学位論文内容の要旨
（Summary of dissertation）

博士の専攻分野の名称　博士（医　学）　氏 名　許 哲 源
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（Title of dissertation）

PTEN loss is a possible mechanism of trastuzumab resistance in extramammary Paget disease
（PTEN 欠失は乳房外 Paget 病においてトラスツズマブ抵抗性のメカニズムに関与している可能性がある）

【Background and Objectives】Extramammary Paget disease (EMPD) is a rare cutaneous intraepithelial adenocarcinoma primarily affecting the genital region in elderly patients. It is believed that malignant cells originate from apocrine glands in the epidermis and subsequently invade the dermis. Typically diagnosed as carcinoma *in situ*, EMPD has a favorable prognosis when treated with wide local resection. However, prognosis worsens significantly in cases with metastasis. Current treatment options for advanced EMPD, such as docetaxel monotherapy and low-dose 5-fluorouracil/cisplatin combinations, demonstrate limited effectiveness, highlighting the urgent need for innovative systemic therapies to improve clinical outcomes. Human epidermal growth factor receptor 2 (HER2), a receptor tyrosine kinase encoded by the *ERBB2* gene on chromosome 17q12, is implicated in the progression of EMPD. Research has focused on the HER2-PI3K/MAPK signaling pathways due to the similarity of EMPD's Paget cells to those in Paget’s disease of the breast. Approximately 90% of EMPD patients show no significant differences in HER2 protein overexpression or *ERBB2* gene amplification between primary tumors and lymph node metastases, suggesting HER2's role in the pathogenesis of metastasis. While trastuzumab, an HER2-targeted therapy, has improved outcomes in HER2-positive breast cancers, acquired resistance remains a significant challenge in metastatic cases. In EMPD, HER2 overexpression ranges from 15% to 65%. Notably, an EMPD patient with the *ERBB2* S310F mutation exhibited acquired resistance to trastuzumab, indicating the necessity for further research into overcoming treatment resistance and developing effective therapies for this aggressive cancer.

【Materials and Methods】A patient-derived xenograft (PDX) model of EMPD harboring the *ERBB2* S310F mutation was established, following a previous methodology. Tissues from a 78-year-old female patient, whose primary lesion had been excised 12 years earlier, were used to establish this model. Initially, surgically resected tissue from the patient was implanted into non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice, resulting in the formation of a tumor nodule exceeding 10 mm in diameter within five months. The EMPD-PDX tumors exhibited similar morphology and protein expression to the patient's original tumor and metastatic lymph nodes. Once the tumor reached 500 to 1000 mm^3, it was transplanted into subsequent generations, consistently retaining the pathogenic *ERBB2* mutations. In a subsequent phase of the study, an EMPD-PDX model with acquired trastuzumab resistance (TR) was developed. EMPD tumors were implanted into the lateral flanks of NOD/SCID mice and treated with escalating doses of trastuzumab over two months. Tumor volume was monitored bi-weekly, and upon exceeding 1000 mm^3, the tumors were advanced to next generation. Resistance was further induced through continuous trastuzumab treatment for an additional eight weeks, with

serial passaging resulting in a TR strain. Genomic DNA was extracted from both trastuzumab-sensitive (TS) and TR EMPD-PDX tumors, with quality assessed via NanoDrop and electrophoresis. Targeted amplicon exome sequencing was performed on 160 cancer-related genes using the Illumina MiSeq platform. Data analysis utilized the GenomeJack bioinformatics pipeline. For total RNA extraction, tumor samples were homogenized, and RNA was reverse transcribed into cDNA for quantitative PCR analysis using specific primers for genes *PTEN* and *CDH1*, normalizing results against *GAPDH*. Antibodies for immunoblotting and immunohistochemistry were sourced from various suppliers, and protein lysates underwent SDS-PAGE followed by transfer to PVDF membranes. The Allred scoring system was employed for quantifying FOXA1 expression in histopathological analyses, using formalin-fixed, paraffin-embedded sections from both TS and TR tumors.

【Results】Tumors carrying the *ERBB2* S310F mutation were propagated in NOD/SCID mice over several generations. These tumors, implanted into the lateral flanks of mice, were subjected to escalating doses of trastuzumab to induce resistance, with tumor growth monitored regularly. When tumors exceeded 1000 mm^3, they were harvested, propagated to subsequent generations, and drug resistance was induced through continuous trastuzumab treatment. Genetic profiling was conducted on TS and TR tumors. TR tumors exhibited a loss of *PTEN* and *CDH1* genes, accompanied by reduced mRNA expression and diminished PTEN protein levels. These findings were confirmed by histological and immunohistochemical analyses, which showed a significant reduction in PTEN protein expression in TR tumors. Further immunoblotting demonstrated an inverse correlation between PTEN expression and the activation of the Akt pathways, as well as the downregulation of p27. We then explored different treatment strategies for the TR EMPD-PDX model. HER2-targeted therapies were tested, with lapatinib demonstrating a marked inhibition of tumor growth, while trastuzumab had no effects in TR tumors. Cyclin-dependent kinase 4/6 inhibitors, abemaciclib and palbociclib, showed significant efficacy in suppressing tumor growth. Cytotoxic chemotherapy, including eribulin and docetaxel, proved highly effective, with a single dose of trastuzumab deruxtecan (T-DXd) leading to complete tumor eradication and no relapse over 14 days. PTEN inhibitors BpV (HOpic) or SF1670 were administered with trastuzumab in TS EMPD-PDX models. The results showed that while trastuzumab effectively suppressed tumor growth in TS models, the addition of PTEN inhibitors partially restored the effect of TR, suggesting that PTEN loss plays a significant role in the development of drug resistance. Moreover, we investigated a clinical case of a 73-year-old Japanese male patient treated with trastuzumab plus docetaxel, whose treatment became less effective over time. Immunohistochemical analysis of biopsies taken before and after chemotherapy revealed no significant changes in PTEN or E-cadherin expression, suggesting that additional mechanisms, aside from PTEN loss, may contribute to TR in clinical settings.

【Discussion】Prior studies have shown that HER2 overexpression in EMPD correlates with *ERBB2* mutations, with some patients responding to anti-HER2 treatments. However, the development of acquired resistance remains a challenge, as indicated by consistent PTEN loss in our model and their prevalence in Cowden syndrome, which predisposes individuals to various malignancies. PTEN is commonly associated with TR in breast cancer, and EMPD resembles Paget's disease of the breast. Our TR EMPD-PDX model constantly displayed PTEN loss, affirming their role in resistance mechanisms not only in breast cancer but also in EMPD. The study also explored the effects of targeted therapies and cytotoxic agents in TR EMPD-PDX models. Lapatinib showed effectiveness in patients with low PTEN tumors. CDK4/6 inhibitors, abemaciclib and palbociclib, demonstrated partial tumor growth suppression. Docetaxel, eribulin, and T-DXd exhibited high efficacy against TR EMPD-PDX tumors, indicating potential treatment options despite TR.

【Conclusion】Our study successfully establishes a TR EMPD-PDX model that mirrors the characteristics of naïve tumors, particularly in terms of HER2 expression and PTEN loss. This model underscores the critical role of PTEN loss in driving TR in EMPD. We also demonstrate the potential of various therapeutic strategies. These findings suggest that targeted interventions addressing the underlying resistance mechanisms may significantly enhance treatment efficacy and outcomes for patients with TR EMPD.