# 学 位 論 文 の 要 旨

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学　位　論　文　名　A Comparative Analysis of Usual- and Gastric-Type Cervical Adenocarcinoma in a Japanese Population Reveals Distinct Clinicopathological and Molecular Features with Prognostic and Therapeutic Insights

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## 論 文 内 容 の 要 旨

### INTRODUCTION

Cervical cancer is a significant global health issue, ranking fourth in incidence and mortality among women. Endocervical adenocarcinoma (ECA) accounts for 20–25% of cases, with incidence rising particularly in developed countries. In 2020, the World Health Organization introduced a two-tiered classification based on human papillomavirus (HPV) status: HPV-associated and HPV-independent types. Usual-type cervical adenocarcinoma (UCA) is the most common HPV-associated subtype, whereas gastric-type cervical adenocarcinoma (GCA) is the most prominent HPV-independent variant. Cervical adenocarcinoma includes diverse morphological subtypes with distinct HPV associations. UCA is strongly linked to high-risk HPV, especially types 16 and 18, while GCA is almost always HPV-negative. First described in 2007, GCA often presents with nonspecific clinical features, subtle cytologic abnormalities, and a deeply infiltrative growth pattern, leading to delayed detection. Its prevalence varies regionally, representing 10–25% of ECAs in Japan, about 10% in Western countries, and 3.8% worldwide. Histologically, it is defined by pale eosinophilic or clear cytoplasm and expression of gastric-type mucins such as MUC6 and HIK1083. Clinically, GCA shows more aggressive behavior and poorer prognosis than UCA.

Molecular studies further distinguish the two: GCA frequently harbors mutations in *TP53*, *STK11*, *KRAS*, *ARID1A*, and *PIK3CA*, while UCA more often involves cell-cycle genes including *TP53*, *KRAS*, and *ERBB2/3*. This study compares clinicopathological, immunohistochemical, and molecular features of GCA and UCA in a Japanese cohort to identify prognostic biomarkers and therapeutic strategies.

## MATERIALS AND METHODS

This study analyzed 110 cervical cancer tissue samples collected from Shimane University School of Medicine (2014–2017) and Seirei Hamamatsu Hospital (2003–2022), comprising 94 UCA and 16 GCA cases. Diagnoses were confirmed by two gynecologic pathologists, and clinicopathological data were retrieved from medical records. All patients provided written informed consent. For immunohistochemistry, FFPE sections were deparaffinized, rehydrated, and subjected to antigen retrieval. Slides were incubated overnight with antibodies against p53, p16, PD-L1, PD-1, CD8, ARID1A, ARID1B, c-Myc, and PTEN followed by secondary antibodies, DAB detection, and hematoxylin counterstaining. Positive controls were included, and immunoreactivity was independently assessed by a blinded pathologist. IHC scoring combined staining intensity and percentage of positive tumor cells, with defined cutoffs for each marker. For genetic analysis, DNA was extracted from FFPE tissues, and Sanger sequencing targeted hotspot regions of *KRAS* (exon 2), *PIK3CA* (exon 9), and *BRAF* (exon 15). PCR products were sequenced, analyzed with Mutation Surveyor, and validated using COSMIC.

Statistical analyses were conducted using chi-square or Fisher's exact test, Kaplan-Meier survival with log-rank test, and Cox regression, with $p < 0.05$ considered significant. The study protocol was approved by the Research Ethics Committee of Shimane University.

## RESULTS AND DISCUSSION

This study included 110 cervical adenocarcinoma patients, comprising 94 usual-type adenocarcinoma (UCA) and 16 gastric-type adenocarcinoma (GCA). UCA exhibited HPV-associated morphology with glandular, papillary, or cribriform architectures, pseudostratified columnar cells, and diffuse p16 expression. GCA was HPV-independent, with irregular lobular glands, pale or eosinophilic cytoplasm, minimal nuclear atypia, and patchy or absent p16 expression. Clinically, GCA patients were more often diagnosed at advanced FIGO stages (III/IV: 37.5% vs. 13.8%), had higher recurrence rates (37.5% vs. 13.8%), greater vaginal invasion (37.5% vs. 13.8%), and worse overall survival (31.3% vs. 8.5%) than UCA, highlighting its aggressive phenotype. Kaplan-Meier analysis confirmed significantly poorer progression-free survival (PFS, $p = 0.014$) and overall survival (OS, $p = 0.032$) in GCA. Immunohistochemical profiling revealed higher rates of aberrant p53 in GCA (25% vs. 4.2%)

and higher p16 positivity in UCA (87.2% vs. 43.8%). ARID1A loss and PTEN loss were more frequent in GCA, whereas ARID1B and c-Myc expression showed no significant differences. Molecular analysis identified *KRAS* (25% vs. 14.8%) and *BRAF* (13.3% vs. 5.3%) mutations more frequently in GCA, while *PIK3CA* mutations were more common in UCA (31.6% vs. 7.7%). In UCA, p16 positivity correlated with improved PFS and OS, younger age, lower recurrence, and fewer distant metastases, indicating its diagnostic and prognostic value. In GCA, high ARID1B expression was associated with shorter PFS and OS, suggesting a role in tumor aggressiveness, likely via chromatin remodeling mechanisms. PD-L1 expression, although limited, correlated with distant metastasis and worse survival, indicating potential utility as a therapeutic biomarker for immune checkpoint inhibitors.

Overall, GCA demonstrates distinct clinicopathological, molecular, and immunological profiles compared to UCA, with aggressive clinical behavior, advanced stage at diagnosis, and poor survival outcomes. The divergent molecular alterations including *KRAS*, *BRAF*, and *PIK3CA* mutations, p53 and p16 expression patterns, and ARID1B overexpression support its classification as an HPV-independent, high-risk subtype. Integrating immunohistochemical and molecular markers may aid in early diagnosis, prognostic assessment, and development of targeted therapies, including immunotherapy, for this aggressive cervical adenocarcinoma subtype.

## CONCLUSION

This comparative study underscores distinct differences between GCA and UCA in clinical presentation, molecular alterations, and immune profiles. GCA demonstrated more aggressive features and poorer survival than UCA. Immunohistochemically, GCA showed p16 negativity and aberrant p53 accumulation, supporting an HPV-independent pathway, whereas UCA exhibited strong p16 expression and wild-type p53, consistent with an HPV-associated pathway. Furthermore, *KRAS* and *BRAF* mutations were more frequent in GCA, while *PIK3CA* alterations predominated in UCA. Remarkably, ARID1B and PD-L1 expression in GCA were linked to adverse features and poorer survival, indicating possible prognostic relevance. Overall, these findings provide clinical implication for subtype-specific molecular and immunotherapeutic strategies in cervical adenocarcinoma.