Microarray Analysis of Metabolically Engineered NS0 Cell Lines Producing Chimeric Antibody

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Abstract: Large scale gene expression provides a powerful approach to the characterisation of cells transcriptional state. Thousand of genes can be monitored in single experiments generating an unprecedented volume of data. In animal cell technology, this information can be used to assign functions to previously unassociated genes, identify potential process variable targets and generate snapshots of transcriptional activity in response to any environmental factor or cellular trigger. We have used a mouse array representing 15000 genes to assess the expression profile of mouse myeloma cell line NS0 and GS-NS0 producing chimeric antibody. Comparisons of gene profiles were also made with proliferation-controlled (over-expressing $p21^{CIP1}$) and apoptosis resistant (over-expressing bcl-2) cell lines. There were 19 genes up regulated and 32 genes down regulated in the apoptosis resistant cell line compared to the parental producing cell line. As for the proliferation-controlled cell line, 54 and 147 genes were up and down regulated respectively. Gene ontology was used to understand the biological relevance of differences in gene expression data. Distinct expression signatures, indicative of observed differences in physiology and productivity between the cell lines, were identified. Our study highlights the potential of microarray technology for the analysis recombinant cell lines as affected by product expression, genetic modification and environmental conditions.

Key words: PGS- NS0, microarray, transcriptomics, metabolic engineering, proliferation control, apoptosis, gene ontology

R. Smith (ed.), Cell Technology for Cell Products, 47–52.

1. INTRODUCTION

As cell culture process technology matures, there is an increasing use of mammalian cells for the production of biologics. This has led to greater interest in cell engineering where research like genetic modification of cell lines and multi-cistronic expression are now gaining attention. In addition, new high throughput technologies like whole genome and proteome analysis are now being harnessed in effort to improve productivity and product efficacy (Korke *et al*., 2002). These tools are complementary to cell culture engineering as they allow the elucidation of physiological mechanisms within cells.

The use of large scale gene expression analysis in mammalian cell culture have been limited mainly to the study of the consequence of changes in environmental conditions. In this study we examine the effect of over-expression of single gene on the gene expression profile of NS0 cell line. we compare the parental wild type mouse plasmacyoma cell line NS0 to NS0-GS producing chimeric antibody, NS0-GS over-expressing $p21^{CIP1}$ cyclin-dependent kinase inhibitor (cdki) and NS0-GS over-expressing bcl-2 anti-apoptotic protein. Single gene expression can alter physiological events drastically (Mayford *et al*., 1995; Sapirstein and Bonventre, 2000) but such alteration is a consequence of the manifestation of interacting gene expression at various regulatory hierarchies. To study the transcriptional profile of cells and subsequently use of gene ontology (Ashburner *et al*., 2000; Lomax and McCray, 2004); www.geneontology.org), and to look for gene pattern alterations we have employed a cDNA array containing 15000 mouse genes.

2. METHODOLOGY

2.1 Cell culture

Four cell lines were used for gene profiling. The wild type mouse plasmacyoma/myeloma cell line (WT), NS0 6A1 transfected with the glutamine synthetase (GS) expression system and expressing the gene for a human-mouse chimeric cB72.3 IgG4 antibody (Bebbington C.R. *et al*., 1992) supplied by LONZA Biologics (Slough, U.K), NS0 6A1 bcl-2 (Tey B.T *et al*., 2000) and NSO 6A1 p21(Watanabe S. *et al*., 2002). Cells were grown in spinner flask at 37^0C before being harvested for analysis.

2.2 RNA isolation, labeling and hybridization

Cells were harvested, lysed and the total RNA extracted using 1ml of Trizol (Invitrogen, Rockville MD, USA) before the RNA was cleaned up using the RNeasy Midi kit (Qiagen, Santa Clara, USA). The mRNA from the NS0 cells were then separately reverse transcribed to 1st strand cDNA. The second strand is then generated using the Klenow fragment of DNA polymerase 1 to incorporate dCTP linked fluorescent dyes (Cy3 or Cy5). The differently labelled cDNA were combined Cy3 labeled pellet and/or Cy5 labeled pellet in 25 µl of hybridisation buffer. Hybridisation was done overnight and the arrays were washed.

2.3 cDNA description and image analysis

cDNA microarrays of 15247 unique oligo (dT)-primed cDNA clones ("NIA mouse 15K"), from Minoru Ko (NIH)(Tanaka T.S *et al*., 2000), were generated at HGMP RC Hinxton, UK. Normalization was done using a web-based resource called GEPAS (gepas.bioinfo.cnio.es) (Herrero J *et al*., 2003), The differentially expressed gene lists were then submitted to FatiGO (fatigo.bioinfo.cnio.es)(Al-Shahrour F *et al*., 2004) where significant gene associations of Gene Ontology were found. P-values of less than 0.05 as well as false discovery rates (independent adjusted P-value) of less than 0.2 were used as criteria for selection of significant associations(Benjamini Y and Hochberg Y, 1995).

3. RESULTS AND DISCUSSION

Clustering genes by their gene ontology allows us to subdivide genes accord to 3 categories as seen in the Figure 1. A summary of the associated is given in Table 1. It is noted that associations within the 6A1 bcl-2 cell line point towards certain biological and metabolic functions being up regulated in order for to maintain the cellular processed. Genes associated with the cell cycle are down regulated as a result and this clearly explains the commonly observed effect of cell cycle arrest in bcl-2 over expressing cell lines(Tey B.T *et al*., 2000). commonly observed effect of cell cycle arrest in bcl-2 over expressing cell lines(Tey B.T *et al*., 2000).

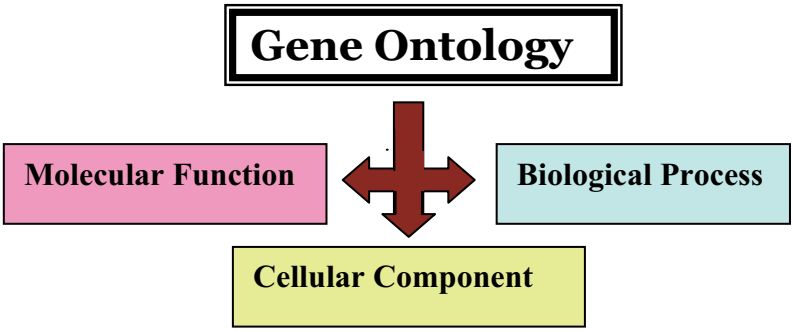

Figure 1. Gene Ontology categories.

Both 6A1 p21 and 6A1 bcl-2 have similar associations, showing increased gene expression for cell homeostasis, ion homeostasis and less cell proliferation. However, as stated before, the physiological effect on specific productivity is rather opposite. Despite having a larger number of genes being differentially expressed, the 6A1 p21 cells do not show other significant associations with the cell line. Differentially expressed gene patterns within the WT cell line reveal a higher number of lipid metabolism genes. There are also more genes that are localized in the cell membrane and endoplasmic reticulum while being less associated with the nucleus and chromosome.

Comparing gene changes between cell lines allows us to differentiate genes associated with bcl-2 and p21 and eliminate common features resulting arising from single gene over-expression. Upon closer inspection, it becomes obvious that in 6A1 p21 cells, there are a significant number of genes being down regulated compared to the WT. Most of these genes are associated with various functions including homeostasis, biosynthesis and enzyme activities. Similarly, compared with the 6A1 bcl-2 cells, 6A1 p21 down regulated genes are significantly different. However, 6A1 p21 up regulated genes do not show any significant patterns, possibly due to the fact that as the cell stop proliferating, reducing certain physiogical aspects associated with cell division. The use of associations across cell lines thus allows for a better understanding of the relative levels of differential gene expression. This helps overcome the limitations of associations within cell lines

Table 1. Summary of associations derived after statistical analysis.

Differential gene expression associations based on Gene Ontology						
	up regulated // down regulated	**down regulated // up regulated**	**up regulated // up regulated**	**down regulated //down regulated**	**up regulated //all genes**	**down regulated // all genes**
bcl-2	Biological process: cell homeostasis, cell ion homeostasis				Biological process: polyol metabolism, respiratory tube development, lactation, acylglycerol metabolism, neutral lipid metabolism, glycerolipid metabolism, epithelial cell differentiation, alcohol biosynthesis Molecular function: glycerol-3-phsophate dehydrogenase activity	Cellular component: chromosome, replication fork Biological process: cell proliferation
WT	Cellular component: cytoplasm, integral to membrane	biological process: nucleoside, nucleotide nucleobase and nucleic acid metabolism, regulations of metabolism Cellular component: nucleus, chromosome			Biological process: steriod metabolism, sterol metabolism, lipid biosynthesis, Cellular component: endoplasmic reticulum	
p21	Biological process: cell homeostasis, cell ion homeostasis Cellular component: membrane	Biological process:cell proliferation				Molecular function: binding Biological process: cell proliferation

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