

The effect of tumor suppressor Pdc4 knockdown on gene expression on mouse fibroblast cells and mouse melanoma cells

Eriko Iwata¹, Noriko Yoshikawa¹, Kana Nishikaze¹, Satomi Kagota², Kazumasa Shinozuka², Kazuki Nakamura¹

¹Dept.Pharmacol.I, Mukogawa Women's Univ., ²Dept.Pharmacol.II, Mukogawa Women's Univ.

Purpose: Programmed cell death 4 (Pdc4) is a novel tumor suppressor gene which is known to act as a negative regulator of protein translation and malignant transformation. However, the tumor suppressor mechanism of Pdc4 remain unclear. In order to elucidate the mechanism of inhibition of tumor malignancy by Pdc4, we conducted the knockdown of Pdc4 in cells.

Methods: In this study, we used three mouse cell lines. The C57BL/6J-emb and the NIH3T3 are normal fibroblast cells, the B16-F0 are low-metastatic melanoma cells. We established the Pdc4 knockdown cells using siRNA systems. mRNA was extracted from Pdc4 knockdown C57BL/6J-emb and B16-F0, we performed DNA microarray analysis. To confirm the results of microarray analysis, we performed real-time PCR analysis.

Results: The mRNA levels of Pdc4 in each cell line were significantly decreased after 24 hr or 48 hr exposure to siPdc4. Among 23,474 mouse genes, microarray analysis identified 3 significantly up-regulated and 10 significantly down-regulated genes in both Pdc4 knockdown C57BL6J-emb and B16-F0 (ratio \geq 2 [up and down]). Real-time PCR showed that the mRNA levels of Skp2 which was identified down-regulated gene, significantly decreased in Pdc4 knockdown C57BL/6J-emb and NIH3T3 compared with the control cells.

Summary/Conclusion: These results suggest that Skp2 might be one of genes involved in the tumor suppression by Pdc4.