Comparative analysis of NRF2-responsive gene expression in AcPC-1 pancreatic cancer cell line

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Abstract NRF2 is a nuclear transcription factor activated in response to oxidative stress and related with metabolizing of xenotoxic materials and ABC transporter mediated drug resistance. We studied the expression of mRNAs under the siRNA-mediated knockdown of NRF2 and tBHQ-treated condition in AsPC-1 metastatic pancreatic cancer cell line to understand the AsPC-1 specific role(s) of NRF2 and further to investigate the relationship between drug resistance and metastatic plasticity and mobility of AsPc1. Here we show that the genes of aldo–keto reductases, cytochrome P450 family, aldehyde dehydrogenase, thioredoxin reductase, ABC transporter and epoxide hydrolase responsible for drug metabolism or oxidative stress concisely responded to NRF2 stabilization and knockdown of NRF2. In addition the expression of PIR, a candidate of oncogene and KISS1, a suppressor of metastasis were affected by NRF2 stabilization and knockdown. Our result provide comprehensive understanding of NRF2 target genes of drug response, oxidative stress response and metastasis in AsPC-1 metastatic pancreatic cancer cell line.

Keywords NRF2 · tBHQ · AsPC-1 · Pancreatic cancer · Oxidative stress · Drug metabolism

Introduction

Every cell is inevitably exposed to extracellular and intracellular oxidative stress, every moment (Finkel 2011; Ma 2010). The nuclear factor erythroid 2-related factor 2 (NRF2 or NFE2L2) is a master transcription factor that activates a battery of genes which have roles in oxidative stress responses, detoxifications, and drug resistances (Bryan et al. 2013; Ma 2013; Mitsuishi et al. 2012; Niture et al. 2014). NRF2 binds to a DNA element, named antioxidant response element (ARE), in the promoter regions of its target genes to activate transcription of these genes (Nguyen et al. 2003). The target genes of NRF2 includes (a) antioxidant genes such as NAD(P)H dehydrogenase [quinone] 1 (NQO1), heme oxygenase (decycling) 1 (HMOX1), superoxide dismutase [Cu–Zn] (SOD1), and glutamate-cysteine ligase catalytic subunit (GCLC); (b) detoxification genes including glutathione S-transferase A3 (GSTA3) and thioredoxin reductase 1, cytoplasmic (TXNRD1); (c) and drug resistance genes such as ATP-binding cassette sub-family G member 2 (ABCG2) and ATP-binding cassette, sub-family C (CFTR/MRP), member 5 (ABCC5) (Malhotra et al. 2010; Nguyen et al. 2003).

Reactive oxygen species (ROS), which are produced by various exogenous or endogenous sources, are double-edge swords. Under tight cellular control, ROS act as important signaling molecules to regulate diverse cellular functions including transcriptional regulation and signal transduction (Corcoran and Cotter 2013; Finkel 2011; Jennings et al. 2013; Ma 2010; Ray et al. 2012). On the contrary uncontrolled production of ROS causes various human diseases.
through DNA damage and impaired cellular functions via oxidative stress (Acharya et al. 2010; Caputo et al. 2012; Kakehashi et al. 2013; Kryston et al. 2011; Saeidnia and Abdollahi 2013; Storr et al. 2013). As an ROS sensor, the level of NRF2 is tightly regulated by a set of proteins through proteasome-dependent proteolysis. The well-known negative regulator of NRF2 is the Kelch-like erythroid cell-derived protein with CNC homology-associated protein 1 (KEAP1). KEAP1 binds and destabilized NRF2 through ubiquitin-dependent proteasomal degradation under normal reducing condition (Bryan et al. 2013; Mitsuishi et al. 2012; Niture et al. 2014; Storr et al. 2013). NRF2 stability is also regulated by the CR6-interacting factor 1 (CRIF1) under both reducing and oxidative stress conditions (Kang et al. 2010) and the glycogen synthase kinase 3β (GSK3β)/β-transducin repeat-containing protein (β-TrCP) axis (Chowdhry et al. 2013; Rada et al. 2011; Rada et al. 2012). It has been reported that stability of NRF2 is also regulated by competitive protein–protein interaction to inhibit NRF2-KEAP1 binding by various proteins such as p21 (Chen et al. 2009), the Wilms tumor gene on X chromosome (WTX) (Camp et al. 2012), p62 (Komatsu et al. 2010), the partner and localizer of BRCA2 (PALB2) (Ma et al. 2012), the dipeptidyl peptidase III (DPP3) (Hast et al. 2013), and the breast cancer susceptibility gene 1 (BRCA1) (Gorrini et al. 2013).

NRF2 functions as either a protector against tumorigenesis or oncogene (DeNicola et al. 2011; Kensler and Wakabayashi 2010; Loboda et al. 2008; Muller and Hengstermann 2012). Stability and activity of NRF2 is important in human diseases, especially in cancers. While NRF2 decreases tumor susceptibility in most carcinogenesis models, constitutive activation of NRF2 may enhance tumor proliferation and/or confer drug resistance in lung, pancreatic as well as colorectal cancer cells (Arlt et al. 2013; Bryan et al. 2013; Duong et al. 2014b; Homma et al. 2009; Hong et al. 2010; Lister et al. 2011; Mitsuishi et al. 2012; Niture et al. 2014; Singh et al. 2008; Storr et al. 2013; Yamadori et al. 2012). Indeed, NRF2 is up-regulated in many types of tumors through somatic mutations that block KEAP1-dependent regulation of NRF2 stability (Mitsuishi et al. 2012; Niture et al. 2014; Storr et al. 2013). Targeting NRF2 either by RNA interference or by small molecules inhibited tumour growth and increased efficacy of chemotherapy (Singh et al. 2008) or EGF-driven proliferation (Yamadori et al. 2012) in non-small cell lung cancer models and reduced the proliferation and drug-resistance in human lung cancer cells (Homma et al. 2009) or human pancreatic cancer cells (Arlt et al. 2013; Duong et al. 2014b; Hong et al. 2010; Lister et al. 2011). Additionally in primary murine cell models, oncogenes including K-Ras, B-Raf, and Myc increased the transcription of Nrf2 gene to activate antioxidant and detoxification program preferable for oncogenesis (Kang et al. 2014). Under these conditions, genetic targeting of K-RasG12D-driven Nrf2 impaired in vivo tumorigenesis (Kang et al. 2014). Taken together, genome-wide analysis of NRF2-responsive genes in specific cancer types will give insights on the context-dependent roles of NRF2. In this work we delineated NRF2-responsive genes in As-PC1 pancreatic cancer cell lines established from metastatic cancer cell in ascites fluid (Chen et al. 1982).

Materials and methods

Cell culture and reagents

AsPC-1 cells were obtained from the Korean Cell Line Bank (Seoul, Korea) and maintained in RPMI-1640 media (HyClone, Logan, UT) supplemented with 20 % FBS (Invitrogen, Carlsbad, CA) and 100 U/ml penicillin/streptomycin (Welgene, Daegu, Korea). The cells were cultured in a humidified 5 % CO2 incubator at 37 °C. The cell viability and cell counting were assessed by the Luna Automated Cell Counter (Logos Biosystems, Gyeonggi-do, Korea). Tert-butyldihydroquinone (tBHQ) was purchased from Sigma (St. Louis, MO) and stored at −20 °C dissolved in DMSO with small aliquots.

siRNA transfection

For NRF2 knockdown, exponentially proliferating cells were transfected with synthesized control siRNA (5′-gag-gagcgcagcagcagaa-3′) or NRF2 specific siRNA (5′-gag-guaagcggagaaaaac-3′) (Hong et al. 2010), both purchased from Bioneer (Daejeon, Korea) using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol.

Cell cycle analysis

Cell cycle analysis was carried out by propidium iodide staining and laser detection of FL2 signal using FACSCalibur (BD Science, Franklin Lakes, NJ), and the data were analyzed by CellQuest Pro software (BD Science). After treatment (72 h for siRNA and 16 h for tBHQ treatment respectively), cells were washed with PBS, fixed 70 % ethanol, and stained with propidium iodide solution (20 μg/ml) containing RNaseA (100 μg/ml) after removal of ethanol.

RNA extraction

Total RNA from AsPC-1 cell lines were prepared with the RNeasy Mini kit (Qiagen, Valencia, CA) according to the
Fig. 1  Cell cycle analysis of NRF2 siRNA-treated or tBHQ-treated AsPC-1 cell line.  

**a**  A representative image of FL2 histogram of FACS analysis. The figure in the lower right quadrant is combined FACS analysis images with notion of 2 and 4 N nuclear ploidy.  

**b**  Immuno blot analysis of tBHQ-treated (100 μM 16 h) or siRNA-treated samples (72 h). AsPC-1 cells were seeded in 6-well plates and treated with tBHQ (or DMSO) or NRF2 siRNA (or control siRNA). Cells were harvested and whole cell lysates were prepared, electrophoresed and transferred onto PVDF membranes. Immunoblotting was performed with indicated antibodies and Erk-1 was used as loading control.
manufacturer’s protocols. The purity and integrity of RNA sample was evaluated by determining the OD260/230 ratio, 28S/18S ratio, peak pattern and electrophoretic migration patterns on Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA).

Western blot analysis

After 72 h of siRNA treatment or 16 h of tBHQ treatment, AsPC-1 cells were lysed in 10 mM Tris–HCl (pH 7.0), 100 mM NaCl, 1 % triton X-100, 1 mM DTT, 20 µg/ml aprotinin, 2.5 µg/ml leupeptin, and 0.5 mM PMSF. Lysates were resolved on 10 % sodium dodecyl sulfate–polyacrylamide by gel electrophoresis (SDS-PAGE) and transferred onto 0.45 µm pore size Polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, MA), and immunoblotted with following antibodies: Cyclin B1 antibody (CST#4135, Cell Signaling Technology, Danvers, MA), Cyclin D1 (CST #2922, Cell Signaling Technology), NRF2 (sc-103032, Santa Cruz Biotechnology, Santa Cruz, CA), Erk-1 (sc-94, Santa Cruz Biotechnology), Cyclin A (sc-239, Santa Cruz Biotechnology). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit (sc-2004, Santa Cruz Biotechnology) or anti-mouse antibodies (sc-2005, Santa Cruz Biotechnology) were used as secondary antibodies.

cDNA microarray analysis

The cDNA microarray analysis was carried out with fluorescence labeling of cRNA and hybridization using 4 × 44 K Human whole genome microarray (Agilent technologies, Palo Alto, CA) for tBHQ treated cells. For cDNA microarray analysis of NRF2 siRNA treated cell, Illumina Biochip system (HT-12) was used. For each microarray three RNA samples of independent experiment were used.

Statistical analysis

Data were analyzed by either Student’s t test (tBHQ treated sample) or LPE test (siRNA treated sample) (Jain et al. 2003) and the results have been expressed p values and mean values.

Results and discussion

The AsPC-1 pancreatic cancer cell line, used in this work had been established from metastatic abdominal ascites fluid cells originated from metastatic pancreatic cancer (Chen et al. 1982). It contains well known mutations of pancreatic
| Probe ID (Agilent 44 k) | Gene symbol | Fold change | p Value | Gene name |
|------------------------|-------------|-------------|---------|-----------|
| A_23_P415015           | ATL2        | 10.528      | 5.52E−04| Atlastin GTPase 2 |
| A_33_P3416588          | RIT2        | 10.341      | 6.05E−04| Ras-like without CAAX 2 |
| A_23_P83134            | GAS1M       | 4.752       | 1.13E−03| Growth arrest-specific 1 |
| A_33_P3257155          | SMAP1       | 4.697       | 4.41E−05| Small ArfGAP 1 |
| A_24_P129341           | AKR1B10D, O | 4.694       | 8.83E−04| Aldo–keto reductase family 1, member B10 |
| A_23_P93641            | AKR1B10D, O | 4.652       | 8.91E−04| Aldo–keto reductase family 1, member B10 |
| A_33_P3276268          | FCER1G      | 4.621       | 1.31E−04| Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide |
| A_23_P258190           | AKR1B1D, O  | 4.588       | 9.03E−04| Aldo–keto reductase family 1, member B1 |
| A_23_P80570            | AADACD      | 4.488       | 9.31E−04| Arylacetamide deacetylase (esterase) |
| A_33_P3416588          | GRK5B, M    | 4.433       | 1.06E−03| G protein-coupled receptor kinase 5 |
| A_33_P3380992          | AKR1B15D    | 4.415       | 9.39E−04| Aldo–keto reductase family 1, member B15 |
| A_23_P3254751          | LOC100131355| 3.703       | 1.67E−03| Hypothetical protein LOC100131355 |
| A_23_P258190           | AKR1B1D, O  | 3.005       | 1.59E−03| Aldo–keto reductase family 1, member C1 |
| A_23_P32143            | ZNF767      | 2.919       | 1.76E−04| Zinc finger family member 767 |
| A_33_P3350853          | LOC202781   | 2.885       | 1.74E−04| Hypothetical LOC202781 |
| A_23_P96623            | OPN1MW      | 2.879       | 2.65E−03| Opsin 1 (cone pigments), medium-wave-sensitive |
| A_33_P3396956          | C1orf172    | 2.874       | 1.87E−03| Chromosome 1 open reading frame 172 |
| A_23_P67453            | TNNI3       | 2.846       | 2.95E−04| Troponin I type 3 (cardiac) |
| A_23_P46238            | CELA2A      | 2.823       | 2.08E−03| Chymotrypsin-like elastase family, member 2A |
| A_24_P943949           | LRRCSB      | 2.775       | 3.48E−03| Leucine rich repeat containing 8 family, member B |
| A_23_P125042           | ZNF222      | 2.763       | 3.62E−03| Zinc finger protein 222 |
| A_33_P3265394          | WDR74       | 3.071       | 2.51E−03| WD repeat domain 74 |
| A_23_P257971           | AKR1C1D, O, M| 3.005      | 1.59E−03| Aldo–keto reductase family 1, member C1 |
| A_23_P32143            | ZNF767      | 2.919       | 1.76E−04| Zinc finger family member 767 |
| A_33_P3350853          | LOC202781   | 2.885       | 1.74E−04| Hypothetical LOC202781 |
| A_23_P96623            | OPN1MW      | 2.879       | 2.65E−03| Opsin 1 (cone pigments), medium-wave-sensitive |
| A_33_P3396956          | C1orf172    | 2.874       | 1.87E−03| Chromosome 1 open reading frame 172 |
| A_23_P67453            | TNNI3       | 2.846       | 2.95E−04| Troponin I type 3 (cardiac) |
| A_23_P46238            | CELA2A      | 2.823       | 2.08E−03| Chymotrypsin-like elastase family, member 2A |
| A_24_P943949           | LRRCSB      | 2.775       | 3.48E−03| Leucine rich repeat containing 8 family, member B |
| A_23_P125042           | ZNF222      | 2.763       | 3.62E−03| Zinc finger protein 222 |
| A_33_P3265394          | WDR74       | 3.071       | 2.51E−03| WD repeat domain 74 |
| A_32_P180741           | TNK2        | 2.690       | 3.52E−03| Tyrosine kinase, non-receptor, 2 |
| A_24_P68908            | LOC344887   | 2.600       | 2.11E−03| Similar to hCG2041270 |
| A_33_P3314401          | CLDN16      | 2.580       | 3.96E−03| Claudin 16 |
| A_33_P3365117          | AKR1C1D, O, M| 2.563      | 4.45E−03| Aldo–keto reductase family 1, member C1 |
| A_24_P152968           | AKR1C1D, O, M| 2.562      | 4.45E−03| Aldo–keto reductase family 1, member C1 |
| A_23_P63432            | RHBDL2      | 2.509       | 2.89E−03| Rhomboid, veinlet-like 2 (Drosophila) |
| A_33_P3294277          | CYP4F3D     | 2.489       | 2.59E−03| Cytochrome P450, family 4, subfamily F, polypeptide 3 |
| A_23_P28697            | HAAO        | 2.394       | 4.38E−03| 3-hydroxyanthranilate 3,4-dioxygenase |
| A_24_P678418           | DICER1-AS   | 2.378       | 2.74E−03| Hypothetical locus FLJ45244 |
| A_23_P46222            | TRIM46      | 2.370       | 2.85E−03| Tripartite motif containing 46 |
| A_33_P3389363          | C1orf54     | 2.364       | 3.17E−03| Chromosome 19 open reading frame 54 |
| A_23_P502047           | CHRD        | 2.345       | 3.99E−03| Chordin |
| A_33_P507010           | CYP4F2D     | 2.340       | 4.52E−03| Cytochrome P450, family 4, subfamily F, polypeptide 2 |
| A_33_P3315239          | ZNF7        | 2.337       | 4.01E−03| Zinc finger protein 7 |
| A_33_P3336287          | SEC61A2     | 2.322       | 4.20E−03| Sec61 alpha 2 subunit (S. cerevisiae) |
| A_23_P301521           | KIAA1462    | 2.275       | 6.97E−03| KIAA1462 |
| A_33_P3420090          | PATE2       | 2.272       | 1.54E−03| Prostate and testis expressed 2 |
| A_23_P218793           | XPNPEP3     | 2.187       | 3.38E−03| X-prolyl aminopeptidase (aminopeptidase P) 3, putative |
| A_33_P3265714          | C2orf61     | 2.184       | 1.05E−02| Chromosome 2 open reading frame 61 |
| A_33_P3252381          | PCA3        | 2.167       | 1.36E−03| Prostate cancer antigen 3 (non-protein coding) |
| A_33_P3378915          | ARHGEF18    | 2.164       | 3.58E−03| Rho/Rac guanine nucleotide exchange factor (GEF) 18 |
| A_33_P3397520          | KRTAP10-12  | 2.137       | 4.86E−03| Keratin associated protein 10-12 |
| A_24_P307135           | TNXB        | 2.111       | 7.60E−03| Tenascin XB |
cancer including, KRAS (p.G12D), TP53 (p.C135fsP35), SMAD4 (p.R100T), and other mutations common in cancers as well: COL2A1 (c.915 + 3A > G), FBXW7 (p.R465C), HEY1 (p.I178V), KIF5B (p.Q467K), MLL (p.P3536H), RNF43 (p.S720*) (Deer et al. 2010). The relative expression level of NRF2 between various pancreatic cancer cell lines including immortalized human pancreatic ductal epithelial cell lines (HPDE) using GEO2R analysis with pre-deposited microarray data (Thu et al. 2014) at NCBI Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/geo2r/?acc=GSE40099&platform=GPL6480) is presented in supplementary Fig. 1. NRF2 was reported to be increased in pancreatic cancer cell lines and the nuclear level of NRF2 in AsPC-1 cell line has been reported to be relatively higher than in immortalized pancreatic ductal epithelial cells (Hong et al. 2010; Lister et al. 2011).

An antioxidant tBHQ increases the level of NRF2 protein by stabilization and stimulates the expression of oxidative stress metabolizing genes (Hirose et al. 1993; Li et al. 2005). Prior to cDNA microarray we tested whether tBHQ or NRF2 siRNA treatment can change the cell cycle of AsPC-1 cell line. As shown in Fig. 1a no apparent change in cell cycle distribution was observed along with no accumulation of sub G1 population. Immunoblot analysis also revealed that no apparent change of cell cycle marker proteins including cyclin B1 and cyclin D. The level of NRF2 protein was shown to be increased in tBHQ treated cells and decreased in NRF2 siRNA treated sample (Fig. 1b).

To identify changed genes upon treatment of 100 μM tBHQ, we used the Agilent 44 k whole genome cDNA array chip. We also used the Illumina HT-12 whole genome cDNA array chip for NRF2 siRNA mediated gene expression analysis. Three independent RNA samples were used in these experiments. After removal of marginal or absent signal spots, 20,312 positive spots were obtained from tBHQ-treated sample and 16,423 positive spots were obtained from NRF2 siRNA-treated sample. Hierarchical cluster image of NRF2 siRNA treatment samples reveals that the gene expression pattern of three siRNA-treated sample and three control siRNA-treated samples are adequately clustered (Fig. 2a). Figure 2b shows the hierarchical cluster image of cDNA microarray of tBHQ-treated sample indicating three independent samples share concordant RNA expression pattern.

Further statistic tests after normalization of positive spots provide statistically significant 533 array sets from tBHQ-treated samples (supplementary Table 1) and 189 array sets from NRF2 siRNA-treated samples (supplementary Table 2). Table 1 shows a list of genes which show more than two fold increase of expression (p < 0.05) after treatment of tBHQ (57 genes). Among them AKR1B10, FCER1G, AKR1B1, AKR1B15, AADAC, GRK5, HDAC9, AKR1C1, CYP4F3, CYP4F2, ALDH3A1, FANCD2, TXNRD1 and SLC7A11 are classified as members of drug response genes or oxidative stress response genes according to gene ontology (Table 1). The lists of genes decreased by tBHQ treatment are listed in Table 2. Four genes classified as drug response or oxidative stress response genes were identified: PDE7A, TGM1, CYTH1 and EPS15. The list of top 50 genes which were decreased by NRF2 siRNA treatment are presented in Table 3. The listing is arbitrary but these genes showed more than 40 % reduction in expression. The siRNA mediated knockdown of NRF2 significantly reduced the expression of oxidative stress or drug response genes including, AKR1B10, ALDH1A1, HGD, TFF1, GPX2, ALDH3A1, PPPI1B, AKR1C4, ABCB6, ABCC3, NFE2L2, EPHXI, ASGR1, SLC2A5, LGA5L1 and MTR (Table 3). The expression of NRF2 itself was significantly (p < 0.001, 50 % reduction) decreased by the treatment of siRNA reflecting the reliable quality control of siRNA treatment. On the contrary to NRF2 siRNA treatment the change of

Table 1 continued

| Probe ID (Agilent 44 k) | Gene symbol | Fold change | p Value | Gene name |
|------------------------|-------------|-------------|---------|------------|
| A_33_P3259548          | WDR5B       | 2.097       | 4.74E-03| WD repeat domain 5B |
| A_23_P38190            | ORMDL3      | 2.084       | 4.14E-03| ORM1-like 3 (S. cerevisiae) |
| A_23_P3956             | CIQTFN1     | 2.069       | 3.97E-03| C1q and tumor necrosis factor related protein 1 |
| A_33_P3238433          | ALDH3A1D, O | 2.063       | 3.96E-03| Aldehyde dehydrogenase 3 family, memberA1 |
| A_23_P345678           | FANCD2D, O, M | 2.046    | 5.27E-03| Fanconi anemia, complementation group D2 |
| A_33_P355120           | TXNRD1D, O  | 2.042       | 4.10E-03| Thioredoxin reductase 1 |
| A_33_P325851           | LOC389791   | 2.032       | 5.19E-03| Hypothetical LOC389791 |
| A_33_P3242623          | SLC7A11D, M | 2.011       | 4.31E-03| Solute carrier family 7, member 11 |
| A_24_P223163           | NAF1        | 2.006       | 4.47E-03| Nuclear assembly factor 1 homolog (S. cerevisiae) |

The fold increased/decreased values are mean of three independent samples. Superscripts were assigned to drug response genes (D), oxidative stress response genes (O) and metastasis (M) related genes according to gene ontology. These gene symbols are presented in bold style.
Table 2: Top 50 gene records with decreased expression (p < 0.05) in tBHQ-treated AsPC-1 cells

| Probe ID (Agilent 44 k) | Symbol     | Fold change | p Value  | Gene name                                                                 |
|------------------------|------------|-------------|----------|---------------------------------------------------------------------------|
| A_23_P337849           | CELF3      | 0.398       | 1.10E−02 | CUGBP, Elav-like family member 3                                            |
| A_24_P322229           | RASL10B    | 0.466       | 7.41E−03 | RAS-like, family 10, member B                                              |
| A_33_P3213512          | COQ5       | 0.468       | 1.24E−02 | Coenzyme Q5 homolog, methyltransferase (S. cerevisiae)                     |
| A_23_P66027            | ALOX15BM   | 0.475       | 1.30E−02 | Arachidonate 15-lipoxygenase, type B                                       |
| A_33_P3356004          | UCKL1-AS1  | 0.542       | 3.18E−02 | UCKL1 antisense RNA 1 (non-protein coding)                                |
| A_33_P3247678          | LOC100130876 | 0.550    | 7.40E−03 | Uncharacterized LOC100130876                                             |
| A_33_P3245679          | LOC100129940 | 0.554   | 2.98E−02 | Uncharacterized LOC100129940                                             |
| A_23_P146325           | ASAP1-IT1  | 0.566       | 9.59E−02 | ASAP1 intronic Transcript 1 (non-protein coding)                           |
| A_32_P110016           | LOC727869  | 0.567       | 3.94E−02 | Uncharacterized LOC727869                                                 |
| A_23_P59988            | SLC35G5    | 0.567       | 2.72E−02 | Solute carrier family 35, member G5                                       |
| A_33_P3248136          | TRIP12     | 0.573       | 1.24E−02 | Thyroid hormone receptor interactor 12                                    |
| A_24_P360529           | PDE7A      | 0.589       | 3.23E−02 | Phosphodiesterase 7A                                                      |
| A_23_P108055           | C3orf51    | 0.597       | 1.85E−02 | Chromosome 3 open reading frame 51                                         |
| A_33_P3544880          | LOC142937  | 0.622       | 1.05E−02 | Uncharacterized protein BC008131                                           |
| A_33_P3576797          | LOC158863  | 0.622       | 1.17E−02 | Uncharacterized LOC158863                                                 |
| A_24_P314597           | KIAA0319L  | 0.631       | 1.73E−02 | KIAA0319-like                                                             |
| A_33_P3247299          | LOC645427  | 0.632       | 2.14E−02 | Uncharacterized LOC645427                                                 |
| A_33_P3256500          | ATXN2      | 0.636       | 2.07E−02 | Ataxin 2                                                                   |
| A_33_P3248265          | LTB        | 0.647       | 2.48E−02 | Lymphotoxin beta (TNF superfamily, member 3)                               |
| A_33_P3522511          | KIAA0485   | 0.649       | 3.41E−02 | Uncharacterized LOC57235                                                  |
| A_33_P3319134          | LOC100506191 | 0.649  | 3.60E−02 | Uncharacterized LOC100506191                                             |
| A_24_P693321           | LOC100190986 | 0.649  | 6.81E−03 | Uncharacterized LOC100190986                                             |
| A_23_P65618            | TGM1D      | 0.653       | 2.67E−02 | Transglutaminase 1                                                        |
| A_33_P3249259          | TGM6       | 0.656       | 1.57E−02 | Transglutaminase 6                                                        |
| A_23_P108032           | RPL23AP32  | 0.658       | 4.13E−02 | Ribosomal protein L23a pseudogene 32                                     |
| A_33_P3337777          | LOC100129387 | 0.661  | 3.27E−02 | Uncharacterized LOC100129387                                             |
| A_33_P326142           | C7orf54    | 0.663       | 3.05E−02 | Chromosome 7 open reading frame 54                                        |
| A_33_P3335840          | WDR33      | 0.666       | 3.53E−02 | WD repeat domain 3                                                        |
| A_33_P3324137          | PRO0628    | 0.668       | 1.90E−02 | Uncharacterized LOC29053                                                  |
| A_33_P3393010          | PKDCC      | 0.669       | 2.08E−02 | Protein kinase domain containing, cytoplasmic homolog (mouse)              |
| A_33_P3321372          | CNTNAP3    | 0.673       | 1.20E−02 | Contactin associated protein-like 3                                        |
| A_33_P3250018          | HCF2C      | 0.673       | 4.56E−02 | Host cell factor C2                                                       |
| A_33_P3762913          | LOC100216546 | 0.677  | 3.29E−02 | uncharacterized LOC100216546                                             |
| A_33_P3223990          | TPM3       | 0.680       | 3.66E−02 | Tropomyosin 3                                                             |
| A_33_P3503937          | LOC284581  | 0.683       | 1.31E−02 | Uncharacterized LOC284581                                                 |
| A_33_P3357382          | POGZ       | 0.685       | 1.94E−02 | Pogo transposable element with ZNF domain                                  |
| A_33_P3276913          | TTC3       | 0.685       | 3.02E−02 | Tetratricopeptide repeat domain 3                                          |
| A_33_P3363091          | VAC14      | 0.685       | 2.87E−02 | Vac14 homolog (S. cerevisiae)                                              |
| A_33_P3356525          | FLJ45482   | 0.686       | 1.22E−02 | Uncharacterized LOC645566                                                 |
| A_33_P3310751          | LOC100132249 | 0.690  | 4.21E−02 | Uncharacterized LOC100132249                                             |
| A_33_P3345743          | PFN1P2     | 0.691       | 2.36E−02 | Profilin 1 pseudogene 2                                                   |
| A_23_P6561             | EBLN2      | 0.692       | 1.29E−02 | Endogenous Bornavirus-like nucleoprotein 2                                 |
| A_23_P59613            | FZD9M      | 0.692       | 1.63E−02 | Frizzled family receptor 9                                                |
| A_33_P3397795          | C14orf135  | 0.694       | 1.31E−02 | Chromosome 14 open reading frame 135                                       |
| A_33_P3304533          | RNF207     | 0.696       | 2.21E−02 | Ring finger protein 207                                                   |
| A_33_P3380405          | CYTH1D     | 0.699       | 1.98E−02 | Cytohesin 1                                                                |
| A_33_P3538279          | PRO2852    | 0.699       | 2.61E−02 | Uncharacterized protein PRO2852                                           |
### Table 2 continued

| Probe ID (Agilent 44 k) | Symbol       | Fold change | p Value     | Gene name                                      |
|------------------------|--------------|-------------|-------------|------------------------------------------------|
| A_23_P60793            | ASMTL-AS1    | 0.703       | 3.95E-02    | ASMTL antisense RNA 1 (non-protein coding)     |
| A_33_P3371752          | EPS15O,M     | 0.704       | 1.52E-02    | Epidermal growth factor receptor pathway substrate 15 |
| A_33_P3355371          | TTC9C        | 0.704       | 3.17E-02    | Tetratricopeptide repeat domain 9C             |

The fold increased/decreased values are mean of three independent samples. Superscripts were assigned to drug response genes (D), oxidative stress response genes (O) and metastasis (M) related genes according to gene ontology. These gene symbols are presented in bold style.

### Table 3 Top 50 gene records with decreased expression (p < 0.05) in NRF2 siRNA-treated AsPC-1 cells

| Probe ID(lumina) | Symbol       | Fold change | p Value (LPE t-test) | Gene name                                      |
|------------------|--------------|-------------|---------------------|------------------------------------------------|
| ILMN_1672148     | AKR1B10O,O   | 0.241       | 0.00E+00            | Aldo–keto reductase family 1, member B10 (aldose reductase)* |
| ILMN_1709348     | ALDH1A1D,O,M | 0.253       | 0.00E+00            | Aldehyde dehydrogenase 1 family, member A1      |
| ILMN_2096372     | ALDH1A1D,O,M | 0.358       | 4.86E-12            | Aldehyde dehydrogenase 1 family, member A1      |
| ILMN_2198239     | HGD         | 0.393       | 5.28E-08            | Homogentisate 1,2-dioxygenase (homogentisate oxidase) |
| ILMN_1794928     | C6orf117     | 0.410       | 1.82E-07            | Chromosome 6 open reading frame 117             |
| ILMN_1729117     | COL5A2       | 0.418       | 1.54E-08            | Collagen, type V, alpha 2                       |
| ILMN_1811347     | TFF3M        | 0.426       | 0.00E+00            | Trefoil factor 3 (intestinal)                   |
| ILMN_1781745     | C9orf152     | 0.445       | 1.43E-06            | Chromosome 9 open reading frame 152             |
| ILMN_1722489     | TFF1D,O,M    | 0.445       | 1.24E-10            | Trefoil factor 1                                |
| ILMN_1800091     | RARRES1      | 0.465       | 1.40E-06            | Retinoic acid receptor responder (tazarotene induced) 1 |
| ILMN_2133205     | GPX2O        | 0.469       | 3.59E-10            | Glutathione peroxidase 2 (gastrointestinal)      |
| ILMN_1702503     | ALDH3A1D,O   | 0.481       | 3.84E-06            | Aldehyde dehydrogenase 3 family, memberA1*      |
| ILMN_2412336     | AKR1C2       | 0.488       | 3.94E-05            | Aldo–keto reductase family 1, member C2         |
| ILMN_2304495     | PPP1R1B,O    | 0.489       | 1.57E-05            | Protein phosphatase 1, regulatory (inhibitor) subunit 1B |
| ILMN_1654873     | ARSD         | 0.491       | 5.26E-05            | Arylsulfatase D                                 |
| ILMN_1772951     | ST6GALNAC1   | 0.492       | 1.06E-07            | ST6 (α-N-acetyl-neuraminyl-2,3-β-galactosyl-1, 3)-N-acetylgalactosaminide α-2,6-sialyltransferase 1 |
| ILMN_1687757     | AKR1C1O      | 0.506       | 1.81E-04            | Aldo–keto reductase family 1, member C4         |
| ILMN_2193980     | ABCB6O       | 0.509       | 5.44E-06            | ATP-binding cassette, sub-family B (MDR/TAP), member 6 |
| ILMN_2161330     | SPDEFM       | 0.513       | 2.61E-03            | SAM pointed domain containing ets transcription factor |
| ILMN_1667814     | ABCC3O       | 0.518       | 3.96E-06            | ATP-binding cassette, sub-family C (CFTR/MRP), member 3 |
| ILMN_1790909     | NFE2L2D,O    | 0.519       | 6.27E-04            | Nuclear factor (erythroid-derived 2)-like 2     |
| ILMN_1680652     | SELENBP1     | 0.520       | 3.74E-04            | Selenium binding protein 1                      |
| ILMN_1756685     | DEPDC6       | 0.523       | 6.27E-04            | DEP domain containing 6                         |
| ILMN_1704353     | IGSF3        | 0.525       | 6.27E-04            | Immunoglobulin superfamily, member 3            |
| ILMN_1743620     | RARRES1      | 0.528       | 1.47E-03            | Retinoic acid receptor responder (tazarotene induced) 1 |
| ILMN_1752932     | MPZL2        | 0.532       | 2.94E-03            | Myelin protein zero-like 2                      |
| ILMN_1701025     | EPHX1O       | 0.535       | 4.13E-05            | Epoxide hydrolase 1, microsomal (xenobiotic)     |
| ILMN_1680738     | CSorf13      | 0.543       | 6.93E-03            | Chromosome 5 open reading frame 13              |
| ILMN_1653956     | LOC644624    | 0.545       | 6.70E-03            | PREDICTED: hypothetical LOC644624              |
| ILMN_1769013     | ASGR1O       | 0.545       | 2.13E-04            | Asialoglycoprotein receptor 1                   |
| ILMN_1748352     | CTSL2M       | 0.547       | 3.16E-03            | Cathepsin L2                                   |
| ILMN_1659984     | MEP1A        | 0.550       | 3.94E-05            | Meprin A, alpha (PABA peptide hydrolase)        |
| ILMN_1736042     | ME1          | 0.551       | 2.91E-03            | Malic enzyme 1, NADP(+)–dependent, cytosolic    |
| ILMN_1779015     | ZNF467       | 0.554       | 1.05E-03            | Zinc finger protein 467                        |
| ILMN_1761247     | PIRM         | 0.561       | 1.83E-02            | Pirin (iron-binding nuclear protein)            |
| ILMN_2255579     | RAB37        | 0.565       | 6.27E-04            | RAB37, member RAS oncogene family              |
| ILMN_1726114     | SLC45A3      | 0.566       | 1.96E-06            | Solute carrier family 45, member 3             |
Table 3 continued

| Probe ID | Illumina Symbol | Fold change | p Value (LPE t-test) | Gene name |
|----------|-----------------|-------------|---------------------|-----------|
| ILMN_1671337 | SLC2A5<sup>D, O</sup> | 0.566 | 6.19E−03 | Solute carrier family 2 (facilitated glc/fruc transporter), member 5 |
| ILMN_2278335 | LOC441282 | 0.567 | 4.28E−04 | Similar to aldo–keto reductase family 1, member B10 |
| ILMN_1712305 | CYBRD1 | 0.572 | 6.27E−04 | Cytochrome b reductase 1 |
| ILMN_2383383 | PIR<sup>M</sup> | 0.576 | 1.74E−02 | Pirin (iron-binding nuclear protein) |
| ILMN_1657547 | CCDC34 | 0.578 | 2.33E−04 | Coiled-coil domain containing 34 |
| ILMN_2383383 | PI3M | 0.579 | 1.74E−02 | Pirin (iron-binding nuclear protein) |
| ILMN_1723978 | LGALS1D<sup>O, M</sup> | 0.579 | 5.76E−03 | Lectin, galactoside-binding, soluble, 1 |
| ILMN_1802100 | ADAM28 | 0.581 | 3.29E−02 | ADAM metallopeptidase domain 28 |

The fold changes are mean of three independent samples. Superscripts were assigned to drug response genes (D), oxidative stress response genes (O) and metastasis (M) related genes according to gene ontology. These gene symbols are presented in bold style.

Table 4 Top 50 gene records with increased expression (p < 0.05) in NRF2 siRNA-treated AsPC-1 cells

| Probe ID | Illumina Symbol | Fold change | p Value (LPE t-test) | Gene name |
|----------|-----------------|-------------|---------------------|-----------|
| ILMN_1796094 | CD36<sup>D, O, M</sup> | 4.476 | 4.78E−25 | CD36 molecule (thrombospondin receptor) |
| ILMN_1784863 | CD36<sup>D, O, M</sup> | 3.416 | 4.13E−13 | CD36 molecule (thrombospondin receptor) |
| ILMN_1656501 | DUSP5 | 2.664 | 1.24E−08 | Dual specificity phosphatase 5 |
| ILMN_1679262 | DPYSL3<sup>M</sup> | 2.389 | 7.67E−11 | Dihydropyrimidinase-like 3 |
| ILMN_1693789 | ALPP3<sup>O</sup> | 2.296 | 1.82E−07 | Alkaline phosphatase, placental (Regan isozyme) |
| ILMN_1700144 | ITGA10 | 2.241 | 7.76E−06 | Integrin, alpha 10 |
| ILMN_1787691 | CITED4 | 2.157 | 5.80E−06 | Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 4 |
| ILMN_2108735 | EEF1A2 | 2.094 | 6.43E−03 | Eukaryotic translation elongation factor 1 alpha 2 |
| ILMN_1813386 | CORO6 | 2.073 | 4.73E−05 | Coronin 6 |
| ILMN_2368530 | IL32M | 2.042 | 1.06E−07 | Interleukin 32 |
| ILMN_1776861 | HAP1 | 2.039 | 2.25E−04 | Huntingtin-associated protein 1 |
| ILMN_2317581 | SHANK3 | 2.023 | 2.48E−05 | SH3 and multiple ankyrin repeat domains 3 |
| ILMN_2317580 | SHANK3 | 1.950 | 1.28E−03 | SH3 and multiple ankyrin repeat domains 3 |
| ILMN_2049417 | TMEM86B | 1.920 | 8.22E−04 | Transmembrane protein 86B |
| ILMN_1778010 | IL32<sup>M</sup> | 1.919 | 2.26E−04 | Interleukin 32 |
| ILMN_2197460 | REEP6 | 1.915 | 4.15E−03 | Receptor accessory protein 6 |
| ILMN_1710553 | TMEM151A | 1.900 | 2.61E−03 | Transmembrane protein 151A |
| ILMN_1678086 | CCDC74A | 1.894 | 2.68E−03 | Coiled-coil domain containing 74A |
| ILMN_1778401 | HLA-B<sup>D, O, M</sup> | 1.878 | 8.82E−05 | Major histocompatibility complex, class I, B |
| ILMN_1709659 | TMEM151A | 1.868 | 1.74E−02 | Transmembrane protein 151A |
| ILMN_1734707 | CHST13 | 1.857 | 3.42E−03 | Carbohydrate (chondroitin 4) sulfotransferase 13 |
| ILMN_1794501 | HAS3<sup>M</sup> | 1.840 | 1.28E−03 | Hyaluronan synthase 3 |
| ILMN_1674580 | TRIM36 | 1.834 | 1.67E−03 | Tripartite motif-containing 36 |
| ILMN_1761912 | MGAT1 | 1.824 | 1.29E−02 | Mannosyl (alpha-1,3,4)-glucopyranosyltransferase 1, 2-N-acetylgalcosaminyl transferase |
| ILMN_1679267 | TGM2<sup>D, O, M</sup> | 1.818 | 1.28E−03 | Transglutaminase 2 |
| ILMN_2136971 | FABP3<sup>D, O, M</sup> | 1.815 | 5.45E−03 | Fatty acid binding protein 3, muscle and heart |
NRF2 expression by the tBHQ treatment was not significant (data not shown) since tBHQ stabilized NRF2 protein but had no effect on the mRNA level of NRF2. The array results of increased genes under the NRF2 activated status (tBHQ treatment) and decreased genes by the NRF2 siRNA treatment seem to coincide. We listed top 50 gene records with increased expression upon NRF2 siRNA treatment in Table 4. Ten genes classified as drug response or oxidative stress response genes (O) and metastasis (M) related genes according to gene ontology. These gene symbols are presented in bold style

| Probe ID | Symbol | Fold change | p Value (LPE t-test) | Gene name |
|----------|--------|-------------|----------------------|-----------|
| ILMN_2077680 | CLDND2 | 1.814 | 2.15E-03 | Claudin domain containing 2 |
| ILMN_1669362 | IGBP6 | 1.811 | 3.15E-06 | Insulin-like growth factor binding protein 6 |
| ILMN_2361737 | TRIM36 | 1.809 | 2.94E-03 | Tripartite motif-containing 36 |
| ILMN_1805842 | FHL1 | 1.796 | 1.82E-03 | Four and a half LIM domains 1 |
| ILMN_2390853 | CTSH | 1.780 | 2.68E-03 | Cathepsin H |
| ILMN_1676712 | LOC65553 | 1.778 | 1.28E-03 | PREDICTED: hypothetical LOC65553 |
| ILMN_2171384 | CXCL5 | 1.766 | 1.17E-02 | Chemokine (C-X-C motif) ligand 5 |
| ILMN_1780057 | RENBP | 1.764 | 6.43E-03 | Renin binding protein |
| ILMN_2188264 | CYR61 | 1.759 | 5.85E-03 | Cysteine-rich, angiogenic inducer, 61 |
| ILMN_1782305 | NR4A2 | 1.744 | 9.73E-03 | Nuclear receptor subfamily 4, group A, member 2 |
| ILMN_1792538 | CD7 | 1.740 | 3.63E-02 | CD7 molecule |
| ILMN_1705814 | KRT80 | 1.738 | 9.51E-03 | Keratin 80 |
| ILMN_1721876 | TIMP2 | 1.733 | 3.53E-02 | TIMP metalloproteinase inhibitor 2 |
| ILMN_1655915 | MMP11 | 1.725 | 2.02E-02 | Matrix metalloproteinase 11 (stromelysin 3) |
| ILMN_1656361 | LOC201175 | 1.722 | 1.43E-02 | Hypothetical protein LOC201175 |
| ILMN_1785646 | PMP22 | 1.720 | 4.71E-02 | Peripheral myelin protein 22 |
| ILMN_1748844 | CNKSR3 | 1.713 | 1.29E-02 | CNKSR family member 3 |
| ILMN_2360415 | PRNP | 1.713 | 2.15E-02 | Prion protein (PRNP)2 |
| ILMN_1814296 | TRPM6 | 1.711 | 2.15E-03 | Transient receptor potential cation channel, subfamily M, member 6 |
| ILMN_1667295 | VASN | 1.706 | 1.84E-02 | Vasorin |
| ILMN_1727466 | KCNMB4 | 1.700 | 5.76E-03 | Potassium large conductance calcium-activated channel, subfamily M, beta member 4 |
| ILMN_2405009 | NBL1 | 1.695 | 2.38E-02 | Neuroblastoma, suppression of tumorigenicity 1 |
| ILMN_1801246 | IFITM1 | 1.694 | 6.27E-04 | Interferon induced transmembrane protein 1 (9–27) |
| ILMN_2339955 | NR4A2 | 1.688 | 3.79E-02 | Nuclear receptor subfamily 4, group A, member 2 |

The fold changes are mean of three independent samples. Superscripts were assigned to drug response genes (D), oxidative stress response genes (O) and metastasis (M) related genes according to gene ontology. The relationships with NRF2 were reported previously as follows: AKR1B10 (Agyeman et al. 2012; Nishinaka et al. 2011), ALDH3A1 (Agyeman et al. 2012), TXNRD1 (Sakurai et al. 2005), AKR1C4 (Ebert et al. 2011), ALDH1A1 (Duong et al. 2014a), PIR (Hubner et al. 2009), GPX2 (Banning et al. 2005), UGDH (Loignon et al. 2009), SRXN1 (Soriano et al. 2008), ME1 (Thimmulappa et al. 2002), ABCB6 (Campbell et al. 2013), EPHX1 (Su et al. 2014), NQO1 (Agyeman et al. 2012; Loignon et al. 2009; Thimmulappa et al. 2002), and ABCC3 (Adachi et al. 2007). Interestingly, we identified three new genes including ALDH3A2, ASPH, and KISS1 as NRF2-responsive genes in this study. To date no study has been reported the relationship of NRF2 with ALDH3A2, ASPH, and KISS1. KISS1 is a protein with 145 amino acid residues and its role is known as an inhibitor of metastasis (Ji et al. 2013). Overexpression KISS1 inhibits metastatic colony formation in ovarian cancer cell lines (Jiang et al. 2005). However, the role of KISS1 in pancreatic
cancers has not yet been elucidated. Previously, a report displayed that NRF2 deficient mice showed higher number of pulmonary metastasis than wild-type mice (Satoh et al. 2010). ShRNA mediated knockdown of NRF2 also enhanced cellular plasticity and motility in HepG2 cell (Rachakonda et al. 2010). However, in esophageal squamous cancer cell line NRF2 suppression downregulated the migration and invasion (Shen et al. 2014). Currently, the potential role of NRF2 in regulation of metastasis is under active investigation.

Conflict of interest Authors declare no conflict of interest.

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| Symbol | Probe ID agilent | Probe ID Illumina (ILMN) | Fold change (TBHQ) | p Value | Fold change (SiRNA) | p Value (LPE t test) | Gene name |
|--------|-----------------|-------------------------|--------------------|---------|--------------------|---------------------|-----------|
| AKR1B10D, O | A_24_P129341 | 1672148 | 4.694 | 8.83E-04 | 0.241 | 0.00E+00 | Aldo–keto reductase family 1, member B10 (aldose reductase) |
| ALDH3A1D, O | A_33_P3238433 | 1702503 | 2.063 | 3.96E-03 | 0.481 | 3.84E-06 | Aldehyde dehydrogenase 3 family, member A1 |
| TXNRD1D, O | A_33_P3351120 | 1717056 | 2.042 | 4.10E-03 | 0.631 | 2.46E-03 | Thioredoxin reductase 1 |
| PIRM | A_23_P137035 | 1761247 | 1.982 | 4.53E-03 | 0.561 | 3.84E-06 | Pirin (iron-binding nuclear protein) |
| GPX2D, O | A_23_P3038 | 2133205 | 1.971 | 3.64E-03 | 0.649 | 3.59E-10 | Glutathione peroxidase 2 (gastrointestinal) |
| AKR1C4O | A_33_P3272291 | 1687757 | 1.900 | 5.30E-03 | 0.506 | 1.81E-04 | Aldo–keto reductase family 1, member C4 (chlordecone reductase; 3-alpha hydroxy steroid dehydrogenase, type I; dihydriodiol dehydrogenase 4) |
| UGDH, O | A_33_P3396607 | 1729563 | 1.856 | 5.80E-03 | 0.619 | 4.86E-02 | UDP-glucose 6-dehydrogenase |
| ALDH1A1D, O, M | A_23_P83098 | 1709348 | 1.823 | 6.21E-03 | 0.253 | 0.00E+00 | Aldehyde dehydrogenase 1 family, member A1 |
| SRXN1O | A_23_P320113 | 1804822 | 1.779 | 6.92E-03 | 0.689 | 4.00E-02 | Sulfiredoxin 1 |
| ME1 | A_23_P8196 | 1736042 | 1.771 | 7.33E-03 | 0.551 | 2.91E-03 | Malic enzyme 1, NAD(P)(+)-dependent, cytosolic |
| ABCB6O | A_23_P5441 | 2193980 | 1.575 | 1.27E-02 | 0.509 | 5.44E-06 | ATP-binding cassette, sub-family B (MDR/TAP), member 6 |
| EPHXI, O | A_23_P34537 | 1701025 | 1.538 | 1.46E-02 | 0.535 | 4.13E-05 | Epoxide hydrolase 1, microsomal (xenobiotic) |
| HGD, O | A_23_P205164 | 2198239 | 1.518 | 1.58E-02 | 0.393 | 5.28E-08 | Homogentisate 1,2-dioxygenase |
| NQO1D, O, M | A_23_P206661 | 1720282 | 1.496 | 1.72E-02 | 0.659 | 1.65E-02 | NAD(P)H dehydrogenase, quinone 1 |
| ALDH3A2O, O | A_33_P3336617 | 1794825 | 1.463 | 1.99E-02 | 0.618 | 1.60E-02 | Aldehyde dehydrogenase 3 family, member A2 |
| ASPH | A_24_P295245 | 2352934 | 1.375 | 3.11E-02 | 0.615 | 2.20E-02 | Aspartate beta-hydroxylase |
| ABCC3O, O | A_23_P207507 | 1677814 | 1.330 | 4.90E-02 | 0.518 | 3.96E-06 | ATP-binding cassette, sub-family C (CFTR/MRP), member 3 |
| KISS1M | A_23_P124892 | 1669404 | 0.771 | 4.70E-02 | 1.534 | 3.29E-02 | KiSS-1 metastasis-suppressor |

The fold change values are mean of three independent samples. Superscripts were assigned to drug response genes (D), oxidative stress response genes (O) and metastasis (M) related genes according to gene ontology. These gene symbols are presented in bold style.

Table 5 List of statistically significant overlapping genes between two microarray data (tHBQ mediated activation of NRF2 and siRNA mediated depletion of NRF2)
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