学位論文の要旨

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論文内容の要旨

INTRODUCTION

Ovarian seromucinous tumors (SMBTs) are relatively rare and their carcinogenesis is largely unknown. Previously, SMBTs in the ovary were considered a subtype of mucinous borderline tumors (MBT). Although SMBTs are believed to be a subtype of MBTs, SMBTs clinically resemble serous borderline tumors (SBTs) in that both tumors typically show papillary projection inside cystic spaces grossly and present hierarchical branching with broad fibrous stroma microscopically. In 2014, the WHO presented several modifications to female tumors and classified SMBTs as a new category distinct from MBTs. SMBTs have now been identified as a new morphological group that is believed to be derived from or associated with endometriosis (30-50%), in a number of cases that are difficult to find in patients with SBTs or MBTs.

Very few reports have suggested that numerous genetic alterations are associated with tumorigenesis in SMBTs. Recently, the first comprehensive attempt was undertaken investigating the molecular underpinning of SMBTs through the application of next generation sequencing. This report found that SMBTs' signatures consisted of frequent somatic mutations in the *KRAS* (100%), *PIK3CA* (60.7%), and *ARID1A* (14.3%) genes, with *TERT* promoter mutations and DNA mismatch repair deficiencies being consistently absent. Another paper confirmed that the loss of ARID1A expression, a surrogate for *ARID1A* mutations, has been reported in one-third of these tumors, a frequency similar to that seen in ovarian endometrioid carcinomas, supporting their close relationship. A further study which focused on *KRAS* and *PTEN* mutations of 16 samples reported that *KRAS* mutation was 69% and no *PTEN* mutation was observed. Molecular biological analyses of SMBTs have revealed several molecular characteristics. However, Molecular profiling of SMBTs in Japanese patients is yet to be performed. In this study, we aimed to assess the molecular features of SMBTs in Japan.

MATERIALS AND METHODS

Formalin-fixed, paraffin-embedded (FFPE) tissue samples from 23 SMBTs were used in this study. The diagnoses were made based on conventional histopathological examination of sections stained with hematoxylin and eosin. Tumors were categorized according to the World Health Organization subtype criteria by several pathologists in each hospital. Tumors were staged according to the International Federation of Gynecology and Obstetrics classification system. The resected specimen of each case was centrally reviewed by a gynecological pathologist and gynecologic oncologist. The study protocol was approved by the Research Ethics Committee of Shimane University.

Paraffin-embedded tissues were serially sectioned to a thickness of 10 mm. Genomic DNA for mutation analysis was extracted from FFPE samples using a commercially available kit (Qiagen, Inc., Valencia, CA, USA).

Extracted DNA was amplified by polymerase chain reaction (PCR) using primers for exon 2 of *KRAS*, exon 15 of *BRAF*, exons 9 and 20 of *PIK3CA*, and exon 20 of *ERBB2* using genomic DNA obtained from microdissected formalin-fixed paraffin-embedded tissue. We focused on analyzing exons reported to harbor the majority of mutations in each gene.

The thermal cycle profile for all gene amplification was as follows: one cycle at 95°C for 30s followed by 40 cycles at 55 °C and extension at 72°C for 15s. All polymerase chain reaction PCR-amplified products were sequenced at Beckman Coulter (Danvers, MA, USA) and analyzed using the Mutation Surveyor DNA Variant Analysis Software (Tokyo, Japan). We confirmed the pathogenicity of each mutation using the Catalogue of Somatic Mutations in Cancer (COSMIC).

The expression levels of ARID1A, p53, and PTEN were evaluated by immunohistochemical analysis (IHC). FFPE sections (3 μm thick) were dewaxed in xylene and hydrated in graded alcohol solutions. After antigen retrieval in sodium citrate buffer, the slides were incubated overnight at 4 °C with antibodies at the following dilutions: 1:50 p53 (M7001; Dako, Carpinteria, CA, USA), 1:100 ARID1A (Sc-32761; Santa Cruz Biotechnology, Santa Cruz, CA, USA), and 1:200 PTEN (138G6; Cell Signaling Technology, Danvers, MA, USA). Loss of ARID1A and PTEN expression in tumor cell nuclei was used as a surrogate for the presence of PTEN/ARID1A loss-of-function mutations. Similarly, p53 immunoreactivity was used as a surrogate for the presence of p53 loss/overexpression of functional mutations. Briefly, ARID1A and PTEN immunoreactivity was scored by two investigators as follows: 0, undetectable; 1+, weakly positive; 2+, moderately positive; and 3+, intensely positive. The loss of ARID1A or PTEN staining intensity (0+) was considered negative. Weak-to-moderate immunoreactivity was considered p53 normal expression. Strong and diffuse nuclear p53 immunoexpression or the complete absence of p53 staining was interpreted as likely indicating a p53 mutation.

RESULTS AND DISCUSSION

The prevalence of *KRAS*, *BRAF*, *PIK3CA*, and *ERBB2* mutations was 4.3% (1/23), 8.6% (2/23), 8.6% (2/23), and 17.3% (4/23), respectively. Overexpression or loss of p53 expression occurred in 26% (6/23), loss of ARID1A expression in 4.3% (1/23), and in none of the cases was loss of the expression of PTEN observed.

In this study, we first described the molecular alterations of SMBTs in the Japanese population. Very recently, Wu et al. performed mutational analysis on 28 SMBTs in Taiwan and reported that somatic mutations in the *KRAS, PIK3CA, ARID1A,* and *PTEN* genes were 100, 60.7, 14.0, and 3.6%. They demonstrated that *KRAS* was mutated in all SMBTs, whereas *PIK3CA* mutations occurred frequently. Compared with their report, the current frequency of these alterations in these genes was much lower. Considering the current findings and the previous study, we hypothesized that the difference in prevalence may be due to a difference in genetic background between the Japanese population and other ethnicities or the difference in methods between direct sequencing and next generation sequencing. SMBTs are known to be associated with endometriosis, and previous studies have identified it in between 45% and 71% of patients , including three cases in which endometriosis was directly contiguous with SMBTs . The current frequency of SMBTs with endometriosis is much lower than that reported in previous reports . Therefore, it may also be due to the fact that the frequency of SMBTs with concurrent endometriosis is significantly lower than that of report of Wu et al.

Loss of ARID1A expression 8/24 (33%), and one case had loss of ARID1A expression in synchronous endometriosis in 24 SMBTs were reported. A significant number of SMBTs that exhibit loss of expression in ARID1A and their common association with endometriosis reveals that these tumors are closely related to endometrioid carcinomas. However, in the current study, the loss of ARID1A was only 4.3%, which was quite lower compared to the study in previous reports. Besides, immunohistochemical staining for p53 have been found positive (mutant p53) in 26.0% (6/23) of SMBTs. Previously, we have constructed mutational profiles of endometriosis-related ovarian neoplasms (ERONs) and observed that *ARID1A* mutation was 95%, while *p53* was 36.8%, suggesting that some ERONs occurring based on *p53* alterations were similar to the current findings in SMBTs.

CONCLUSION

The current findings suggest that alterations in *KRAS/BRAF/PIK3CA* and *PTEN* are rare carcinogenic events in Japanese SMBTs. The high frequency of positive p53 staining along with a low frequency of loss of ARID1A staining suggests that carcinogenesis of SMBTs could be related to the alteration of *p53* rather than that of *ARID1A*. The fact that the *ERBB2* mutation is the highest event suggests its important role in the tumorigenesis of Japanese SMBTs.