



HOKKAIDO UNIVERSITY

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学 位 論 文 審 査 の 要 旨

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STUDY OF MAMMAGLOBIN 1 AS A REGULATOR IN TRASTUZUMAB RESISTANT CELLS'
AGGRESSIVENESS
(トラスツズマブ耐性細胞の攻撃性における調節因子としてのマンマグロビン1に関する研究)

博士学位論文審査等の結果について（報告）

The Human Epidermal growth factor Receptor 2 (HER2), belongs to Epidermal Growth Factor Receptor (EGFR) superfamily, is one of the most studied signal transduction pathways in cancer. Amplification or overexpression of HER2 is detected in 23% to 26% of primary breast tumors. Trastuzumab (Herceptin), a recombinant humanized monoclonal antibody to HER2, which has been considered as the first-gate therapy for HER2-positive breast cancer patients. However, the effectiveness remains low due to due to acquired or de novo resistance.

In this study, Ms. Kusumastuti established resistant cells by long-term treatment with trastuzumab. These cells showed higher proliferation, invasion, and migration abilities than the wild-type cells. Mammaglobin 1 (MGB1), cyclin D1, E1, A2, and phosphorylated NF- κ B (p-p65) were upregulated in resistant cells. These proteins regulate cell proliferation, migration, and invasion of resistant cells. The depletion of MGB1 decreased cyclin and p-p65 expression. Cyclin D1 and A2, but not E1 expression, were affected by p-p65 downregulation.

In chapter 1 of the doctoral dissertation, general introduction about cancer cell, hallmark of cancer, cancer resistance and therapy, and key factors and molecules related of this study are introduced.

In chapter 2, the examinee explained the material and method used in this study briefly. First, she established SKBR3 breast cancer resistant cells, then checked the resistance. After confirming the resistant cells, she investigated the characteristic of resistant cells relative to wild-type cells by MTT assay, trans-well migration and invasion assay, senescence assay, cell death assay, and plasmid transfection. Moreover, she also checked the essential factors and their relation using siRNA (gene knock-down) and western blotting (protein expression).

In chapter 3, she showed and explained the comparison result of resistant cells and wild-type cells to ensure the specificity the cells used as a resistant model. Resistant cells were obtained through chronically exposed wild-type cells to trastuzumab. Resistant cells have low sensitivity to trastuzumab

treatment, higher MKI67 and less PTEN mRNA level than those are wild-type cells. These characteristics prove the resistance of the cells. Then, she checked the other phenotype of the resistant cells that related to aggressiveness (proliferation, migration, and invasion ability). The resistant cells were more aggressive than that of wild-type cells. Along with increment of aggressiveness, Mammaglobin 1 (MGB1) gene was upregulated in resistant cells. This gene was important to regulate resistant cells' proliferation, migration, and invasion ability.

In chapter 4, because depletion of MGB1 decreased cell viability (in chapter 3), the possible mechanisms related to this phenomenon were investigated. She checked the cell cycle regulator cyclin D1, cyclin E1, and cyclin A2. All cyclins were upregulated in resistant cells. All cyclins were downregulated when MGB1 was silenced. She also checked the cell death and senescence as other possible mechanisms by trypan blue assay and β -galactosidase assay, respectively. Decreasing MGB1 in resistant cells induced neither cell death nor cell senescence. These results suggested that MGB1 regulates resistant cells' viability through cell proliferation control rather than apoptosis or senescence induction. Cyclins were also responsible to migration and invasion regulation in resistant cells. Decreasing cyclins by siRNA was decreased migration and invasion ability, it suggested that cyclins involved in resistant cells' aggressiveness regulation. The regulation of cyclins by MGB1 linked through Nf- κ B, especially p65. Resistant cells were proved to upregulate p-p65. She further checked the role of p65 in resistant cells' aggressiveness. p65 was involved in resistant cells' aggressiveness regulation. She also checked the role of MGB1 in resistance process and cyclins expression. By using plasmid transfection, she established MGB1-overexpressing cells. Interestingly, MGB1 did not induce resistance to trastuzumab and trigger cyclins expression. These results indicated that play a role after cells getting resistance.

In chapter 5, she checked if MGB1 involved in antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) mechanism. Failure to stimulate immune-mediated mechanisms to eliminate tumor cells is one of the characteristics of trastuzumab-resistant cancer cells. ADCC and ADCP are the dominant immune-based antitumor effects of trastuzumab. The results revealed that ADCC and ADCP might still occur even if MGB1 is depleted. However, further experiments should be conducted to confirm this hypothesis. In any case, she concluded that MGB1 could be a promising therapeutic target for HER2-positive breast cancer patients with trastuzumab resistance.

In conclusion, Ms. Kusumastuti found that MGB1 increased after breast cancer cells gained trastuzumab resistance and promoted aggressiveness through cyclin and NF- κ B regulation.

Therefore, we acknowledge that the examinee is qualified to be granted a Doctorate of Life Science from Hokkaido University.