Data in Brief

Gene expression profiling can predict the fate of HeLa cells exposed to X-ray irradiation with or without protoporphyrin accumulation

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ABSTRACT

Protoporphyrin IX (PpIX) enhances the generation of reactive oxygen species in cells following physicochemical interactions with X-rays. To evaluate the use of porphyrins as radio-sensitizers in radiotherapy, the transcriptomic effects of PpIX and/or X-ray irradiation were investigated in HeLa cells. Microarray experiments were performed using Agilent-014,850 Whole Human Genome Microarray 4x44K G4112F (GEO#: GSE61805). We selected the condition corresponding to 1 μg/mL PpIX exposure prior to 3 Gy-irradiation of cells, and collected cells 24 h post irradiation. X-ray exposure at a dose of 3 Gy did not affect cell survival 24 h post irradiation, regardless of the concentration of PpIX. Approximately 50% cells exposed to X-ray irradiation alone (XT) and 70% cells exposed to PpIX treatment for 6 h before X-ray irradiation (PpIX-XT) lost clonogenic ability. Based on p-values (p < 0.01), we selected genes for functional analysis. The majority of the regulated genes in the XT and PpIX-XT groups were related to cell cycle arrest. Furthermore, transcriptome analysis of the cells collected 24 h post irradiation revealed the fate of the cells that lost clonogenic ability due to cell cycle arrest.

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1. Direct link to deposited data

Deposited data can be found here: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE61805.

2. Experimental design

Protoporphyrin IX (PpIX) enhances the generation of reactive oxygen species in cells following physicochemical interactions with X-rays [1]. To evaluate the use of porphyrins as radio-sensitizers in radiotherapy, the transcriptomic effects of PpIX and/or X-ray irradiation were investigated in HeLa cells. The present study employed microarray analysis to quantify genes that were upregulated or downregulated as a result of PpIX treatment prior to X-ray irradiation (PpIX-XT), X-ray irradiation alone (XT), and PpIX treatment alone (PpIXT). The changes in gene expression were determined by comparing the expression levels in treated cells to those in the non-treatment (NT) group. The selected genes were classified according to their biological functions and properties.

3. Materials and methods

3.1. Cell culture

The HeLa cell line was supplied by the Riken Cell Bank (Tsukuba, Japan). The cells were cultured in DMEM containing 10% fetal bovine
serum in a 5% CO₂ humidified incubator at 37 °C. The medium was supplemented with 100 units/mL penicillin and 100 μg/mL streptomycin.

3.2. X-ray irradiation

HeLa cells were irradiated (3 Gy) using 160-kV X-rays (Faxitron® Cabinet X-ray System Model CP-160, Wheeling, IL, USA). A Unidos® E Universal Dosimeter (PTW, Freiburg, Germany) was used to measure the dose rate. The resulting dose rate was 1 Gy/min.

3.3. Cell viability assay

The cell proliferation reagent 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H5-tetrazolio]-1,3-benzene disulfonate (WST-1) was used in a WST-1 cell viability assay to assess cellular responses to PpIX treatment and X-ray irradiation.

3.4. Clonogenic ability

Clonogenic survival ability was evaluated using a colony-forming assay based on the methods of Franke et al. [2].

3.5. Sample preparation and microarray analyses

RNA was extracted from cells using the Qiagen RNeasy Mini Kit (Qiagen GmbH, Germany), according to the manufacturer’s guidelines. The quality of the purified RNA was verified using an Agilent® 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). RNA concentration was determined using a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Fluorescent cyanine-3-cytidine triphosphate (CTP)-labeled cRNA was used for hybridization to human oligo microarray slides (Agilent Technologies) at 65 °C for 17 h. The hybridized microarray slides were washed according to the manufacturer’s instructions and were scanned with an Agilent DNA Microarray Scanner (#G2565BA, Agilent Technologies) at a resolution of 5 μm. The scanned images were analyzed quantitatively using Agilent Feature Extraction Software version 9.5.3.1 (Agilent Technologies).

3.6. Microarray data analysis

Data were normalized by quantile normalization and were analyzed using GeneSpring GX software version 10.0.1 (Agilent Technologies). The Gene Ontology (GO) Database (http://www.geneontology.org/) was used to functionally categorize the gene expression profiles. GO terms were obtained from Agilent Technologies eArray [3]. Microarray cDNA probes were classified according to GO terms for different biological processes.

3.7. Statistical analysis

The Student’s t-test was used to assess the significance of each gene in the microarray. Genes with p-values < 0.01 compared to the NT group were selected and assigned to the PpIXT, XT, and PpIX-XT groups. After excluding overlapping probes, genes with significantly different expressions in each group (PpIXT, XT, and PpIX-XT) were analyzed using the functional annotation chart in the Database for Annotation, Visualization and Integrated Discovery (DAVID Bioinformatic Resources 2007, National Institute of Allergy and Infectious Disease) [4-6].

Table 1

| Condition | NT | PpIXT | XT | PpIX-XT |
|-----------|----|-------|----|---------|
| 1 h post irradiation | 100 ± 7 | 108 ± 9 | 98 ± 22 | 110 ± 10 |
| 24 h post irradiation | 100 ± 10 | 108 ± 23 | 96 ± 8 | 117 ± 19 |
| 72 h post irradiation | 100 ± 14 | 109 ± 7 | 74 ± 6 | 68 ± 4 |

The data correspond to the mean ± SD at 1 h (n = 6), 24 h (n = 4), and 72 h (n = 4) post irradiation. Statistical significance (p < 0.01) relative to the experiment performed without PpIX at the same irradiation dose is indicated by (TT). Statistical significance (p < 0.01) relative to the experiment performed without irradiation at the same PpIX concentration is indicated by (+).

Table 2

| Condition | NT | PpIXT | XT | PpIX-XT |
|-----------|----|-------|----|---------|
| Colony forming ability (%) | 100 ± 5 | 96 ± 2 | 52 ± 8 | 31 ± 8 |

Statistical significance (p < 0.01) relative to the experiment performed without PpIX at the same irradiation dose is indicated by (TT). Statistical significance (p < 0.01) relative to the experiment performed without irradiation at the same PpIX concentration is indicated by (+).

Fig. 1. Correlation matrix of gene expression profiles in HeLa cells after X-ray treatment or PpIX plus X-ray treatment. Pairwise correlations of gene expression levels between and in individuals are shown. Probe sets with normalized signals were used to calculate correlations between 12 arrays using Pearson’s correlation coefficient. The color scale at the bottom indicates the strength of the correlations.

Fig. 2. Summary of genes showing altered expression with p-values < 0.01 in the PpIX-XT (PpIX treatment prior to 3 Gy X-ray irradiation), XT (3 Gy X-ray irradiation alone), and PpIXT (PpIX treatment alone) groups, versus the expression in the NT (non-treatment) group.
4. Biological significance

To evaluate the gene expression profiles indicating the effects of X-ray irradiation with and without PpIX, we examined the biological condition of the cells. Cell viability was analyzed by the WST-1 assay at 1 h, 24 h, and 72 h post irradiation (Table 1). The percent survival was expressed in reference to non-irradiated control cells. Table 2 shows the effect of PpIX on clonogenic survival with 3 Gy irradiation. Clonogenic survival is expressed in reference to non-irradiated control cells. For the microarray analysis, we selected the condition corresponding to 1 μg/mL PpIX exposure prior to 3 Gy irradiation of cells, and the cells were collected 24 h post irradiation. X-ray exposure at a dose of 3 Gy did not affect cell survival at 24 h post irradiation, regardless of the concentration of PpIX. Approximately 50% cells in the XT group and 70% cells in the PpIX-XT group lost clonogenic ability and were destined for cell death.

5. Genomics data

We evaluated the variation in microarray gene expression profiles using Pearson’s correlation coefficients both within and between treatment groups for the correlation analysis. Correlation coefficients for 12 microarrays were obtained using normalized signals. A color-coded pairwise correlation matrix is shown in Fig. 1. Every array showed high correlation regardless of the different treatment.

Based on the p-values, we selected genes showing altered expression in each treatment group with reference to the NT group (Fig. 2). Functional analysis revealed that the majority of the regulated genes in the XT and PpIX-XT groups were related to cell cycle arrest (Fig. 3). The combined effect of PpIX and X-ray irradiation sensitized HeLa cells to X-ray treatment.

6. Conclusion

By transcriptome analysis of the cells collected 24 h post irradiation, we determined the fate of the cells that lost clonogenic ability due to cell cycle arrest. The cells exposed to PpIX prior to irradiation had more difficulty avoiding death than did cells that were exposed to only X-ray irradiation.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

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