Project: Microarray Validation of Cardiovascular Risk Factors

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Introduction

The broad application of cDNA and oligonucleotide microarrays has demonstrated that generating gene profiles does not guarantee targets for diagnosis or therapy. The major hurdle is to derive biologically relevant information from an extensive list of differentially regulated genes *(Moreau Y, Trends Genet 2003)*. Biostatistical analysis is used to identify statistically significant changes in gene expression, but statistics alone cannot assess the biological relevance of a differentially regulated gene. Not surprisingly, many gene profiling experiments yield uninterpretable data consisting of long lists of genes. The success and quality of a gene profiling experiment depends upon well-designed secondary and tertiary screens to determine the biological function of large numbers of candidate genes. Such screens must be highly specific for a particular biological question. Simple cell-culture assays are often inadequate models of complex problems such as cardiovascular disease. And cellular assays are both laborious and costly. Yet typical animal models such as mice are equally unsuitable for validating large numbers of candidate genes. We aim to address this fundamental question: how can one effectively close the information gap between a microarray project and the genetic basis of a human disease? An additional problem is the low probability of events typical of most multifactorial diseases, including cardiovascular disease. Large populations of patients must be studied over long periods of time, to generate a sufficient number of primary and secondary end-points that correlate with changes in gene expression. Obviously, such studies are prohibitively expensive. To circumvent this difficulty, we will target a select group of patients at high risk for cardiovascular disease. In Germany, more than 50,000 patients suffer from end-stage renal disease. Despite advances in hemodialysis, the mortality of these patients is high (approx. 15% per year), with more than 50% of patient deaths caused by cardiovascular events. Since all relevant patient data are precisely recorded under close clinical supervision, this group is particularly well suited for correlating gene profiles with morbidity and mortality due to cardiovascular disease.
C. elegans has a limited lifespan of 14-18 days. Its genome, development and cellular architecture are precisely defined. Hence, this organism is exceptionally suitable for investigating the effects of genetic changes. Over the last years, several *C. elegans* genes have been isolated that modulate the lifespan of this organism (reviewed in *Coffer P, Sci STKE 2003)*. For example, elimination of daf-2, a homologue of the insulin/IGF-1 receptor, prolongs the lifespan of *C. elegans*. DAF-2 activates the phosphatidylinositol kinase AGE-1 which phosphorylates PDK-1. Mutation of either kinase prolongs the lifespan, underlining the importance of the insulin-like signalling cascade, a pathway conserved between *C. elegans* and humans, in lifespan control. Essential for the life-prolonging effects is transcriptional activation of genes induced by Forkhead. Recent experiments indicate that inhibition of TOR kinase also prolongs lifespan *(Vellai T et al., Nature 2003)*. Lastly, gene expression in *C. elegans* can be efficiently reduced using RNAi, making it the ideal model system to evaluate large number of genes for their effect on complex biological programs.

Results/Project Status

We proposed to profile the gene expression of a population at high risk for cardiovascular disease and subsequently validate differentially regulated genes in *C. elegans*. The project consists of three steps: 1. Identification of candidate genes that are differentially regulated in patients with increased cardiovascular risk. 2. Modulation of lifespan by potential candidate genes in C. elegans. 3. Validation of potential target genes in humans. We demonstrated the feasibility of this approach using peripheral blood mononuclear cells (PBMC) and comparing the gene profile (7.5 K) of a small number of hemodialysis patients with a pre-dialysis population and healthy age-matched controls. Statistical analysis revealed that each group forms a distinct cluster, with approximately 150 genes differentially regulated in ESRD patients compared to the control group. These preliminary results demonstrate that PBMC of hemodialysis patients display a unique expression pattern of genes that differentiates this population from normal controls. Notably, TOR and Forkhead, two genes that modulate the lifespan of *C. elegans*, were differentially regulated in ESRD patients. We have now completed the data and sample collection of 330 dialysis patients, and established a protocol to amplify the extracted mRNA. The 35k cDNA clones (RZPD) will be purified by October 2005, and arrrayed on two sets of glass sides. Meanwhile, we examined the effect of different signalling cascades on the lifespan of *C. elegans*. Using either RNAi or mutant strains generated in our laboratory, we characterized the important role of the insulin-like signalling cascade, a pathway highly conserved in both *C. elegans* and humans. We found that deletion of the peptide transporter PEPT1 reduces the activity of TOR, and increases the stress tolerance and lifespan of *C. elegans*. We identified the stress-sensor of the insulin signalling cascade, the serum- and glucocorticoid-response kinase SGK; mutations of this kinase cause the same increase in lifespan as mutations of DAF-2, AGE-1, and PDK-1. Our work also revealed that the lifespan of *C. elegans* can be effectively manipulated using RNAi. To use this tool in high-throughput screens, we generated an RNAi plasmid library of 17.010 clones.

Fig 1: *Preparation of the 35k Chip using a Hamilton Workstation*

Lit.: ***1.*** *Simons M, Gloy J, Ganner A, Bullerkotte A, Bashkurov M, Kronig C, Schermer B, Benzing T, Cabello OA, Jenny A, Mlodzik M, Polok B, Driever W, Obara T, Walz G. Inversin, the gene product mutated in nephronophthisis type II, functions as a molecular switch between Wnt signaling pathways. Nat Genet. 2005; 37(5):537-43.* ***2.*** *Hoppe T, Cassata G, Barral JM, Springer W, Hutagalung AH, Epstein HF, Baumeister R. Regulation of the myosin-directed chaperone UNC-45 by a novel E3/E4-multiubiquitylation complex in C. elegans. Cell. 2004;118(3):337-49.*