Gene expression dynamics of glioblastoma cells in plasma-activated medium and plasma-activated Ringer's lactate solution

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Abstract: We have previously reported the similarities and differences of plasma-activated medium (PAM)-treated and plasma-activated Ringer's lactate solution (PAL)- treated glioblastoma cells in terms of cell viability, induction of intracellular reactive oxygen speicies. In this study, we further investigated the similarities and differences of PAM-treated and PAL-treated glioblastoma cells in gene expression dynamics. Our results suggest that PAM and PAL activate different signalling pathways for cell death.

Keywords: gene expression, Plasma-activated medium, Plasma-activated Ringer's lactate solution.

1. Introduction

We have previously proposed plasma-activated medium (PAM) and plasma-activated Ringer's lactate solution (PAL) for the future promising option for cancer treatments [1-5]. Both PAM and PAL exhibited anti-tumor effects on glioblastoma cells, however, PAM induced more reactive oxygen species (ROS) on glioblastoma cells comparing with PAL [2]. These results suggest that intracellular molecular mechanisms of cell death are different between PAM-treated and PAL-treated glioblastoma cells. In this study, we investigated gene expression dynamics by quantitative real-time PCR methods to elucidate the differences of intracellular molecular mechanisms.

2. Materials and Methods

U251SP cells (human glioblastoma cell line) were grown in Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS) and penicillin (100 U/mL)-streptomycin (100 μg/mL; P/S) under an atmosphere of 5% CO_2 at 37°C.

3×10^5 cells were plated in 3 mL medium on a 6-well plate. On the following day, 8 mL DMEM and Ringer's lactate solution in a 60 mm dish were treated with plasma (L = 3 mm, 2.0 slm), and the medium of the cells in the 6-well plate was replaced with each 3 mL of PAM and PAL. After 2 h, 3 mL culture medium was replaced with PAM and PAL.

1 h, 4 h, or 24 h after PAM/PAL treatments, cells were collected, and total RNA was extracted from the cells. Reverse transcription was performed to synthesize cDNA.

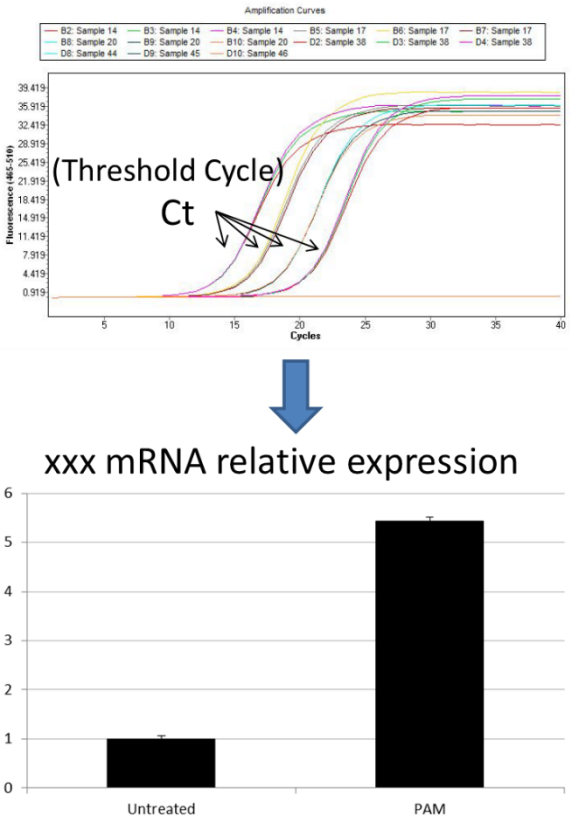

Fig. 1. Analyses of gene expression dynamics using a quantitative real-time PCR method.

Quantitative PCR was conducted using LightCycler®480 SYBR Green I Master (Roche Diagnostics) and monitored in real-time using the LightCycler®480 PCR system (Roche Diagnostics) with the method of $2^{-\Delta\Delta}$CT (Fig. 1). Expressions of all target genes were normalised to GAPDH as reference.

3. Results and Discussion

First, gene expression dynamics of antioxidant genes were investigated in both PAM-treated and PAL-treated glioblastoma cells. Interestingly, those antioxidant genes that we chose were not changed by PAM and PAL treatments. Next, we investigate the dynamics of immediate early gene expression of PAM-treated and PAL-treated glioblastoma cells. PAM induced some immediate early gene expression, while PAL did not. Genes related in oxidative stress or any other stress were also induced in PAM-treated glioblastoma cell. Based on these data, each cell death mechanism of PAM-treated and PAL-treated glioblastoma cells was revealed.

4. Conclusion

Gene expression dynamics revealed the similarities and differences of intracellular molecular mechanisms between PAM-treated and PAL-treated glioblastoma cells.

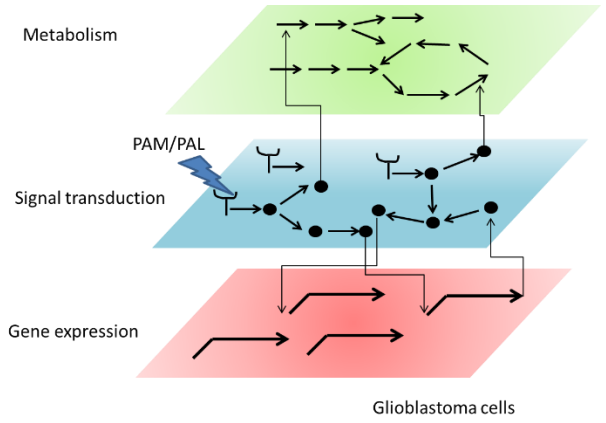

Fig. 2. Gene regulatory network, signalling network, and metabolic network affected by PAM/PAL treatments.

In the future, interactions between gene regulatory network and signalling network should be elucidated in both PAM-treated and PAL-treated glioblastoma cells. Biological network which consists of gene regulatory network, signalling network, and metabolic network should be comprehensively understood in PAM-treated and PAL-treated glioblastoma cells.

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