論 文 要 旨

Gene Regulation by Antitumor *miR-204-5p* in Pancreatic Ductal Adenocarcinoma: The Clinical Significance of Direct RACGAP1 Regulation

（癌抑制型マイクロ RNA(miR-204-5p)が制御する膵臓癌：RACGAP1 の臨床的意義）

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【序論及び目的】

MicroRNAs (miRNAs) are small non-coding RNAs (19–22 nucleotides, single stranded) that act to fine-tune the expression of protein-coding and non-coding RNAs in a sequence-specific manner. As a unique characteristic of miRNAs, a single miRNA regulates a vast number of RNA transcripts within a cell. Therefore, it is possible to identify novel RNA networks based on miRNA regulation using the latest genome analysis strategies in a given cell type. Accumulating evidence has shown that aberrantly expressed miRNAs act as oncogenes or tumor suppressors in human cancer cells and are involved in cancer pathogenesis.

RNA-sequencing analyses of miRNA expression signatures revealed that *miR-204-5p* was significantly downregulated in pancreatic ductal adenocarcinoma (PDAC) tissues. Although a tumor suppressor function of *miR-204-5p* has been reported in several cancers, *miR-204-5p* regulation of RNA networks in PDAC is still obscure. Here, we aimed to investigate the antitumor roles of *miR-204-5p* and to identify *miR-204-5p*-regulated oncogenes involved in PDAC pathogenesis. Comprehensive gene expression analyses and *in silico* database searches revealed that 25 putative targets are regulated by *miR-204-5p* in PDAC cells. Among these targets, high expression of seven genes (*RACGAP1*, *DHRS9*, *AP1S3*, *FOXC1*, *PRP11*, *RHBDL2* and *MUC4*) was significantly associated with a poor prognosis of patients with PDAC according to analyses of The Cancer Genome Atlas (TCGA) database. In this study, we focused on *RACGAP1* (Rac GTPase-activating protein 1) and performed further cell functional analyses. Our present data may provide new insights into the potential mechanisms of PDAC aggressiveness.

【材料及び方法】

In the present study 24 PDAC clinical samples were collected from PDAC patients who underwent resection in our hospital, as controls 17 pancreatic tissue specimens were collected from noncancerous regions. Gene expression analyses were conducted using total RNA extracted from cryopreserved PDAC tissues, and immunohistochemistry was performed using paraffin embedded PDAC tissues. We also used two PDAC cell lines in this study: SW1990 cells and PANC1 cells. In this study the procedure for qRT-PCR and for miRNA or siRNA transfection into cells have been used. The mature miRNA or siRNA were used for transfection of miR-204-5p, negative control miRNA and two RACGAP1 siRNAs. As functional analysis, XTT assay, invasion and migration assays were performed. For western blot and IHC detection of RACGAP1 expression, an anti-RACGAP1 antibodies and an anti-GAPDH

antibody was used as an internal loading control for wester blotting.

【結　果】To confirm the miRNA expression signature, expression levels of *miR-204-5p* in normal pancreatic tissues (n = 17), PDAC tissues (n = 24), and cell lines (SW1990 and PANC-1) were evaluated. The expression level of *miR-204-5p* was significantly downregulated in PDAC specimens. Additionally, the expression levels of this miRNA in the two cell lines were lower than those in normal pancreatic tissues. PDAC cell proliferation was not affected by miR-204-5p on the proliferation, migration and invasion of PDAC cells.

Significant associations were detected between upregulated expression of seven genes (RACGAP1, DHRS9, AP1S3, FOXC1, RHBDL2, MUC4 and PRR11) and had a poor prognosis in patients with PDAC i.e $p < 0.05$.

We analyzed clinicopathological factors of *miR-204-5p* and *RACGAP1* expression (*miR-204-5p*; *RACGAP1*). The recurrence of *RACGAP1* showed high expression with a significant difference ($p < 0.0015$). Immunostaining revealed expression of the RACGAP1 protein in PDAC lesions but a lack of expression in noncancerous epithelial tissues

Finally, we identified 64 candidate genes were identified that were affected by *RACGAP1* in PDAC cells (Table 3). Among these genes, high expression of 12 genes (*MMP28, CEP55, CDK1, ANLN, S100A14, SLC6A14, TRIM29, TMPRSS4, SERPINB3, CAPN8, MELK, FAR2*) were significantly associated with poor prognosis in patients with PDAC by TCGA analysis

【結論及び考察】

miR-204-5p was downregulated in PDAC clinical specimens and acted as an antitumor miRNA by targeting several oncogenes involved in PDAC pathogenesis. *RACGAP1* was directly regulated by antitumor *miR-204-5p*, and high expression of *RACGAP1* significantly predicted a shorter survival in patients with PDAC. Knock down assay of *RACGAP1* Overexpression enhanced PDAC cell migration and invasion, suggesting *RACGAP1* as a possible therapeutic target for PDAC patients. Our approach to identify antitumor miRNAs and their regulated target genes in PDAC has potential value for the development of new therapeutic strategies.

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