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NEW FUNCTIONS OF ACBP IN REGULATION OF VESICULAR TRAFFICKING?

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Madam, Sir,

This month, an article on a microarray study is published for the first time in Cellular and Molecular Biology. The technology, being improved over last 20 years, allows the survey on gene expression in a cell or tissue by hybridizing a labeled mRNA or cDNA population on a slide grafted with a set of gene specific probes. Sizes of Microarrays vary. Today Arrays from several Providers are supplied that represent the entire human transcriptome. Comparison of chips representing different experimental conditions, after signal to noise ratio subtraction, genders hybridization intensity variations that can be related to differential gene expression between the studied experimental conditions. Such a global approach in biology allows the discovery of new gene regulations and new gene functions. Gene-Expression profiling on microarrays today has become a reference in pharmacological and clinical studies.

In their (recently published) paper (of this issue), Vock et al. report their study on an siRNA mediated knock down of human acyl-CoA binding protein (ACBP) in HepG2 cells cultures, applied to expression profiling on DNA microarrays.

Acyl-CoA binding protein (ACBP) is a lipid binding protein, originally isolated as diazepam binding inhibitor (DBI) from rat brain with its ability to displace diazepam from gamma aminobutyric acid receptors (3). The ubiquitously expressed ACBP shows a high degree of structural and functional conservation (5) and various tissue specific functions in the regulation of glucose induced insulin secretion from pancreatic beta cells (1), modulation of monocyte mediated inflammation (7,8), release of cholecystokinin from intestine (4)and the stimulation of steroid synthesis (6). Observation of modified acyl-CoA composition, strongly diminished levels of the very-long chain acyl-CoA C26:0 and impaired sphingolipid synthesis, aberrant membrane structure and vesicle accumulation in an ACBP-knock-out yeast cells lead to the suggestion of a rôle of ACBP in vesicular trafficking (2).

In a previous paper the authors had reported, in HepG2 and Hela cells, an activity of ACBP in transcriptional activation of HMG-CoA Synthetase (HMGCS1) and cholsterogenic HMG-CoA reductase (HMGCSR), two genes encoding ratelimiting enzymes of the cholesterol synthesis pathway (9).

Expression profiling experiments were done in triplicate and each microarray hybridization was done form RNA preparations pooled from 3 wells. Expression intensities for the 50,000 probe-sets from Affymetrix Hu133 2.0 Gene Chips were calculated and subsequently filtered for significant expression, applying Affymetrix

MAS5 software. A second filter was applied, selecting probe-sets, presenting a minimum fold change of 50% between studied experimental conditions. Submission of the filtered list of probe-sets to a single sided permutation t-test ($p \leq 0.05$) yielded 64 candidates for differentially expressed genes. False discovery rate was estimated at 1%. Mentioning of the number of probe-sets left after reduction through the two applied filters would allow readers to fully appreciate the statistical power of the presented work.

Candidates obtained from experimental expression profiling were then submitted to Genomatix BiblioSphere Pathway Edition (BSPE), to identify putative functional relations. BSPE provides statistical analysis of every visible network towards overrepresented and underrepresented gene classes. Statistical analysis is calculated by z-scoring (www.genomatix.de). The process yielded two couples of genes for which a gene-interaction was signaled by the text-mining tool, which are early growth response 1 (EGR1) and thrombospondin 1 (THBS1), and ras homolog gene family member B (RhoB) and Rhophilin-2 (Rhpn2), which were all down-regulated. The four genes were validated by quantitative PCR. In their conclusion the authors very much focus on the the RhoB and Rhpn2 and their function in endosomal transport and signaling, which could be related more generally to vesicular trafficking via actin cytoskeleton assembly.

The presented work, shows at the same time the advantages and difficulties of new genome wide technology approaches, meaning the possibility of hypothesis free research, on one hand, that confronts the scientist with a multitude of unexpected results, on the other hand. It has been shown in the past, that in such a case it is a fruitful strategy to try to select genes by functional networks, because common genes of a given pathway that are co-regulated, have been experienced to represent candidates of higher robustness, and text mining is a powerful approach to identify such genes. In the case of the here referred study, identified functional networks of gene-candidates were limited to two genes per network.

In summary, this microarray study generated 64 new candidates for transcriptional regulation through ABPC, or other interactions that are influenced by ACBP. Candidates obtained through statistical analysis of the triplicate experiment will need to be validated through new and independent approaches. Furthermore, a text-mining approach associated two couples of candidates into two functional networks. If validated, one of these couples provides evidence for a role of ACBP in vesicular trafficking apllied to endosomal transport and/or signaling. Cellular studies need to be done to confirm the role of these functions in ACBP mediated vesicular trafficking and potentially connect them with previously identified functions of this gene.

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