GENE EXPRESSION PROFILING (TRANSCRIPTOMICS) OF BLOOD IN DUCHENNE MUSCULAR DYSTROPHY

BIOMARKERS CONFERENCE
10 December 2009
Paris, France

Brenda Wong, MD
Pediatric Neuromuscular Program
Cincinnati Children’s Hospital Medical Center
OVERVIEW

• Introduction
• Duchenne Muscular Dystrophy (DMD)
• Gene expression profiling in blood
• Gene expression profiling in blood of subjects with DMD
• Corticosteroid on blood gene expression in DMD
• Conclusion
INTRODUCTION

• Hereditary Neuromuscular Disorders: heterogenous phenotypes, now with accurate genetic definition and pathophysiology

• With groundbreaking clinical trials – a need for unbiased, more sensitive outcome measures to detect early small changes in a short time
INTRODUCTION

Biomarker:

“A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.”

Biomarkers Definition Working Group, NIH Clin Pharmacol Ther 2001;69:89-95
DUCHENNE MUSCULAR DYSTROPHY

• 1852
  Meryon described progressive weakness only affected males with normal spinal cord and defective muscle membrane

• 1861
  Duchenne described paraplegie hypertrophique de l’enfance de cause cerebrale
DUCHENNE MUSCULAR DYSTROPHY

• Best known and most severe form of childhood muscular dystrophy
• Estimated to affect 1 in 3500 live-born males
• Mutations of the dystrophin gene on Xp21.2
• Loss of independent walking 6-12 years, cardiopulmonary dysfunction in teens and death in 20s
• Current treatment: corticosteroids
• Landmark clinical trials – Ataluren/PTC 124 for nonsense mediated DMD; Exon skipping
• Clinical biomarker discovery for DMD:
  – Diagnostic: Genotype-phenotype correlation and disease course
  – Pathophysiologic: disease mechanisms and common pathways
  – Therapeutic: pharmacogenetic, pharmacodynamic, pharmacokinetic and safety
• -omics – transcriptomics, proteomics, metabolomics
Genomic Profiling using blood instead of muscle

? potential for providing a biological marker for DMD
Concept of Gene Expression Profiling: Two Different Leukemias

Golub et al. Science 286: 531
The Future of Genomic Profiling of Neurological Diseases Using Blood

Frank R. Sharp, MD; Huichun Xu, MD; Lisa Lit, BS; Wynn Walker, PhD; Michelle Apperson, MD, PhD; Donald L. Gilbert, MD; Tracy A. Glauser, MD; Brenda Wong, MD; Andrew Hershey, MD, PhD; Da-Zhi Liu, PhD; Joseph Pinter, MD; Xinhua Zhan, MD; Xinshe Liu, MD; Ruiqiong Ran, MD, PhD

Sequencing of the human genome and new microarray technology make it possible to assess all genes on a single chip or array. Recent studies show different patterns of gene expression related to different tissues and diseases, and these patterns of gene expression are beginning to be used for diagnosis and treatment decisions in various types of lymphoid and solid malignancies. Because of obvious problems obtaining brain tissue, progress in genomics of neurological diseases has been slow. To address this, we demonstrated that different types of acute injury in rodent brain produced different patterns of gene expression in peripheral blood. These animal studies have now been extended to human studies. Two groups have shown that there are specific genomic profiles in the blood of patients after ischemic stroke that are highly sensitive and specific for predicting stroke. Other recent studies demonstrate specific genomic profiles in the blood of patients with Down syndrome, neurofibromatosis, tuberous sclerosis, Huntington disease, multiple sclerosis, Tourette syndrome, and others. In addition, data demonstrate specific profiles of gene expression in the blood related to different drugs, toxins, and infections. Although all of these studies are still preliminary basic scientific endeavors, they suggest that this approach will have clinical applications to neurological diseases in humans.

Arch Neurol. 2006;63:1529-1536
Genomic Profiling/Transcriptomics

- Gene profiles/signatures – solid tumors? Predict response to specific types of therapy
- Blood genomics – obtaining tissues not practical
- PAX tubes – immediate stabilization of RNA as soon as blood is drawn into tubes
- Variables affecting gene expression in blood:
  - age and sex – children: higher expression of genes associated with immunoglobulins
  - genetic diseases (NF, TS) with specific profiles
RNA Expression Profiling of Blood from Humans

Whole Blood ➔ Apply RNA to MICROARRAYS
STORE -70°C ➔ GeneSpring, Partek and NCI public software to analyze data
Isolate RNA ➔ DAVID, KEGG and others for pathways

Established and Reliable Technology
We analyze the transcriptome how?

“Affymetrix Oligonucleotide Array”
54,000 probes
47,000 transcripts and variants - ? 25,000 genes
Genomic Microarray Analysis

1. Affymetrix U133 2.0 Plus microarray chips used
   1. Chip runs of 7 patients
      1. 2 conditions
      2. 2 control samples
   2. Batches of approximately 20 patients
2. Normalization to controls to eliminate batch to batch variation
3. Identification of outliers due to within sample variation (D-Chip)
4. Identification of gene difference (Significance Analysis of Microarrays – SAM 1.0 and SAM 2.0)
5. ANOVA analysis for significance with multiple testing correction (Genespring)
6. Cluster analysis (Genespring)
7. Gene class identification (Genespring)
Gene expression in blood of subjects with Duchenne muscular dystrophy

• 34 DMD subjects (boys)
• 14 on steroids
• Ages 3-20 yrs
• 21 healthy controls – age and gender matched
Cluster analysis of genes [with $\geq 2.5$-fold change] (Y-axis) regulated in blood of DMD compared to healthy age matched males)
Signaling pathways activated in blood of DMD subjects

Most significant KEGG pathways for the 191 genes (unpaired t test, fold change ≥ 2.0, FDR <0.05), DMD vs controls

- Leukocyte Transendothelial Migration (p 0.01)
- Regulation of Actin Cytoskeleton (p 0.03)
- Antigen Processing and Presentation (p 0.06)
- Neurodegenerative Disorders (p 0.06)
Leukocyte Trans-endothelial Migration
Regulation of Actin Cytoskeleton
Gene expression in blood of subjects with Duchenne muscular dystrophy

- The 59 probes with a >[2.5]-fold change separated DMD from controls using cluster analyses.

- Almost all of the genes regulated in peripheral blood were different from the genes reported to be regulated in diseased muscle of subjects with DMD.

- It is proposed that the genes regulated in blood of subjects with DMD are indicative, at least in part, of the immune response to the diseased DMD muscle.
Corticosteroid effects on blood gene expression in Duchenne muscular dystrophy
Lit, Sharp, Apperson, Liu, Walker, Liao, Xu, Ander, & Wong
*The Pharmacogenomics Journal, 2009*

Questions:

• Would patients on steroids have differences in blood gene expression compared with patients not on steroids?

• Would patients on deflazacort (DEFL) have differences in blood gene expression compared with patients on prednisone (PRE)?
Corticosteroid effects on blood gene expression in Duchenne muscular dystrophy
Lit, Sharp, Apperson, Liu, Walker, Liao, Xu, Ander, & Wong
The Pharmacogenomics Journal, 2009

Sample:
34 DMD Subjects
Age 9 yrs +/- 3 yrs
34 Caucasian
3 Other

Steroid profiles:
14 Chronic steroids (DMD-STEROID)
Mean time on steroids 43.9 months
20 never administered steroids (DMD)
6 Prednisone (PRED)
8 Deflazacort (DEFL)
Methods:
• 15 ml blood – PAXtube (preserves RNA)
• Isolate RNA
• Hybridize to Affymetrix U133-Plus 2.0 GeneChip
• Over 54,000 probesets
• 20,000+ known genes

Analyses:
• Genespring 7.2 software
• Significance 1.5-fold difference between groups
• Student t-test, P <= 0.05
• DAVID to assess co-regulation of pathways/biological significance
• PAM (Prediction Analysis of microarrays to identify set of minimum genes that stratify groups
• Comparisons:
  • DMD:DMD-STEROIDS
  • PRED:DEFL

**On steroids – most classified correctly**


**No steroids – all Ss classified correctly**

DEFL – all classified correctly

PAM – 496 probes separate DEFL(blue)/PRED(pink) (100% correctly). X-axis: subject id (across the top). Y-axis: probability of classifying subjects.

Pred – all classified correctly
CONCLUSIONS

Of note – The immune system and muscle:

1. WMS Geneva 12 Sep 2009 James Tidball: Regulating interactions between the immune system and muscle for the treatment of Muscular Dystrophy

2. “Genes involved in inflammatory immune responses mediated by chemokine and cytokine signaling pathways as the most significant amongst the list ..” Microarray analysis of mdx mice expressing high levels of utrophin: Therapeutic implications for dystrophin deficiency. Baban D, Davies K. NMD 2008;18:239-247


- Our pilot studies in DMD transcriptomics show that gene expression profiling in blood of DMD subjects should be further explored as a potential biomarker for monitoring the therapeutic and adverse effects of new treatments or new strategies of clinical management for DMD.