

Identification of genes involved in the astrocyte response to beta amyloid

Introduction

Alzheimer's disease is one of the most devastating neurological disorders. Recently, research into the mechanisms of this neurodegenerative disease have begun to provide new insights. In this study, rat cortical type-I astrocytes were cultured on beta-amyloid peptide fragment 25-35 for 12 hours, 1, 3, and 5 days. These samples were harvested at the indicated times and hybridized to the Affymetrix U34 Neuro GeneChip. This array contains ~1200 genes specifically of interest in neuroscience research. Statistical analysis was performed with GeneSifter. This microarray analysis system was also used to map differentially expressed genes to candidate biological terms and pathways. Several clear patterns of gene expression were discovered which correlated with genes involved in signal transduction.

Analysis Methodology

The raw data is available from the NCBI Gene Expression Omnibus (GEO) (<http://www.ncbi.nlm.nih.gov/projects/geo/>), GDS519. Initial analysis, to remove genes that were not significantly expressed, was carried out by applying ANOVA on the entire data set, followed by the Benjamini-Hochberg method for estimating the false discovery rate (Benjamini and Hochberg, 1995). Genes lower than 1.5 fold change for at least one time point were not analyzed further. A quality control criteria was selected to view only those genes flagged as "P" by the Affymetrix MAS5 algorithm. This filtering reduced the

dataset to 173 genes that are significantly expressed.

This subset was further analyzed using the partition clustering method, partitioning around medoids (PAM). Using $k=2$, the data clearly separated into two clusters, differing primarily at the 12 hour time point (Figure 1). k was chosen to maximize the mean silhouette width (Kaufman and Rousseau, 1990). These two clusters were further examined using the Ontology Report and KEGG Report in GeneSifter.

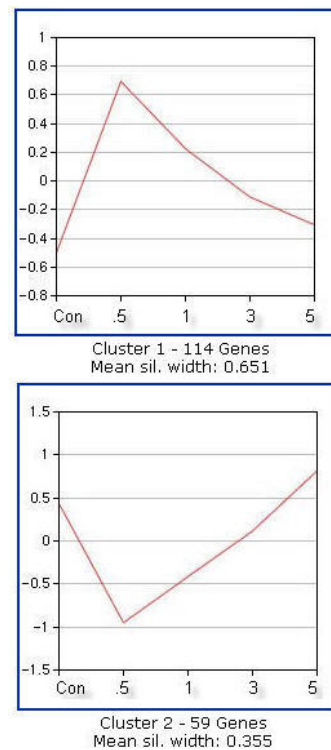


Figure 1. Filtered data clustered using partition clustering. We initially filtered the dataset from ~1,200 genes of raw data to 173 differentially expressed genes. We then subjected this subset to partition clustering using the PAM algorithm. We made several choices for k , ranging from 2-10. The highest mean silhouette width was obtained with $k=2$. The two resulting clusters differ primarily at the 0.5 day time point.

Conclusions

Utilizing these tools, further analysis indicated that several gene families involved in signal transduction were highly overrepresented at the 12 hour time point. In particular, these genes are involved in phosphorylation, kinase activity, and adenyly nucleotide binding. Along with other terms in the list (Figure 2), this leads us to suggest that PKA-mediated signaling may play a role in astrocyte response to α -beta.

A.

Ontology	List	Array	z-score
catalytic activity	27	141	4.28
adenyly nucleotide binding	16	79	3.36
ATP binding	16	77	3.47
nucleotide binding	16	85	3.02
purine nucleotide binding	16	85	3.02
transferase activity	15	64	3.88
kinase activity	13	59	3.33
phosphotransferase activity, alcohol group as acceptor	13	57	3.47
transferase activity, transferring phosphorus-containing groups	13	58	3.4
protein kinase activity	12	52	3.37

B.

Ontology	List	Array	z-score
transporter activity	5	210	-2.1
growth factor binding	3	7	4.68
insulin-like growth factor binding	3	5	5.73
enzyme regulator activity	2	11	2.06
glycosaminoglycan binding	2	9	2.42
kinase regulator activity	2	4	4.19
protein kinase regulator activity	2	4	4.19
sodium:potassium-exchanging ATPase activity	2	7	2.92
3',5'-cAMP binding	1	2	2.96

Figure 2. Z-score report of molecular function gene ontology (GO) terms. A z-score report was generated in GeneSifter which displays only those terms with a z-score greater than 2 or less than -2 , the top ten of which are shown. Of the genes in the cluster, "List" indicates the number that had that ontology term. "Array" refers to the number of genes on the array (U34 Neuro) that were in the current ontology. Part A displays the GO terms of genes represented in the upregulated cluster (114 genes). Part B displays those genes in the downregulated cluster (59 genes).

References

Benjamini, Y. and Hochberg, Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. Roy. Stat. Soc. B.*, 57: 289–300.

Kaufman L, Rousseeuw PJ: *Finding Groups in Data: An Introduction to Cluster Analysis*. New York: Wiley; 1990.