Microarrays could be key to safe gene therapy

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Using DNA microarrays to identify changes in gene expression could help improve the safety of human gene therapy by giving scientists prior warning of adverse reactions, report US researchers.

Jackie Stilwell and Jude Samulski at the University of North Carolina (http://www.med.unc.edu/genether/) compared the gene expression profiles of two viruses that are commonly used in gene therapy clinical trials and found that the viral vectors alone might influence gene expression.

Viral vectors can affect gene expression

In order to see the effect of viral infection on a cell, the researchers examined the gene expression profile of lung cells that had been infected with one of two viral vectors, adenovirus (Ad) or adeno-associated virus (AAV). By using DNA microarrays, which can monitor the whole genome on a single chip, they were able to obtain a molecular snapshot of the interactions of thousands of genes at once.

They found that AAV, unlike Ad, has a minimal effect on gene expression. However, Ad elevates the levels of genes encoding cytokines and chemokines, which are involved in inflammation or in cellular stress responses. Microarrays represent 'a relatively inexpensive way to get a molecular fingerprint of the state of a cell treated with a gene therapy vector', said Stilwell, whose data are published in the journal *Molecular Therapy* [1].

The findings correlate well with those obtained from animal studies; thus, the use of microarrays could have important implications for the development of gene therapy vectors. 'Currently, toxicity testing is mostly done in animals and in clinical studies, which can be costly and difficult with the current regulations. We have demonstrated that this testing can be markedly reduced, by using microarrays,' said Stilwell. 'DNA microarrays don't replace clinical trials, but they could give indications cautioning the researcher before something unexpected happens in the clinic,' she added.

Different vectors for different jobs

The analysis uncouples for the first time the molecular steps involved in viral infection and viral gene expression. To disentangle these events, the researchers compared gene expression changes after infection with different forms of each virus. Once again, exposing the cell to AAV, either as an intact virus, as a recombinant vector shell or as an empty capsid – the protein coat of the virus devoid of DNA – produced a minimal response from the genes.

By contrast, the same experiments using the Ad virus produced strikingly different results. Exposure to intact or recombinant virus activated gene expression from genes involved in inflammation and cell-stress response. However, when the same cells were infected with the empty capsid version of Ad, the expression of these genes was reduced significantly. These results demonstrate that the virus shell alone induces a cellular signature as part of infection, regardless of the modifications to the viral vector DNA template.

The results provide a useful database of viral vector information that can benefit other researchers and clinicians involved in human gene therapy. For example, any modification to these vectors can be tested using microarrays and their molecular profiles observed before their use in the clinic. As the technology for examining changes in gene and protein expression improves, this database can be expanded.

Commenting on the choice of viral vector Stilwell said, 'Ad vectors can be very effective in cancer therapies where the goal is to kill the cells that are being infected but they may need to be modified more before they are ready for clinical use.' AAV vectors have been used in clinical trials to replace defective genes in cystic fibrosis and hemophilia. 'Since AAV is not toxic it is great to use to treat diseases where you are replacing a defective gene in a cell that you want to survive over the long term,' said Stilwell.

Gene therapy for Canavan's

Currently, Samulski's group are involved in a gene therapy clinical trial for Canavan's disease – a rare, inherited neurodegenerative disease, which is common in Ashkenazi Jews. Using an AAV vector the defective gene, ASPA, was replaced in seven patients. This was the first FDA approved AAV vector

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based clinical trial conducted by an academic institution in the USA. The group will start a second trial this year.

However, more work is required on AAV vectors and the next step for Stilwell is to detect the cellular signature that result from infection with other serotypes of AAV and to test the AAV vector in other cell types. She also plans to reproduce her research *in vivo*.

Reference

1 Stilwell, J.L. and Samulski, R.J. (2004) Role of viral vectors and virion shells in cellular gene expression. *Mol. Ther.* 9, 337–346

Moving forward with reverse vaccinology

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Reverse vaccinology is a valuable starting point when searching for novel vaccine candidates, says Rino Rappuoli, vice president and chief scientific officer at biotech leaders Chiron Corporation in Sienna, Italy (http://www.chiron.com/).

Using classical vaccinology, pathogens are grown *in vitro* and these, or components of these, are then used to develop vaccines. It is an approach that has led to the development of many important vaccines. But it cannot deal with pathogens that do not grow *in vitro*. Reverse vaccinology takes advantage of the growing number of genome sequences available for many microorganisms. These can be analysed to identify genes encoding various protein antigens on the surface of pathogens, which can then be used as targets for recombinant vaccine development.

Genome-based vaccine discovery

The genome-based vaccine discovery approach was first applied to meningococcus B, a bacterium that is a major cause of sepsis and meningitis and cannot be grown *in vitro*. Computer analysis of the meningococcus-B genome predicted over 600 potential vaccine candidates of which 350 were expressed in *Escherichia coli*, purified and used to immunize mice. Of these, 29 were found to induce bactericidal antibodies, thereby leading to protective immunity.

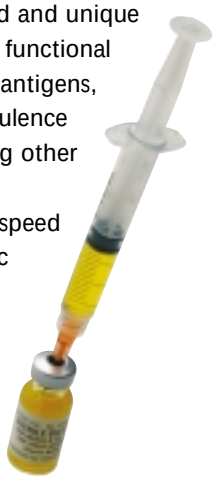
Similarly, reverse vaccinology enabled the development of vaccines against Hepatitis B and C viruses, which also cannot be tackled by conventional vaccinology. A recombinant vaccine against hepatitis B is now routinely used for universal immunization of children and a vaccine against hepatitis C is currently in clinical trials.

Proteins and polysaccaharides

Although reverse vaccinology is now becoming a standard technology, it does not provide a universal solution for vaccine development, says Rappuoli. 'Reverse vaccinology does not allow the discovery of vaccines which are not protein based,' he said, 'For instance, many infant vaccines are based on polysaccharides, which are conjugated to carrier proteins.'

The situation is different for protein antigens, says Rappuoli. 'Reverse vaccinology is a great tool in the early phase of vaccine development,' he told delegates at the 11th International Congress on Infectious Diseases (ICID) in Cancun, Mexico (http://www.isid.org/11th_icid/).

Biocomputing can be used to analyze the genome of a single pathogen, but it can also be used to compare multiple genomes to shed light onto conserved and unique families of proteins, functional domains of protein antigens, and evolution of virulence mechanisms. Among other advantages of this technology are the speed with which genomic sequences can be compared at a reasonable cost, in comparison with traditional strategies.



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