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Genetic and Clinical Characteristics of Phyllodes Tumors of the Breast

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Abstract

PURPOSE: Phyllodes tumors (PTs) of the breast are rare, accounting for less than 1% of all breast tumors. Among PTs, malignant PTs (MPTs) have malignant characteristics and distant metastases occur in about 20% to 30% of MPTs. However, there is no effective treatment for MPTs with distant metastasis, resulting in an abject prognosis. We performed targeted deep sequencing on PTs to identify the associations between genetic alterations and clinical prognosis. *METHODS:* We performed targeted deep sequencing to evaluate the genetic characteristics of PTs and analyzed the relationships between clinical and genetic characteristics. *RESULTS:* A total of 17 PTs were collected between 2001 and 2012. Histologic review was performed by pathologists. The samples included three benign PTs, one borderline PT, and 13 MPTs. The most frequently detected genetic alteration occurred in the *TERT* promoter region (70.6%), followed by *MED12* (64.7%). *EGFR* amplification and *TP53* alteration were detected in four MPTs without genetic alterations in *MED12* and *TERT* promoter regions. Genetic alterations of *RARA* and *ZNF703* were repeatedly found in PTs with local recurrence, and genetic alterations of *SETD2*, *BRCA2,* and *TSC1* were detected in PTs with distant metastasis. Especially, MPT harboring *PTEN* and *RB1* copy number deletion showed rapid disease progression. *CONCLUSIONS:* In this study, we provide genetic characterization and potential therapeutic target for this rare, potentially lethal disease. Further large-scale comprehensive genetic study and functional validation are warranted.

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Introduction

Phyllodes tumors (PTs) of the breast are rare, accounting for less than 1% of all breast tumors [1]. In contrast to invasive carcinoma of breast, PTs develop in the mesenchymal fibroepithelial tissues [2]. Based on the pathologic degree of stromal cellularity, atypia, stromal overgrowth, status of tumor border and mitoses, the World Health Organization categorized PTs as benign, borderline, and malignant [2,3].

Malignant phyllodes tumors (MPTs) frequently result in tumor recurrence and distant metastasis compared to benign and borderline PTs [1–3]. Approximately 20% to 30% of MPTs follow a distantly metastatic disease course [2]. Treatment of MPTs is complete surgical excision, as for other breast cancers [4,5], and adjuvant radiation therapy has benefit in some cases of MPT [6]. MPTs with distant metastasis show considerable morbidity with rapid progression and are treated with chemotherapy. However, chemotherapy for metastatic MPTs is rarely effective, and these tumors have a dismal prognosis [1].

Recent genetic studies provided information on genetic alterations and therapeutic clues for MPT. Somatic mutation of mediator complex subunit 12 (*MED12*), the mediator complex between transcription factors and the RNA polymerase II initiation complex, was frequently observed in PTs [7]. Nearly all somatic mutations occurred in exon 2 of *MED12* and were more frequently detected in benign PTs compared with MPTs [8,9]. In addition, MPTs have been reported to be associated with other somatic mutations in *TP53*, *RB1,* and *EGFR* [7].

In addition, next-generation sequencing revealed complex genetic alterations of PTs. Somatic mutation in *TERT* was reported to cooperate with *MED12* alteration in PTs, and mutations in other genes including *TP53, EGFR, NF1, CDKN2A, CDKN2B,* and *RARA* were also observed [10,11]. In these studies, MPTs had more genetic alterations than benign and borderline PTs [7], but there was no difference in the number of mutations between primary and metastatic lesions [11].

In spite of comprehensive genetic studies of MPTs, associations between genetic alterations and clinical prognosis have not been revealed. Even though MPTs have malignant characteristics, distant metastasis does not occur in 80% of MPTs [1,2]. However, there is no effective treatment for MPTs with distant metastasis, and patients with these tumors have a dismal prognosis.

In this study, we performed targeted deep sequencing on MPTs and analyzed the relationships between genetic alterations and clinical characteristics, including prognosis.

Materials and Methods

Patients

This study involved prospective explorative analysis of patients with PT of the breast at Samsung Medical Center and Seoul National University Hospital. Women who were diagnosed with PT of the breast by diagnostic examination and pathologic review and received curative surgery were enrolled. All patients provided written informed consent, and study approval was obtained from the Institutional Review Board of Samsung Medical Center, Seoul, Korea (IRB No: SMC 2017–04-073).

Pathologic Grade

Experienced pathologists reviewed all pathology specimens to determine benign, borderline, or malignant PT. They also described the following tumor characteristics: pathologic grade, stromal cellularity, atypia, stromal overgrowth, status of tumor border, mitosis per 10 high power fields (10HPF), tumor size, and necrosis.

DNA Extraction

Unstained sections (4 mm) of tumors consisting of more than 75% malignant cells were dissected under microscopy by comparison with an H&E-stained slide, and genomic DNA was extracted using a Qiagen DNA FFPE Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. After extraction, DNA concentration and 260/280-nm and 260/230-nm ratios were measured by spectrophotometry (ND1000, NanoDrop Technologies, ThermoFisher Scientific, MA). Each sample was then quantified using a Qubit fluorometer (Life Technologies, Carlsbad, CA). Libraries were prepared from samples with total genomic DNA yield >10 ng.

Targeted Deep Sequencing Using a Customized Cancer Panel (CancerSCAN™)

Genomic DNA (250 ng) from each tissue was sheared in a Covaris S220 ultrasonicator (Covaris, Woburn, MA) and used with CancerSCAN™ probes and a SureSelect XT reagent kit HSQ (Agilent Technologies) for construction of a library according to the manufacturer's protocol.

This panel is designed to enrich exons of 381 genes (Supplementary Table 1), covering 366.2 kb of the human genome. After enriched exome libraries were multiplexed, the libraries were sequenced on a HiSeq 2500 sequencing platform (Illumina). Briefly, a paired-end DNA sequencing library was prepared through gDNA shearing, end-repair, A-tailing, paired-end adaptor ligation, and amplification. After hybridization of the library with bait sequences for 27 hours, the captured library was purified and amplified with an index barcode tag, and the library quality and quantity were assessed. Sequencing of the exome library was performed using the 100-bp paired-end mode of the TruSeq Rapid PE Cluster Kit and TruSeq Rapid SBS Kit (Illumina).

Variant Detection Using the Customized Cancer Panel

Sequence reads were mapped to the human genome (hg19) using Burrows-Wheeler Aligner (BWA) [12]. Duplicate read removal was performed using Picard and SAMtools [13]. Local alignment was optimized using the Genome Analysis Toolkit (GATK) [14]. Variant calling was performed only in regions targeted in CancerSCAN™. To detect single nucleotide variants, we integrated the results of three kinds of variant caller, which increased the sensitivity [15–17]. Pindel was used to detect indels [18]. Copy number variations were calculated for targeted regions by dividing the read depth per exon by the estimated normal reads per exon using an in-house reference.

Results

Clinicopathological characteristics

A total of 17 PTs were collected between 2001 and 2012 (Table 1). Histologic review by pathologists revealed three benign PTs, one borderline PT, and 13 MPTs. All patients were female, and the median age was 45.7 years (range 26.2-72.2). All patients underwent curative surgery, and two patients received adjuvant radiotherapy.

All specimens were primary breast PT tissues except for one metastatic lung tissue. Disease recurrence occurred in five patients;

Table 1. Clinicopathologic Characteristics (N = 17)

Characteristics	N (%)
Age at diagnosis (median)	45.8
<40 years	5 (29.4)
40–60 years	9 (53.0)
≥60 years	3 (17.6)
Grade	
Malignant	13 (76.4)
Borderline	1 (5.9)
Benign	3 (17.7)
Mitosis (/10HPF)	
≥20	5 (29.4)
10–20	7 (41.2)
<10	4 (23.5)
Unknown	1 (5.9)
Cellularity	
High	7 (41.2)
Moderate	7 (41.2)
Low	2 (11.8)
Unknown	1 (5.9)
Necrosis	
Present	5 (29.4)
Absent	1 (5.9)
Unknown	11 (64.7)
Stromal atypia	
Present	15 (88.2)
Absent	1 (5.9)
Unknown	1 (5.9)
Stromal overgrowth	
Present	4 (23.5)
Absent	3 (17.6)
Unknown	10 (58.8)
Tumor border	
Infiltrating	5 (29.4)
Pushing	6 (35.3)
Well-defined	5 (29.4)
Unknown	1 (5.9)
Radiotherapy	
Yes	3 (17.6)
Chemotherapy	
Yes	2 (11.8)
Recurrence	
Local recurrence	3 (17.6)
Distant metastasis	2 (11.8)
No	12 (70.6)

three with local recurrence and two with lung metastasis (Supplementary Figure 1). One patient with lung metastasis received palliative chemotherapy and radiotherapy, but she died due to disease progression.

Genetic Alterations in Phyllodes Tumors

Among 381 genes included, the most frequently detected site of genetic alteration was the *TERT* promoter region (12 of 17 cases, 70.6%) (Figure 1). The major alteration was a G>A transition in the promoter region (12 of 13 cases), and one intra-chromosomal translocation was detected. Genetic alteration of *MED12* was also frequently detected (11 of 17, 64.7%). Nonsynonymous single nucleotide variants (SNVs) of *MED12* were detected in seven PTs and non-frame shift indels in four PTs. Genetic alterations of *TERT* and *MED12* were not mutually exclusive, and 10 cases of PT had genetic alterations in both genes.

Three benign PTs harbored both *TERT* promoter and *MED12* genetic alterations, whereas other genetic alterations were not commonly observed. One borderline PT had only genetic alteration in the *TERT* promoter.

After *MED12* and *TERT*, frameshift deletion of *RAD50* was the most commonly detected genetic alteration, followed by *SETD2* (Figure 2). Genetic alteration of *RAD50* and/or *SETD2* was detected in only borderline and malignant PTs, not in benign PTs.

We found four MPTs that did not have genetic alterations in both *MED12* and *TERT* promoter regions. For these four PTs, *EGFR* amplification was detected in two and *TP53* alteration in three cases. *PIK3CA* H1047R mutation was observed in one PT without *EGFR* or *TP53* genetic alteration. Genetic alterations of *ARID1B*, *DNMT3A*, *FGFR4,* and *RAD50* were also observed in more than one PT.

Genetic Characterization of Malignant Phyllodes Tumor According to Tumor Recurrence

Of 12 MPTs, five experienced tumor recurrence (Table 1 and Figure 1). No recurrence was observed in benign and borderline PTs. Three cases were local recurrence, and two were pulmonary metastasis. All primary tumors with metastasis had genetic alteration of *MED12* or *TERT* promoter.

Besides these two genes, all MPTs with local recurrence had genetic alteration of *RARA*, but other genetic alterations were not repeatedly observed. Two PTs with pulmonary metastasis had a similar genetic landscape of *BRCA2* nonsynonymous SNV, *SETD2* and *TSC1* genetic alterations. One PT had *NRAS, PALB2,* and *PTCH2* SNVs, and the other had *NCOR1* and *PDGFRB* SNVs and copy number deletions in *PTEN* and *RB1*.

Lastly, we compared genetic alterations between primary tissue and lung metastasis (SMC1411 and SMC1412). This analysis showed a similar genetic profile between the two samples, but more genetic alterations, including *ASXL1*, *DNMT,1* and *ZNF217*, were observed in the pulmonary metastatic lesion.

Discussion

In this study, genetic alterations of *TERT* promoter and *MED12* were the most frequently observed alterations in all subtypes of PT, as in previous genetic studies. We also found that *EGFR* amplification and/or *TP53* alteration were possible driver mutations in PTs without *TERT* and *MED12* alterations. Genetic alteration of *RAD50*, a DNA repair gene, was also frequently detected in PTs, and *BRCA2* SNVs were only detected in MPT with recurrence. Breast mesenchymal fibroepithelial tumors comprise benign fibroadenomas and PTs. While fibroadenoma commonly occurs, PT is rarely detected [19]. Large scaled genetic studies for mesenchymal fibroepithelial tumors showed that *MED12* was the most commonly mutated gene in both fibroadenomas and PTs [20,21]. Other genetic studies also reported that a *MED12* mutation was one of the most common and ancestral genetic alteration in PT [8,22,23]. Among these studies, about 60% of fibroadenomas had a *MED12* somatic mutation [21]. In terms of PTs, one showed that 70% of PTs had a *MED12* genetic alteration regardless of histologic grade [22], and others reported that *MED12* genetic alteration was more frequently detected in benign and borderline PTs compared with MPTs [8,20,23].

Recent comprehensive genomic profiling of MPTs using a next-generation sequencing technique showed that approximately 50% of MPTs had *MED12* short variants [11]. Regardless of PT grade, 72% of PTs had *MED12* short variants [20]. In this study, we sequenced the entire exons of *MED12* and found that 7 of 12 MPTs had *MED12* alterations. Especially, we could specify that 5 of 7 were nonsynonymous SNVs, and two were non-frame shift deletions. All genetic events of *MED12* occurred in exon 2.

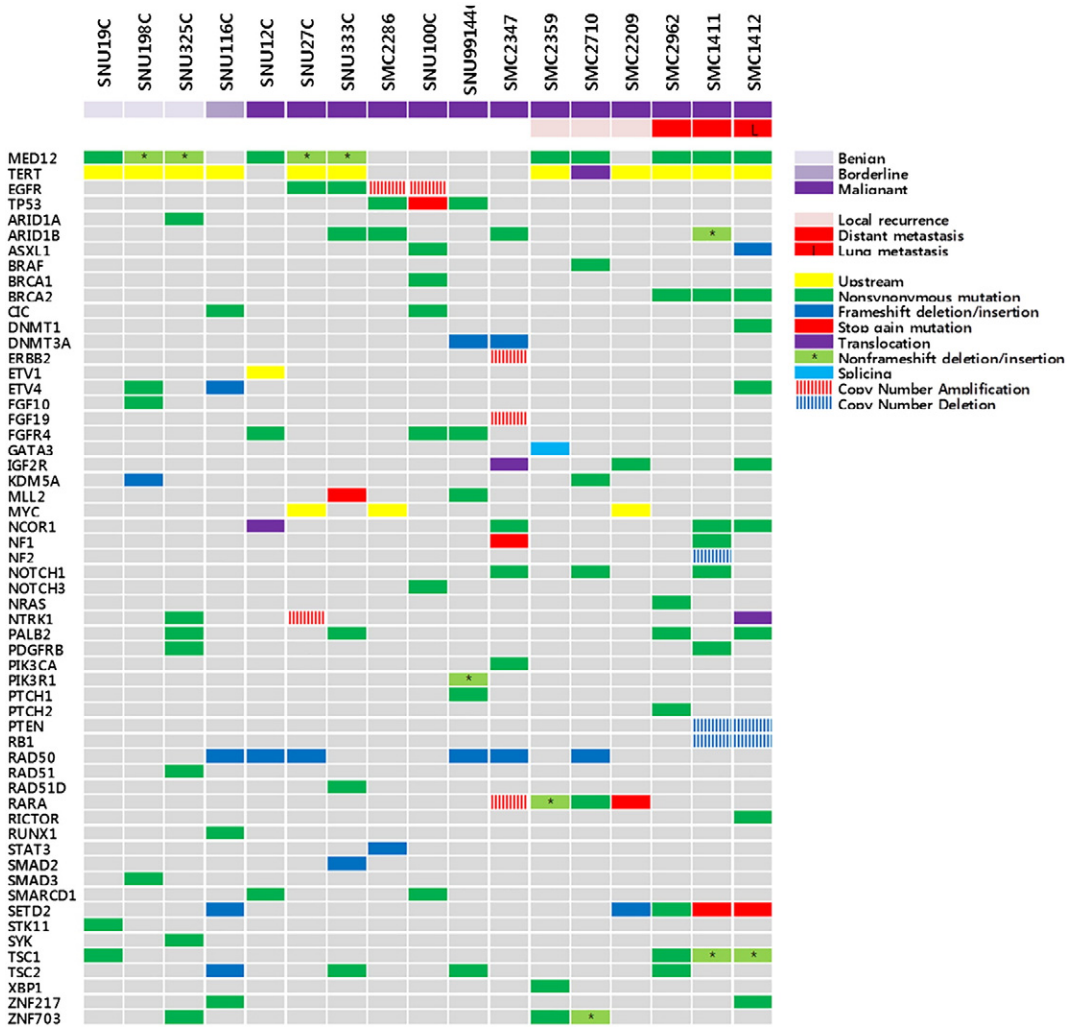

Figure 1. Genetic landscape of phyllodes tumors (N = 17).

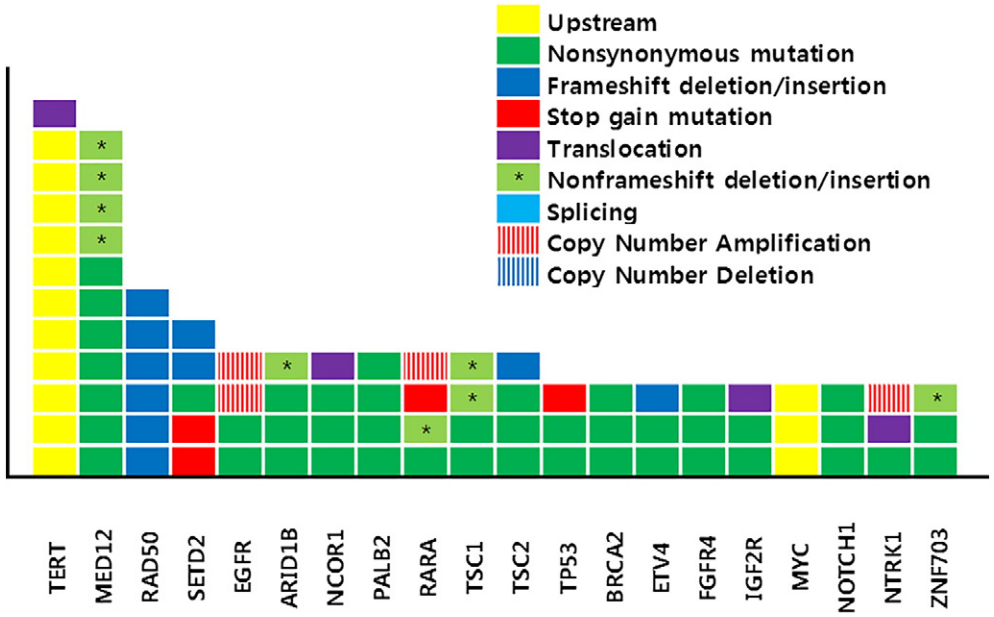

Figure 2. Frequency of genetic alterations identified in phyllodes tumors (N = 17).

In addition to *MED12*, the *TERT* promoter was a repeatedly mutated region in PTs. A previous study reported that 11 of 18 tested malignant PTs had genetic variants in the *TERT* promoter [11], and another study showed that 60–70% of PTs had *TERT* genetic alterations [10]. Our study yielded similar results. All benign and borderline PTs and 7 of 12 MPTs had genetic aberration in the *TERT* promoter. In addition, all *TERT* genetic alterations were found together with *MED12* variants with the exception of one MPT.

MPTs without *TERT* and *MED12* genetic alterations had variant genetic alterations. *EGFR* amplification and *TP53* and *DNMT3A* mutations were repeatedly observed, and we suggest that these mutations possibly initiate tumorigenesis in the absence of *TERT* and *MED12* alterations. One malignant PT without any of the above genetic alterations had *PIK3CA* H1047R SNV. In a previous study, *PIK3CA* mutation was present in malignant PT without *MED12* alteration [11,20]. They showed that genetic alterations of *NF1, RB1, PIK3CA, EGFR* and *TP53* occurred in only borderline and malignant PTs [20]. Therefore, we suggest that this malignant PT might have similar genetic characteristics to invasive carcinoma of the breast. In terms of *TP53*, a previous study reported that mutations were frequently detected regardless of the status of *MED12* or *TERT* [11,20]. However, we did not find *TP53* co-mutation with *MED12* or *TERT* promoter alterations.

Lastly, we attempted to identify genetic characteristics associated with tumor recurrence. Genetic alterations of *RARA* and *ZNF703* [11] were repeatedly found in PTs with local recurrence, and genetic alterations of *SETD2*, *BRCA2,* and *TSC1* were detected in PTs with distant metastasis. Previous genomic study of fibroepithelial tumor of breast already reported *RARA*, *SETD2* and other genetic mutations being potential therapeutic targets [20]. But they did not consider clinical outcome of PTs. In our study, one case of MPT harboring *PTEN* and *RB1* copy number deletion had rapid disease progression despite repeated metastatectomy and palliative chemotherapy and radiotherapy. Interestingly, targeted agents for these genetic alterations already exist. PARP inhibitor targeting *BRCA2* and mTOR/AKT inhibitor for *TSC1* would be potential target agents for the treatment of metastatic MPTs [24,25].

In this study, we performed genetic analysis of PTs using targeted deep sequencing. We also characterized the genetic aberrations of metastatic PTs, and the identified genetic alterations are candidate therapeutic targets of small molecules. In spite of the small sample size, our study provides insight into not only the genetic characterization, but also therapeutic guidelines for this rare and potentially lethal disease. Further large-scale comprehensive genetic studies and functional validation will provide a fundamental understanding of the genetic characteristics of phyllodes tumor and clues to effective therapeutic strategies.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tranon.2017.10.002.

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