideas, bringing us ever closer to cures. Conference sessions focused on particular diseases: diabetes, cancer and blood diseases, heart and muscle disease, and neurodegeneration and spinal cord injury. Other sessions discussed how to take this critical research “from bench to bedside.” Attendees had the opportunity to learn about the current, cutting-edge work of NYSCF’s post-doctoral fellows presented in poster
sessions. The “Fifth Annual NYSCF Translational Stem Cell Research Conference” will take place in New York City on October 12–13, 2010.

Acknowledgments

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[Correction statement added after online publication 10 March 2010: Darja Marolt’s name was originally spelled incorrectly.]

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The New York Stem Cell Foundation
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Conflicts of interest
The authors declare no conflicts of interest.

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