Cell-based arrays

UHN Microarray Centre

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Introduction

The next step in decoding the human genome is to elucidate the function of all genes. High throughput techniques, including cell-based microarrays, will allow for the rapid annotation of genomes with accurate information about the biological function of each gene.

Cell-based microarrays are a variation on the standard microarray format, first described by B. Palsson[13]. Essentially, expression plasmids or RNA are spotted on microarray slides and used to transfect cells with the addition of a transfection reagent. Cultured cells in medium are added to the array, adhere to the spots and become transfected by internalising the nucleic acid. The result is a living cell array of localised transfected cells in a monolayer of nontransfected cells. This method has been called "reverse transfection" by Ziauddin and Sabatini as cells are added on top of the nucleic acid instead of *vice versa*[3].

General Reverse Transfection Method (taken from Ziauddin & Sabatini)[3]

1. Create Plasmids
2. Microarray printed: Robotic arrayer prints plasmid DNA/gelatin solution on
3. Reverse transfection: Put transfection reagent on coverslip, then place microarray (plasmid side down) on top; incubate 10-20 minutes, remove transfection reagent by vacuum aspiration; place slide (printed side up) in tissue culture dish; pour in actively growing cells; grow cells for 40 hours; fix cells in paraformaldehyde/sucrose in PBS.
4. Immunofluorescence: Permeabilise cells with 0.1% Triton X-100 in PBS for 15 minutes; probe with primary and Cy3-labelled secondary antibodies.
5. Imaging: Image at high resolution (5µm) with laser fluorescence scanner; photograph with conventional microscope; measure fluorescent intensity and quantify signal intensity

Advantages of cell-based microarrays

1) Allow for high-throughput screening of specific proteins
2) Stable and can be converted, when needed, into arrays of cell clusters expressing the encoded gene product
3) Automated interpretation of cell images will allow for quantitative and reproducible identification of cellular phenotypes
4) Allow sparing use of rare cell lines

Disadvantages of cell-based microarrays

1) Need for further development of automated image analysis and data management
2) Currently works best with adherent cells and cells that are easily transfected
3) Sensitivity issues (due to small number of cells per feature)

Each feature of an array can consist of 30-500 transfected cells, depending on the competency and size of the cells used. At the end of the experiment, microarrays are fixed and prepared for immunofluorescence and then analysed for immunostaining, viability, and cell morphology.

Cell-based microarrays are most effective with cells that are adherent and easily transfected[6]. Other limitations include the fact that the technique is technologically challenging; access to libraries of expression clones, RNAi reagents, or other compounds; and sensitivity of the assay, considering the small number of cells

per feature[6]. One limitation of the reverse transcription technique is the need for extensive posttransfection processing including fixing and permeabilising the cells and multiple antibody incubation steps[4]. Instead of using antibodies, transient or stable expression of reporter constructs that express green fluorescent protein (GFP) or luciferase could allow monitoring of gene expression as well as pathway activation. Although, there are concerns that the attachment of a reporter tag may perturb gene function[1].

Technological advances over the next few years should negate some of these limitations.

Applications

Cell-based microarrays can be used as an alternative to protein microarrays for identification of small molecule targets and for discovery of gene products that alter cellular physiology. Highly representational protein microarrays are difficult to make because of the large numbers of individually purified proteins required. Also, it is unclear how long proteins printed on an array will be stable once the array is printed[3]. The cell-based microarray technique bypasses the constraints of protein immobilisation and, because proteins are translated, folded, and interact within the cellular environment, the expressed proteins are functional and stable.

Sabatini *et al*. performed a Proof of Principle experiment involving the screen of cDNAs in expression vectors looking for genes whose overexpression led to apoptosis, increased tyrosine phosphorylation, or affected cell-cell adhesion in mammalian cells[2].

RNA interference (RNAi) is the process in which single or double stranded RNA leads to the degradation of the encoded mRNA and thus suppresses gene expression. RNAi is a post-transcriptional method of gene silencing, originally discovered in *C. elegans*[11]. RNAi is different from a permanent knock-out, as the protein is not completely eliminated. This allows selective "knock-down" of a particular gene and is useful for the dissection of molecular pathways and determination of epistatic relationships[3]. RNAi also allows for the study of genes that would be lethal as knockouts[1]. The discovery of a gene that confers lethality when knocked-down only in cells that are overexpressing an oncogene could provide an in road for new cancer therapies[2]. Most published RNAi microarray methods are proof of concept work. Studies are limited by the difficulty of assembling a large number of siRNAs known to silence their target genes and the need for sophisticated high resolution microarray scanners to examine thousands of spots on a cell microarray.

In addition to loss-of-function studies, Bailey *et al.* have shown that lentivirus-infected cell microarrays (LICM) can be used for both loss-of-function and over-expression screens in a broad range of mammalian cell types, including primary non-dividing cells[5]. The main hurdle for LICM is the requirement for high-titer virus obtained by concentrating virus-containing supernatant, a process that is time-consuming and difficult to automate, not to mention the need for dedicated laboratory space required for such viral work.

Cell-based microarrays have been used for monitoring the gene function of signalling proteins. The serum response enhancer (SRE) element is responsive to activation of several signaling pathways including the mitogen-activated protein (MAP) kinase pathway[4]. Webb *et al.* have successfully coupled the green fluorescence protein (GFP) gene to the SRE element as a model system for monitoring MAP kinase signaling activity on cell arrays[4].

Cell microarrays can also be used for subcellular localisation of tagged proteins. Conrad and colleagues have used cell arrays to phenotype cells using GFP-tagged proteins[8]. In their study, eleven different subcellular patterns were classified, each characterising a distinct phenotype, by identifying the localisation of GFP-tagged marker proteins[8]. This study combined cell arrays, screening microscopy, and machine-learning-based classification methods to automate a high-throughput cell phenotype screening and reported an overall prediction accuracy of more than 80% for the eleven localisation classes.

Cell-based microarrays have been implemented in screening drug targets. Mishina *et al*. have demonstrated the use of reverse transfection technology for the analysis of ligand-induced activation of G protein-coupled receptors, a superfamily of proteins that also include important drug targets[7]. The authors point out the

features of cell-based microarrays for this application including the rapid generation of a desired phenotype through transfection, the ability to combine multiple plasmids at each feature, a consistent environment across multiple data points, and the compatibility with standard microplates and several semiautomated or automated systems[7].

Delehanty and colleagues have demonstrated the potential for cell-based microarrays to be used for the comparative functional analysis of single-chain antibodies expressed on the plasma membrane of mammalian cells[12]. In their study, they were able to use cell arrays to differentiate the relative binding affinities of the native single-chain antibody and three binding site mutants whose affinities span almost 3 orders of magnitude. The significance of this work is that expression of recombinant antibody fragments on the surface of cells is emerging as a therapeutic strategy, particularly in cancer treatment.

Image Analysis

Currently, conventional microscopes designed for high throughput imaging and high resolution DNA microarray scanners are being used for image acquisition. CellProfiler Cell Image Analysis software (www.cellprofiler.org) is designed for biologists to quantitatively measure phenotypes from thousands of images automatically[10]. CellProfiler software is free and open source. The unbiased quantitative measurement of the shape, size, and intensity of cellular staining is essential for cell-based microarrays to be used efficiently and effectively.

Future of cell-based microarrays

One area that is in need of improvement involves image acquisition and analysis. Software packages for collecting images of transfected cell populations, automated analysis of each image, and data management are also being developed. In addition, technological improvements in microarray surface chemistries and transfection efficiencies are underway and should advance the cell-based microarray platform[6]. Also, in order for this platform to be successful for loss-of-function applications, verified mammalian genome-wide RNAi libraries will be needed.

Eventually, the cell-based microarray platform may evolve to accommodate assays on living cells in real time, for example real-time analysis of transient phenotypes such as morphological changes and cellular calcium ion flux[2].

Cell-based microarray technology is still in its infancy but growing rapidly due to technological advances and an interest in ulitizing the cell array platform for the high-throughput screening of functional proteins.

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