Data in Brief

Transcriptional profiling of apoptosis-deficient Drosophila mutants

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Data for searching transcriptional alterations in Drosophila apoptosis-deficient mutants.

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Direct link to deposited data.

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE47853.

Experimental design, materials and methods

Drosophila preparation

Flies were maintained on a standard diet containing 4% yeast, 4% cornmeal, 10% glucose and propionic acid. All flies were kept in 25 °C with 60% humidity in the alternate 12 h light and dark cycle. As a model of apoptosis-deficient mutant, we utilized a hypomorphic allele of Drosophila apaf1 ortholog, darkcd4 mutants, in which both developmental and stress-induced apoptosis were remarkably diminished [2,3]. For the precise control of genetic background, we have backcrossed darkcd4 mutants six generations into w1118 control strains.

RNA extraction, purification, and quality verification

All flies were collected within one day after adult eclosion and incubated for five days for adult maturation with free access to food and mating. Five male flies were collected in one sampling tube and immediately frozen in liquid nitrogen. Flies were homogenized in TRIzol reagent (Invitrogen) by Multi-Beads Shocker (Yasui Kikai) set to 1500 rpm, 15 s × 3 cycles, and total RNA was extracted as reported [4].

Total RNA was then purified using RNeasy Plus Micro Kit (Qiagen), and cRNA yield (more than 0.825 μg) and labeling efficiency (6 pmol/μg) were validated by NanoDrop spectrophotometer (Thermo Fisher Scientific). Four independent RNA samples of high quality, which had two sharp peaks of 18S and 28S ribosomal RNA [5] were subjected to microarray analysis (Fig. 1).

Experimental procedures for microarray analysis

Cyanine-3 (Cy3)-labeled cRNA was prepared from 50 ng of total RNA by Low Input Quick Amp Labeling Kit, One-Color (Agilent Technologies) according to the manufacturer’s instruction. cRNA was purified by RNeasy Mini Kit (Qiagen), and cRNA yield (more than 0.825 μg) and labeling efficiency (6 pmol/μg) were validated by NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific). 600 ng of Cy3-labeled cRNA was then fragmented in a 30-minute incubation at 60 °C.
Fig. 1. An example of RNA quality validation by Agilent 2100 Bioanalyzer. Drosophila total RNA contains two main peaks, 18S and 28S ribosomal RNA.

Table 1
List of differentially expressed entities in darkcd4 (up-regulated, fold change > 1.5, p < 0.05).

| Probe name | Gene symbol | p-Value          | Corrected p-value | Fold change | Entrez gene ID |
|------------|-------------|-----------------|-------------------|-------------|----------------|
| A_09_P137560 | CG6484 | 1.21E−07 | 7.59E−04 | 826.2603 | 36994           |
| A_09_P077411 | CG3397 | 3.84E−07 | 0.001204653 | 36632 | 41454           |
| A_09_P057606 | CG30091 | 3.29E−06 | 0.005706159 | 259.83295 | 36806           |
| A_09_P023946 | CG6639 | 7.32E−06 | 0.009384108 | 54.593582 | 41454           |
| A_09_P012361 | Dro | 9.64E−06 | 0.010505327 | 84.814806 | 36635           |
| A_09_P164825 | CG3397 | 5.14E−05 | 0.020792374 | 54.593582 | 41454           |
| A_09_P109390 | CecC | 2.58E−04 | 0.04110067 | 50.98296 | 43599           |
| A_09_P205050 | Atta | 3.83E−05 | 0.017613623 | 45.11442 | 36636           |
| A_09_P029291 | CG18563 | 1.37E−04 | 0.036148455 | 44.8299 | 36636           |
| A_09_P057606 | CG30091 | 1.37E−04 | 0.036148455 | 44.8299 | 36636           |
| A_09_P022946 | CG6639 | 7.32E−06 | 0.009384108 | 54.593582 | 41454           |
| A_09_P012361 | Dro | 9.64E−06 | 0.010505327 | 84.814806 | 36635           |
| A_09_P164825 | CG3397 | 5.14E−05 | 0.020792374 | 54.593582 | 41454           |

(continued on next page)
Table 1 (continued)

| Probe name | Gene symbol | p-Value | Corrected p-value | Fold change | Entrez gene ID |
|------------|-------------|---------|-------------------|-------------|----------------|
| A_09_P052476 | ple     | 1.31E – 04 | 0.03720452 | 2.1828754 | 38746 |
| A_09_P074666 | CG8449 | 2.99E – 04 | 0.04508278 | 2.1645174 | 41628 |
| A_09_P030576 | Fer1HCH | 3.29E – 04 | 0.04758134 | 2.0818182 | 40415 |
| A_09_P017531 | Spz2 | 2.59E – 04 | 0.04411067 | 2.0289657 | 2768666 |
| A_09_P033336 | Prs2 | 1.37E – 04 | 0.03614845 | 1.9307616 | 36705 |
| A_09_P218510 | pot | 1.75E – 04 | 0.03921483 | 1.8158448 | 31254 |
| A_09_P074856 | CG9312 | 4.37E – 04 | 0.01565035 | 1.7286412 | 41686 |
| A_09_P112520 | CG31664 | 3.71E – 04 | 0.04772616 | 1.7204942 | 33359 |
| A_09_P064416 | yellow-c | 3.59E – 04 | 0.04295666 | 1.7025788 | 34879 |
| A_09_P079531 | CG1927 | 4.83E – 05 | 0.02035598 | 1.7006992 | 38262 |
| A_09_P171400 | CG9760 | 1.11E – 05 | 0.01031309 | 1.7006477 | 39388 |
| A_09_P030571 | Fer2LCH | 4.83E – 05 | 0.02035598 | 1.6878323 | 44965 |
| A_09_P078611 | Idg4 | 1.69E – 04 | 0.03921483 | 1.6784037 | 31926 |
| A_09_P121595 | Wnt2 | 2.12E – 04 | 0.04714648 | 1.6761436 | 35975 |
| A_09_P079536 | CG14787 | 1.32E – 04 | 0.03614845 | 1.6502483 | 31096 |
| A_09_P145165 | CG9284 | 7.03E – 05 | 0.02556935 | 1.6346469 | 50130 |
| A_09_P054936 | CG10646 | 7.53E – 05 | 0.02636249 | 1.6294572 | 39424 |
| A_09_P032636 | Spred | 1.96E – 04 | 0.04165185 | 1.5928276 | 36643 |
| A_09_P154000 | Idg4 | 3.09E – 04 | 0.04593384 | 1.5830332 | 31926 |
| A_09_P020526 | spz3 | 2.86E – 04 | 0.04534591 | 1.5688794 | 34077 |
| A_09_P027505 | CG9449 | 3.00E – 04 | 0.04582735 | 1.5068132 | 40117 |
| A_09_P025405 | Fer2LCH | 2.94E – 04 | 0.04582735 | 1.5040354 | 44965 |
in a reaction mixture containing 1 × Agilent fragmentation buffer and 2 × Agilent GE blocking agent. After fragmentation, 2 × Agilent GE hybridization buffer HI-RPM was added to the sample and then hybridized to SurePrint G3 custom microarray 8 × 60K (G4102A#040871) for 17 h at 65 °C in a rotating Agilent hybridization oven (Agilent Technologies). Slides were scanned after washing on the SureScan Microarray Scanner using AgilentG3_GX_1Color_HighSensitivity (Agilent Technologies). Feature Extraction Software 10.7.3.1 (Agilent Technologies) was used with default parameters (protocol GE1_107_Sep09 and Grid: 040871_D_F_20120511) to obtain background-subtracted and spatially-detrended Processed Signal intensities. Data quality was evaluated by Evaluation Metrics for GE1_QCMT_Sep09 in the QC Report.

Data processing and analysis

Extracted text data were processed using GeneSpring GX12.1 (Agilent Technologies). Non-uniform or saturated probes as well as population outliers were compromised and quantile normalization was applied to each data set as the following setting: Threshold raw signal 1.0, Algorithm, Percentile Shift, Percentile Target, 75. Baseline was corrected by the median of all samples. Probes from all samples with intensity less than 20% were filtered out, resulting in 25,083 validated entities. These data from four independent samples for wild type and darkcd4 flies were subjected to statistical analysis by unpaired student’s t-test with Benjamini Hochberg FDR correction. We obtained differentially expressed 188 (p < 0.05) or 481 entities (p < 0.1), and subsequent cut-off by fold change > 1.5 yielded 149 (Tables 1, 2) or 321 entities, respectively. GO analysis of these entities clearly demonstrated that immune-related genes were drastically elevated in darkcd4 mutants, while no GO term was enriched significantly for down-regulated genes. dFoxO target genes such as thor or lip3 were also significantly induced in darkcd4 mutants (Table 1). Reduction in dark expression was confirmed as three entities corresponding to dark were downregulated 6.0-, 4.5- and 3.5-fold compared to control (Table 2). Drosophila gnmt, the gene of our interest from metabolome analysis [1], was also included in the list of upregulated genes (p < 0.1), as two probes indicated 3.1- and 3.2-fold increase in darkcd4 mutants.

Discussion

Here we described a transcriptomic profiling of Drosophila apoptosis-deficient mutants, darkcd4. As reported recently, necrotic wing cells triggered spontaneous immune response in apoptosis-deficient mutants at this stage. Our well-controlled microarray data delineated the phenotypes observed in darkcd4 mutants and helped us clarify the systemic responses against necrotic cells. As far as we know, this is the first microarray analysis to describe transcriptional changes in apoptosis-deficient mutants in Drosophila. It is interesting that many other genes are also down- or up-regulated in these mutants, and this dataset may be useful for revealing novel and unexpected phenotypes triggered in response to necrosis or other functions of Dark/caspase.

Conflict of interest

The authors declare no conflicts of interest.

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