Gene Expression Profiling in ALS: Past, Present & Future

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Overview

• Introduction

• Past
  – From pooling patient samples..
  – …to profiling individual cell types

• Present
  – Profiling classes of RNA
  – Profiling cellular compartments

• Future
  – Integral to clinical trials
  – Informing diagnosis, prognosis & treatment
Introduction

- Gene expression profiling
- Transcriptomics
  - cDNA arrays
  - Microarrays
    - 3’IVT
    - Exon
    - Exon & splice site (HTA)
  - RNaseq
- Quantitative measure of multiple RNA transcripts at time of sampling
Uses in ALS

- Disease mechanisms
- Monitoring progression
- Identifying diagnostic and prognostic biomarkers
Past: GEP in ALS

Human spinal cord homogenates; pooled RNA; antioxidant and neuroinflammation DEG

Differential expression of 14 genes in amyotrophic lateral sclerosis spinal cord detected using gridded cDNA arrays

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Molecular signature of late-stage human ALS revealed by expression profiling of postmortem spinal cord gray matter

Fernando Dangond,1 Daechae Hwang,2 Sandra Camelo1, Piera Pasinelli,3 Matthew P. Frosch,4 Gregory Stephanopoulos,2 George Stephanopoulos,2 Robert H. Brown, Jr.,3 and Steven R. Gullans5
Past: GEP in ALS

Human spinal cord homogenates; pooled RNA; antioxidant and neuroinflammation DEG

sALS and fALS spinal cord distinguished; pro–inflammatory & RNA transcription DEG

SOD1 mouse models:
Inflammation, metal ion dysfunctions & apoptosis

Molecular signature of late-stage human ALS revealed by expression profiling of postmortem spinal cord gray matter

Differential expression of inflammation- and apoptosis-related genes in spinal cords of a mutant SOD1 transgenic mouse model of familial amyotrophic lateral sclerosis
SOD1–ALS & NRF2

- NSC34 cell line
- Vector only, nSOD1, G93A SOD1
- 268 genes differentially expressed
- NRF2 pathway
Immunohistochemistry revealed decreased protein Nrf2 immunoreactivity in MNs ALS v Controls.

q-PCR revealed decreased Nrf2 mRNA in MNs ALS v Controls.
Neuroprotective effect of Nrf2/ARE activators, CDDO ethylamide and CDDO trifluoroethylamide, in a mouse model of amyotrophic lateral sclerosis.

Neymotin A, Calingasan NY, Wille E, Naseri N, Petri S, Damiano M, Liby KT, Risingson R, Sporn M, Beal MF, Kiaei M.

Department of Neurology and Neuroscience, Weill Medical College of Cornell University, New York-Presbyterian Hospital, New York, NY 10065, USA.
Original Contribution

*S[+] Apomorphine is a CNS penetrating activator of the Nrf2-ARE pathway with activity in mouse and patient fibroblast models of amyotrophic lateral sclerosis\textsuperscript{☆}

Richard J. Mead\textsuperscript{a,1}, Adrian Higginbottom\textsuperscript{a,1}, Scott P. Allen\textsuperscript{a}, Janine Kirby\textsuperscript{a}, Ellen Bennett\textsuperscript{a}, Siân C. Barber\textsuperscript{a}, Paul R. Heath\textsuperscript{a}, Antonio Coluccia\textsuperscript{b}, Neelam Patel\textsuperscript{a}, Iain Gardner\textsuperscript{c}, Andrea Brancale\textsuperscript{b}, Andrew J. Grierson\textsuperscript{a}, Pamela J. Shaw\textsuperscript{a,*}

E

F

\begin{figure}
\centering
\includegraphics[width=0.4\textwidth]{E.png}
\caption{Forelimb stride length (mm)}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=0.4\textwidth]{F.png}
\caption{Hind-limb stride length (mm)}
\end{figure}
ALS & PTEN/PI3K

“Surviving” motor neurones at post-mortem express cell survival genes

Phosphatase and tensin homologue/protein kinase B pathway linked to motor neuron survival in human superoxide dismutase 1-related amyotrophic lateral sclerosis

Janine Kirby,1,* Ke Ning,1,* Laura Ferraiuolo,1 Paul R. Heath,1 Azza Ismail,1 Su-Wei Kuo,1 Chiara F. Valori,1 Laura Cox,1 Basil Sharrack,2 Stephen B. Wharton,3 Paul G. Ince,3 Pamela J. Shaw1,* and Mimoun Azzouz1,*
ALS & PTEN/PI3K

PTEN/PI3K also implicated in C9ORF72–ALS through transcriptomic analysis
(G4C2)n Cell Model

Isogenic inducible NSC34 model with 10, 51 & 102 (G4C2) interrupted repeats

Matt Stopford
(G4C2)n Cell Model

Isogenic inducible NSC34 model with 10, 51 & 102 interrupted repeats
- (G4C2) 102 rpts v NSC34 sham (+5d Tet)
  - Protein transport, vesicle-mediated transport
  - RNA metabolism
  - PI3K/AKT pathway
PTEN Knockdown Rescues Toxicity

Q–PCR: PTEN knockdown in sham & (G4C2)102

Cell Viability Assay: Knockdown of PTEN in induced (G4C2)102 cells rescues cell viability
Spinal cord MNs with TDP–43 pathology revealed significant dysregulation of splicing
- Differentially spliced genes enriched for ribonucleotide binding
- Also enriched for ALS genetic loci (15/24 loci diff. spliced)

Mutant TARDBP fibroblasts also showed splicing dysregulation
- Differentially spliced genes enriched for ribonucleotide binding
- More diff. spliced genes in TARDBP–ALS v SOD1–ALS & SALS
RNA Splicing

- C9ORF72–ALS spinal cord MNs – 3’IVT arrays
- Lymphoblastoid cell lines – exon arrays
- Weighted gene co-expression network analysis (WCGNA) performed
RNA Splicing

Isolated C9ORF72–ALS spinal cord MNs

C9ORF72–ALS LCLs

54% “RNA processing” gene DE in MNs also DE in LCLs
Past: Disease Mechanisms

• Investigating disease mechanisms
  - NRF2 pathway
  - PTEN/PI3K/AKT pathway

These pathways are viable targets for therapeutic invention

  - Further evidence for dysregulated RNA splicing
Uses in ALS

• Disease mechanisms
  – NRF2 pathway
  – PTEN/AKT pathway
  – RNA splicing

• Monitoring progression

• Identifying diagnostic and prognostic biomarkers
LCM of MNs G93A SOD1 mouse; very few changes in presymptomatic; increase in cell growth / maintenance genes

No widespread induction of cell death genes occurs in pure motoneurons in an amyotrophic lateral sclerosis mouse model

Florence E. Perrin, Gaëlle Boisset, Mylene Docquier, Olivier Schaad, Patrick Descombes and Ann C. Kato

Microarray Analysis of the Cellular Pathways Involved in the Adaptation to and Progression of Motor Neuron Injury in the SOD1 G93A Mouse Model of Familial ALS

Laura Ferraiuolo, Paul R. Heath, Hazel Holden, Paul Kasher, Janine Kirby, and Pamela J. Shaw

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Changes in presymptomatic SOD1 G93A mice due to homogenous background; late-stage transcriptional repression
Monitoring Progression

LCM of MNs G93A SOD1 mouse; very few changes in presymptomatic; increase in cell growth / maintenance genes

No widespread induction of cell death genes occurs in pure motoneurons in an amyotrophic lateral sclerosis mouse model

Florence E. Perrin¹, Gaëlle Boisset¹, Mylene Docquier², Olivier Schaad², Patrick Descombes² and Ann C. Kato¹,*

Evidence of reduced metabolic support in astrocytes, with reduced lactate levels and p75 activation.

Changes in presymptomatic SOD1 G93A mice due to homogenous background; late-stage transcriptional repression
Urinary p75_{ECD}
A prognostic, disease progression, and pharmacodynamic biomarker in ALS

ABSTRACT

Objective: To evaluate urinary neurotrophin receptor p75 extracellular domain (p75_{ECD}) levels as disease progression and prognostic biomarkers in amyotrophic lateral sclerosis (ALS).

Methods: The population in this study comprised 45 healthy controls and 54 people with ALS, 31 of whom were sampled longitudinally. Urinary p75_{ECD} was measured using an enzyme-linked immunoassay and validation included intra-assay and inter-assay coefficients of variation, effect of circadian rhythm, and stability over time at room temperature, 4°C, and repeated freeze-thaw cycles. Longitudinal changes in urinary p75_{ECD} were examined by mixed model analysis, and the prognostic value of baseline p75_{ECD} was explored by survival analysis.

Results: Confirming our previous findings, p75_{ECD} was higher in patients with ALS (5.6 ± 2.2 ng/mg creatinine) compared to controls (3.6 ± 1.4 ng/mg creatinine, p < 0.0001). Assay reproducibility was high, with p75_{ECD} showing stability across repeated freeze-thaw cycles, at room temperature and 4°C for 2 days, and no diurnal variation. Urinary p75_{ECD} correlated with the revised ALS Functional Rating Scale at first evaluation (r = −0.44, p = 0.008) and across all study visits (r = −0.36, p < 0.0001). p75_{ECD} also increased as disease progressed at an average rate of 0.19 ng/mg creatinine per month (p < 0.0001). In multivariate prognostic analysis, bulbar onset hazard ratio (HR) 3.0, p = 0.0035), rate of disease progression from onset to baseline (HR 4.4, p < 0.0001), and baseline p75_{ECD} (HR 1.3, p = 0.0004) were predictors of survival.

Conclusions: The assay for urinary p75_{ECD} is analytically robust and shows promise as an ALS biomarker with prognostic, disease progression, and potential pharmacodynamic application. Baseline urinary p75_{ECD} provides prognostic information and is currently the only biological fluid-based biomarker of disease progression. Neurology® 2017:88:1-7
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Present:
miRNA Biomarkers

- Non-coding single stranded 22-mer RNA
  - Regulate gene expression; multiple targets
  - Stable and detectable in blood
  - Reflect physiological conditions
miRNA Biomarkers

- 27 SALS patients, 25 controls & 36 ALS mimics
- Taqman Low Density Arrays (TLDA cards) 750 miRNAs
- Validated miRNA changes in 2\textsuperscript{nd} cohort using mirScript
- Investigated miRNA changes over disease progression
miRNA Biomarkers

- **Diff Exp miRNA** ($p<0.05$)
  - sALS v Con 12
  - sALS v Myopathy 28
  - sALS v Non-IM 25

- **Validation cohort**
  - 23 sALS v 22 Controls
  - 12 miRNAs tested
  - 3 validated
miRNA Biomarkers

- miR-206 ↑
- miR-143-3p ↑
- miR-374b-5p ↓
- No change in response due to riluzole or site of disease onset (B v UL v LL)
miRNA Biomarkers

• Longitudinal Study
  - Diagnosis v >3mths later (n=21 ALS)
  - miR–143–3p increased with progression
  - miR–374b–5p decreased with progression

![Graph showing relative concentration of miRNAs](image-url)
Profiling Cellular Compartments

- Total RNA
- Nuclear v Cytoplasmic
Cyto v Nuclear

- Fibroblast samples
  - 4 controls

  - Exon arrays
    - Gene level
    - Exon level

- Nuclear v cytoplasmic RNA
Cyto v Nuclear

(A) Cytoplasmic MT vs. Cytoplasmic CON
- FC $\geq 1.2$
- P-value $\leq 0.05$
- 702 gene
  - 426 up
  - 276 down

(B) Nuclear MT vs. Nuclear CON
- FC $\geq 1.2$
- P-value $\leq 0.05$
- 685 gene
  - 345 up
  - 340 down

Afnan Al Sultan
Cyto v Nuclear

DEG:
- mRNA splicing
- Transcription

Afnan Al Sultan

DEG:
- Mitotic nuclear division
- DNA replication
- mRNA splicing
Missense v Truncation

- Fibroblast samples
  - 4 controls
  - 3 truncating TARDBP (p.Y374X)
- Cytoplasmic RNA
- RNA sequencing
Present: RNA–Seq

- Not limited by predefined probes
- Identification of novel transcripts & RNA species
- Wider dynamic range
- Sequence specific information

Challenges:
- Volumes of data
- Analysis pipelines
• Missense DEG
  - Regulation of RNA metabolic process
  - Cell adhesion

• Truncation DEG
  - Vesicle mediated transport
  - Response to organic substance

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Profiling Cellular Compartments

- Total RNA
- Nuclear v Cytoplasmic
- Translatome
Profiling Cellular Compartments

- Total RNA
- Nuclear v Cytoplasmic
- Translatome
- Axon v Cell Body

Wen-Yo Tu & Paul Heath
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Future: Clinical Trials

- Collect DNA & RNA
- Clinical data – MUNE/MUNIX/Imaging/Cognitive ability
- Systems biology approach

- Sub-classify patients
  - Responders v non-responders
  - Correlations with clinical, biochemical & genetic data
Future: Clinical Care

• Diagnostic & Prognostic Biomarkers
  – Skyline Diagnostics AMLprofiler
    • Replaced 7 different tests, 3–4 weeks
    • Single Affymetrix based assay
      – Assesses prognostic markers t(16;16)/inv(16), t(8;21) and t(15;17)
      – CEBPA double mutations & NPM1 A/B/D mutations
      – BAALC and EVI1 expression

• Can be completed in 3 days
Future...

Considerations...

RNA-seq

Total RNA

Cytoplasmic

Translatome

Axonal

Microarrays

Nuclear

Cell body

mRNA

miRNA

xRNA
Future GEP?

ALS
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Wen-Yo Tu
Theresa Walsh
Helen Wollff
Mbombe Kazoka
Lee Tuddenham
Any Questions?