Fgl2/fibroleukin: A Biomarker for Tolerance Induction in Transplantation

Gary A. Levy, MD
Director University of Toronto Transplantation Institute
Toronto Ontario Canada

Organ transplantation remains the most effective treatment for patients with end-stage organ failure; however, the shortage of donor organs and need for long-term immunosuppressive therapy limits its effectiveness. The discovery of biomarkers that predicts transplantation tolerance would be useful to identify patients in whom immunosuppression could be reduced or discontinued and could also have implications for patients with autoimmune disease. We have established an animal model of tolerance by transplanting fully HLA mismatched hearts from BALB/c (MHC haplotype H2d) into C3H (MHC haplotype H2k) recipients. Tolerance was induced through a short initial regimen of rapamycin. Eleven of twelve (11/12) heart grafts survived for greater than 100 days post-transplant, and histology showed normal cardiac structure with no evidence of rejection. These hearts contained CD3+CD4+CD25+FoxP3+ T regulatory cells which were strongly positive for FGL2/fibroleukin. By ELISA, tolerant animals had high levels of plasma FGL2. In contrast, cardiac grafts without treatment were rejected within 10 days post-transplantation and heart histology showed marked mononuclear cell infiltrates, vasculitis with thrombosis and cardiomycyte necrosis and plasma levels of FGL2 were reduced or absent. Specific tolerance was confirmed by the observation that skin grafts from BALB/c donors were accepted, whereas grafts from a third party (C57BL/6) were rejected within 15 days. *In-vitro,* T-lymphocytes from tolerant mice specifically exhibited lower proliferation and cytotoxicity to donor targets than rejecting controls. To examine the mechanism of tolerance and rejection, cytoplasmic proteins from transplanted tolerant and rejecting hearts were extracted and separated by 2D gel electrophoresis in order to identify specific protein biomarkers of rejection and tolerance. Gel images were analyzed, and differentially expressed proteins between normal and rejecting hearts were subjected to MALDI-TOF mass spectrometry for protein identification. Forty four (44) proteins were found up-regulated and 52 proteins were down-regulated in rejecting hearts and 38 proteins were upregulated and 42 downregulated in tolerant hearts. Of interest, galectin-1 was increased 2-3 fold in rejecting hearts, whereas heat shock proteins (HSP-10) levels were decreased. In tolerant mice, a consistent finding was the finding of high levels of the immunoregulatory protein FGL2/fibroleukin. As it has been reported that FGL2 is an effector of all classes of T regiulatory cells, we hypothesized that this *in vivo* graft acceptance without need for immunosuppression (tolerance) could be due to a suppression of reactive T-cells through a T-regulatory cell mediated mechanism through production of FGL2, a potent immunoregulatory protein. To examine the immunoregulatory effects of FGL2, we demonstrated that in vitro FGL2 through binding to the inhibitory FcγRIIB receptor prevented dendritic cell maturation, inhibited T cell proliferation and induced B cell apoptosis. In vivo, recombinant FGL2 prevented rejection of fully mismatched heart transplants. Furthermore, transgenic mice which constitutively overexpressed FGL2 accepted fully mismatched heart allografts without the need for immunosuppression. Studies are now underway to confirm the relevance of these observations in humans to human disease. Supported by grants the Canadian Institutes of Health Research, the Heart and Stroke Foundation of Canada, the Physician Services Inc. and Canada Foundation for Innovation.

Validation of a 16-Gene Expression Signature in Non-Small-Cell Lung Cancers from FFPE Samples

Sung-Liang Yu, 俞松良, Ph.D.
Assistant professor, Department of Clinical Laboratory Science and Medical Biotechnology, College of Medicine, National Taiwan University, Taiwan

Non-small-cell lung cancer (NSCLC) causes more deaths than any other type of cancer worldwide. The recurrence rate among patients with early-stage NSCLC is about 40% within 5 years even after receiving combined surgical and chemo therapies. The current tumor progression classification system is not an adequate measurement for treatment prognosis. Here we developed a multiplexed, quantitative biomarker assay method to validate the association between a 16-gene expression signature and the risk of overall or relapse-free survival. A multiplex of 16 NSCLC biomarkers, previously identified from a microarray study, and a reference gene was developed and evaluated. This multiplex was used to analyze 77 surgically resected, formalin-fixed, paraffin-embedded (FFPE) samples of NSCLC containing major histology types of adenocarcinoma (62%) and squamous cell carcinoma (26%). Using as little as 5ng of total RNA extracted from FFPE slices, we quantified the expression level of each gene with a linear correlation coefficient > 0.99. Risk scores were calculated

based on the expression levels of the 16 genes. Patients with a low-risk, 16- gene signature had longer survival time than those with a high-risk 16-gene signature (p=0.001, log-rank test). In addition, patients with high-risk, 16-gene signature had shorter relapse free survival time than those with a low-risk, 16-gene signature (p=0.006, log-rank test). As a measure of the multiplex assay as an independent prognostic indicator, the hazard ratios (adjusted by age, gender, stage, and histology) were calculated as 2.2 (95% CI= 1.31-3.66, p=0.003) and 1.8 (95% CI= 1.11-2.92, p=0.0183) for overall survival and relapse-free survival, respectively.

Technical progress impacting the derivation and characterization of human induced pluripotent stem cells

Knut Woltjen
Manager, Ontario Human iPSC Facility, Hospital for Sick Children, MaRS Centre, Toronto, Canada

Transgenic expression of four defined transcription factors (c-Myc, Klf4, Oct4 and Sox2) is sufficient to reprogram somatic cells to induced pluripotent stem cells (iPSCs), gaining resemblance to embryonic stem cells (ESCs) in their potential to differentiate into a spectrum of adult cells types. This technical advance circumvents legal, moral, and ethical issues currently impeeding human ESC-based regenerative medicine. Human iPSC technology, beyond holding future potential for autologous cell transplantation therapies, has opened up the possibility of producing *in vitro* drug screening panels and achieving more accurate human disease models. In collaboration with leading international research institutions, the Ontario Human iPSC Facility is developing best practices in reprogramming technologies with a focus on the establishment and banking of normal and patient-specific primary fibroblast cell lines, and the subsequent generation and characterization of research-grade hiPSCs. The Facility has established access to a large resource of paediatric patient samples and has so far generated and banked hiPSCs from patients bearing neurological, musculoskeletal, cardiac and lung disorders. Technical advances impacting primary hiPSC induction and the quality of derivative products – such as non-viral reprogramming factor delivery via *piggyBac* (PB) transposition, a technique which allows high-fidelity reprogramming and downstream removal of the reprogramming transgenes – are being adapted and applied. Furthermore, the Facility is developing streamlined characterization protocols including multiplex gene expression profiling and flow cytometry-based assays to evaluate hiPSC clones prior to teratoma analysis and downstream applications. The Ontario hiPSC Facility also serves an important role in providing researchers training and direct access to cutting edge iPS technologies for independent research endeavours. Researchers interested in initiating collaboration or placing service requests are encouraged to contact contact@ontarioips.ca or visit www.ontarioips.ca.

Personalized Genetic Device for Life Science Research and Novel Multiplex Quantitative PCR for Gene Expression

Tiffany Jiang, Ph.D.

Asia Pacific Marketing Manager, Beckman Coulter

As life science research has been making tremendous progresses over time, we have entered post Human Genome Project era in the 21st century. In this era, new medicine and drug discovery will be making more impacts on our lives. The current focus of research studies are on genes, their expression products and the interactions of those products in cells. Those studies will help to further understand the mechanism of diseases, enable us to develop new and effective treatments and drugs. This presentation will discuss the trend of gene expression and highlight a novel method (XP-PCR) to improve linearity and efficiency using a multiplex quantitative approach. This technology is widely applied by tumor research centers (MD Anderson, MSK, etc.) for prognosis prediction of Glioblastoma Multiforme and prostate cancer with FFPE samples, SBRCT tumor typing. In addition, recent publications in Nature Medicine and PNAS illustrated the importance of accurate quantitative gene expression analysis for immunotherapy and cellular analysis. Scientific results in the area of stem cell, plant, animal and Pharmaceutical researches will be discussed as well.