Meeting report

A burst of energy in metabolic disease research

Jaswinder K Sethi

Address: Department of Clinical Biochemistry, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QR, UK.
E-mail: jks30@cam.ac.uk

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A report on the 'Diabetes Mellitus' and 'Adipogenesis and Obesity' joint Keystone Symposia, Banff, Canada, 4-10 March 2004.

The genomic era has injected a fresh burst of energy into the study of complex metabolic diseases. Over 900 delegates congregated at the foothills of the Rockies in Banff, Canada to learn about the latest developments in research into obesity and the commonly associated disorder, type 2 diabetes mellitus. Allen Spiegel (National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, USA) kicked off the line up and introduced the major initiatives that the National Institutes of Health (NIH) have recently established to support diabetes and obesity research. These include the establishment of large-scale, multidisciplinary, collaborative efforts that combine and coordinate information generated from genomics, proteomics, stem-cell biology and bioinformatics. In particular, members of the following consortia were well represented by the speakers at the meeting: the Beta Cell Biology Consortium and Endocrine Pancreas Consortium [http://www.cbil.upenn.edu/EPConDB]; the Diabetes Genome Anatomy Project [http://www.diabetesgenome.org]; the Nuclear Receptor Signaling Atlas [http://www.nursa.org] and the Mouse Metabolic Phenotyping Centers [http://www.mmpc.org]. Other initiatives that are clearly beginning to make an impact on diabetes and obesity research include the identification of obesity genes in invertebrates, and interdisciplinary research bridging neurobiology and behavioral science. Some of the major new developments that dominated the conference stemmed from these initiatives and are discussed in this report. It was refreshing, however, to note that these collaborative efforts were not limited to US-based research groups and often appear to be truly global collaborations.

Molecular control of adipogenesis and obesity

Gary Ruvkun (Harvard Medical School, Boston, USA) highlighted the fact that important lessons for human disease can be learnt from the biology of invertebrates. He and his colleagues have used genome-wide RNA interference (RNAi) to identify genes involved in fat deposition in *Caenorhabditis elegans*. Although these worms do not have a defined adipose-like tissue, they do accumulate triglycerides in identifiable depots in response to nutritional and environmental cues. Indeed, there are evolutionary ancient mechanisms to regulate feeding and fat storage, even in these simple organisms. Of the 16,757 genes Ruvkun and co-workers suppressed by RNAi, inactivation of 261 led to reduced fat accumulation but was also accompanied by larval arrest, embryonic lethality or sterility, so these genes were not studied further. The team was able to identify 305 genes whose inactivation reduced body fat content and 112 that led to increased fat storage; all of these sequences are available at Wormbase [http://www.wormbase.org]. Many of these fat-regulating genes have mammalian homologs that have already been implicated in fat metabolism and storage. Approximately 150 have not previously been linked to fat biology in mammals, however. Hence, Ruvkun and colleagues have identified 150 potentially novel mammalian targets for regulating triglyceride storage.

Also looking at evolutionarily common ancestors, Gökhan Hotamisligil (Harvard School of Public Health, Boston, USA) reminded attendees that the geneticist's favorite subject, the fruit fly *Drosophila*, has a rudimentary fat body that has many parallels with mammalian adipose tissue. The fact that the fat body plays a major role in host defense and immune function has provided a novel perspective of the biological functions of mammalian adipose tissue. The functional parallels in immune biology and energy metabolism were further echoed in talks by Stuart Weisberg (Columbia University, New York, USA) and Steven Shoelson (Joslin Diabetes Center, Boston, USA). Weisberg described how

adipose-tissue expression profiles from various models of adiposity led him and his colleagues to the finding that adipose tissue from obese rodents and human subjects not only carries the hallmarks of an 'inflamed' organ, but is also infiltrated by bone-marrow derived macrophages. Furthermore, macrophages are also present in adipose depots that coat skeletal muscle. Weisberg postulated that these cells might be the most likely source of the key inflammatory cytokines that have been linked to obesity-related insulin resistance. Previous work by Shoelson and others has implicated inhibitor of kappaB kinase β (IKKβ) in mediating cytokine-induced insulin resistance. To further identify the tissues in which IKKβ activity could be relevant, Shoelson has generated a new series of tissue-specific, genetically modified murine models. Preliminary phenotyping and expression profiling appears to suggest that the liver and adipose tissue are involved in the insulin-sensitizing effects of IKKβ inhibition *in vivo*, but the β cells and skeletal muscle show little change. Could the liver and adipose tissue be the organs in which the immune system impacts on insulin sensitivity in obese diabetics?

Diabetes mellitus: molecular signaling, genes and therapeutics

The pathophysiology of the common polygenic form of type 2 diabetes results from early onset insulin resistance coupled with functional defects in insulin secretion by pancreatic islet β cells. Islet transplantations offer a potentially valuable therapeutic approach for the treatment of diabetes, but significant obstacles must be overcome to combat the shortage of islet tissue. This requires a greater understanding of the ontogeny of the endocrine pancreas. Only then will it be possible to isolate the appropriate progenitor cells, and to expand and differentiate them *in vitro* prior to transplantation. To this end, the Endocrine Pancreas Consortium and the Beta Cell Biology Consortium were established. Klaus Kaestner (University of Pennsylvania, Philadelphia, USA) presented the tremendous progress that has been made towards constructing and sequencing cDNA libraries that are enriched in rare transcripts expressed during pancreatic development. The figures currently stand at 15 mouse cDNA libraries containing more than 70,000 transcripts, and seven human libraries containing more than 110,000 transcripts. Kaestner's group has used cluster analysis to identify approximately 14,000 representative expressed sequence tags (ESTs) from these libraries, and has then used these to make the 'PancChip' microarray. Kaestner reported that version 5.0 of this chip is now available from the Endocrine Pancreas Consortium, and the first human pancreas microarray, termed hPancChip1.0, is also being developed.

Using a different approach, Duncan Odom (Whitehead Institute/Massachusetts Institute of Technology, Cambridge, USA) combined the power of genome-wide promoter arrays and chromatin immunoprecipitation (ChIP) systematically to identify the genes in human liver and pancreatic islets that are directly regulated by the transcriptional regulators HNF1α, HNF4α and HNF6 and transcribed by RNA polymerase II. In so doing, his group has dissected the tissue-specific regulatory networks orchestrated by the three transcription factors in both liver and pancreas development. The model they obtained provides a plausible mechanism for the less common form of diabetes that is caused by HNF4α deregulation.

Another computational approach was taken by the Beta Cell Biology Consortium to identify several hundred human cell-surface antigens that may allow the detection of progenitor pancreatic cells. These antigens are currently being used by the Consortium for conventional and genetic immunization to create a new collection of antisera that will be made available (within the next year), for further characterization by the community. These new developments are set to substantially increase our understanding of the ontogeny of the endocrine pancreas.

Insulin resistance and type 2 diabetes - a problem in the power plant

Previously, researchers hypothesized that mitochondrial dysfunction in insulin-target tissues might be correlated with the common form of human type 2 diabetes, but this could not be attributed to the deregulated expression of a single gene. This conclusion was re-iterated by two speakers: Vamsi Mootha (Broad Institute, Cambridge, USA) and Mary-Elizabeth Patti (Joslin Diabetes Center, Boston, USA), who described their independent studies of the differential expression profiles of control, prediabetic and diabetic human skeletal muscle. Reasoning that the differential expression of groups of related genes might be co-ordinately altered in diabetic muscle, both groups tested this hypothesis using novel, innovative computational approaches together with publicly available gene-ontology lists.

Mootha and colleagues developed an iterative statistical approach they called gene set enrichment analysis (GSEA), which uses previously defined gene sets - based on biological pathways - to increase the signal relative to noise, and to improve the statistical power of any observed changes. In contrast, Patti and colleagues annotated their data using programs such as GeneSpring (from Silicon Genetics Inc.), UnCHIP (from Harvard University, USA) and Onto-Express (from Wayne State University, Detroit, USA). They then used GenMAPP, MaPPFinder (both from the Gladstone Institutes, University of California, San Francisco, USA) and Onto-Express to integrate the expression data with known pathways, and to determine confidence levels for differential expression within ontology groups. The highest scoring gene sets contained many genes that are members of the mitochondrial oxidative phosphorylation pathway; all of these oxidative phosphoryation genes showed a modest but consistent decrease in expression in diabetic tissue, and it seems

that a subset is co-regulated across many tissues and highly expressed in insulin-responsive, glucose-disposal tissues. Using motifADE - a generic tool that combines gene-expression patterns with promoter analysis - Mootha and colleagues were able to hypothesize the identity of key regulators in this transcriptional network. Subsequent experimental evidence from more traditional molecular and pharmacological approaches confirmed that these players are, in fact, nuclear respiratory factor-1 (NRF-1) interacting with peroxisome proliferation-activated receptor-γ (PPAR-γ) coactivator-1 (PGC1α) to co-regulate estrogen-related receptor α (ERRα) and GA-repeat binding protein α (GABPα). Together these factors control the expression of the oxidative phosphorylation genes. Patti and colleagues also found that expression of NRF-1 was reduced in diabetic subjects only, but the expression of PGC1α and PGC1β was decreased in both diabetic and pre-diabetic subjects, a finding that suggests that they may be good candidates for diagnosing an individual's risk of developing type 2 diabetes.

The role of proper mitochondrial function in insulin action may not be limited to skeletal muscle. Evidence presented by Silvia Corvera (University of Massachusetts Medical School, Worcester, USA) supported this notion by demonstrating that mitochondrial biogenesis and remodeling occurs during adipose tissue development. She also suggested that mitochondria might play a major role during the development of obesity and in the actions of the insulin sensitizer rosiglitazone.

Signaling pathways and diabetes

To identify novel candidate proteins that may be involved in the signals that regulate insulin-sensitive glucose uptake, Michael Czech (University of Massachusetts Medical School, Worcester, USA) and co-workers have looked at differential expression of genes in insulin-sensitive compared to insulin-resistant states, as well as of genes that are selectively expressed in differentiated adipocytes and muscle, the two principle tissues in which insulin stimulates glucose uptake. In addition, he has taken a proteomic approach to identify proteins that are intimately associated with vesicles containing the insulin-responsive glucose transporter-4 (Glut4) or with the signaling serine/threonine protein kinase B (also known as Akt). Complementing these approaches with gene silencing by RNAi, Czech's group has unveiled 200 candidate proteins including some previously known suspects, such as the receptor interacting protein RIP140. The novel candidate proteins that were mentioned in Czech's talk include WNK1, a protein kinase involved in ion transport in the kidney, an exocytosis-related protein, EHD1, and a SNARE protein involved in the control of synaptic vesicle fusion.

Czech also described his use of RNAi technology to systematically knock-down key signaling molecules and screen for their requirement in insulin-stimulated glucose uptake in an *in vitro* adipocyte model system (3T3-L1 cells). To date, two pathways have been postulated to mediate insulin-stimulated glucose uptake: the conventional phosphatidylinositol 3-kinase/protein kinase B (PI3K/PKB)-dependent pathway, which may recruit downstream effectors such as protein kinase C (PKC) λ and ζ; and the novel CAP-Cbl-Crk-TC10 pathway, which is independent of PI3K activity. Both pathways are activated by insulin receptor tyrosine kinase and perturbations have been shown to modify insulin-stimulated Glut4 translocation. Using specific gene inactivation, Czech's group could not demonstrate a requirement for the CAP-Cbl-Crk--TC10 pathway or signals dependent on PKC λ and ζ. Instead, they have found that both Akt1 and Akt2 are absolutely required for normal insulin-stimulated glucose transport, with Akt2 playing a predominant role. That Akt2 is also relevant to human diabetes was emphasized by Stephen O'Rahilly (Cambridge University, UK), who announced the discovery of a naturally occurring, but rare, dominant-negative mutation in the human *Akt2* gene. This mutation was associated with a dominantly inherited form of severe insulin resistance. It is the first example of an inherited gene defect in a post-receptor signaling molecule causing human diabetes and was identified from the group's severe insulin resistance (SIR) cohort (now numbering 250 subjects). These reports now firmly establish a role for Akt2 in normal insulin-stimulated glucose uptake.

In conclusion, the past few years have seen an avalanche of new technologies being successfully applied to understanding complex disease states such as obesity and diabetes. Transgenics and knockout mouse technology has now been joined by transcriptomics, proteomics and genome-wide RNAi, all of which, when coordinated, are proving to be very powerful research approaches. Good times are indeed ahead for obesity and diabetes research as the way is being paved for multidisciplinary approaches to uncover the underlying causes of these multi-factorial diseases. Abstract books from the meeting can be ordered online from [http://www.keystonesymposia.org]

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