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### 主 論 文

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(ミャンマーの乳癌症例ではヒト乳癌ウイルス (HMTV) の陽性率は低い)

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### 副 論 文

Molecular Subtypes of Breast Cancers from Myanmar Women: A Study of 91 Cases at Two Pathology Centers

(ミャンマー乳癌の分子サブタイプ : 2 施設 9 1 症例の検討)

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# 主 論 文

## Low prevalence of human mammary tumor virus (HMTV) in breast cancer patients from Myanmar

(ミャンマーの乳癌症例ではヒト乳癌ウイルス (HMTV) の陽性率は低い)

### **Introduction**

Breast cancer is a heterogeneous disease featured by distinct histomorphologic appearances and clinical behaviors. Although being the most prevalent cancer of women worldwide, little was known about its etiology. Like some other cancers, viruses might play a role. Mouse mammary tumor virus (MMTV) is a retrovirus that causes majority of murine mammary tumors. In 1995, viral sequences 90%–95% identical to MMTV were detected in 39% of human breast cancers in the United States. Subsequently, a nearly complete proviral structure of 9.9-kb size, which was 95% homologous to MMTV, was successfully identified from human breast cancers and designated as human mammary tumor virus (HMTV). Earlier studies reported HMTV prevalence rates of more than 30% in breast cancer cohorts in United States, Italy and Australia. So it seemed like HMTV involvement in human breast cancer is ubiquitous. However, later studies in United Kingdom, Austria, Germany and Sweden could not identify HMTV. We regarded this as an interesting point to explain why characteristics of breast cancers are diverse in different geographic regions. HMTV prevalence in Myanmar breast cancers has not been investigated yet though that of some other Asian countries have been revealed. We planned to investigate HMTV status in Myanmar breast cancer to gather important facts about its etiology as well as to contribute more epidemiological data to the interesting story of role of HMTV in human breast cancers.

### **Materials and Methods**

#### Study subjects

A set of 58 formalin-fixed paraffin-embedded breast cancer specimens collected from Myeik General Hospital (Myeik City, Myanmar) and Sakura Specialist Hospital (Yangon City, Myanmar) were investigated.

### DNA extraction

Genomic DNA was extracted from two to four 10- $\mu$ m-thick sections of breast cancer paraffin blocks.

### Semi-nested PCR for HMTV

Semi-nested PCR was performed with primers 5F and MR1 (1<sup>st</sup> PCR) and primers 5F and 2NR (2<sup>nd</sup> PCR) to amplify 189-bp HMTV sequence. PCR products were electrophoresed on gel and examined under ultraviolet light to identify positive band.

### Confirmation of HMTV sequence

For confirmation, positive band of PCR product was isolated from gel, purified and sequenced. Sequence alignments were performed by BLAST (NCBI).

### Exclusion of murine DNA contamination

Exclusion of murine DNA contamination is an essence for reporting of genuine existence of HMTV in human samples. To fulfill this, mouse-specific mitochondrial (mt) DNA semi-nested PCR was performed on HMTV-positive sample.

### Positive controls

To ascertain that experiment protocols were valid, we applied reliable positive controls. DNA extracted from RCB0526:Jyg-MC(A) murine mammary tumor cell line with high MMTV expression, was used as positive control for HMTV PCR. DNA extracted from the 4T1 murine cancer cell line was applied as positive control for mouse mtDNA PCR.

### Ethics

The study protocol was approved by the Ethics Committee of Okayama University and the Ethics Review Committee of Department of Medical Research of Yangon City (Myanmar).

## **Results**

### One case of Myanmar breast cancers revealed positive band by HMTV PCR

Only one out of 58 samples (1.7%) was positive for HMTV. The PCR reaction was confirmed by repeating the semi-nested PCR using newly extracted DNA from the paraffin block.

### Sequencing of the positive band revealed HMTV sequence

The complete 189-bp PCR product sequence was 98.9% homologous to the reference HMTV sequence (GenBank accession No. AF243039).

### Homology with other HMTV sequences at GenBank

The sequence was again 98.9% identical to another HMTV sequence from Vietnam (GenBank AY161347). On the other hand, homology to sequence of positive control (GenBank AK145002) was only 92%.

### Murine DNA contamination was excluded by mouse mtDNA PCR

Mouse mtDNA semi-nested PCR revealed no amplifiable murine DNA in positive sample. Sensitivity of this PCR was evaluated to be sensitive enough to amplify target DNA amount as low as 0.8pg.

### Characteristics of HMTV-positive case

The HMTV-positive case was identified as invasive carcinoma of no special type (NST) with histological grade III advanced breast cancer (T3, N1, M0).

## **Discussion**

To the extent of our knowledge, studies on the prevalence of HMTV sequences in human breast cancers have been conducted in 17 countries, including Myanmar (present study). Reported prevalence varied widely from 0% to 78% according to geographic regions. Studies in North and South America, Australia and Mediterranean countries revealed prevalence as high as 78% (range: from 12% to 78%; average: 49.4%). Conversely, zero prevalence was

reported in Central and Northern European nations. As for Asia, the same zero prevalence was revealed in one study in Japan and three studies in Iran. Near zero prevalence was shared between Vietnam and Myanmar (this study). Both Vietnamese study and ours detected only a single sequencing-confirmed HMTV-positive case with resultant prevalence of 0.8% and 1.7% respectively. More intriguingly, the two sequences identified shared 98.9% homology. We would like to suggest that these two South-East Asian countries might share some similar etiologic backgrounds.

We were concerned with studies in Pakistan and China which reported relatively high HMTV prevalence (20.0% and 16.8% respectively). After thorough review, it was revealed that confirmation by sequencing was conducted in only 2 out of 16 positive cases in the Pakistani study and never conducted in the Chinese study. A recent study in Iran claimed that 12% of their cases were HMTV-positive, but it, again, lacks the essential proof by sequencing. These data appear to be contingent and necessitate further confirmatory works.

Interestingly, Asian countries with zero or low HMTV prevalence like Japan, Vietnam and Myanmar have much lower breast cancer incidence if compared to countries with high HMTV prevalence. This difference suggests that these Asian countries might have been less affected by HMTV-related breast cancers while the latter countries suffered additional cases probably related to HMTV.

## **Conclusion**

In conclusion, our study disclosed HMTV prevalence in Myanmar breast cancer patients as the first report. In agreement with other Asian studies, the prevalence of HMTV in Myanmar was quite low. Frequency of positivity and sequence of HMTV identified closely resembled those from Vietnam. It is assumed that HMTV does not play a significant role in breast cancer carcinogenesis in most Asian populations.

## 副 論 文

### Molecular Subtypes of Breast Cancers from Myanmar Women: A Study of 91 Cases at Two Pathology Centers

(ミャンマー乳癌の分子サブタイプ：2施設91症例の検討)

Breast cancer is the most frequent cancer in Myanmar women. Molecular subtyping and the derived surrogate subtyping, based on immunohistochemistry markers of hormonal receptors, human epidermal growth factor receptor 2 (HER2) and Ki-67, has been a breakthrough in breast cancer pathology and management. Predominant subtypes were found to have some variations across racial/geographic differences. Histopathological characteristics and molecular subtypes in breast cancers from Myanmar were never comprehensively reported.

In this study, 91 breast cancers from Myanmar women were investigated. Immunohistochemistry tests were performed with antibodies to estrogen receptor (ER), progesterone receptor (PgR), HER2, Ki-67, cytokeratin (CK)5/6 and CK14. Immunohistochemistry-based molecular subtyping was conducted.

Ages of 91 cases ranged from 30 to 81 years with mean age of 51.3. Most of the cancers (94.5%) were invasive carcinoma of no special type (NST). Mitoses were frequent and not a single case of low histologic grade was spotted (grade II: 46.2%; grade III: 53.8%). Approximately 94% of cases were more advanced than stage I with lymph node metastases as frequent as 57.1%. Mean Ki-67 index was 33.9% (range; 4%-90%). Tumors with ER positive, PgR positive, and HER2 positive were 57.1%, 37.4%, and 28.6%, respectively. Subtyping revealed: luminal A (11.0%), luminal B (HER2<sup>-</sup>) (39.6%), luminal B (HER2<sup>+</sup>) (6.6%), HER2 (22.0%), triple negative (TN)-basal-like (12.1%), TN-null (8.8%).

Literature search of molecular subtype patterns revealed that subtype distribution of Myanmar breast cancers was comparable to those of other Asian countries: HER2 and TN subtypes were more common compared to Western countries.

In conclusion, we reported the comprehensive clinicopathological profile of breast cancers from Myanmar women. Myanmar breast cancers were revealed as aggressive tumors, as evidenced by high frequency of subtypes with poor prognosis and high Ki-67 index.

## Relevance to “Main research paper”

Breast cancers are well characterized as highly heterogeneous in clinical behavior although vast majority are just occupied in the usual histologic type of invasive carcinoma (NST). The main research paper has focused on detection of HMTV as a measure to explore the etiologic background of Myanmar breast cancers. Meanwhile, the supplementary research paper gave special attention to clinicopathologic profiling and molecular subtyping which provided fundamentally important background data of Myanmar breast cancers, that were never comprehensively reported yet. In addition, the finding of low prevalence of HMTV in Myanmar in parallel with zero or low prevalence of HMTV in other Asian countries highlighted the necessity to check whether breast cancer molecular subtyping patterns of Asian countries are similar. The supplementary research paper provided the information that subtype distribution of Myanmar breast cancers was also comparable to other Asian countries.

Therefore, although direct citation was not made at publications, main and supplementary research papers were complementary to each other giving essentially important knowledge about Myanmar breast cancer which is a leading cause of morbidity and mortality in Myanmar women.