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専　　攻　　名	医学専攻
学位論文題名	Molecular analysis of ascitic fluid cytology reflects genetic changes of malignancies of the ovary equivalent to surgically resected specimens. （腹水細胞診を用いた分子生物学的解析は外科的切除標本と同等に卵巣癌の遺伝子変異を反映する）
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学位論文内容の要旨

Objectives

Epithelial ovarian cancer (EOC) is common and fatal gynecologic malignancy in Japan with 13,049 new cases and 4,784 deaths were estimated to have occurred in 2018. The treatment for advanced stage EOC includes primary debulking surgery followed by adjuvant chemotherapy or neoadjuvant chemotherapy followed by interval debulking surgery. In addition, highly invasive surgery is sometimes required on these patients to make a definitive diagnosis.

Ascitic fluid cytology (AC) is a minimally invasive, rapid, and clinically useful method for diagnosis, but it is difficult to evaluate the nature of the tumor by morphological features alone. In the present study, we determined the concordance rate of molecular profiles between surgically resected tissue samples and peeling samples of AC in patients with EOC and evaluated its application.

Methods

Patients (n = 369) with ovarian, fallopian tube, and peritoneal carcinoma were treated at our hospital from 2008 to 2021. We performed a molecular biological analysis on 33 patients from whom written informed consent for genomic analysis was obtained. In all cases, a diagnosis was based on perioperative intraperitoneal findings and pathological evaluation. The median age was 62 (range 41–78) and included ovarian (n = 23), fallopian tube (n = 2), and peritoneal (n = 8) carcinomas. The histological subtypes of these 33 patients included 31 serous carcinomas, one clear cell carcinoma, and one endometrioid carcinoma. All patients were staged according to International Federation of Gynecology and Obstetrics (FIGO) 2014 criteria and clinical data. Almost all patients (n = 32) were diagnosed with stage III or IV disease.

In each case, we extracted DNA from formalin-fixed paraffin-embedded (FFPE) tissue and

AC and compared for quantity and quality of the DNA. Surgically resected FFPE tissue and AC samples were analyzed by next-generation sequencing using an *in house* generated gynecologic oncology panel (52 genes) and compared the concordance rate between their molecular profiles.

Results

The quantity and quality of extracted DNA from FFPE and AC was determined by quantitative real-time PCR using two sets of primers that amplify long- and short-length amplicons. The mean concentration of long-length DNA and the mean relative quantification value were 47.9 ng/μL (range, 0.4–483.6 ng/μL) and 0.30, respectively, in DNA extracted from FFPE, whereas they were 6.0 ng/μL (range, 0.0–51.8) and 0.31, respectively, in DNA extracted from AC. As expected, there was a significant difference in the extracted DNA (long/short) amount between FFPE and AC ($p < 0.01$). However, there was no difference in “quality” of the extracted DNA, which was represented by an RQ value ($p = 0.85$). This indicated that archival cytological specimens could be utilized for genomic analysis.

We identified 159 mutations (54 oncogenic and 105 non-oncogenic mutations) in 66 DNA samples (33 FFPE tissues and 33 AC) from 33 patients. Of the 159 mutations, 57 (35.8%) were shared between surgically resected FFPE tissue and AC. However, the concordance rate of the molecular profiles between the two was significantly higher for oncogenic compared with non-oncogenic mutations (85.1% vs. 10.5%, $p < 0.01$). In fact, AC covered all ($n = 46$) oncogenic mutations detected in surgically resected specimens and identified additional mutations ($n = 8$).

In AC, *TP53* mutation was found in 94% (31/33) of the cases, in particular, *TP53* mutation was found in all cases (31/31) of serous carcinoma. Other mutations that were frequently detected in AC were *BRCA1* (21%) and *ARID1A* (15%). In FFPE tissue, *TP53* mutations were also found in 94% (31/33), which was similar to that of AC, and *BRCA1* (15%). All 33 cases had at least one shared mutation between AC and FFPE tissue.

Discussion

Few papers have compared the genomic profiles of ascites and resected tissues in ovarian cancer. Most of the studies focused on ”hot spot” mutations or only *BRCA*-related abnormalities. In the present study, the genomic concordance rate of oncogenic mutations, not only *BRCA,* but 52 potentially mutated genes between AC and FFPE tissues, was very high. In other words, we can understand the genomic characteristics of tumors in detail through a genomic analysis of AC. This may be useful, of course, for cases in which it is difficult to obtain surgical or biopsy specimens.

In the present study, although the quantity of extracted DNA was limited, the quality obtained from AC samples was similar to that of surgically resected FFPE and the sequence metrics were comparable. All oncogenic mutations detected by FFPE were also identified in AC. In addition, eight oncogenic mutations not detected by FFPE were identified in AC. This may reflect that surgically resected specimens are localized lesions, whereas ascitic fluid may yield a more comprehensive picture of the whole abdominal cavity.

Conclusion

We found that genomic analysis of AC can identify all the genetic changes associated with EOC to understand tumor characteristics without interventional surgery or biopsy and may play an important role in developing personalized precision medicine.

論文審査結果の要旨

野崎氏が執筆したCancer Cytopathology誌（Impact factor（2021):4.354）に掲載された論文について審査を行った。本研究では、「侵襲性の高い卵巣組織生検試料と同等に、腹水試料における遺伝子検査が病的バリアントの検出に有効である」という仮説の検証のため、卵巣における腫瘍組織と腹水との両試料について次世代シーケンサーNGSによるオンコパネル（52遺伝子）検査を研究方法とし、がん遺伝子ならびに非がん遺伝子のバリアントを検出、比較検証にて考察を行ったものである。本研究は過去に同じ研究はなく、卵巣がん腹水試料の遺伝子検査が従来から行われている腫瘍組織生検試料と比して、同等以上のがん遺伝子バリアントを検出できることを示している。研究対象者は県立中央病院にて治療を受けたstage ⅢおよびⅣの卵巣がん患者であり、卵巣がん種は漿液性が大半である。論文は権威ある雑誌にて査読および受理をされており、得られた知見については疑義を生じるものはないと審査員にて判断した。

最終試験においては、論文の研究仮説を立てた臨床背景と研究の新奇性・独創性、腹水を検査試料とする妥当性など研究の意義、サンプリング期間、得られた知見の臨床応用の可能性、本研究における野﨑氏の役割、遺伝子検査から生じる生殖細胞系列遺伝子バリアント検出の可能性（2次的所見）への遺伝カウンセリング体制、他施設にて試料収集と解析が行われた研究であるため当該施設における倫理委員会の審議・承認状況、などを審査することとした。