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Downregulation of miRNA-31 induces taxane resistance
in ovarian cancer cells through increase of receptor tyrosine kinase MET.
(miRNA-31 の発現低下により、レセプターチロシンキナーゼ MET の
抑制が解除され、卵巣癌のタキサン耐性が誘導される。)

【Background and Objectives】 Ovarian cancer is one of the most aggressive female reproductive tract tumors. Paclitaxel (PTX) is widely used for the treatment of ovarian cancer. However, ovarian cancers often acquire chemotherapeutic-resistance to this agent. MicroRNAs (miRs) are endogenous non-coding RNAs of approximately 23 mer which play important roles in regulation of gene expression. Mature form of miRs silence gene expression by binding to the 3'-UTR of target mRNAs and initiate translational repression or cleavage of cognate mRNAs. In this study, we aimed to investigate the correlation between microRNAs(miRs) and sensitivity to PTX in chemoresistant ovarian cancer.

【Materials and Methods】 We performed microarray analysis to identify miRs which potentially cause the resistance to taxanes such as PTX. Human KF ovarian cancer cells and KFr13 cisplatin-resistant KF ovarian cancer cells were kindly provided by Prof. Yoshihiro Kikuchi (National Defense Medical College). SK-OV-3 and OVCAR-3 were obtained from the ATCC. RMG-1 was obtained from Health Science Research Resources Bank. TU-OM-1 was kindly provided by Dr. Junzo Kigawa (Tottori University School of Medicine). To establish PTX-resistant KFr13 (KFr13Tx), cells were cultured with 2 nM of PTX, then the PTX concentration was gradually increased to 30 nM. To assess the biological role of miR-31, pre-miR-31 was transfected into ovarian cancer cell-line using Lentiviral miR RNAi Expression System. We examined a murine xenograft tumor model and compared the tumor weight and survival with control to investigate the effects of miR-31 on PTX sensitivity *in vivo*. Tumor specimens from patients with ovarian cancer were obtained from Hokkaido University Hospital under institutional review board-approval. Informed consent was obtained from each patient. All samples were obtained at the initial surgery.

【Results】 Fifty-five miRs were found to be downregulated below the half amount, while two miRs were upregulated more than two-fold in KFr13Tx cells compared to wild type KFr13. Among these miRs, miR-31 was negatively correlated with IC₅₀ values of PTX between six cell lines including KFr13, RMG-1, SK-OV-3, OVCAR-3, KF and TU-OM-1. The levels of miR-31 was also significantly suppressed in KFr13Tx compared with KFr13. We next established KFr13Tx cells overexpressing three different levels of miR-31 and found that the ovarian cancer cells with higher amounts of miR-31 exhibited lower cell viability after incubation with PTX. Conversely, inhibition of miR-31 expression in KFr13 by anti-miR-31 oligonucleotides increased cell viability after incubation with PTX. It is notable that miR-31 introduction did not change the sensitivity to other agents such as carboplatin, irinotecan, doxorubicin and gemcitabine and these

results suggested that the decrease of miR-31 caused PTX-specific resistance. Among the candidates of miR-31 target molecules, the 3'UTR region of MET was found to be isolated from the Ago2-dependent immunoprecipitated RNA fraction of KFr13Tx overexpressing miR-31. As expected, the expression level of MET was increased in KFr13Tx cells compared to that in KFr13 cells. Conversely, an

increase of MET protein levels was observed after introduction of anti-miR-31 oligonucleotides into KFr13. The same tendency was also observed in other cell lines used in the PTX sensitivity experiment mentioned above. Normalized luciferase activity revealed that miR-31 significantly suppressed the activity of luciferase combined with wild-type MET 3'-UTR in KFr13Tx overexpressing miR-31, whereas no difference was observed with the control luciferase vector. Furthermore, miR-31 did not affect luciferase with MET 3'-UTR possessing a mutation in the putative miR-31 binding site. *In vivo* study, the weight of the subcutaneous tumors was similar in control and miR-31 overexpressing KFr13 cells without PTX treatment. In contrast, under treatment with PTX, miR-31 overexpressing tumors were significantly smaller than that of mock expressing tumors. mice with intraperitoneal injection of miR-31 overexpressing tumor survived longer during the treatment with PTX. The weights of subcutaneous tumor derived from drug resistant

KFr13Tx control were reduced by the combination of MET inhibitor SU11274 with PTX.

Furthermore, overall survival of the mice with the KFr13Tx control was found to be improved by the combination treatment of MET inhibitor with PTX. The expression levels of miR-31 were lower in chemo-resistant tumors compared to sensitive ones in 12 women with serous adenocarcinomas with FIGO Stage IIIC or IV who underwent surgery as their initial treatment. Moreover, the lower expression of miR-31 strongly correlated with reduced overall survival in Stage IIIC patients who

were treated with PTX and carboplatin. In agreement with our *in vitro* data, the higher protein levels of MET were correlated with lower levels of miR-31 in the cohort analysis of the tumors.

【Discussion】 Recently, versatile roles for miRs have been identified in various human cancers and there are several reports about the correlation between miRs and chemoresistance. In this study, we compared the profiles of miRs between PTX-resistant and their chemosensitive parental cells, and demonstrated a role for miR-31 in ovarian cancer chemoresistance. miRs profiling and the identification of miR targets is a fruitful approach to identify the molecular basis of cancer cell phenotypes.

【Conclusion】 The present study suggests that downregulation of miR-31 induces resistance of ovarian cancer to taxanes like PTX through upregulation of MET. Our findings provide a basis for clinical studies to determine if miR-31 expression levels are a marker of chemosensitivity in ovarian cancer patients, and if MET kinase inhibitors can rescue the PTX response in women with chemo-resistant ovarian cancers.