UHNMAC News

The University Health Network Microarray Centre Newsletter – Autumn 2006

UHN Microarray Centre

Welcome to the Autumn 2006 edition of the UHNMAC News. This newsletter will be published quarterly and will provide general UHNMAC news, a review of a recent journal article, and a feature report pertaining to microarray technology.

ANNOUNCEMENTS

Agilent Certified Service Provider

The UHNMAC is the first Agilent certified service provider in Canada. Please visit www.microarrays.ca for more information about services using the Agilent platform.

Updated website

The UHNMAC website, www.microarrays.ca, now provides more on-line support, access to presentations and posters presented at recent conferences, and current information about our products and services.

SEMINARS

- BRI Microarray Meeting (Montreal) - October 12-13, 2006
- Drop by our booth at the UHN Research Day - November 20, 2006 - and learn more about our services
- Please check our website for upcoming seminars hosted by the UHNMAC

FEATURE ARTICLE & REVIEW

Villeneuve, D.J., Hembruff, S.L., Veitch, Z., Cecchetto, M., Dew, W.A., and Parissenti, A.M. **cDNA microarray analysis of isogenic paclitaxel- and doxorubicin-resistant breast tumor cell lines reveals distinct drug-specific genetic signatures of resistance.** Breast Cancer Research and Treatment, 2006, 96:17-39.

Resisting Arrest - using microarrays to decipher genetic signatures of drug resistance for tumor cell lines

One of the goals in cancer treatment is to provide patients with "tailor-made" chemotherapeutic drug regimens, an approach that will result in more effective and efficient treatment with fewer side effects. cDNA microarray analysis is widely used to classify tumours based on their expression profiles and predict patient prognosis to specific cancers. However, one of the problems with predicting patient prognosis is the inability to differentiate between genes that affect patient prognosis and

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FEATURE REPORT

Array Comparative Genomic Hybridisation

Array comparative genomic hybridisation (aCGH) is a microarray-based technique used to identify chromosomal copy number changes (deletions, amplifications, and micro-amplifications) genome-wide. aCGH can also be used to characterise various carcinomas and to identify and map disease genes involved with schizophrenia, depression, autism, and mental retardation. This technique has been used successfully for the analysis of tumour samples and cell lines and for analysis of single copy gains and losses in specific chromosomal regions, telomeres, and entire chromosomes. aCGH provides rapid

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As always, general questions about microarrays can be addressed to help@microarrays.ca, orders for UHNMAC array products can be placed at orders@microarrays.ca, and questions about any of our services can be addressed to geneservice@microarrays.ca. If you have any suggestions for newsletter articles or questions you'd like addressed, please contact general@microarrays.ca.

GENETIC SIGNATURE OF RESISTANCE

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genes that play a role in the response to certain drugs. From a clinical standpoint, identifying genes whose expression correlates with tumour cell sensitivity to specific chemotherapeutic agents would better enable us to predict patient response and deliver more personalised medicine. From a research standpoint, identifying these genes would provide insight into the possible mechanisms through which tumours acquire resistance to certain drugs and would also advance chemotherapeutic drug discovery.

Villeneuve *et al*. used cDNA microarrays to identify drug-specific changes in gene expression that accompany the establishment of resistance to paclitaxel or doxorubicin, two common chemotherapeutic agents, in highly controlled *in vitro* studies. It is thought that the genes identified in the genetic signatures may allow for prediction of patient response to paclitaxel and doxorubicin. The significance of this study is that it is the first microarray analysis to compare gene expression amongst a series of isogenic drug-resistant tumour cell lines, such that drug-specific genomic signatures of resistance could be obtained and verified by Q-PCR and immunoblotting experiments.

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This study used the MCF-7 breast tumour cell line, which has well-characterised estrogen receptors and has been used extensively to model breast cancer cell growth, derived from epithelial tissue. Using cDNA microarray analysis of wildtype MCF-7 breast tumour cells and isogenic paclitaxel-resistant ($MCF\text{-}7_{TAX}$) or doxorubicin-resistant ($MCF\text{-}7_{DOX}$) derivative cell lines, Villeneuve *et al*. identified drug-specific changes in gene expression that accompany the establishment of resistance to paclitaxel and doxorubicin.

Of the genes identified as “resistant”, the majority were drug-specific and coded for proteins involved in drug transport, drug metabolism, growth, survival, and cell death. Of the 35 genes identified by microarray and Q-PCR experiments to comprise the paclitaxel resistance signature, 11 genes were found to be “highly paclitaxel-specific”. And of the 33 genes identified to comprise the doxorubicin resistance signature, 11 genes were found to be “highly doxorubicin-specific”.

Although Villeneuve *et al*. set out to find drug-specific genetic signatures of resistance to paclitaxel and doxorubicin, they found the $MCF\text{-}7_{DOX}$ cell line has significant cross-resistance to paclitaxel. Since one third of genes that have changing expression upon selection for resistance to paclitaxel exhibit similar changes in expression for resistance to doxorubicin, and vice versa, these genes could prove to be general instead of specific predictors of drug resistance. It would be interesting to repeat this set of experiments using the UHN Human 19k array (which contains 19,008 ESTs), as a larger and more diverse genetic signature could have been found for each drug-resistant cell line if an array representing more genes was used.

While this study was the first to compare gene expression amongst a series of isogenic drug-resistant tumour cell lines using microarray analysis, another study has also demonstrated that studies using MCF-7 cells could be used to support clinical data suggesting the importance of certain genes in the acquisition of resistance to another chemotherapy drug (docetaxel) and in the prediction of response to this drug in breast cancer patients[2]. Another study by Villeneuve *et al*. is currently underway to examine the utility of the identified genes as biomarkers for prediction of resistance to anthracyclins and taxanes in breast cancer tumours.

By identifying genes that play a role in the response to certain drugs, researchers will learn more about the mechanisms through which tumours acquire resistance to certain drugs and, eventually, predict patient response to certain drugs.

David J. Villeneuve is a Research Associate in the laboratory of Dr. Amadeo Parissenti at the Sudbury Regional Hospital in Sudbury, Ontario, Canada. Dr. Amadeo Parissenti is a Chair in Cancer Research (Regional Cancer Program of the Sudbury Regional Hospital) and Professor (Division of Medical Sciences, Northern Ontario School of Medicine, Sudbury, ON, and at Laurentian University, Sudbury, ON). The focus of Dr. Parissenti’s research is tumour biology.

References:

1. Villeneuve, D.J., Hembruff, S.L., Veitch, Z., Cecchetto, M., Dew, W.A., and Parissenti, A.M. cDNA microarray analysis of isogenic paclitaxel- and doxorubicin-resistant breast tumor cell lines reveals distinct drug-

specific genetic signatures of resistance. Breast Cancer Research and Treatment, 2006, 96:17-39.

2. Iwao-Koizumi, K., Matoba, R., Ueno, N. Kim, S.J., Ando, A., Miyoshi, Y., Maeda, E., Noguchi, S., Kato, K. Prediction of docetaxel response in human breast cancer by gene expression profiling. Journal of Clinical Oncology, 2005, 23:422-431.

ARRAY CGH

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genome-wide analysis at high resolution and the information provided by aCGH experiments can be directly linked to physical and genetic maps of the human genome. Traditional CGH has limited resolution and data analysis requires a high level of cytogenetic expertise.

Genome-wide arrays cover the entire genome allowing for identification of chromosomal changes in suspected and novel regions. Disease-specific CGH arrays survey regions of the genome that are often altered in certain disease states and chromosome-specific arrays contain elements specific to a particular chromosome. The disadvantage of these arrays is that they require a priori knowledge of the regions of interest.

In terms of the spotted elements, there are two main types of aCGH arrays. One type is spotted with PCR amplified cDNA or bacterial artificial chromosome (BAC), that are usually in the range of 150 to 200kb. Another type is spotted with synthetic oligonucleotides in the range of 25-85 bp in length. BAC clones are used for aCGH because of their high resolution and the fact that they provide stronger signal intensities than cDNA clones (due to their large size). However, arrays made from BAC clones (or cDNA) are subject to PCR contamination and BAC array data may contain mapping inaccuracies of the clones to the human genome. The advantage of oligonucleotide arrays is that the arrays themselves are easier to make (PCR and clone management is not required). However, oligonucleotides are likely to cross-hybridise with multiple genomic loci. To increase the signal-to-noise ratio, assays (which involve PCR amplification of sample genomic DNA) have been developed to reduce the genomic complexity of the sample prior to hybridisation.

If an aCGH array has a 1Mb resolution it means the array has approximately one clone (one element spotted on the array) per megabase of the genome. A tiling array is one that contiguously covers the genome using overlapping clones. The very high resolution of this type of array allows gains or losses of 40-80 kb regions to be detected.

A marker-based array is one that consists of elements that do not sequentially overlap. The resolution of this type of array is dependent on the distances between the clones and the size of the clones.

aCGH Service at UHNMAC

The UHNMAC now offers an aCGH service using the Agilent platform. Please visit our Agilent Expression Service page or contact us at agilent@microarrays.ca for more information. Details about the Agilent aCGH arrays can be found on Agilent's website.

Agilent's Human Genome CGH (Kit 44A and Kit 44B) array contains more than 40,000 60-mer oligonucleotides that represent both coding and non-coding sequences. This array provides an average spatial resolution of 35 kb. For direct labelling, 3µg genomic DNA is required. Using phi29 amplification, 100ng of input genomic DNA can be labelled without a complexity reduction step.

Agilent also offers Human and Mouse Genome CGH 244k arrays and will soon be offering a Rat Genome CGH array.

aCGH Survey

Please visit http://www.surveymonkey.com/s.asp?u=875302380951 to complete our survey on aCGH. The survey is designed for all researchers; those who are currently using the aCGH technique and those who are not. We'd appreciate your feedback!