Theme 7
Applying new science and technology

Plenary 7.0

Using Genomics and Systems Biology to Address Complex Problems:
Pancreatic Beta Cell Apoptosis in Diabetes Mellitus

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Summary
In the present text the author briefly discusses the use of the systems biology approach to understand complex human diseases, taking as an example type 1 diabetes mellitus (T1DM). In the course of this discussion, a definition of the systems biology approach is presented and its potential impact on the 3Rs commented.

Keywords: systems biology, microarray analysis, genomics, cytokines

Introduction

It will be discussed here the use of the systems biology approach to understand complex human diseases, taking as an example type 1 diabetes mellitus (T1DM). In the course of this discussion, a definition of the systems biology approach will be presented and its potential impact on the 3Rs commented.

Type 1 diabetes mellitus (T1DM) is one of the most prevalent chronic diseases in children and adolescents, with an incidence ranging from 4-30 cases per 100,000 persons-year in Europe. The global prevalence of T1DM is increasing, and there is no cure for the disease – affected patients require life-long insulin therapy. Unfortunately, exogenous insulin therapy is not a perfect substitute for endogenous insulin production, and the chronic elevation in blood glucose levels may lead to the so called chronic complications of diabetes, including blindness, renal failure, gangrene, myocardial infarction and stroke.

Accumulating evidence indicates that pancreatic beta cells, the only physiological source of insulin production, die by apoptosis in early T1DM. Apoptosis is an active, gene directed process, and recent observations by our group (s. "selected references" below) suggest that beta cell fate following exposure to immune mediators is a complex and highly regulated process, depending on the duration and severity of perturbation of key interacting gene networks. In line with this possibility, genetic susceptibility to T1DM might also depend on gene networks, explaining why it has been so difficult to pinpoint "diabetes susceptibility genes". This departs from the traditional view of phenomena, based on the study of signalling pathways by intuitive inferences based on the study of individual pathway components. Identification of complex and interacting gene/protein patterns poses a formidable challenge, but the sequencing of the human genome, and of the genome of several other species, makes it possible to address it by the use of new high throughput technologies, such as microarray analysis and proteomics. The increasing use of massive parallel analysis of gene/protein expression is emphasising that interpathway cross-talk reflects levels of complexity that cannot be adequately explained by studying individual pathways in isolation. In other words, to fully understand the abnormal cell responses during a pathological stage we need a global multivariate strategy, as proposed by the systems biology approach.

The systems biology approach seeks to devise models based on the comprehensive, qualitative and quantitative analysis of all

constitutive parts of a cell or tissue with the ultimate aim of explaining biological phenomena through the interaction of all its cellular and molecular components. This is based on the analysis of large scale datasets, such as global gene or protein expression. The model is then refined through introduction of perturbations in the system and additional rounds of large scale gene/protein analysis. Systems biology thus turns into an interactive process, in which researchers devise models based on large datasets, make predictions based on the model, and then perform additional large scale experiments to test/validate the prediction and further refine the model.

Against this background, we are utilising microarray analysis and detailed promoter studies to clarify the pattern and regulation of gene expression in primary rat beta cells and in human islets exposed for different time points to the pro-apoptotic cytokines interleukin-1β (IL-1β + interferon-γ (IFN-γ). Nearly 2000 cytokine-induced genes were identified, and the picture emerging from these findings is that beta cells are not passive bystanders of their own destruction. Beta cells respond to cytokine-mediated damage by triggering several genes involved in defense/repair and endoplasmic reticulum stress, by decreasing their most differentiated functions and their capacity for growth and regeneration, and by inducing expression of diverse cytokines and chemokines. Several of these effects of cytokines depend on the activation of the transcription factors NF-κB and STAT-1, and by blocking NF-κB or STAT-1 activation we prevented both cytokine and dsRNA (double stranded RNA) + cytokine-induced rat beta cell death. Subsequent experiments, combining NF-κB blocking and microarray analysis, suggested that NF-κB functions as a "master switch" of IL-1β effects on beta cells, controlling diverse networks of transcription factors and effector genes that contribute to beta cell apoptosis. STAT-1 probably plays a similar role for IFN-g-induced genes. This hypothesis was further investigated by time course and cluster analysis of gene expression in cytokine-treated insulin-producing INS-1 cells, and by "*in silico*" and molecular biology analysis of the promoter regions of genes located in different clusters. Based on the data obtained by our different microarray analysis, we are presently constructing a "Beta Cell Gene Expression Bank", which is already accessible at http://t1dbase.org/cgi-bin/enter_bcgb.cgi. The ultimate goal of this open access resource is to identify and annotate all genes expressed in rat, mouse and human beta cells.

By combining functional studies with microarray analysis, performed with or without targeted perturbations of the system (following the systems biology approach), we hope to eventually understand the complex mechanisms regulating the cytokine-induced beta cell "decision" to undergo apoptosis. This information may point out to new approaches to prevent beta cell death in early T1DM.

These new approaches to understand biological phenomena may have an impact on the 3Rs. Thus:

1. By allowing us to obtain massive information from limited amounts of tissue, it increases the information obtained from a given number of animals, thus decreasing the number or animals required for the experiments. For instance, in the last few years our own laboratory decreased by more than 80% the number of rats and mice utilised in research (these are the only models utilised by our laboratory). A caveat is that in the present phase of exploration and discovery of the genome, there may be an increase in the generation and use of transgenic animals to characterise the function of newly discovered genes.

2. The use of computer models, based on the systems biology approach, may eventually decrease the need of using animals. Computer models of complex and integrated biological systems are as good as the data used to make them. Unfortunately, our present understanding of the interaction between the multiple cellular and molecular components of biological phenomena is limited and fragmentary, making it very difficult to model complex biological responses in a reliable and dynamic way. Thus, additional work on *in vitro* and *in vivo* experimental models is required to accumulate sufficient information for adequate computer modelling. Of note, and in our view, key events detected by computer modelling will, in most cases, need to be confirmed in biological systems.

Conclusion

In conclusion, systems biology is a novel and vibrant field, which is creating new rules as it moves ahead. The challenge it poses is enormous, but it seems that for the first time we have the tools and the adequate experimental approach to tackle biological problems at its real level of complexity.

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