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学位論文題目	MDM4 as a prognostic factor for patients with gastric cancer with low expression of p53:immunohistochemical study of p53, MDM2, and MDM4（p53 低発現胃がん患者の予後因子としての MDM4：p53,MDM2,MDM4 の免疫組織化学的検討）
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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, Xiaochen Zhang describes the prognostic significance of MDM4 expression in patients with gastric cancer. The content is summarized as follows:

（**目的　Purpose**）The purpose of this study is to investigate the prognostic relationship among p53, MDM2, and MDM4 using clinical data and tumor tissue samples. In addition, the author evaluates the effect of MDM4 overexpression on tumor growth properties and sensitivity to cytotoxic drugs in an *in vitro* assay.

（**対象と方法　Materials and Methods**）The author retrospectively collects the clinical data and tumor tissue samples of patients with stage I–IV GC who had undergone surgery or received chemotherapy at the University of Tsukuba Hospital between January 2006 and December 2018. She extracts age, sex, Eastern Cooperative Oncology Group performance status, TNM stage, primary tumor size, histologic type, serum CEA level, adjuvant chemotherapy, and survivals from the medical records. The author investigates the p53, MDM2, and MDM4 expressions of the tumor tissue samples using IHC staining with anti-TP53 antibody (DO-7, ready-to-use) (Dako, Glostrup, Denmark), anti-MDM2 antibody (2A10, diluted 1:80) (Abcam, Cambridge, UK) and anti-MDM4 antibody (2D10F4, diluted 1:500) (Thermo Fisher Scientific, Waltham, MA, USA). For the positive control for p53, MDM2, and MDM4, the author uses paraffin-embedded lung cancer, retinoblastoma, and colon cancer tissues, respectively. Negative controls are obtained by replacing of the primary antibody with PBS. The author determines

the expression status as follows: p53 was defined as high expression if more than 25% of the tumor nuclei were obviously stained, and MDM2 and MDM4 were defined as high expression if more than 50% of the tumor nuclei were stained. Overall survival (OS), disease-free survival and progression-free survival (PFS) are estimated using the Kaplan–Meier method and compared using the log-rank test. Hazard ratios (HRs) and their 95% confidence intervals (CI) adjusted for other variables are calculated using the Cox proportional hazards model to examine the association between patient background factors and survivals. The author maintains NUGC4 human gastric cancer cell line with wild-type TP53 in RPMI 1640 medium supplemented with 10 % fetal bovine serum. MDM4 cDNA is isolated using the 3× FLAG-MdmX/pcDNA3.1 plasmid and subcloned in a lentivirus expression plasmid. The author produces MDM4-infectious recombinant viruses using a ViraPower Bsd Lentiviral Support Kit and 293FT cells and transduced in NUGC4 cells. The author seeds cells in 24-well plates with 500 µl complete medium at a density of 2,000 cells per well, and cultured for 7 days. Relative viable cell numbers are determined by crystal violet staining every 24 h after seeding. The author performs a soft-agar colony-formation assay and counted colonies consisting of more than 50 cells. She also analyzes the 50% inhibitory concentration (IC50) of MDM4-overexpressed NUGC4 cells to 5-Fluorouracil (5-FU), cisplatin, and oxaliplatin using the MTT assay.

（結果　**Results**）The author analyzes the p53, MDM2, and MDM4 expressions in 146 patients with stage I-III and in 95 patients with stage IV gastric cancer. Of these, p53, MDM2, and MDM4 are highly expressed in 27 (18%), 73 (50%), and 59 (40%) patients with stage I-III, respectively, and in 41 (43%), 67 (71%), and 24 (25%) patients with stage IV disease, respectively. The author shows that high expression of p53, but not MDM2 or MDM4, was associated with poor PFS and OS by multivariate analyses in both of patients with stage I-III disease and patients with IV disease. In patients with low p53 expression, however, high expression of MDM4, but not MDM2, is associated with poor OS by multivariate analyses in patients with stage I-III (HR 2.68, 95% CI 1.12–6.44) and IV (HR 6.21, 95% CI 2.20 - 17.58) diseases. The author shows that MDM4-NUGC4 cells expressed a nearly 24-fold higher level of MDM4 than that of control cells. She also shows that in the conventional plate culture, both control and MDM4-NUGC4 cells grew similarly, with a doubling time of 48 h, and that in the soft-agar culture, MDM4-NUGC4 cells formed two-fold more colonies than the control cells (193 ± 37 vs. 101 ± 13, $p<0.02$). The IC50 of 5-FU and oxaliplatin in the MDM4-NUGC4 cells are 5.3-fold and 3.5-fold higher than those for the control cells, respectively. The IC50 for cisplatin is similar in MDM4-NUGC4 and control cells.

（考察　**Discussion**）Although the author does not find any relationship between MDM4 expression and prognosis when all the patients were analyzed, those with high MDM4 expression have significantly shorter OS than those with low expression of MDM4 among patients with low expression of p53, which is considered a surrogate for wild-type p53. Among patients with stage IV disease, those with low expression of p53 and high expression of MDM4 have similar OS to those with high p53 expression, a surrogate for mutated p53. This may suggest that normal p53 function is almost suppressed by highly expressed MDM4. The author shows that MDM4 overexpression endowed NUGC4 cells with resistance to 5-FU and oxaliplatin but not to cisplatin. Although the mechanism of its different effects on oxaliplatin and cisplatin sensitivity remains unknown, this indicates that oxaliplatin or cisplatin can be reasonably selected for chemotherapy of gastric cancer using MDM4 expression in tumor samples as a biomarker.

審査の結果の要旨
Abstract of assessment result

（**批評　General Comments**）

This is the first report indicating that MDM4 induces drug resistance phenotype in cancer cells with wild-type p53, leading to poor overall survival in patients with gastric cancer. This study also suggests that chemotherapy regimen for gastric cancer can be optimized using MDM4 expression in tumor cells as a biomarker. These results prompt researchers to plan clinical studies of MDM4 expression and gastric cancer chemotherapy as well as basic studies on the precise mechanisms of MDM4 and drug resistance.

（**最終試験の結果　Assessment**）

The final examination committee conducted a meeting as a final examination on February 25, 2021. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

（**結論　Conclusion**）

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.