2018 Medicine by Design Symposium Report

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Abstract:

Medicine by Design's third annual symposium took place on December 4, 2018, at Toronto's MaRS Discovery District, and attracted more than 300 researchers in regenerative medicine from across the University of Toronto (U of T) and its affiliated hospitals. Speakers included four leading international experts and U of T researchers whose work is supported by Medicine by Design, a U of T initiative that seeks to accelerate translation of stem cell discoveries into new patient therapies. Seventy-five graduate students and post-doctoral fellows presented their research the largest poster session since the symposium launched three years ago.

The theme of this year's symposium was "Cell and gene therapy," and presentations covered a wide range of topics: from the application of cutting-edge tools to probe basic molecular mechanisms in stem cell biology to the practical challenges of developing cell therapies. With this report, we provide an overview of the talks, which were grouped into four sessions: muscle engineering, neural engineering, immune engineering and gene engineering.

Muscle Engineering:

A detailed understanding of developmental signalling pathways has led to protocols for generating diverse cell types from pluripotent stem cells (PSCs) for research, drug discovery and therapy. The muscle engineering session covered the differentiation of PSCs into different cardiac cell fates, transplantation strategies for restoring cardiac function and how macrophages could be used for endogenous repair in the skeletal muscle.

Stephanie Protze, a principal investigator at University Health Network (UHN) presented a method she developed as a post-doctoral fellow at UHN working with Gordon Keller to differentiate human (h)PSCs into sinoatrial node (SAN)-like pacemaker cells (SANLPCs) (Protze et al., 2017). Unlike electronic pacemakers, which must be replaced every few years, the biological pacemakers could potentially last for a person's lifetime and therefore may offer a long-term solution for patients with arrhythmias and other heart disorders. *In vitro* differentiated SANLPCs lacked the expression of the cardiomyocyte marker NKX2-5 as expected, exhibited typical pacemaker action potentials and the expected higher beating rates compared to ventricular cardiomyocytes. Following engraftment, the SANLPC effectively paced the rat heart. In her new lab, Protze will investigate whether these cells can act as pacemakers in the pig heart. The pig is considered a relevant pre-clinical model because its heart size and structure are comparable to the human heart. Protze also reported on her team's efforts to generate atrioventricular node (AVN)-like pacemaker cells (AVNLPCs) by first using single cell RNA sequencing to identify AVN markers, such as NKX2-5 and TBX3, and then using those markers to enrich for the *in vitro* differentiated AVNLPCs.

Cardiomyocyte replacement has shown promise for heart remuscularization and restoration of function following myocardial infarction (MI) in a variety of animal models, as previously presented by **Michael**

Laflamme (UHN) at the inaugural Medicine by Design symposium (Laflamme et al., 2007; Shiba et al., 2012; Shiba et al., 2016). This year, Laflamme showed their recent work on engrafting cardiomyocytes derived from human embryonic stem cells (hESC) into the pig model of MI. His team was able to grow large numbers of hESC-CMs in bioreactors to generate grafts as large as one cubic centimetre with cardiomyocyte purity (~80% cardiac troponin T positivity) mirroring that of the input cells injected at the site of injury. While the grafts matured over time, evoked minimal cellular rejection and displayed vascularization, graft-related arrhythmias were observed as had been previously reported in non-human primate studies. The arrhythmias persisted for 3-4 weeks at 250 beats per minute, compared to 90 for vehicle recipients, and were no longer detected by 4 weeks. Electroanatomical mapping (EAM) revealed a focal mechanism of graft automaticity in hESC-CMs that could be caused by contaminating pacemaker cells, poor coupling or initial graft immaturity. Laflamme stressed that improving the phenotype of the input cardiomyocytes may eliminate the incidences of graft-related arrhythmias and is the focus of their ongoing work.

Endogenous repair has emerged as an attractive possibility for treating skeletal muscle injury and disease. Central to this process are immune macrophage cells because they can adopt pro- and anti-inflammatory phenotypes and drive both tissue inflammation and repair. **Bénédicte Chazaud** (University of Claude Bernard Lyon 1, France) showed that skewing the proinflammatory Ly6C^{pos} macrophages to the anti-inflammatory Ly6C^{neg} state reduces scar formation and improves myogenesis after injury in the mouse model of Duchenne muscular dystrophy (DMD), known as *dmx* mice (Juban et al., 2018). Proinflammatory macrophages secrete TGF-ß complexed with LTBP4, a gene known to be associated with DMD, to promote collagen secretion from muscle fibroblastic cells which leads to scarring. Metformin-induced inhibition of AMPK reduced *ltbp4* expression and TGF-ß1 production *in vitro* and *in vivo,* while macrophage transcriptional profiling revealed a shift toward an anti-inflammatory phenotype. Importantly, metformin treatment resulted in greater muscle strength in treated animals. Both pro- (Ly6C^{pos}) and anti-inflammatory (Ly6C^{neg}) macrophages express *tgf ß1* but the protein is only secreted by the Ly6C^{pos}, suggesting posttranslational regulation that remains to be elucidated. Depending on the type of muscle injury, Chazaud proposed modulating the inflammatory phenotype of tissue macrophages as a promising strategy to drive repair.

Neural Engineering:

Advances in *in vitro* neural fate specification and 3D culture promise to accelerate our understanding of diverse neurological conditions and the development of therapeutic strategies to treat these conditions, from Parkinson's to diseases of the enteric system and deafness.

Lorenz Studer (Memorial Sloan Kettering Cancer Center) gave an overview of his lab's long-term efforts to develop *in vitro* differentiated dopamine neurons (DA) as replacement tissue for Parkinson's disease patients whose neurons are selectively lost. His team has successfully used small-molecule inhibitors against six developmental pathways to differentiate hPSCs into functional neurons and drive functional repair in the mouse, rat and monkey models of Parkinson's disease (Kriks et al., 2011). They have also established a clinical-grade protocol for manufacturing cell products for human studies. With 10 billion DA cryopreserved and ready for transplant, with no detectable PSC contaminants, a Phase 1 trial is expected to commence in 2019, pending FDA approval. The lab is also working to implement optogenetic tools to enable grafted cells to switch from an "on" or "off" state in freely moving animal models and to use CRISPR screens to identify genes that could help boost survival of engrafted cells (Steinbeck et al., 2015). Studer also stressed the need for a more flexible regulatory approval process to

facilitate the translation of improved “next gen” differentiation protocols from the lab into clinical practice.

Furthermore, Studer presented data on generating neural crest-derived enteric neural progenitors. Migration defects in these cells lead to fatal Hirschprung disease, caused by the lack of innervation in the distal gastrointestinal tract (Fattahi et al., 2016). In another project, the lab has made strides in differentiating hPSCs into glia by transiently overexpressing the transcription factor NFIA (unpublished data). NFIA increases glial competency by reducing DNA methylation associated with neuronal fates. The resulting astrocytes not only mimicked primary astrocytes *in vivo*, but also were able to integrate long-term (10 months) into cortical layers in mice after grafting. Studer’s future work will no doubt help elucidate the role of glia in brain development and disease.

In the auditory system, glial cells have demonstrated potential as a therapeutic cell source in the treatment of disabling hearing loss, which affects about five per cent of the world’s population. **Alain Dabdoub** (Sunnybrook Research Institute) presented data demonstrating that auditory neurons could be produced by reprogramming glial cells from the cochlea of the inner ear. The primary auditory neurons (PANs) transmit sound signals from the ear to the brain and are often lost due to noise damage, disease or old age. Overexpression of the transcription factors ASCL1 and NeuroD1 *in vitro* reprogram glial cells to an induced neuron phenotype characterized by Tau-GFP expression and a transcriptional profile typical of PANs (Noda et al. 2018). The induced neurons connect to cochlear hair cells and nuclear neurons when co-cultured with each respective tissue, although it remains to be determined if these synapses are functional. Ongoing single cell RNA sequencing work seeks to define the transcriptional states the glia transition through during reprogramming into neurons. Dabdoub’s team is also developing an *in vivo* mouse model in which PANs are eliminated by ouabain, a toxic compound, before applying gene therapy to endogenously reprogram glial cells to rescue hearing loss.

Over the past few years, efforts to model human brain development and disease have focused on brain organoids, which display key structural features that are not present in animal models. **Yun Li** (The Hospital for Sick Children) presented her work using brain organoids to study human brain pathologies associated with the PTEN-AKT-mTOR growth factor signalling cascade that regulates cortex development. Mutations in PTEN have been associated with increased brain size, or megalencephaly. Li was able to replicate this phenotype in human and mouse PTEN -/- organoids, consistent with PTEN’s role as a negative regulator of AKT-mTOR-induced cell proliferation and differentiation (Li et al., 2017). Intriguingly, increased neural progenitor proliferation and expansion led to increased cortical folding in human organoids only, supporting the notion that organoids appropriately model human corticogenesis. Inhibition of Akt resulted in smaller organoids, consistent with an earlier finding that Akt loss leads to microcephaly (Boland et al., 2007). Together these findings demonstrate that spatial and temporal control of the PTEN-AKT-mTOR signalling pathway is vital to regulating human neural development and brain pathologies.

Immune Engineering:

Immune cell engineering has led to some of the most promising advances in cancer therapy including the recently approved CAR-T immunotherapy, in which T cells are engineered to express anti-CD19 chimeric antigen receptor to target B-cell malignancies. Among the remaining challenges are antigen escape by cancer cells, which leads to relapse, as seen in several clinical trials, as well as off-target toxicity.

Yvonne Chen (University of California, Los Angeles) presented her work engineering single-chain, bispecific CARs capable of triggering T cell activation when either CD19 or CD20, another pan B-cell marker, is present on the tumour cell to reduce probability of antigen escape (Zah et al., 2016 A). In the mouse Raji tumour model, bispecific CARs demonstrated their efficiency in eradicating tumour cells and preventing spontaneous relapse for more than six months, in contrast to animals treated with conventional CARs, which relapsed due to antigen escape (Zah et al., 2016 B and unpublished data). Chen also presented COVERT (Cytoplasmic Oncoprotein Verifier and Response Trigger), a method they developed to target intracellular tumour markers to reduce off-target effects commonly associated with the extracellular targets (Ho et al., 2017). The COVERT method applies conditionally active granzyme B (GrB), which is kept inactive through an N-terminal SUMO1 peptide and triggers apoptosis upon activation. Cleavage of SUMO1 by SENP1, a SUMO-specific protease, highly expressed in some cancers, activates the enzyme and triggers cell death. Chen is looking to apply COVERT to enable T cells to selectively target tumour cells expressing the right protease. This may also allow the targeting of solid tumours that resist conventional immunotherapy.

Recently described innate lymphoid cells (ILCs) play important roles in inflammation, host defence and tissue remodelling, and thus have several potential applications in the development of cell therapies. **Sarah Crome** (UHN) identified a regulatory $CD3^{-}CD56^{+}$ ILC population (regulatory ILC) that is capable of directly regulating T cells and is associated with poorer clinical outcomes in ovarian cancer patients (Crome et al., 2017). These regulatory ILCs inhibited T cell expansion and altered $CD4^{+}$ and $CD8^{+}$ tumour-associated T cell cytokine production. Transcriptome analysis and phenotypic characterization determined that these regulatory ILCs have low cytotoxic activity, produce IL-22, and have a distinct gene expression profile from other ILCs. NKp46 is highly expressed in these cells and is required for T cell suppression, as demonstrated using a knockout mouse and anti-NKp46 antibodies in *ex vivo* T cell cultures with regulatory ILCs. In B7-$H3^{-/-}$ mice, a regulatory ILC population that limited T cell-induced tissue destruction of beta islet cells was also observed and, similar to human regulatory ILCs, expressed IL-22 and NKp46. Going forward, Crome is exploring how to exploit regulatory and tissue repair functions of various human ILC populations.

Gene Engineering:

The gene engineering session covered transcriptional regulation of pluripotency and cell fate specification, epigenetic editing and synthetic biology approaches for generating desired cell populations.

The study of gene regulatory networks in mammalian cells has been revolutionized by CRISPR gene editing technology, which enables the rapid and robust deletion of specific regulatory DNA regions to screen target genes of interest. Jennifer Mitchell (Department of Cell and Systems Biology, U of T) applied CRISPR to investigate transcriptional networks in pluripotency by deleting enhancer elements individually or in tandem from so-called enhancer clusters (ECs), also known as super enhancers. A number of ECs have been computationally predicted but their functional relevance remains unclear. Mitchell investigated the role of ECs in regulating target gene expression through partial or complete deletion of enhancer elements. The data suggest that separate elements within the ECs and individual enhancers act redundantly to fine-tune expression of target genes (Moorthy et al., 2017). Furthermore, different ECs can be employed in different cell populations. Whereas Sox2 expression is regulated by a downstream EC in mouse ES cells, a more proximal enhancer regulates Sox2 expression in ES-derived neural stem cells (Zhou et al., 2014; and unpublished data). A better understanding of transcriptional regulation in PSCs and progenitors could be applied to enhance generation of desired cell types *in vitro.*

It could also reveal disease mechanisms given that the majority of disease-associated single nucleotide polymorphisms have been mapped to noncoding regions where regulatory elements reside.

Enhancers and promoters are subject to epigenetic regulation with a growing list of diseases associated with changes in DNA methylation. Rudolf Jaenisch (Massachusetts Institute of Technology) argued that the reversible nature of epigenetic regulation makes it an attractive therapeutic target. To this end, his lab developed tools to investigate and edit epigenetic markers in regulatory sequences of genes of interest (Stelzer et al., 2015; Liu et al., 2016). When applied in the context of disease, the targeted demethylation of the 5'UTR of FMR1 gene, which is hypermethylated and repressed in Fragile X syndrome, restored FMR1 expression in patient-derived iPSCs (Liu et al., 2018). Furthermore, neurons derived from those cells had a normal phenotype and maintained their demethylated status when transplanted into mouse brains. However, given the repetitive nature of the target GGC sequence, off-target effects are a concern, with more than 1700 off-target sites and 29 genes changing their methylation state, as revealed by Chip-Seq. Jaenisch is now working to develop a similar approach to target Rett syndrome, another neurological disorder. Unlike Fragile X, Rett syndrome primarily affects females (males typically die before birth) who carry a mutant copy of the MECP2 gene while the other working copy is inactivated due to hypermethylation of the silenced X chromosome. Reactivation of the hypermethylated wildtype copy of MECP2 is a potential therapeutic approach after mouse studies found the disorder to be reversible. To this end, Jaenisch showed a MECP2 dual colour reporter mouse ES line, which will allow them to demethylate the silenced MECP2 promoter and test if MECP2 can be activated in differentiated neurons. While epigenetic editing holds therapeutic promise, Jaenisch noted that its applicability will depend on the type of disorder (developmental versus reversible) and the number of cells that would have to be targeted.

Laura Prochazka (Institute of Biomaterials and Biomedical Engineering, U of T) presented her ongoing work as a post-doctoral fellow in Peter Zandstra's lab, where she is developing modular synthetic gene circuits for a programmable cell behaviour. Having previously developed a bow-tie type of circuit for HEK293 cells, she reported on applying the same principles to hPSCs to increase the efficiency of *in vitro* derived endodermal cells and eventually pancreatic progenitors (Prochazka et al., 2014). The bow tie architecture consists of a sensor input module, that recognizes a set of miRNAs, a computational module that integrates the signal in a predicted way and an output module that changes cell behaviour. Using a computational model and whole genome miRNA Seq data, Prochazka determined the minimal inputs and circuit configuration that guide hPSCs through two decision-making steps from hPSC to definitive endoderm to pancreatic progenitors. Using a newly developed platform for rapid construction and testing of these gene circuits, she optimized and validated circuit performance in hPSC and definitive endoderm.

Special Speaker:

Finally, one talk was dedicated to machine learning and its emerging potential in drug discovery. Alán Aspuru-Guzik (departments of Chemistry and Computer Science, U of T) argued that artificial intelligence is also necessary if we are to fully explore the vast chemical space, comprising more molecules than there are visible atoms in the universe, to identify potential therapeutics (Sanchez-Lengeling and Aspuru-Guzik, 2018). This becomes possible through iterative cycles of algorithm-driven selection of potential compounds, from all possible small and medium-sized molecules, and experimental validation. The main challenge, Aspuru-Guzik said, is the lack of negative data in the literature. Because machine learning is only as good as the data it learns on, the missing negative results bias the algorithms towards experiments that reached desired goals.

Conclusion:

Since its founding four years ago, Medicine by Design has brought together researchers from diverse disciplines from across the university and its affiliated hospitals to develop treatments for some of the world's most common diseases. The third annual symposium reflected the large breadth of regenerative medicine research happening in Toronto to fulfill this mandate. The presentations covered a wide range of topics, from basic insights into stem cell function to technology development and translation of discoveries into potential new treatments. We look forward to learning about new insights coming out of these collaborations in the coming years.

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