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Data in Brief

Molecular profiling of MPS1 gene silencing in U251 glioma cell line

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ABSTRACT

Aneuploidy has been recognized as a common characteristic of cancers. Aneuploidy frequently results from errors of the mitotic checkpoint, the major cell cycle control mechanism that acts to prevent chromosome missegregation. Mutation of the genes that control chromosome segregation during mitosis may explain the high rate of chromosomal instability and aneuploidy, a characteristic of most solid tumors, including glioblastoma (GBM) (Gordon et al., 2012 [1]; Singh et al., 2012 [2]). Monopolar spindle 1 (*MPS1*) is an essential spindle assembly checkpoint kinase that is overexpressed in several human cancers (Kilpinen et al., 2010 [3]; Mills et al., 1992 [4]; Yuan et al., 2006 [5]). In our previous publication, we have shown the role of MPS1 kinase in DNA repair and enhanced radiosensitivity in GBM (Maachani et al., 2015 [6]). Here, we provide methodological and analytical details of that study, to compare mRNA expression profile of si*MPS1*-silenced U251 cells with untransfected control, and siRNA control (siNeg) at 6, 24, and 48 h after transfection. The raw data of this study is deposited in Gene Expression Omnibus under the accession number GSE57091.

Specifications	
Organism/cell line/tissue	*Homo sapiens*/immortalized cell line from Glioblastoma patient derived U251/brain
Sex	Male
Sequencer or array type	GeneChip Human Genome U133A 2.0 Array (Affymetrix)
Data format	Raw and processed
Experimental factors	Control (untransfected), siNegative and siMPS1 transfected U251 cells at 6, 24, and 48 h after transfection
Experimental features	Microarray analysis was conducted in duplicate for each experimental condition to determine the changes in full transcriptome
Consent	N/A
Sample source location	National Cancer Institute Frederick Tumor Repository, MD, USA

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57091.

2. Experimental design, materials and methods

2.1. Cell lines

For this study, U251 (National Cancer Institute Frederick Tumor Repository) human glioblastoma (GBM) cells were cultured in a Dulbecco's Modified Eagle Medium (DMEM; Gibco®) supplemented with 10% fetal bovine serum (FBS; Gibco®). Cells were grown and maintained in a humidified atmosphere at 37 °C and 5% (v/v) CO_2.

2.2. Cell transfections

U251 cells were transfected with 2-pmol siMPS1 using RNAi Max lipid transfection reagent (Invitrogen). Briefly, siRNA was complexed with lipid in DMEM media for 15 min at room temperature. Two-thousand cells suspended in DMEM containing 20% FBS were added and continued incubating at room temperature for additional 15 min. Plates were maintained at 37 °C/5% CO_2. Untransfected cells and wells transfected with negative (All star siNegative [siNeg], Qiagen) control siRNAs were used as controls.

siMPS1: 5′ TTGGACTGTTATACTCTTGAA 3′ (SI00071624, Qiagen Inc., Germantown, MD).

2.3. RNA isolation, purification, and quality control

Total RNA was isolated after 6, 24 and 48 h post-siRNA transfection. The TRIzol reagent (Life Technologies™) was used to extract the total cellular RNA from duplicate samples at each time point. Extracted RNA was purified using the RNeasy kit (Qiagen®) and quantified using the Agilent 2100 bioanalyzer (Agilent Technologies; Table 1). All RNA samples presented A260/A280 ratios above 1.8, and RNA integrity numbers (RIN) above 8 (except for 1 control sample), as recommended for microarray analysis.

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Table 1
RNA purity and quality assessment for microarray experiments.

Sample no.	Sample ID	ng/μl	A260	A280	260/280	260/230	RIN
1	Control 1, 6 h	28.39	0.71	0.379	1.87	0.61	9.2
2	Control 2, 6 h	28.65	0.716	0.365	1.96	0.07	6.3
3	Control 1, 24 h	19.45	0.486	0.229	2.12	0.05	8.8
4	Control 2, 24 h	15.27	0.382	0.175	2.18	0.04	9
5	Control 1, 48 h	15.08	0.377	0.192	1.97	0.37	9
6	Control 2, 48 h	9.99	0.25	0.116	2.15	0.57	8.7
7	siNeg 1, 6 h	30.76	0.769	0.408	1.88	0.77	8.2
8	siNeg 2, 6 h	26.05	0.651	0.322	2.02	0.74	8.5
9	siNeg 1, 24 h	14.17	0.354	0.185	1.91	0.51	8.2
10	siNeg 2, 24 h	17.26	0.432	0.229	1.88	0.52	8.3
11	siNeg 1, 48 h	22.33	0.558	0.272	2.05	0.7	9.3
12	siNeg 2, 48 h	16.21	0.405	0.18	2.25	0.11	9
13	siMPS1 1, 6 h	30.71	0.768	0.38	2.02	0.11	8.9
14	siMPS1 2, 6 h	33.68	0.842	0.419	2.01	1.31	8.3
15	siMPS1 1, 24 h	15.9	0.397	0.208	1.91	0.14	8.6
16	siMPS1 2, 24 h	20.38	0.51	0.273	1.87	0.33	8.7
17	siMPS1 1, 48 h	14.64	0.366	0.165	2.22	0.81	9.1
18	siMPS1 2, 48 h	21.22	0.53	0.269	1.97	0.34	9

2.4. Microarray experiments and gene expression analysis

2.4.1. cRNA preparation, labeling, purification and quality control analysis

RNA was processed for use on Affymetrix (Santa Clara, CA, USA) GeneChip Human Genome U133A 2.0 Arrays, according to the manufacturer's GeneChip 3′ IVT Express kit user manual. Briefly, 250 ng of total RNA containing spiked in Poly-A RNA controls was used in a reverse transcription reaction (GeneChip 3′ IVT Express Kit; Affymetrix) to generate first-strand cDNA. After second-strand synthesis, double-stranded cDNA was used in a 16 h in vitro transcription (IVT) reaction to generate aRNA (GeneChip 3′ IVT Express Kit; Affymetrix).

2.4.2. Hybridization and washing

12 μg of fragmented aRNA was used in a 200-μl hybridization cocktail containing added hybridization controls. 130 μl of mixture was hybridized on arrays for 16 h at 45 °C. Standard post-hybridization wash and double-stain protocols (FS450; GeneChip HWS kit, Affymetrix)

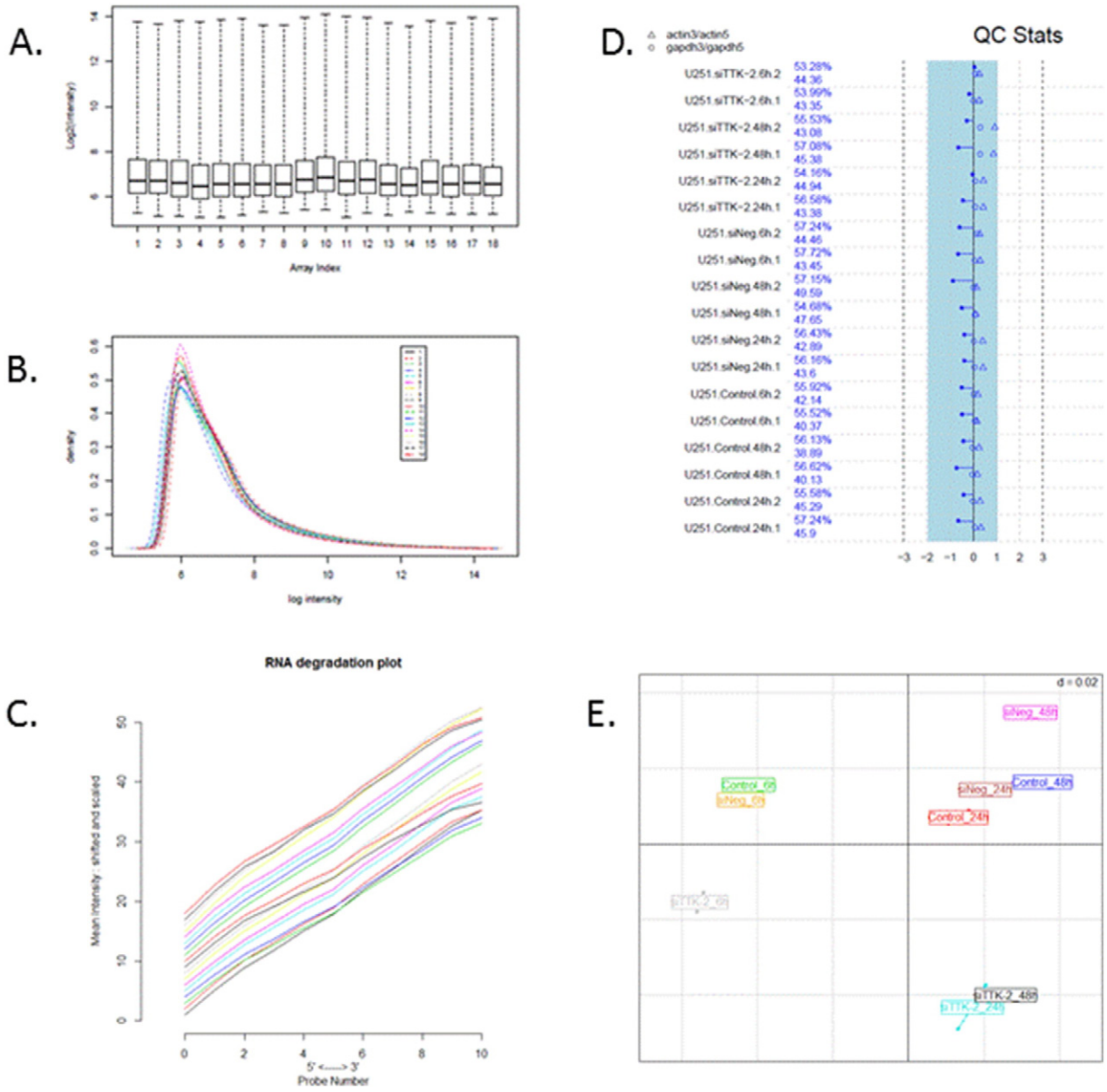

Fig. 1. Quality control measures for the data set. (A, B) Boxplot and histograms of pm intensities. (C) RNA degradation plot. (D) QC plot of 3′:5′ ratios, background levels, and percent present calls for spiked-in and control genes. (E) Principal component plot of duplicate data. Lines extending from sample labels show replicate samples of that treatment.

were used on an Affymetrix GeneChip Fluidics Station 450. Arrays were scanned on an Affymetrix GeneChip scanner 3000.

2.4.3. Quality control and data analysis

Microarray data pre-processing and analysis was performed using packages from the Bioconductor software platform (http://www.bioconductor.org) [7]. Affymetrix CEL files were normalized using MAS 5.0 signal algorithm using simpleaffy package [8]. Data quality was assessed using various quality control measures as suggested by Affymetrix platform QC metrics (Fig. 1). The boxplots and histogram of pm intensities of each were similar (Fig. 1A, B). RNA degradation was examined and all samples were determined to be adequate (Fig. 1C). For each array, ratios of 3′ vs. 5′ends of control probes for GAPDH and β-actin were plotted (Fig. 1D). The plot also shows percentage of present gene calls and background levels along with scale factors. The percent present calls (53–57%) and average background levels (38–49) showed a similar distribution indicating general agreement with other samples. The scale factors were within 3-fold from the average of all samples indicating a good measure of intensity. A principal components analysis (PCA) was performed and the first two principal components plotted. Replicates of each sample clustered together. Additionally, samples were seen to be clustered by early time (6 h) and late times (24 and 48 h) post-transfection. Importantly, control and siNeg transfected samples clearly grouped differently from siMPS1 transfected samples. These results indicate that siMPS1 transfection leads to time dependent gene expression changes.

2.4.4. Annotation

The mapping between probe identifiers and gene symbols was done using hgu133adb annotation package from Bioconductor [7]. Probeset level data was collapsed into gene level by custom algorithm. Some of the probesets of a given gene were designed to correspond to alternately spliced variants, and taking the overall average of all probesets would lead to incorrect estimation. To prevent this effect, our algorithm computes pairwise correlations of all the probesets for a given gene, and signal from probesets with maximum correlation will be averaged.

2.4.5. Differential expression

Intensity values were log2 scaled, and *Z* score transformed. A filter with standard deviation of 1.5 was implemented to remove invariant genes. To find differentially expressed transcripts between the untransfected control, siNeg and siMPS1 transfected U251 at each of the time points measured, we used one-way analysis of variance (ANOVA) and p values are adjusted with the post-hoc Scheffe method. Paired comparisons were estimated by the Tukey honest significant difference method, applied to the fitted ANOVA model. Genes changed ($P \leq .05$) between control, siNeg, and siMPS1 were considered for further analysis. Paired comparisons were performed for each time point.

2.4.6. Molecular profiling and functional enrichment analysis

To characterize the molecular mechanism underlying the enhanced radiosensitivity exhibited by GBM cells after MPS1 inhibition, we used gene expression profiling of *MPS1*-silenced U251 cells. Two-way hierarchical clustering of mRNA expression profile of si*MPS1*-silenced U251

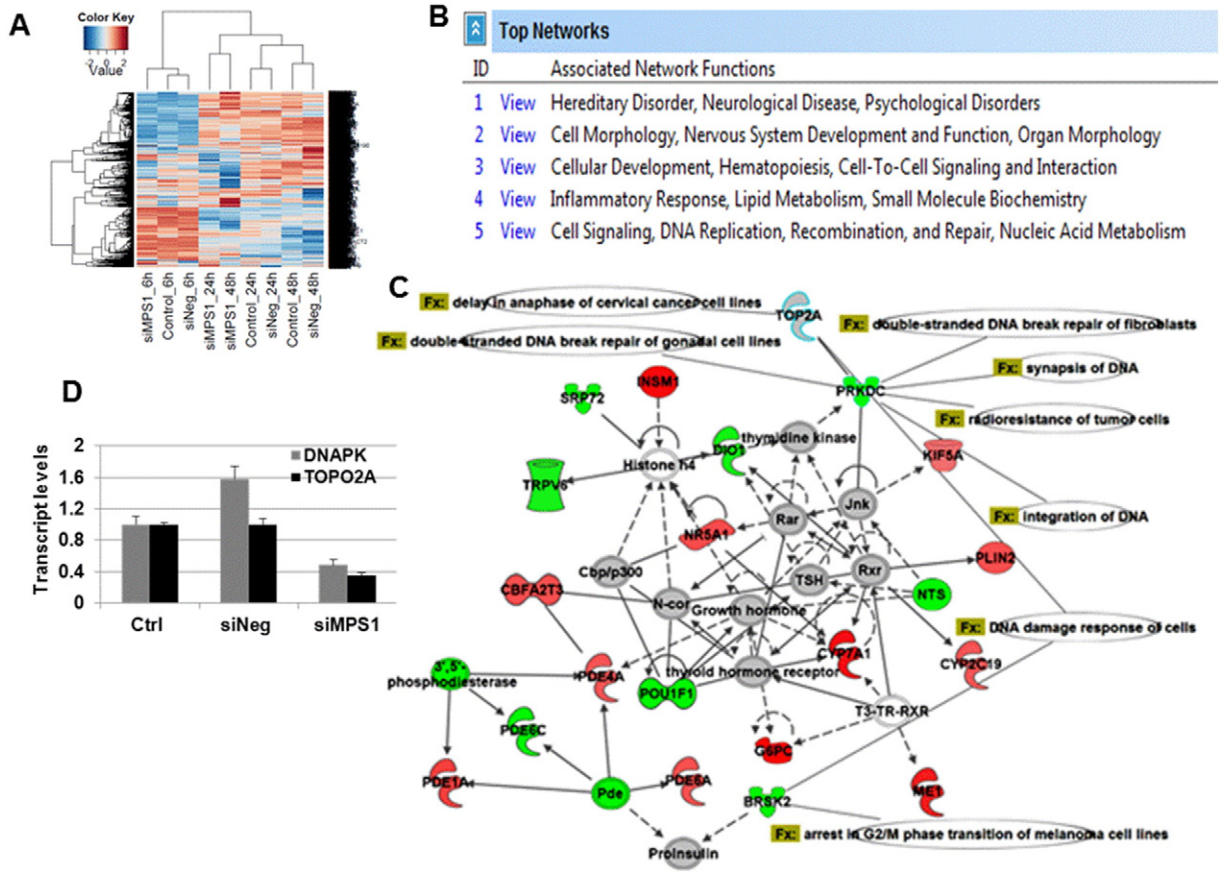

Fig. 2. Molecular profiling reveals changes in genes associated with DNA replication, recombination and repair. (A) Two-way hierarchical clustering representation of mRNA expression profile of siMPS1 silenced U251 cells compared to siNeg transfected or untransfected cells (Control) at 6, 24 and 48 h post-transfection. Values shown derived using a cutoff *p*-value <0.05. Up-regulation, red; down-regulation, blue. (B, C) Ingenuity pathway analysis of top networks generated from processing mRNA targets from siMPS1 transfected cells, (B) associated network functions and (C) molecular network showing involvement of DNA damage and repair molecules. (D) U251 cells were transfected with either siNeg or siMPS1 and 48 h post-transfection expression of *DNAPK* and *TOPO2A* was assessed using gene specific RT-PCR (GAPDH normalized). Untransfected cells (Ctrl) are also shown. Data presented are the mean ± the standard deviation from three independent experiments.
Figure adapted from original publication [6].

cells compared with siNeg-transfected or untransfected cells (control) at 6, 24, and 48 h after transfection is shown (Fig. 2A). Values were derived using a cutoff $P \leq 0.05$ and fold change of ≥ 1.33. We observed 237, 391, and 891 genes as differentially expressed in siMPS1-silenced U251 cells compared with siNeg-transfected cells at 6, 24, and 48 h, respectively. Ingenuity pathway analysis of these differentially expressed genes identified enrichment for genes associated with the neurological disease, nervous system development, DNA replication, recombination, and repair pathway (DRRRp; Fig. 2B and C). DRRRp was one of the top identified pathways (IPA score, 29), as well as the top molecular and cellular function with 20 molecules affected by downregulation of *MPS1* (Fig. 2B and C). Genes identified as deregulated following MPS1 loss-of-function that are associated with DNA damage and repair were *DNAPK* [also known as protein kinase DNA-activated (*PRKDC*)], and topoisomerase II alpha (*TOPO2A*).

2.5. Discussion

We describe detailed technical methods to reproduce the analysis of si-MPS1 silenced U251 cells compared with siRNA control (siNeg) and untransfected controls at 6, 24 and 48h using Affymetrix GeneChip Human Genome U133A 2.0 Arrays. Aneuploidy and chromosomal instability are characteristic of solid tumors including GBM [1,2]. MPS1 gene was implicated as an essential spindle assembly checkpoint kinase in several cancers [3–5]. Our earlier study demonstrated prognostic value of MPS1 gene in GBM. Inhibition of MPS1 was shown to alter the expression of transcripts associated with DNA damage, repair, and replication including DNA-dependent protein kinase (PRKDC/DNAPK) [6]. This data can contribute to future investigations examining spindle assembly regulation as a therapeutic intervention in GBM.

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