論文審査の要旨及び担当者

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（論文審査の要旨）

論文題名：*miR-125a-5p* expression was associated with docetaxel resistance through regulation of the *ICIS/MCAK* pathway in esophageal squamous carcinoma cells
（食道扁平上皮癌細胞のドセタキセル耐性に関わる*ICIS/MCAK* 経路における *miR-125a-5p* の関与による制御）

The addition of docetaxel to cisplatin/5-FU (DCF) therapy has been reported to enhance anticancer activity. However, the prognosis of patients with drug resistance is poor. Recent studies have reported the roles of microRNAs (miRNAs) in chemotherapy resistance. This study aimed to investigate whether miRNA expression profiles could be used to predict a tumor's response to docetaxel and to examine the functions of miRNAs in human esophageal squamous carcinoma cell lines. The results of our research showed that downregulation of *miR-125a-5p* enhanced resistance to docetaxel by interfering with the microtubule network via regulation of inner centromere KinI stimulator (*ICIS*) protein expression.

In Thesis Review, the scientific committee asked several questions and provided comments on the study. The first question involved the cell lines used in this study. The author answered that the study initially was conducted with four cell lines (TE4, TE8, TE11, and TE15 cells). After establishment of docetaxel resistance, the results from TE8-docetaxel-resistance (TXTR) and TE11-TXTR cells showed significant differences in IC_{50} compared with the corresponding parent cell lines ($p < 0.05$). At least two cell lines were selected and analyzed in order to provide better support for the findings. Next, the author was asked about the validity of the results in docetaxel-resistant cells. The author answered that after the establishment of docetaxel-resistant cell lines, the process to maintain the resistant cells required exposure to docetaxel at the IC_{50} concentration, and docetaxel resistance was analyzed by WST-8 assays at every step. The scientific committee suggested that another method for achieving this is by the cloning method, which can be used to maintain drug resistance activity and improve the validity of the results. Next, the author was asked about the selected target gene, miRNA, and previous publications. The author answered that they had selected *ICIS* as the target because *ICIS* has an important role in the mechanism of action of docetaxel and that there are currently no other studies that have reported the role of *ICIS* in docetaxel resistance in esophageal cancer. Additionally, the roles of *miR-125a-5p* in mediating genes, molecules, and cancer have been described in previous reports. For example, *miR-125a-5p* mediates mitotic centromere-associated kinesin (*MCAK*) signaling by downregulating this tumor suppressor in colorectal cancer and modulates the expression of E2F2 in gastric carcinogenesis. However, no studies have examined the role of *miR-125a-5p* in docetaxel-resistant esophageal cancer. These were the reasons that the research focused on *ICIS* and *miR-125a-5p*. Fourth, the author was asked about evaluation of the live cell population in apoptosis assays. The author answered that the study used parental cell lines with upregulated *miR-125a-5p* and decreased expression of *ICIS* and *MCAK*; after transfection with a specific inhibitor of *miR-125a-5p*, the expression levels of *miR-125a-5p*, *ICIS*, and *MCAK* were confirmed. Apoptosis assays were performed using a FITC-Annexin V apoptosis detection kit I and BD FACS Aria II flow cytometer after exposure of cells to docetaxel for 72 h. The results indicated that there was a significant increase in the live cell population after transfection with the wild-type miR-inhibitor compared with that after transfection with the negative control following knockdown of *miR-125a-5p*. Finally, the scientific committee commented on and discussed the additional work required for the study. The next experiments will be performed using clinical samples and will focus on the clinical applications of this research, such as identification of the blood concentrations of docetaxel in patients in relation to the IC_{50} of docetaxel in docetaxel-resistant cell lines and confirmation of the role of *miR-125a-5p* in chemosensitivity and chemoresistance.

The results of this study demonstrated the role of *miR-125a-5p* and target gene *ICIS* in modulating docetaxel sensitivity. The identification of miRNA expression profiles in docetaxel-resistant cells could provide a better understanding of the mechanisms through which docetaxel mediates cancer-related phenotypes in esophageal squamous cell carcinoma. For this study, future experiments with clinical samples and analysis of the clinical applications of the data will be important steps for predicting the response to chemotherapy and may facilitate the development of alternative treatment options for patients who would otherwise be resistant to common chemotherapies. Furthermore, these findings suggest that development of novel strategies for targeted therapies is critical to improving outcomes in patients with esophageal squamous cell carcinoma.