MIAME Checklist

Experimental Design

Authors:
David Kuninger, Alistair Wright[a], and Peter Rotwein

Oregon Health & Sciences University
Department of Biochemistry and Molecular Biology
Portland, Oregon 97239-3098

[a]Present address: Dalhousie Medical School
School of Medicine
Halifax, Nova Scotia, Canada, B3H 4R2

Type of experiment:
The primary focus of these studies is to define the transcriptional network regulated by ectopic expression of the transcriptional co-activator protein, p300, in order to define its mechanism(s) of action in promoting muscle cell survival.

Experimental factors:
Our laboratory has generated a murine C2-derived myoblast cell line stably expressing an IGF-II cDNA in antisense orientation (C2AS12 cells). These cells proliferate normally in serum-rich growth medium but progressively die in low-serum differentiation medium. Ectopic expression of p300 (a transcriptional co-coactivator with acetyltransferase activity) prevents the progressive cell death induced by serum withdrawal, however, the mechanism of this action is not understood. Further, over-expression of a mutated form of p300, lacking at protein interaction domain (ΔTAZ2), failed to maintain cell viability although it retains catalytic activity. Wild-type and mutant p300 are delivered using recombinant adenoviruses (Ad-p300 and Ad-p300ΔTAZ2) and their expression is regulated by a second recombinant adenovirus encoding the tetracycline transactivator protein (Ad-tTA), affording regulated expression of p300 forms (Tet-off system) and providing a control for viral load.

Using these reagents the overall experiment was as follows: Three parallel series of C2AS12 cells were infected with (1) wt p300 + tTA, (2) p300ΔTAZ2 +tTA, (3) wt p300 +tTA +doxycycline. Infected cells were grown to confluence followed by transfer to low-serum differentiation medium (T0). Cell were isolated at this point and following 24 (T24) hours incubation for RNA isolation. Companion dishes of cells were included for analysis by immunocytochemistry to ensure regulated transgene expression and cell viabilities.

Number of hybridizations:
6 hybridizations on oligonucleotide arrays (Affymetrix MG_U74Av2)

Hybridization design:

Affymetrix system, single color.

Type of reference used for the hybridizations:
No reference used.

Quality control steps:
Standard Affymetrix control steps. Quality assessement of cRNA was performed on the RNA 6000 LabChip sing the 2100 Bioanalyzer (Agilent, Palo Alto, CA).

Number of replicates:
6 biological replicates representing 3 treatment groups, 2 timepoints and 2 different biological outcomes (cell survival vs apoptotic death).

URL of supplemental we sites/database accession numbers:
When the paper is accepted, it will be deposited in the NIH NCBI Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/)

Samples

Origin of sample:
Murine, C3H strain-derived C2 myoblasts engineered to stably express an antisense IGF-II cDNA (see Stewart and Rotwein, JBC 1996).

Manipulation of samples:
Cells were infected with recombinant adenoviruses encoding either wild type or mutant p300 in the presence or absence doxycline and were incubated in low serum differentiation medium for 24 h.

Protocol for preparing hybridization extract:
Total RNA was isolated from samples using Trizol Reagent (Invitrogen) followed by an additional sodium acetate/ethanol precipation.

Labelling protocol:
Standard Affymetrix protocols (Affymetrix GeneChip Expression Analysis Technical Manual, rev.3. 2001)

External controls (spikes):
Standard Affymetrix external spikes added to hybridization mixture: BioB, BioC, BioD, and cre at 1.5 pm, 5.0 pm, 25 pm and 100 pm, respectively.

Hybridization Procedures and Parameters

Protocol and conditions:
Standard Affymetrix protocols (Affymetrix GeneChip Expression Analysis Technical Manual, rev.3. 2001)

Measurement Data and Specifications

Quantifications based on images:
Original Affymetrix .dat proprietary output files

Type of scanning hardware and software used:
Software-Affymetrix Microarray Suite Software, version 5.0
Scanning hardware- HP GeneArray Scanner

Type of image analysis software used:
Affymetrix Microarray Suite Software, version 5.0

Description of measurements produced by the image-analysis software and measurements used in the analysis:
Probe level measurements produced by Affymetrix Microarray Suite Software, version 5.0 (.cel files)

Complete output of image analysis before data selection and transformation (spot quantitation matrices):
Original Affymetrix output files (.cel files)

Data selection and transformation procedures:
Chip data (.cel files) were normalized using global scaling values (200), and analyzed with Affymetrix Microarray Suite 5.0 software using default parameters except for a Tau setting of 0.015. Filtered pair-wise comparisons were performed with the following criteria: only expression signal values ≥ 100 for Ad-p300 wild type samples were considered and mean fold-change ≥ 4 vs both Ad-p300 +Dox and Ad-p300ΔTAZ2 samples was required for selection.

Final gene expression data table(s) used by authors to make their conclusions after data selection and transformation (gene expression data matrices):
Normalized expression data and final gene list are attached as separate Excel files, titled Ad-p300 ALL Exp.xls and Ad-p300 Final.xls, respectively.

Array Design

Platform type:
Affymetrix oligonucleotide array

Surface and Coating Specifications:
Glass

Array:
Affymetrix MG_U74Av2 array

Features on array:
See www.affymetrix.com

Reporters on the array:
See www.affymetrix.com