**10g Application of Microarrays to Reverse Engineer Attachment Dependence in Mammalian Cell Lines**

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With the advent of high quality glass slides and precision robotics, microarrays have gained widespread recognition as powerful bioinformatics tools. Increasingly, microarray-based experiments have focused on exploring metabolic pathways, biological mechanisms, and genome-wide differences between tumor cells and normal/healthy cells. We have used both cDNA and oligonucleotide microarrays to identify variations in gene expression between similarly derived, but different phenotypic HeLa cell lines. We concentrated on comparing attachment-dependent with attachment-independent HeLa cells; both derived from a common ancestral cell line. Initial work focused on growth characterization in controlled environments, enabling us to prepare samples with cells properly aligned in the same phase of growth; a key prerequisite for meaningful microarray analysis. The microarray data was first normalized using statistical software and then filtered to scour the genome for likely candidates for further investigation. A combination of algorithms such as principal components analysis and k-means clustering were employed for this purpose; organizing genes into distinct groups. Using this approach, six genes were identified as potential targets for further study. Expression ratios obtained by microarray analysis were verified using Real time RT-PCR for the six selected genes previously indicated. Focusing first on a type II membrane protein, we were able to alter the physiology of the attachment-independent HeLa cells using siRNA. Numerous size characterization studies indicated that blocking the expression of this membrane protein in HeLa cells dramatically increased the relative number of multi-cellular aggregate clusters. In addition, we over-expressed a protein associated with attachment, in attachment-independent HeLa cells in order to alter the adhesion properties. We believe our strategy of applying bio-informatics techniques to characterize and manipulate phenotypic behaviors will be used to alter the properties of numerous cell lines for desired biotechnology objectives.