Hotspot mutation profiles of AKT1 in Asian women with breast and endometrial cancers

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Abstract

Background: The V-Akt murine thymoma viral oncogene (AKT) 1 (E17K) is a subfamily of serine/threonine protein kinases that affects the survival, proliferation, and invasion of cancer cells. The clinicopathological features and frequencies in Asian populations with AKT1 mutations in breast and endometrial cancers are unclear. Hence, we aimed to determine the frequencies and relationships between clinicopathological features and AKT1 mutations in Asian women with cancer.

Methods: We extracted DNA from 311 and 143 samples derived from patients with breast and endometrial cancers to detect the AKT1 point mutation (hotspot), E17K. We examined correlations between clinicopathological features and AKT1 mutation status.

Results: The frequency of AKT1 mutations in breast cancer was 7.4%, and they were found more frequently in human epidermal growth factor receptor 2 (HER2)-negative breast cancer subtypes, although this was not statistically significant (P = 0.08). The frequency of AKT1 mutations in endometrial cancer was 4.1%, and the mutations were histologically detected only in endometrioid types. However, AKT1 mutations did not correlate with relapse-free or overall survival of patients with breast or endometrial cancer.

Conclusions: AKT1 mutations are associated with HER2-negative subtype in breast cancer and in endometrial cancer with endometrioid histology. The frequencies of AKT1 mutations in breast and endometrial cancers were similar between Asian and other regional women. The frequency of mutations is too low in both tumor types to talk about predictive significance.

Keywords: AKT1 mutation, Breast cancer, Endometrioid histology, HER2, Endometrial cancer

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Background
The phosphatidylinositol 3-kinase (PI3K)/v-Akt murine thymoma viral oncogene (AKT)/mTOR pathway is activated across many cancer lineages, and it is associated with cancer development and metastasis [1]. Approximately 70% of patients with breast cancer have activated phosphatidylinositol 3-kinase (PI3K). A mutation in PI3K catalytic subunit α is one of the most frequently activated in some cancers [2].

The AKT1 subfamily of serine/threonine protein kinases affects cell survival, proliferation, and invasion. A dominant hotspot mutation is a glutamate 17 to lysine (E17K) substitution, and AKTI pleckstrin homology domain mutations activate serine-threonine kinase. Moreover, the findings of recent clinical trials have suggested that mutated AKT1 is a potent predictive marker of responses to AKT inhibitors [3, 4].

A large-scale retrospective analysis of recent data derived from ~20,000 solid tumours revealed an AKT1 mutation frequency of ~1% [1]. The reported frequencies of AKT1 mutations are between 1.4 and 8.2% [5, 6] in breast cancer and 2.2 and 4.1% in endometrial cancer [7, 8].

However, the clinicopathological features and mutation frequency of AKT1 in Asian populations with breast and endometrial cancers have not been examined. Here, we investigated the frequencies of AKT1 mutations and their associations with clinicopathological features in Asian women with breast and endometrial cancers.

Materials and methods
The pathological and clinical data of the cases used in this study were collected from the medical records in a retrospective manner.

Tissue samples and patient selection
Breast cancer cohort
We obtained 118 frozen tumour surgical specimens and 193 formalin-fixed paraffin-embedded (FFPE) surgical or biopsy tissues that had been preserved in a biobank at the National Cancer Centre Hospital between May 1989 and December 2012. We extracted DNA from the frozen tumour specimens and FFPE tissues using QIAamp DNA Mini Kits and QIAamp DNA FFPE Tissue Kits, respectively (Qiagen GmbH, Hilden, Germany) as described by the manufacturer [9]. Mutations at the AKT1 hotspot detected using peptide nucleic acid-locked nucleic acid (PNA-LNA) clamp methods in samples collected and stored at the National Cancer Center Hospital were analysed by LSI Medience Corporation (Tokyo, Japan) as described previously [10].

Endometrial cancer cohort
We extracted DNA from 142 DNA FFPE surgical tissue samples that had been preserved at the National Cancer Centre Hospital between May 1989 and November 2012 using QIAamp DNA FFPE Tissue Kits (Qiagen) as described by the manufacturer [9]. We detected the AKT1 hotspot mutation using real-time PCR as described [8] with the forward and reverse primer sequences of exon 4 of AKT1 (5′-CACACCCAGTTTCCTGCTG-3′ and 5′-CCTGGTGGCCAAAAGAGGCT-3′, respectively). The PCR cycling program comprised activation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 60 s, and 72 °C for 10 min. The PCR products were sequenced using the BigDye terminator method and an auto-sequencer (Applied Biosystems, Foster City, CA, USA).

Histopathological evaluation
Oestrogen (ER) or progesterone (PgR) receptors were classified as positive or negative based on ASCO/CAP Guideline [11]. In all cases, at least 1% of cells staining was considered positive according to the criteria. Human epidermal growth factor receptor-2 (HER2) positivity was classified according to the guidelines of the American Society of Clinical Oncology/College of American Pathologists [12]. Hormone receptor-positive status was defined as positive for either ER or PgR. The histological and nuclear grade of breast cancer and endometrioid uterine carcinoma were graded as described in previous studies [9, 13] and in a previous report [14], respectively. Pathological evaluations are always performed by at least two expert pathologists (including HY and MY), although the results of diagnoses in institutional practice are used. ND in our Tables is because there was a time when it was not evaluated in the old days, and we could not re-evaluate it sufficiently because of the deterioration of pathological specimens.

Clinical outcome evaluation based on AKT1 mutation
Differences in outcomes between patients with and without AKT1 mutations were assessed using data from 311 and 143 patients in breast and endometrial cancer subgroups. Differences between proportions for categorical variables were analysed using chi-square tests. Postoperative relapse-free (RFS) and overall (OS) survival was analysed using Kaplan–Meier curves. We defined RFS as elapsed time between the day of surgery and disease progression or the last follow-up for operable lesions. We defined OS as elapsed time between the first day of diagnosis and the date of death or the last follow-up. Significant prognostic factors associated with RFS and OS were determined by multivariate analysis using Cox hazard models. Clinically relevant factors for breast (initial stage, histologic grade, ER status, PgR status, HER2...
status, and AKT1 mutation) and endometrial (diagnostic stage, lymphovascular invasion, and AKT1 mutations) cancers were included as covariates in multiple Cox proportional hazards models. All data were statistically analysed using JMP version 11 (SAS Institute, Cary, NC, USA). Statistical significance was set at \( P < 0.05 \).

### Results

**Prevalence of AKT1 hotspot mutation (E17K) in patients with breast cancer**

The overall prevalence of AKT1 mutations was 23 (7.4%) of 311 breast cancer samples.

**Association of AKT1 mutation with clinicopathological parameters in breast cancer**

Table 1 shows the clinicopathological parameters according to AKT1 mutations in the breast cancer samples. Mutated AKT1 was not associated with prevalence, age, histologic subtype, initial stage (7th edition of UICC [the Union for International Cancer Control] TNM stage), tumour grade, lymphovascular invasion, and hormone receptor status. In the 16 patients with the so-called triple negative subtype, which is negative for both ER/PgR/HER2, two patients had AKT1 hotspot mutations. The number of patients with AKT1 hotspot mutation was 13% (3/22). On the other hand, HER2-negative status tended to have more AKT1 gene mutations, although this was not statistically significant (\( P = 0.08 \)).

The median follow-up duration was 73 (range 2.5–450) months. Twenty-eight patients initially had metastases, 148 of the 283 patients who initially had early breast cancer relapsed (137 and 11 with wild-type and mutated AKT1, respectively), and 48 died of breast cancer.

The RFS and OS did not significantly differ between patients with wild-type and mutated AKT1 (Fig. 1). The median relapse-free durations in patients with mutated and wild-type AKT1 were 75 and 79 months, respectively (\( P = 0.77 \)). Multivariate analysis associated only the initial stage with OS (Additional Table 1).

**Prevalence of AKT1 hotspot mutations (E17K) in patients with endometrial cancer**

The overall prevalence of mutated AKT1 in endometrial cancer samples was 6 (4.1%) of 143 (Additional Figure 1).

**Associations between AKT1 mutation with clinicopathological parameters in endometrial cancer**

Table 2 shows the clinicopathological parameters according to AKT1 mutations in the endometrial cancer samples. Mutated AKT1 was not associated with prevalence and age, histological subtype, initial stage (UICC

| Table 1: Characteristics of Asian patients with breast cancer \(N = 311\) |
|---------------------------|---------------------------|---------------------------|
| Total                     | Wild-type \(n = 288\)    | Mutated \(n = 23\)        |
| Median age (range; y)     | 52 (22–90)               | 52 (22–90)               |
| Initial TNM Stage          |                           |                           |
| IA                        | 71                       | 66 (24)                  |
| IB                        | 2                        | 2 (1)                    |
| IIA                       | 68                       | 65 (23)                  |
| IIB                       | 59                       | 53 (19)                  |
| IIIA                      | 46                       | 42 (15)                  |
| IIIB                      | 12                       | 10 (4)                   |
| IIC                       | 16                       | 15 (5)                   |
| IV                        | 28                       | 27 (10)                  |
| ND                        | 10                       | 8 (4)                    |
| Histology                 |                           |                           |
| IDC                       | 284                      | 266 (92)                 |
| ILC                       | 11                       | 9 (3)                    |
| ND                        | 16                       | 13 (5)                   |
| Grade                     |                           |                           |
| 1                         | 31                       | 29 (10)                  |
| 2                         | 146                      | 135 (47)                 |
| 3                         | 116                      | 109 (38)                 |
| ND                        | 18                       | 15 (5)                   |
| Lymphovascular invasion   |                           |                           |
| Positive                  | 130                      | 117 (41)                 |
| Negative                  | 121                      | 115 (40)                 |
| ND                        | 60                       | 56 (19)                  |
| Hormone Receptor          |                           |                           |
| Positive                  | 264                      | 249 (86)                 |
| Negative                  | 47                       | 39 (14)                  |
| HER2 status               |                           |                           |
| Positive                  | 55                       | 54 (19)                  |
| Negative*                 | 256                      | 234 (81)                 |

Bp, partial mastectomy; Bt, total mastectomy; HER2, human epidermal growth factor receptor-2; HR, hormone receptor; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; LVI, lymphovascular invasion; mut, mutation; ND, no data. * HER2 negative includes triple negative and ER+/PR+/HER2- cancers. Differences between proportions for categorical variables were analysed using chi-square tests.

8th ed./FIGO 2018, tumour grade in the histological subtype, or lymphovascular invasion.

The median follow-up duration was 60 (range 0.6–275) months. Six patients initially had metastatic lesions, 18 of the 137 patients who initially had early endometrial cancer, relapsed (16 and 2 patients with wild-type and mutated AKT1, respectively), and 21 patients died of any cause. Three deaths were related to other types of
Fig. 1 Relapse-free survival (RFS) and overall survival (OS) in patients with breast cancer. There were no significant differences in RFS and OS between patients with wild-type AKT1 breast cancer and those with AKT1-mutated breast cancer.

Table 2 Characteristics of Asian patients with endometrial cancer (N = 143)

|                      | Total (N = 143) | AKT1 mutation status (N = 137) | p     |
|----------------------|-----------------|--------------------------------|-------|
|                      |                 | Wild-type (%)                  | Mutated (%) |
| Median age (range; y)| 56 (28–89)      | 56 (28–89)                     | 49 (44–62) | 0.19 |
| Initial TNM Stage    |                 |                                |       |
| IA                   | 54              | 52 (38)                        | 2 (33) |
| IB                   | 27              | 25 (18)                        | 2 (33) |
| II                   | 19              | 19 (14)                        | 0 (0)  |
| IIIA                 | 27              | 16 (12)                        | 1 (17) |
| IIIB                 | 1               | 1 (1)                          | 0 (0)  |
| IIIC                 | 19              | 18 (13)                        | 1 (17) |
| IVB                  | 6               | 6 (4)                          | 0 (0)  |
| Histology            |                 |                                |       |
| Endometrioid         | 131             | 125 (91)                       | 6 (100) |
| Serous               | 3               | 3 (2)                          | 0 (0)  |
| Clear                | 2               | 2 (2)                          | 0 (0)  |
| Carcinosarcoma       | 3               | 3 (2)                          | 0 (0)  |
| Others               | 4               | 4 (3)                          | 0 (0)  |
| Grade in Endometrioid histology |     |                                |       |
|                      |                 |                                |       |
| 1                    | 85              | 80 (58)                        | 5 (83) |
| 2                    | 26              | 25 (18)                        | 1 (17) |
| 3                    | 20              | 20 (15)                        | 0 (0)  |
| Lymphovascular invasion |        |                                |       |
| Positive             | 18              | 62 (45)                        | 2 (33) |
| Negative             | 125             | 75 (55)                        | 4 (67) |

Differences between proportions for categorical variables were analysed using chi-square tests.
cancer or other diseases, and 18 were due to endometrial cancer.

The RFS and OS did not significantly differ between patients with wild-type and mutated AKT1 (Fig. 2). The median RFS in patients with mutated and wild-type AKT1 were 77.8 months and not reached, respectively ($P = 0.13$). The median OS in patients with mutated and wild-type AKT1 was not reached and 183 months, respectively ($P = 0.21$). Multivariate analysis associated the initial stage and AKT1 mutation with OS (Additional Table 2).

**Discussion**

This is the first report of an association between clinico-pathological features and AKT1 mutation status in women with breast and endometrial cancers in Asia.

In the present study, we found AKT1 mutations in 7.3 and 4.1% of breast and of endometrial cancer samples. The reported frequency of AKT1 mutations in breast cancer is between 1.4 and 8.2% [5, 6], and they are associated with histogenesis of hormone receptor positive subtype [15]. Thus, our finding that AKT1 mutations are associated with HER2-negative status in breast cancer agrees with those of a previous study [15]. The TCGA (The Cancer Genome Atlas Program) data showed that in a predominantly Caucasian breast cancer cohort, AKT1 mutations had a frequency of 3% in most hormone receptor positive breast cancers [16]. On the other hand, a report from China also showed that AKT1 mutations were found in 7.1 and 8.2% of breast cancer patients [6, 17], suggesting that AKT1 mutations may be particularly common in Asians.

Previous reports have suggested that activation of the PI3K/AKT pathway is involved in the development of endometrioid adenocarcinoma [7, 8, 18], and that atypical hyperplasia is also associated with AKT1 mutations, leading to benign and (pre) malignant endometrial lesions [19]. In addition, AKT1 mutations were found in atypical hyperplasia, suggesting that AKT1 mutations may be involved in the carcinogenesis of endometrial carcinoma in benign and (pre) malignant endometrial lesions [19]. Louis J.M. van der Putten et al. reported We did not find a relationship between clinico-pathological features and AKT1 mutations because of the rarity of the mutation. However, none of the patients with mutated AKT1 died. A longer follow-up might reveal a correlation between AKT1 mutation status and its prognostic importance.

Our survival results themselves are difficult to say definitively due to the small number of cases. However, recently published results from the AACR Project GENIE, which incorporated a large group of estrogen receptor positive breast cancer patients, reported that AKT1 mutant cases had comparable survival rates compared to AKT1 wild-type controls [20].

Despite these positive findings, this study has three major limitations. The AKT1 mutation is rare, and rates of relapse or death are extremely low. Therefore, we cannot reach any conclusions on the survival based on prognostic factors. Recently, with the development of next generation sequencing (NGS), cancer genomic
profiling including AKT1 are now being evaluated using NGS tests. It is an important limitation that this study only examined AKT1 E17K hotspot mutation. Finally, there is fact that a large proportion of endometrial cancer is associated with a hypermutator phenotype, mainly due to MSI (microsatellite instability) and POLE (DNA polymerase epsilon) mutations. It is hard to distinguish the real AKT1 driver mutations from random mutations caused by this phenotype.

Precision medicine is becoming important in the treatment of cancer, and the AKT1 mutation is a promising target. Active point mutations (hot spots), are limited in breast and endometrial cancers, and E17K is a representative mutation site. The development of systems for detecting single mutations has progressed. Moreover, recent clinical trials have suggested that the AKT1 mutation is a potent predictive marker of a response to AKT inhibitors [3, 4]. Therefore, the AKT1 mutation might be a target of liquid biopsies, particularly in cell-free plasma DNA [21].

In conclusion, the rate of mutated AKT1 in breast cancer was 7.4%, and it correlated with the HER2-negative subtype. The rate in endometrial cancer was 4.1%. AKT1 mutations are associated with HER2-negative subtype in breast cancer and in endometrial cancer with endometrioid histology. The frequencies of AKT1 mutations in breast and endometrial cancers were similar between Asian and other regional women. The frequency of mutations is too low in both tumor types to talk about predictive significance.

Abbreviations
Akt: V-Akt murine thymoma viral oncogene; Bp: partial mastectomy; Bt: total mastectomy; ER: oestrogen receptor; FFPE: formalin-fixed paraffin-embedded; HER2: human epidermal growth factor receptor-2; HR: hormone receptor; IDCI: invasive ductal carcinoma; ILIC: invasive lobular carcinoma; LVI: lymphovascular invasion; mut: mutation; ND: no data; PgR: progesterone receptor

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12885-021-08869-3.

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Authors’ contributions
TS designed the study, TS and JH collected the data. TS statistically analyzed the data. TS, JH, KS, AS, EN, CS, MYunokawa, KY, HY, MYoshida, TKato, TKinoshita, TF, drafted the manuscript and read and approved the final version for publication. TS and JH contributed equally to the study, and all authors significantly contributed to the content of the manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
The study protocol was approved by the National Cancer Center Research Ethics Review Committee at the National Cancer Center Hospital, Tokyo, Japan (approval No. 2014 092). This study was conducted in accordance with the Declaration of Helsinki (2013). The requirement for informed consent was waived by the Institutional Review Board of the National Cancer Center Hospital, owing to the retrospective nature of the study. Patients could refuse to participate in this study in an opt-out form on the website of our institution.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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