Julia Joung

Bioc 158 Final Paper

December 9, 2011

Impact of DNA Microarrays on Drug Development

Until the emergence of DNA microarray technology, the process of drug development was often tedious and time consuming. Developers began with identifying a biochemical pathway implicated in a desired pathophysiological process. An enzyme or receptor, preferably one involved in the rate-limiting step, was then characterized and purified. Most of the time, this enzyme or receptor was screened against collections of structurally diverse small molecules, although occasionally, sufficient information was known about the enzyme's mechanism of action and structure to allow for only particular classes of small molecules to be targeted. Finally, the lead compounds isolated from the screening were optimized for desirable properties such as availability and specificity for the target enzyme through a series of clinical trials that can take over several years (Debouck and Goodfellow, 1999).

In recent years, the development of DNA microarrays has facilitated various steps in the process of drug development that were described previously. DNA microarrays consist of a collection of microscopic DNA spots that contain short segments of genes attached to a solid surface. The short segments serve as probes that can hybridize with a cDNA sample, its target. The hybridization between probe and target can then be measured and quantified by detection of fluorophore-, silver-, or chemiluminescence-

labeled target. Through the measurement of the relative abundance of nucleic acid sequences that have hybridized with their targets, DNA microarrays can be used to measure expression levels of a large number of genes simultaneously. It is a convenient method because it does not depend on DNA sequencing or on PCR-based differential display, both of which require more time (Debouck and Goodfellow, 1999). Ever since its emergence, DNA microarrays have been used in numerous steps in drug development from identifying and characterizing potential drugs and targets to determining drug efficacy, specificity, toxicology and metabolism.

DNA microarrays facilitate the identification of potential drug targets because they allow for identification of multiple potential targets by comparing the differences in expression of thousands of genes between disease and normal cells. Genes can be expressed as a consequence of disease. For instance, an increase or decrease in gene expression or tissue and cell type selectivity can differentiate disease cells from normal cells (Gerhold et al., 2002). Thus any genes that show statistically significant differences between disease and normal cells can potentially become drug targets, increasing the number of drug targets. Additionally, this means that potential therapeutic target genes whose biological functions were previously unknown can be characterized with methods such as individual gene transfection using different vector systems followed by microarray analysis. Fambrough et al. (1999) used this approach to analyze the signaling pathways of platelet-derived growth factor-β receptor and fibroblast growth factor receptor 1. Characterization of therapeutic target genes sheds light on which enzymes or receptors could potentially become ideal targets. Drug developers can now begin to develop drugs without first knowing the mechanism behind the disease.

Similar to developing drugs for unknown diseases, DNA microarrays enable the use of unknown drugs in a process also known as forward pharmacology. With microarrays to measure changes in mRNA before and after drug treatment, one can identify mechanisms of action for previously poorly understood compounds, which can become potential candidates for drugs (Gerhold et al., 2002). Once a database of gene expression profiles is established, one can compare the gene expression profiles of unknown drugs to the database to determine their targets. For instance, Hughes et al. (2000) used yeast as a model organism to moniter more than 6,000 transcripts under 300 different experimental conditions, including 279 knockouts. The expression profiles became a reference database for comparing drug effects to the knockouts. Using the database they established, the group found that dyclonine, a topical anesthetic of unknown action, was implicated in the perturbation of the metabolism of ergosterol, an equivalent of cholesterol in yeast. Likewise, they found that fenpropimorph and haloperidol had similar effects on the same pathway. Hughes et al. (2000)'s work demonstrated that one could use large reference databases to define drug actions. Around the same time, Butte et al. (2000) also used DNA microarrays to determine relationships between unknown drugs and pathways. They discover relationships among genes and chemotherapeutic agents in 60 cancer cell lines. For example, they found a gene-drug relationship linking a leukocyte cytosolic protein to a particular anticancer agent, showing that microarrays can provide valuable insights into drug-pathway interactions that were not previously foreseen.

On top of finding drug-pathway relationships, DNA microarrays can provide a better understanding of the properties of unknown drugs by categorizing them under

well-defined classes of drugs. Many studies have shown that microarrays can readily identify differential effects of several drugs or compounds and classify them to a therapeutic class. The class of the drug can help select for lead compounds based on, for instance, secondary effects during drug screening. Hu et al. (2002) documented differences among transcription profiles of 14 agonists and antagonists of the α1, α2, β1, and β2 adrenergic receptors in vascular smooth muscle cells. Using gene expression profiles and clustering, they tried to distinguish agonists from antagonists for 75 genes. They were successful in clearly differentiating between the α and β adrenergic receptors but failed to differentiate among the subtypes. Even so, the group's ability differentiate between agonists and antagonists proved that classifying drugs based on known gene expression profiles can facilitate an early screening of potential drugs.

Following Hu et al. (2002)'s work on agonist and antagonist classification, Gunther et al. (2003) developed statistical methods, namely the classification tree and random forest, to predict drug efficacy based on gene expression for multiple classes of antidepressant drugs, antipsychotic drugs, and opioid receptor antagonists. Both methods correctly categorized known drugs and even predicted a novel subclass. With information regarding the drug's class, one can predict the physiological consequences of each drug. Gunther et al. (2003)'s approach can be expanded beyond the three classes covered in this study to encompass a large number of drug classes and cellular physiologies. Drug classification with microarrays help prioritize lead compounds early in drug development, saving a significant amount of time.

Besides identifying and characterizing potential drugs and targets, DNA microarrays also play an important role in determining drug efficacy prior to clinical

trials. One can quickly verify the specificity and effect of the drug on particular genes by comparing the gene expression after drug treatment to that of repressed genes. In yeast, genes can be silenced using antisense technology, RNA interference, or knockouts (Gerhold et al., 2002). Marton et al. (1998) compared gene expression patterns of yeast calcineurin knockouts to those of wild type yeast treated with calcineurin repressors. Calcineurin is responsible for ion homeostasis in yeast, specifically adaptation to prolonged mating pheromone treatment and regulation of the onset of mitosis. In humans, it is involved in T-cell activation, apoptosis, cardiac hypertrophy, and transition from short-term to long-term memory. FK506 and CsA are common inhibitors of Calcineurin. Maron et al. (1998)'s study of wildtype yeast grown without inhibitors, with FK506, and with CsA showed that the gene expression of 36 open reading frames changed by more than two fold, which was very similar to that of Calcineurin knockouts. This comparison validated both FK506 and CsA's targets as Calcineurin. To determine the specificity of the inhibitors, the group compared the treatment results to more than 40 other deletion strains or drug treatments thought to produce similar effects to the Calcineurin knockout's, but none of them had statistically significant results, showing that the inhibitors were specific.

In an effort to avoid generalizing from one gene, Marton et al. (1998) performed a similar study on HIS3, an enzyme that catalyzes histidine biosynthesis in yeast. They compared the effects of 3-aminotriazole (3-AT), a competitive inhibitor of HIS3, and a mutant strain without HIS3. Statistical analysis of the DNA microarrays produced a high correlation between the two, supporting their previous study on Calcineurin inhibitors. Their studies showed that microarrays could be used to determine drug efficacy by

comparing the expression profile of an inhibitory drug to that of the drug target's knockout.

DNA microarray analysis of drug efficacy allows for relatively simple and safe drug testing prior to long clinical trials. The results from the analysis can then guide the clinical trials. Tseng et al. (2011) used microarrays to carry out a preclinical drug evaluation of RAF265, a novel B-Raf/vascular endothelial growth factor receptor-2 inhibitor. RAF265 was shown to inhibit the uptake of positron emission tomography (PET) tracers in the A375M(B-Raf(V600E)) human melanoma cell line. DNA microarray analysis of treated tumor xenografts revealed significantly decreased expression of genes regulating glucose and thymidine metabolism as well as changes in apoptotic genes, suggesting that image tracers FDG, 3-dioxy-3-[(18)F]flurothymidine and annexin V could serve as potential imaging biomarkers for RAF265 therapeutic monitoring because RAF265 inhibits these biomarkers' accumulation in tumor xenographs. Following the work of Tseng et al. (2011), the clinical trials will focus on those imaging biomarkers for testing RAF265 therapeutic monitoring.

Aside from determining the efficacy of a drug, the specificity of a candidate drug must also be considered in drug development. Again, DNA microarrays facilitate this process. Marton et al. (1998) developed a method referred to as the "decoder strategy" that can be used to detect a drug's secondary effects. The decoder strategy builds on the premise that inhibition of different targets might have similar expression patterns so a simple comparison would be unlikely to unambiguously identify drug targets. The decoder strategy involves comparing a drug-treated wild type to a panel of different mutant strains to find mutant strains that have similar expression to the drug-treated wild

type. Then, the mutant strains isolated are treated with the drug. If the mutant gene is in the same pathway that the drug affects, then the drug signature of the drug-treated mutant will be slightly different from that of the drug-treated wildtype, namely much fewer gene expression changes after drug treatment. If there are much fewer expression changes, this strategy essentially shows that almost all of the drug effects are due to or dependent on the drug target gene and that the drug is specific.

After testing whether a potential drug is effective and specific, one needs to determine the toxicology of the drug. Traditionally, toxicologist used rodent bioassays to identify potentially hazardous substances. This process was time consuming, expensive, and required high doses of the substance. Even worse, confirmation of hazards in humans is often insensitive because of its retrospective nature and limited sensitivity. The emergence of microarrays allowed for identifying and quantifying the dose of potentially toxic substances and extrapolate from one specie to another. Then, one can establish gene expression 'signature' with known hazardous agents such as polycyclic aromatic hydrocarbons, peroxisome proliferators, oxidant stress, or estrogenic chemicals. By comparing an unknown substance to one of the gene expression 'signatures', one can determine whether the substance is toxic (Afshari et al., 1999).

Using the toxicology of the drug established by DNA microarrays, one can establish the proper dose of the drug that maximizes therapeutic index while minimizing side effects. In addition, microarrays have the potential to determine possible secondary effects resulting from overdose. Marton et al. (1998) performed a DNA microarray analysis on wild type yeast after doubling the concentration of F506, an inhibitor of Calcineurin that was discussed previously. This triggered the inhibition or expression of

many genes that were independent of Calcineurin's pathway. Those genes could potentially be related to side effects resulting from drug overdose. Microarrays done on many different genetic variations can also identify susceptible individuals who may respond to a treatment or have adverse reactions to drugs. The influence of genetic differences on drug response defines pharmacogenomics, an important branch of pharmacology. DNA microarrays seem fairly promising in establishing the ideal dose of a drug for a particular individual without running thousands of potentially harmful clinical trials.

Lastly, DNA microarrays provide an insight on the drug metabolism pathway, a dynamic transcriptional regulatory system. Understanding the drug metabolic pathway is important because drug developers want to avoid selecting a drug candidate that is metabolized by only one polymorphic gene. If that were the case, the drug can accumulate to toxic concentrations in individuals carrying specific polymorphisms who are unable to metabolize the drug. In addition, induction of a drug-metabolizing gene by one drug can cause rapid metabolism of other substrate drugs, something that drug developers will want to avoid. Many groups have attempted to document drug metabolism gene expression patterns for various drugs. Gerhold et al. (2000) used DNA microarrays to determine how the rat liver, where most drug metabolism occurs, responds to drug treatment. They measured gene expression profiles in livers of rats treated with vehicle, 3-methylcholanthrene (3MC), phenobarbital, dexamethasone, or clofibrate and confirmed the data for each gene using quantitative RT-PCR. The responses of drug metabolism genes, including CYPs, epoxide hydrolases (EHs), UDP-glucuronosyl transferases (UGTs), glutathione sulfotransferases (GSTs), sulfotransferases (STs), drug

transporter genes, and peroxisomal genes, to these well-studied compounds agreed well with, and extended, published observations. Gerhold et al. (2000) were able to distinguish cytochrome p-450 genes that are dynamically regulated in response to diet, xenobiotics, and hormonal changes up to a threshold of ~90% DNA identity based on the cytochrome p-450 genes' responses to different drugs tested. The group demonstrated the feasibility of determining drug metabolic pathways with DNA microarrays.

Although the recent discovery of DNA microarrays has the potential to facilitate almost every step in the drug development process, DNA microarrays have a few clear limitations. Sometimes measurements of mRNA do not necessarily reflect protein quantities, enzyme activities, or extranuclear signal transduction, so in some cases one cannot make conclusions on, for example, a drug's secondary effects based solely on DNA microarrays. One would need to conduct supporting tests after microarray analysis. In addition, cells are often subject to homeostatic responses that attempt to restore the original state of the system, which can mask experimental results like a potential gene for drug target (Gerhold et al., 2002). These limitations have yet to be addressed in the use of DNA microarrays in drug development.

In the long term, despite those limitations, DNA microarrays will become more prevalent in the drug development pipeline because it offers a faster and easier route to developing successful drugs. DNA microarrays permit the identification of induced gene products that can be used as surrogate markers to readily follow the effect and dose of a drug, thus avoiding potentially long and dangerous clinical trials. Aside from determining drug efficacy, they can be used to predict adverse events resulting from drugs, such as upregulation of specific liver enzymes. Before using DNA microarray analyses to assess

a drug's secondary effects, one would require a better and full understanding of the toxicological consequences of over and under expression of a given gene, which in turn depend on knowledge of the identity and function of the full complement of human genes. As our understanding of the human genome continues to develop over the next decade, DNA microarrays can potentially become key components of multiple steps in the drug development process, including identifying potential drugs and targets and determining drug efficacy and specificity.

Works Cited

DNA microarrays in drug discovery and development (Debouck and Goodfellow(1999)): http://www.ctu.edu.vn/~dvxe/Bioinformatic/PDF%20Files/Volume21/ng0199supp_48.pdf

Drug target validation and identification of secondary drug target effects using DNA microarrays (Marton et al. (1998)): http://microarray.icmb.utexas.edu/Marton_NatMed_1988.pdf

Application of Complementary DNA Microarray Technology to Carcinogen Identification, Toxicology, and Drug Safety Evaluation (Afshari et al., 1999) http://cancerres.aacrjournals.org/content/59/19/4759.short

Prediction of clinical drug efficacy by classification of drug-induced genomic expression profiles *in vitro* (Gunther et al., 2003) http://www.pnas.org/content/100/16/9608.full

Better Therapeutics through Microarrays (Gerhold et al., 2002) http://www.cmbi.kun.nl/edu/bioinf4/articles/pdf/mar_Gerhold.pdf

Hughes, T.R. et al. Functional discovery via a compendium of expression profiles. Cell 102, 109–126 (2000).

Butte, A.J., Tamayo, P., Slonim, D., Golub, T.R. & Kohane, I.S. Discovering functional relationships between RNA expression and chemotherapeutic susceptibility using relevance networks. Proc. Natl Acad. Sci. USA 97, 12182–12186 (2000).

Fambrough, D., McClure, K., Kazlauskas, A. & Lander, E.S. Diverse signaling pathways activated by growth factor receptors induce broadly overlapping,
rather than independent, sets of genes. Cell 97, 727–741 (1999).

Hu, W.Y., Fukuda, N. & Kanmatsuse, K. Growth characteristics, angiotensin II generation, and microarray-determined gene expression in vascular smooth muscle cells from young spontaneously hypertensive rats. J. Hypertens. 20, 1323–1333 (2002).

Monitoring expression of genes involved in drug metabolism and toxicology using DNA microarrays (Gerhold et al. 2000)
http://physiolgenomics.physiology.org/content/5/4/161.full

Use of DNA microarray and small animal positron emission tomography in preclinical drug evaluation of RAF265, a novel B-Raf/VEGFR-2 inhibitor.Tseng et al. 2011
http://www.ncbi.nlm.nih.gov/pubmed/21390189