Modern Pathology (2021) 34:1320–1332
https://doi.org/10.1038/s41379-021-00787-w

ARTICLE

Genetic differences between benign phyllodes tumors and fibroadenomas revealed through targeted next generation sequencing

Cedric Chuan Young Ng[1] · Nur Diyana Md Nasir[2] · Benjamin Nathanael Loke[2,3,4] · Timothy Kwang Yong Tay[2] · Aye Aye Thike[2,3] · Vikneswari Rajasegaran[1] · Wei Liu[1] · Jing Yi Lee[1] · Peiyong Guan[1,5] · Abner Herbert Lim[1] · Kenneth Tou En Chang[6] · Mihir Ananta Gudi[6] · Preetha Madhukumar[7,8] · Benita Kiat Tee Tan[7,8,9] · Veronique Kiak Mien Tan[7,8] · Chow Yin Wong[7,8] · Wei Sean Yong[7,8] · Gay Hui Ho[7] · Kong Wee Ong[7] · International Fibroepithelial Consortium · George Wai Cheong Yip[4] · Boon Huat Bay[4] · Patrick Tan[3] · Bin Tean Teh[1,3] · Puay Hoon Tan[2,3,4,10]

Received: 20 August 2020 / Revised: 17 February 2021 / Accepted: 17 February 2021 / Published online: 16 March 2021
© The Author(s), under exclusive licence to United States & Canadian Academy of Pathology 2021

Abstract

Breast fibroepithelial lesions are biphasic tumors which comprise the common benign fibroadenomas (FAs) and the rarer phyllodes tumors (PTs). This study analyzed 262 (42%) conventional FAs, 45 (7%) cellular FAs, and 321 (51%) benign PTs contributed by the International Fibroepithelial Consortium, using a previously curated 16 gene panel. Benign PTs were found to possess a higher number of mutations, and higher rates of cancer driver gene alterations than both groups of FAs, in particular *MED12*, *TERT* promoter, *RARA*, *FLNA*, *SETD2*, *RB1*, and *EGFR*. Cases with *MED12* mutations were also more likely to have *TERT* promoter, *RARA*, *SETD2*, and *EGFR*. There were no significant differences detected between conventional FAs and cellular FAs, except for *PIK3CA* and *MAP3K1*. *TERT* promoter alterations were most optimal in discriminating between FAs and benign PTs. Our study affirms the role of sequencing and key mutations that may assist in refining diagnoses of these lesions.

Introduction

Breast fibroepithelial lesions comprise a family of biphasic tumors that display variations in their biology and clinical management. They include the common benign fibroadenomas, as well as the rarer phyllodes tumors, which can be benign, borderline, or malignant [1, 2]. The pathogenesis of breast fibroepithelial lesions is not completely understood,

These authors contributed equally: Cedric Chuan Young Ng, Nur Diyana Md Nasir, Benjamin Nathanael Loke

Members of the International Fibroepithelial Consortium are listed below Acknowledgements

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41379-021-00787-w.

✉ Bin Tean Teh
teh.bin.tean@singhealth.com.sg

✉ Puay Hoon Tan
tan.puay.hoon@singhealth.com.sg

1 Laboratory of Cancer Epigenome, National Cancer Centre Singapore, Singapore, Singapore

2 Department of Anatomical Pathology, Singapore General Hospital, Singapore, Singapore

3 Duke-NUS Medical School, Singapore, Singapore

4 Department of Anatomy, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

5 Quantitative Biology and Medicine Programme, Duke-NUS Medical School, Singapore, Singapore

6 Department of Pathology and Laboratory Medicine, KK Women's and Children's Hospital, Singapore, Singapore

7 Division of Surgery and Surgical Oncology, National Cancer Center Singapore, Singapore, Singapore

8 Department of Breast Surgery, Singapore General Hospital, Singapore, Singapore

9 Department of Surgery, Sengkang General Hospital, Singapore, Singapore

10 Division of Pathology, Singapore General Hospital, Singapore, Singapore

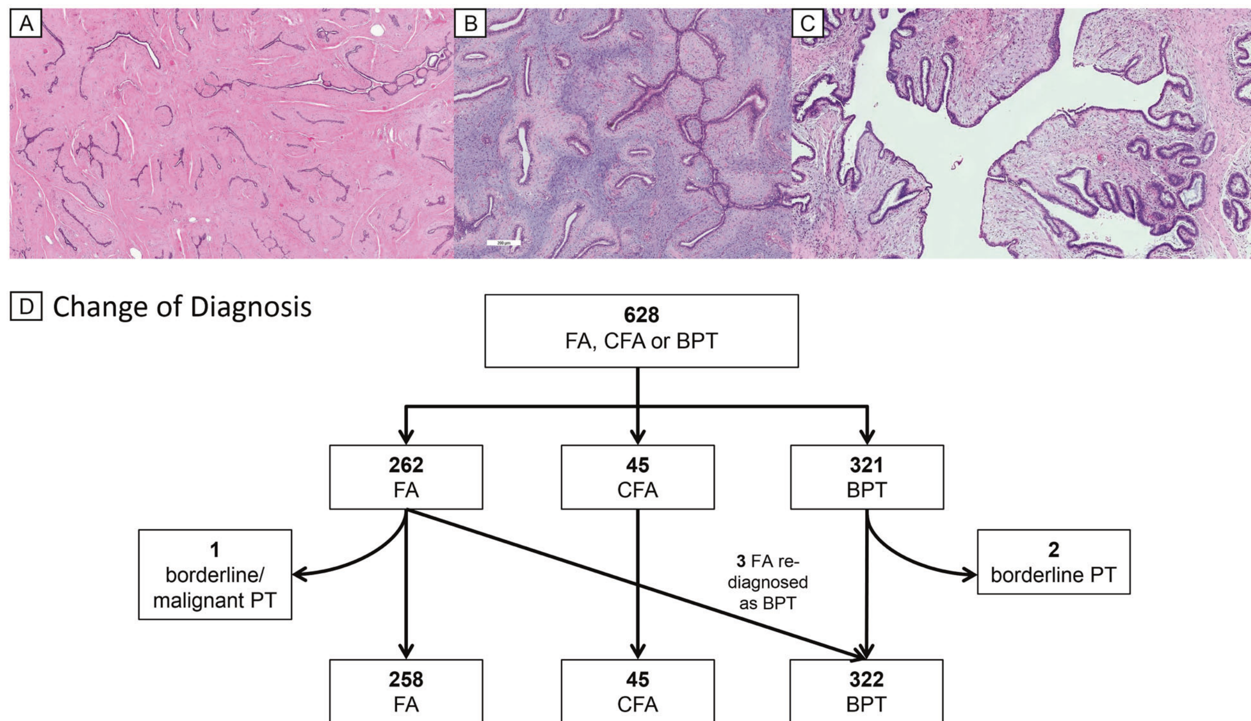


Fig. 1 Histological images of fibroepithelial lesions and our study cohort. A Light microscopy image at low magnification displaying a conventional fibroadenoma with intracanalicular patterns. **B** Cellular fibroadenoma shows slightly increased cellularity. **C** Benign phyllodes tumor with prominent fronds at ×50 magnification. **D** Change in diagnoses after genomic analysis. FA: conventional fibroadenoma, CFA: cellular fibroadenoma, BPT: benign phyllodes tumor.

with a paucity of studies outlining their developmental mechanisms [3, 4]. In recent years, there have been new findings from genomic studies of these tumors [5–10], with *MED12* being the most frequently aberrant gene, where the majority of mutations occur in codon 44 of exon 2 [5, 11, 12]. Genomic features have also suggested that fibroadenomas may be non-obligate precursors of phyllodes tumors [6, 13].

Histologically, fibroadenomas exhibit biphasic proliferation of stromal and epithelial compartments. The cellular variant of the fibroadenoma is characterized by mild to moderately increased stromal cellularity (Fig. 1). Phyllodes tumors also exhibit increased stromal cellularity but are accompanied by an exaggerated intracanalicular growth pattern with leaf-like stromal proliferations often referred to as fronds [14, 15]. Periductal stromal condensation may be observed. Between 60 and 75% of phyllodes tumors are diagnosed as benign, with borderline and malignant varieties at 15–20% and 10–20%, respectively [16, 17]. Phyllodes tumors may grow more rapidly than fibroadenomas on follow-up ultrasonography [18], but they cannot be reliably differentiated by imaging [19–22].

On histology, the differential diagnosis between cellular fibroadenomas and benign phyllodes tumors can prove challenging [3]. This was highlighted in an interobserver study involving ten breast pathologists evaluating 21 histologically challenging fibroepithelial lesions. Individual diagnoses varied from fibroadenoma to borderline phyllodes tumor in 9 (43%) of 21 cases. Of note, diagnoses were split equally (5/5) or nearly equally (6/4) between cellular fibroadenoma and benign phyllodes tumor in four cases [23].

While proliferative parameters such as mitotic activity and Ki67 labeling index have been applied in evaluating cellular fibroepithelial lesions, no consistent thresholds can be used for individual cases. This may restrict the value of such an approach to differentiating lesions within the fibroepithelial spectrum [24–27]. We have previously described a 5-gene RT-PCR signature that can distinguish between fibroadenoma and phyllodes tumor on core needle biopsies. However, the study evaluated mostly conventional fibroadenomas and further investigations into cellular fibroadenomas are needed [28]. We recently published the genomic characterization of an international series of breast fibroepithelial lesions [29]. Here, we delve deeper into specific comparisons among conventional fibroadenomas, cellular fibroadenomas, and benign phyllodes tumors derived from the cohort, to determine key mutations that may assist in refining their diagnoses.

Table 1 Panel of 16 genes interrogated in the study cohort.

MED12	*RARA*	*EGFR*	*PTEN*
TERT promoter	*FLNA*	*RB1*	*ERBB4*
SETD2	*NF1*	*BCOR*	*IGF1R*
KMT2D	*PIK3CA*	*TP53*	*MAP3K1*

Materials and methods

Formalin-fixed paraffin-embedded (FFPE) samples for a total of 628 adult fibroepithelial tumors [262 (42%) conventional fibroadenomas, 45 (7%) cellular fibroadenomas, and 321 (51%) benign phyllodes tumors] were obtained from institutions within the International Fibroepithelial Consortium in our initial study cohort.

DNA extraction and targeted next generation sequencing

One representative paraffin block of the tumor was selected from each case and eight sections of FFPE tissue, each measuring ten microns thick, were cut from the selected blocks. Genomic DNA was then extracted with the QIAamp DNA FFPE extraction kit (Qiagen, Valencia, CA, USA). The DNA yield and quality were determined using the PicoGreen® dsDNA quantitation assay (Thermofisher, Waltham, MA, USA).

The extracted DNA was then subjected to ultra-deep amplicon-based sequencing using the Illumina HiSeq 4000 platform. The assay encompasses a concise curated panel of 16 genes (Table 1) that was established based on the information derived from our group's earlier studies [5, 6]. Sequencing was performed at a depth of at least 100× of the target regions for all samples.

Low-pass copy number variation (CNV) analysis

In total, 45 FFPE tissues comprising 15 conventional fibroadenomas, 15 cellular fibroadenomas, and 15 benign phyllodes tumors were selected for low-pass whole genome sequencing (WGS) to a depth of 5–10×. An additional ten blood samples were chosen as a panel of normal for this experiment. Sequencing was performed on the Illumina HiSeq platform with a paired end configuration of 150 base pairs.

Biocomputational analysis

Biocomputational analysis was done according to methods described in our previous study [29]. The FastQC software package (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was utilized to perform quality checking of the FASTQ sequence files. After removing adapters, trimmed paired reads were mapped to hg19 (hs37d5) [30] using BWA-MEM (v0.7.15-r1140) [31] and sorted/indexed with samtools [32]. FreeBayes (v1.1.0-4-gb6041c6, settings: -m 30 -q 30 -F 0.01 -u) [33] and wANNOVA [34] were applied to call and annotate variants, respectively. Annotated variants were first filtered according to a few criteria: (1) only variants with more than 100X coverage and variant allele frequency of at least 5% were retained; (2) minor allele frequency of the variant in the normal population must be zero; (3) synonymous and non-exonic variants were also excluded, and (4) variants in dbSNP [35] were excluded, unless they are in COSMIC [36] or ClinVar [37, 38]. Manual curation of variants passing these filters was done on the Integrative Genomics Viewer [39] to remove any sequencing artefacts. R (R Foundation for Statistical Computing, Vienna, Austria) and the statistical software SPSS for Windows, version 25 (SPSS, Inc., Chicago, IL, USA) were used for statistical analysis. The relationship between the variant frequencies and the different FEL subgroups was analyzed using the χ^2 and Fisher's exact tests. A p value of < 0.05 was considered to be significant.

For the low-pass CNV, sequencing reads were aligned to human reference genome NCBI GRC Build 37 using BWA-MEM followed by duplicate marking and base-score recalibration using GATK version 4.14 for post alignment data processing.

ichorCNA was used as the method of estimating and accessing copy number alterations in this experiment. Firstly, a panel of normal was generated using the chosen germline samples which were identically processed and sequenced as the FFPE tissues to improve accuracy for estimating copy number alteration while correcting for systematic biases. This was performed with the help of an Rscript included with ichorCNA package. Once a panel of normal was generated copy number alterations were then identified using a bin size of 1 Mb. Downstream processes and illustrations were then analyzed using R.

Measuring sensitivity and specificity of the assay

Mutations of fibroadenomas and benign phyllodes tumors were compared to evaluate the assay's performance. We classified the status of true- and false-positive and true- and false-negative categories based on the comparison groups. We calculated per cent sensitivity as 100 × [true positive/(true positive + false negative)], and specificity as 100 × [true negative/(true negative + false positive)]. Receiver-operating characteristic curves (ROC) and areas under the curve (AUC) were plotted to derive the mutation that could give optimal discrimination.

Table 2 Number of mutations and cancer driver mutations in each of the three tumor types.

	Total ($n = 625$)	Conventional FA ($n = 258$)	Cellular FA ($n = 45$)	Conventional FA and cellular FA ($n = 303$)	Benign PT ($n = 322$)	p value FA vs CFA vs BPT	p value FA and CFA vs BPT	p value CFA vs BPT	p value FA vs CFA
No. of mutations									
0	158 (25%)	91 (35%)	13 (29%)	104 (34%)	54 (17%)	<0.001*	<0.001*	0.004*	0.247
1	213 (34%)	102 (40%)	18 (40%)	120 (40%)	93 (29%)				
2	150 (24%)	45 (17%)	10 (22%)	55 (18%)	95 (30%)				
3	66 (11%)	16 (6%)	3 (7%)	19 (6%)	47 (15%)				
4	24 (4%)	4 (2%)	0 (0%)	4 (1%)	20 (6%)				
5	9 (1%)	0 (0%)	0 (0%)	0 (0%)	9 (3%)				
6	5 (1%)	0 (0%)	1 (2%)	1 (0%)	4 (1%)				
No. of cancer driver mutations									
0	503 (80%)	219 (85%)	35 (78%)	254 (84%)	249 (77%)	0.008*	0.016*	0.886	0.155
1	101 (16%)	35 (14%)	8 (18%)	43 (14%)	58 (18%)				
2	19 (3%)	4 (2%)	2 (4%)	6 (2%)	13 (4%)				
3	2 (0.3%)	0 (0%)	0 (0%)	0 (0%)	2 (1%)				

FA conventional fibroadenoma, *CFA* cellular fibroadenoma, *BPT* benign phyllodes tumor.

*Statistically significant with p value of < 0.05.

Histological review of cases

After genomic analysis, cases with available haematoxylin and eosin (H&E) slides and found to harbor unusual genotypes such as presence of cancer driver mutations (*TP53*, *RB1*, *NF1*, *PTEN*, *PIK3CA*, *EGFR*, *BCOR*, *ERBB4*, *MAP3K1*, *IGF1R*), or those with more than two mutations in non-cancer driver genes were histologically reviewed.

Results

Clinicopathological correlations

Our study cohort initially consisted of 628 cases of conventional fibroadenomas, cellular fibroadenomas, and benign phyllodes tumors. Ethnicity data were available for 605 cases. There were 517 (82%), 88 (14%), and 23 (4%) cases of Asian, Caucasian, or unknown descent, respectively. Three cases were re-diagnosed after histological review as borderline/malignant phyllodes tumors, thus excluding them from this study (Fig. 1). The following calculations for mutation rates are thereby based on the remaining 625 cases.

One hundred and fifty-eight (25%) cases showed no mutations, with 213 (34%), 150 (24%), 66 (11%), 24 (4%), 9 (1%), and 5 (1%) showing 1, 2, 3, 4, 5, or 6 mutations accordingly (Table 2). Benign phyllodes tumors harbored more mutations (mean 1.79), compared to cellular fibroadenomas (mean 1.18) and conventional fibroadenomas (mean 1.03) ($p < 0.001$). No significant differences were found between fibroadenomas and cellular fibroadenomas in terms of the number of mutations ($p = 0.247$). Benign phyllodes tumors were more likely to have a higher number of alterations in cancer driver genes than both groups of fibroadenomas ($p = 0.008$).

Fibroadenomas and benign phyllodes tumors possess distinct mutations

MED12 mutations were observed in 334 (53%) cases, followed by the *TERT* promoter, *KMT2D,* and *RARA* with aberrations in 122 (20%), 89 (14%), and 82 (13%) cases, respectively (Table 3 and Fig. 2). Benign phyllodes tumors showed the highest *MED12* mutation rate with 199/322 (62%) cases harboring alterations in this gene, compared to 113/258 (44%) conventional fibroadenomas and 22/45 (49%) cellular fibroadenomas ($p < 0.001$). Further analysis revealed significant differences in mutation rates for six other genes in benign phyllodes tumors compared to conventional and cellular fibroadenomas (Table 3). These were *TERT* promoter (32 vs 6 vs 4%, $p < 0.001$), *RARA* (17 vs 8 vs 13%, $p = 0.001$), *FLNA* (13 vs 6 vs 4%, $p = 0.002$), *SETD2* (12 vs 4 vs 7%, $p < 0.001$), *RB1* (3 vs 1 vs 0%, $p = 0.025$), and *EGFR* (5 vs 2 vs 4%, $p = 0.027$). Conventional and cellular fibroadenomas did not differ significantly in their mutation spectrum except for *PIK3CA* (2 vs 9%, $p = 0.011$) and *MAP3K1* (1 vs 4%, $p = 0.047$). Across our study cohort, we found *MED12* mutations to be significantly associated with mutations in *TERT* ($p < 0.001$), *RARA* ($p < 0.001$), *SETD2* ($p = 0.012$), and *EGFR* ($p = 0.022$) (Table 4).

Table 3 Frequency of mutations of each gene across conventional fibroadenomas, cellular fibroadenomas, and benign phyllodes tumors.

Gene	Total ($n = 625$)	Conventional FA ($n = 258$)	Cellular FA ($n = 45$)	Benign PT ($n = 322$)	p value FA vs CFA vs BPT	p value FA and CFA vs BPT	p value FA vs BPT	p value CFA vs BPT	p value FA vs CFA
MED12	334 (53%)	113 (44%)	22 (49%)	199 (62%)	<0.001*	<0.001*	<0.001*	0.098	0.528
TERT	122 (20%)	16 (6%)	2 (4%)	104 (32%)	<0.001*	<0.001*	<0.001*	<0.001*	0.647
KMT2D	89 (14%)	39 (15%)	6 (13%)	44 (14%)	0.624	0.732	0.621	0.952	0.757
RARA	82 (13%)	20 (8%)	6 (13%)	56 (17%)	0.001*	0.001*	0.001*	0.498	0.219
FLNA	60 (10%)	15 (6%)	2 (4%)	43 (13%)	0.002*	0.001*	0.003*	0.088	0.714
SETD2	53 (8%)	10 (4%)	3 (7%)	40 (12%)	<0.001*	<0.001*	<0.001*	0.262	0.396
TP53	14 (2%)	4 (2%)	1 (2%)	9 (3%)	0.315	0.422	0.315	0.826	0.745
RB1	13 (2%)	2 (1%)	0 (0%)	11 (3%)	0.025*	0.022*	0.033*	0.209	0.555
NF1	24 (4%)	13 (5%)	0 (0%)	11 (3%)	0.333	0.678	0.330	0.209	0.125
PTEN	8 (1%)	1 (0%)	1 (2%)	6 (2%)	0.121	0.288	0.106	0.869	0.162
PIK3CA	18 (3%)	5 (2%)	4 (9%)	9 (3%)	0.591	>0.999	0.505	0.038*	0.011*
EGFR	22 (4%)	4 (2%)	2 (4%)	16 (5%)	0.027*	0.051	0.025*	0.879	0.200
BCOR	15 (2%)	8 (3%)	1 (2%)	6 (2%)	0.341	0.438	0.341	0.874	0.750
ERBB4	11 (2%)	4 (2%)	1 (2%)	6 (2%)	0.782	>0.999	0.774	0.869	0.745
MAP3K1	13 (2%)	2 (1%)	2 (4%)	9 (3%)	0.096	0.265	0.076	0.547	0.047*
IGF1R	4 (1%)	0 (0%)	0 (0%)	4 (1%)	0.060	0.125	0.073	0.454	NA

FA conventional fibroadenoma, *CFA* cellular fibroadenoma, *BPT* benign phyllodes tumor.

*Statistically significant with p value of < 0.05.

Adjunctive value of genomic analysis in refining diagnosis

Histological review was performed for a subset of cases after genomic analysis of our initial study cohort of 628 fibroepithelial lesions, based on the criteria above. Numbers of cases here for each disease entity are reflective of what was known before the review. Out of 128 cases that underwent review, 6 (4.7%) had their diagnosis changed (Figs. 1 and 3).

Of these six cases, three were revised from the original diagnosis of conventional fibroadenoma to benign phyllodes tumor. The first (FEB551) had missense mutations in *MED12*, *FLNA*, *RARA*, and *ERBB4*. The second (FEB851) had a frameshift mutation in *KMT2D* and a missense mutation in *ERBB4*, while the other (FEB858) had a missense mutation in *MED12*, a nonsense mutation in *RARA,* and a missense mutation in *NF1*.

Two cases of benign phyllodes tumor were rediagnosed to borderline phyllodes tumor. The first (FEB589) harbored a promoter mutation in *TERT* and a missense mutation in *TP53*, while the other (FEB1476) displayed missense mutations in *MED12*, *FLNA,* and *IGF1R*. The last case (FEB941) was reclassified from conventional fibroadenoma to a spindle cell tumor with necrosis consistent with borderline or malignant phyllodes tumor. It disclosed mutations in *MED12*, *TERT* promoter, *RARA*, and *EGFR*.

Copy number variation analysis

CNVs were noted across all three subtypes in chromosomes 1–3, 7, 9–12, 15–16, 18–22, and X; and recurrent chromosomal alterations (occurring in at least two cases) were presented in this study (see Supplementary material, Table S1 and Fig. S1). A high proportion of fibroadenomas, cellular fibroadenomas, and benign phyllodes tumors had amplifications in 7q11.21-q21.11 (15/15 vs 15/15 vs 15/15), 19p13.3-p12 (15/15 vs 15/15 vs 14/15), and 19q13.11-q13.43 (15/15 vs 14/15 vs 14/15); gains in certain chromosomal segments such as 10q23.33-q25.1 (15/15 vs 15/15 vs 15/15), 20p13-p12.3 (15/15 vs 15/15 vs 13/15), and 21q21.1-q22.2 (12/15 vs 14/15 vs 11/15); and chromosomal deletions such as 7q36.1-q36.3 (14/15 vs 14/15 vs 15/15), 10p15.3-p14 (15/15 vs 15/15 vs 15/15), 18p11.32-q21.2 (15/15 vs 13/15 vs 14/15), and 19q11-q13.11 (14/15 vs 15/15 vs 14/15). We did not observe any statistically significant differences between conventional fibroadenoma and cellular fibroadenoma; and when comparing conventional fibroadenoma, cellular fibroadenoma, and benign phyllodes tumors.

Discussion

A diagnosis of fibroadenoma is usually straightforward. However, uncertainty arises in cellular fibroadenomas that

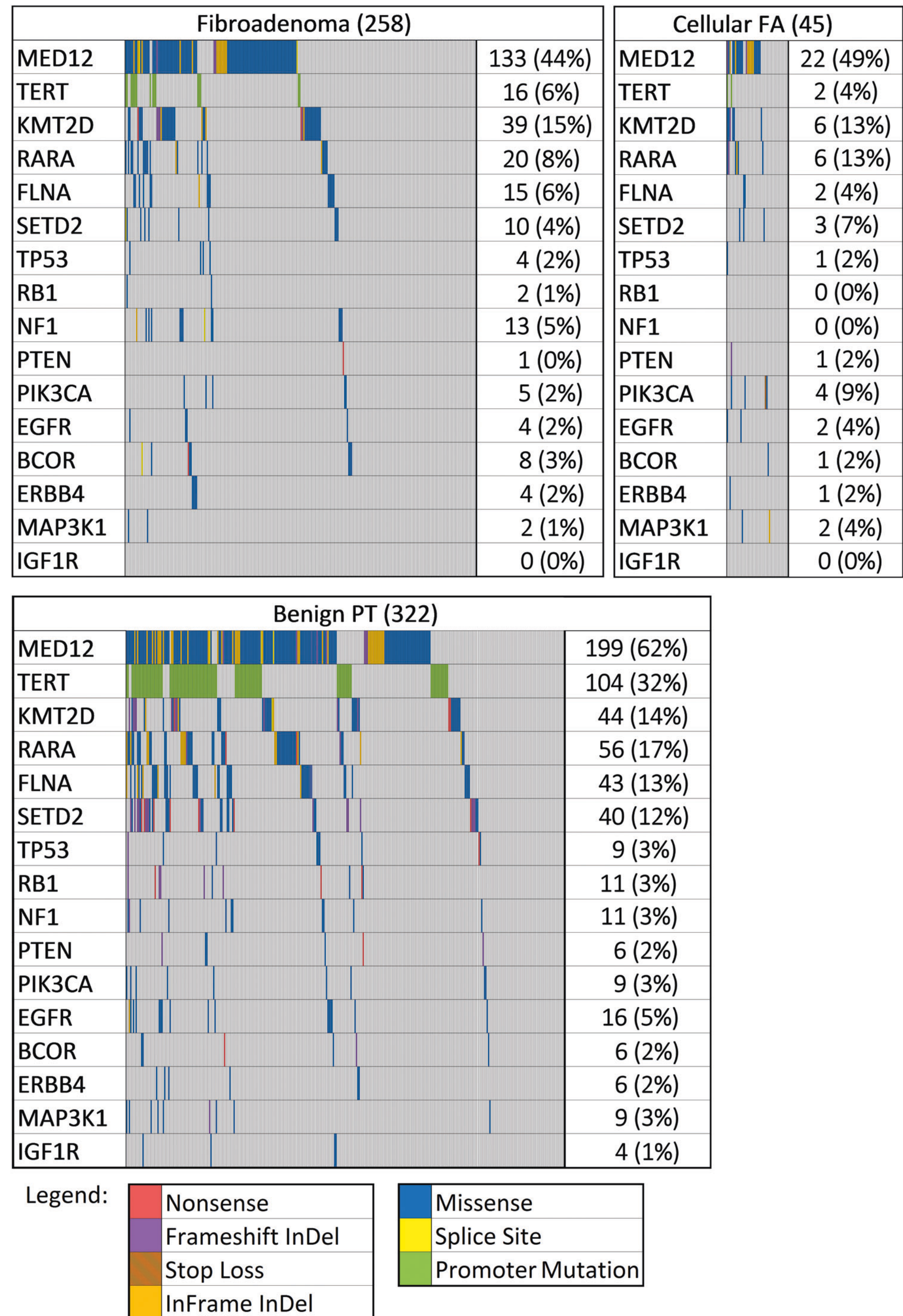


Fig. 2 Waterfall plot of recurrently mutated genetic aberrations. Genomic landscape of conventional fibroadenomas, cellular fibroadenomas and benign phyllodes tumors, and their mutation rates.

possess overlapping features with phyllodes tumors [3]. The extent of leaf-like fronds and stromal cellularity that favor a benign phyllodes tumor over a cellular fibroadenoma remain subjective, and substantial interobserver differences exist. For instance, stromal atypia at the benign end of the fibroepithelial spectrum where benign phyllodes tumors and cellular fibroadenomas both lie is usually mild. Stromal cellularity and mitotic activity of benign phyllodes tumors also overlap with those of cellular fibroadenomas [40, 41]. As a result, distinguishing cellular fibroadenomas from benign phyllodes tumors can pose significant difficulties, even for experienced breast pathologists [23]. In this study, we determined if there were molecular differences between fibroadenomas (in particular cellular fibroadenomas) and benign phyllodes tumors that can be adjunctive aids in overcoming the limitations posed by histological examination.

We observed *MED12* mutations across all three groups of lesions, with the majority (72%) occurring in codon 44

Table 4 Mutation frequencies for the panel of 16 genes sequenced across the entire cohort, arranged in descending order, and their co-mutation with mutant *MED12*.

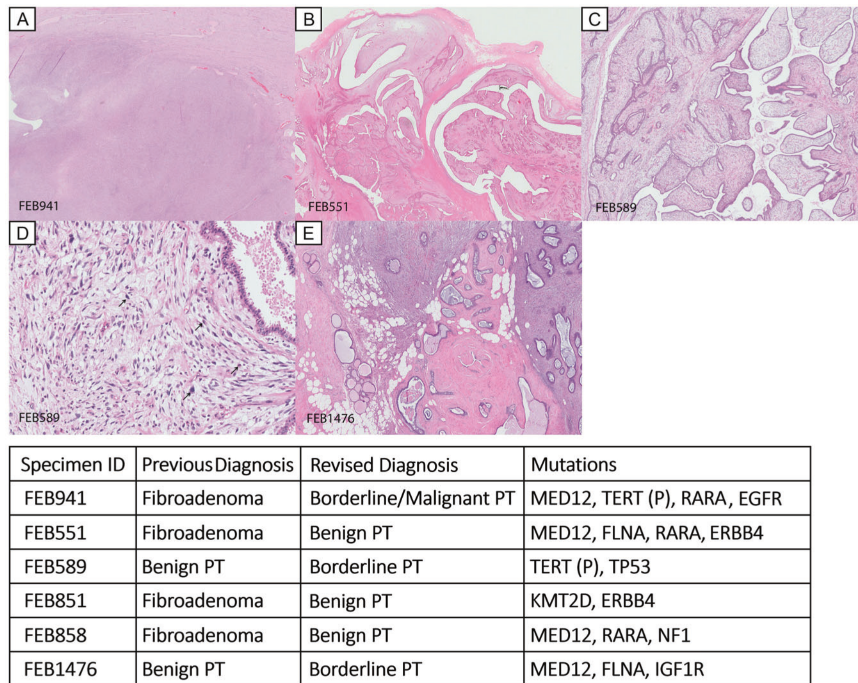
Gene	No. mutated (%)	Cases with mutant MED12 ($n = 334$)	Cases with wild-type MED12 ($n = 291$)	Sig. (2-tailed) for co-mutation with mutant *MED12* (p value)
MED12	334 (53%)	334 (100%)	291 (100%)	N/A
TERT	122 (20%)	86 (26%)	36 (12%)	<0.001*
KMT2D	89 (14%)	52 (16%)	37 (13%)	0.302
RARA	82 (13%)	63 (19%)	19 (7%)	<0.001*
FLNA	60 (10%)	38 (11%)	22 (8%)	0.131
SETD2	53 (8%)	37 (11%)	16 (5%)	0.012*
NF1	24 (4%)	15 (4%)	9 (3%)	0.361
EGFR	22 (4%)	17 (5%)	5 (2%)	0.022*
PIK3CA	18 (3%)	6 (2%)	12 (4%)	0.084
BCOR	15 (2%)	8 (2%)	7 (2%)	0.998
TP53	14 (2%)	7 (2%)	7 (2%)	0.799
RB1	13 (2%)	8 (2%)	5 (2%)	0.551
MAP3K1	13 (2%)	10 (3%)	3 (1%)	0.086
ERBB4	11 (2%)	9 (3%)	2 (1%)	0.056
PTEN	8 (1%)	4 (1%)	4 (1%)	0.848
IGF1R	4 (1%)	4 (1%)	0 (0%)	0.061

*Statistically significant with p value of < 0.05.

(see Supplementary material, Table S2). MED12 was noted to be involved in Wnt/β-catenin signaling, as β-catenin binds with the MED12 subunit in Mediator to activate transcription [42]. This pathway was found to be dysregulated in uterine leiomyomas which reported identical missense *MED12* mutations as fibroadenomas [43]. Thus, the *MED12* gene may be similarly affected in benign fibroepithelial lesions to promote cell proliferation and differentiation via Wnt signaling, which is known to be a fundamental growth control pathway [42].

According to our previous gene expression profiling study on ten fibroadenomas, genes upregulated in *MED12*-mutant fibroadenomas were associated with ER+ breast cancers and estrogen stimulus in ER+ breast cancer cells [5], and MED12 could enhance the function of estrogen receptor alpha (ERα) [44]. *KMT2D* which encodes the epigenetic regulator lysine (K)-specific methyltransferase 2D could directly interact with ERα, causing its recruitment and activation [45]. The retinoic acid receptor alpha which is encoded by *RARA* acts as a transcription factor and is capable of binding with ERα [46]. Activating mutations in *PIK3CA* have been reported to be frequent in ER-positive breast cancer [45]. Meanwhile, ER-EGFR signaling was discovered to be reciprocal: EGFR signaling promotes the activation of ER, and ER signaling promotes the activation of EGFR. Estrogen may induce activation of MAPK and PI3K which are associated with cell proliferation, angiogenesis, and tumor metastasis in non-small cell lung cancer [47]. In terms of immunohistochemical studies, epithelial expression of ER was higher in benign PTs than in borderline and malignant PTs [48], whereas Tan et al. observed significantly higher ER expression in fibroadenomas than in phyllodes tumors [49]. Thus, these results suggest potential involvement of estrogen signaling in the pathogenesis of benign tumors. Luo et al. observed that MED12 could bind to the EGFR promoter and result in EGFR transcription in ovarian cancers [50]. EGFR plays a role in elevating tumor cell survival, motility, and development through the Ras-Raf-Mek-Erk (Ras-MAPK) pathway [51], and its mutations have been described in breast, lung, and colorectal cancers [52–54]. Interestingly, activating mutations such as L858R confer sensitivity to tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib in non-small cell lung cancer, as mutant EGFR binds the inhibitors more tightly than wild-type EGFR [55]. However, we did not detect this mutation in our current cohort of fibroadenomas and benign phyllodes tumors.

From our results, benign fibroepithelial tumors generally had a low incidence of cancer driver mutations, reaffirming their benign nature. The ten cancer driver genes (*TP53*, *RB1*, *NF1*, *PTEN*, *PIK3CA*, *EGFR*, *BCOR*, *ERBB4*, *MAP3K1*, and *IGF1R*) had the lowest incidence of mutations amongst the 16 genes (Table 4). Among the three subcategories of benign fibroepithelial tumors, benign phyllodes tumors also had a higher proportion of tumors having four or more mutations (33/322, 10%) compared to cellular fibroadenomas and conventional fibroadenomas (1/45, 2% and 4/258, 2%, respectively) (Table 2), indicating that they were more genetically complex. However, a statistical difference in mutation rate was found only in two out of the ten cancer driver genes (*RB1* and *EGFR*) when comparing benign phyllodes tumors with fibroadenomas. Taken together, this shows that benign phyllodes tumors on the whole are similar to fibroadenomas (eight out of the ten cancer driver genes showed no significant differences in mutation frequencies), yet at the same time, the higher rate of mutations in *RB1* and *EGFR* seen in benign phyllodes tumors suggests a potential for malignant progression compared to fibroadenomas as both alterations are detected in phyllodes tumors of higher grades [56]. Sixteen conventional fibroadenomas in our cohort harbored *TERT* promoter –124 C > T (chr5:1,295,228C > T) and –146 C > T (chr5:1,295,250C > T) mutations (15/16, 94% and 1/16, 6%, respectively), while two cellular fibroadenomas had alterations in the former (see Supplementary material, Table S3). Yoshida et al. similarly reported *TERT* promoter mutations in fibroadenomas although their proportion was less (4/58, 7%) [57], while four other studies did not detect such alterations among their fibroadenoma



Specimen ID	Previous Diagnosis	Revised Diagnosis	Mutations
FEB941	Fibroadenoma	Borderline/Malignant PT	MED12, TERT (P), RARA, EGFR
FEB551	Fibroadenoma	Benign PT	MED12, FLNA, RARA, ERBB4
FEB589	Benign PT	Borderline PT	TERT (P), TP53
FEB851	Fibroadenoma	Benign PT	KMT2D, ERBB4
FEB858	Fibroadenoma	Benign PT	MED12, RARA, NF1
FEB1476	Benign PT	Borderline PT	MED12, FLNA, IGF1R

Fig. 3 Cases with changed diagnoses and their mutations. A FEB941: this case was designated a fibroadenoma, but on histological review, the tumor comprised sheets of spindle cells areas of necrosis, consistent with a borderline/malignant phyllodes tumor. **B** FEB551: originally labeled as a fibroadenoma, histological review showed well-developed stromal fronds with mild stromal hypercellularity, consistent with a benign phyllodes tumor. **C** FEB589: case that was initially labeled as a benign phyllodes tumor, showed phyllodal architecture with leaf-like stromal fronds. **D** FEB589: higher magnification showed moderate to marked stromal atypia, readily found mitoses (arrows) and mild to moderate stromal hypercellularity, indicating borderline grade. **E** FEB1476: initially diagnosed as a benign phyllodes tumor, histological review favored borderline grade as the tumor borders were irregularly permeative along a relatively long front.

samples [8, 58–60]. When comparing benign phyllodes tumors and cellular fibroadenomas, mutations in *TERT* promoter gene showed the greatest difference in mutation frequency (32 vs 4%) (Table 3), while its sensitivity and specificity were 32% and 96%, respectively (AUC 0.641, $p = 0.002$) (Table 5 and Fig. 4). This shows that the *TERT* promoter gene mutation is probably the most useful in helping to subclassify cellular fibroepithelial tumors that border on cellular fibroadenomas and benign phyllodes tumors. This may also be useful in small biopsies where there is only partial sampling of the tumor and the full architectural features of a phyllodes tumor are not appreciated. Other mutations had AUC values close to the reference line of 0.5 (no real predictability) and *p* values which were not statistically significant, indicating that their utility for diagnostic discrimination may not be optimal. This was similarly observed when comparing both conventional and cellular fibroadenomas vs benign phyllodes tumors, as well as when comparing conventional fibroadenomas with their cellular counterpart.

Most sequencing studies on fibroadenomas and benign phyllodes tumors involved targeted profiling of *MED12* and *TERT* promoter [11, 12, 57, 58, 61–69], while few had done multi-gene assays or exome sequencing [5–8, 59, 60, 70, 71]. To the best of our knowledge, our study has the largest number of fibroadenomas and cellular fibroadenomas profiled. Although most studies reported a lack of alterations in cancer driver genes in the benign group of fibroepithelial lesions, some studies did reveal such mutations. Our previous exome sequencing of eight fibroadenomas showed a singular *RB1* mutation [5], while other investigators found mutant *TP53* [72] and *PIK3CA* [73]. A single case of a pediatric fibroadenoma harboring *PIK3CA* missense mutation, a juvenile fibroadenoma with mutant *MAP3K1*, and a pediatric benign fibroepithelial neoplasm with *PTEN* and *BRCA1* mutations, have been reported [59]. Profiling heterogeneous lesions demonstrated *TP53* mutation in an FA-like area of a malignant PT, and a *PIK3CA* mutation in an FA-like area of a borderline PT [74]. It is not yet known whether presence of such mutations could signal possible malignant transformation of fibroadenomas, predict future progression into phyllodes tumors, or indicate likely recurrence of benign phyllodes tumors. However, it is critical to distinguish benign phyllodes tumors from fibroadenomas, since the former has a likelihood for grade progression upon recurrence and its malignant forms can metastasize, although such occurrences are extremely low [14, 15, 75–83].

Table 5 Sensitivity and specificity of mutation profiling for optimal discrimination, for conventional and cellular FAs vs benign PTs; cellular FAs vs benign PTs; and conventional FAs vs cellular FAs.

Gene	FA + CFA vs BPT				CFA vs BPT				FA vs CFA			
	Sensitivity	Specificity	AUC	*p* value	Sensitivity	Specificity	AUC	*p* value	Sensitivity	Specificity	AUC	*p* value
MED12	62%	55%	0.586	<0.001*	62%	51%	0.564	0.162	44%	51%	0.475	0.586
TERT	32%	94%	0.633	<0.001*	32%	96%	0.641	0.002*	6%	96%	0.509	0.851
KMT2D	14%	85%	0.495	0.819	14%	87%	0.502	0.960	15%	87%	0.509	0.849
RARA	17%	91%	0.543	0.062	17%	87%	0.520	0.671	8%	87%	0.472	0.550
FLNA	13%	94%	0.538	0.103	13%	96%	0.544	0.343	6%	96%	0.507	0.883
SETD2	12%	96%	0.541	0.075	12%	93%	0.529	0.524	4%	93%	0.486	0.765
TP53	3%	98%	0.504	0.853	3%	98%	0.501	0.975	2%	98%	0.497	0.943
RB1	3%	99%	0.514	0.547	3%	100%	0.517	0.708	1%	100%	0.504	0.934
NF1	3%	96%	0.496	0.856	3%	100%	0.517	0.708	5%	100%	0.525	0.590
PTEN	2%	99%	0.506	0.792	2%	98%	0.498	0.970	0%	98%	0.491	0.844
PIK3CA	3%	97%	0.499	0.974	3%	91%	0.470	0.510	2%	91%	0.465	0.457
EGFR	5%	98%	0.515	0.513	5%	96%	0.503	0.951	2%	96%	0.486	0.757
BCOR	2%	97%	0.495	0.814	2%	98%	0.498	0.970	3%	98%	0.504	0.925
ERBB4	2%	98%	0.501	0.960	2%	98%	0.498	0.970	2%	98%	0.497	0.943
MAP3K1	3%	99%	0.508	0.746	3%	96%	0.492	0.860	1%	96%	0.482	0.695
IGF1R	1%	100%	0.506	0.787	1%	100%	0.506	0.892	0%	100%	0.500	1.000

For FAs and CFAs vs BPT, we considered true positive as the number of benign PTs which had the mutation; false negative as the number of benign PTs without the mutation; false positive as the number of FAs and CFAs which had the mutation; and true negative as the number of FAs and CFAs without the mutation. For CFAs vs BPTs, true positive and false negative were the number of BPTs with/without the mutation, while false positive and true negative were CFAs with the mutation present/absent, respectively. When comparing FAs vs CFAs, true positive and false negative were determined by the number of FAs with/without the mutation, while false positive and true negative were CFAs with the mutation present/absent, respectively.

FA conventional fibroadenoma, *CFA* cellular fibroadenoma, *BPT* benign phyllodes tumor.

*Statistically significant with p value of < 0.05.

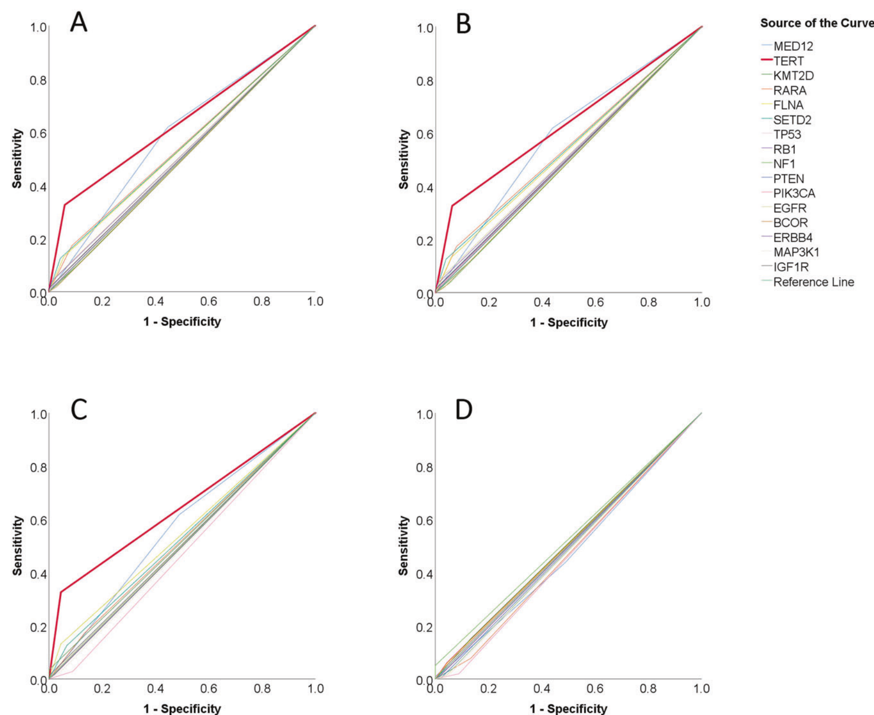


Fig. 4 ROC curves of 16 gene mutations. These were analyzed in **A** combined FAs vs benign PTs; **B** conventional FAs vs benign PTs; **C** cellular FAs vs benign PTs; and **D** conventional FAs vs cellular FAs.

Investigating genetic alterations of paired primary tumors and their recurrent/metastatic lesions could be an area for further exploration to unravel their molecular mechanisms.

When we histologically reviewed fibroadenomas and benign phyllodes tumors with cancer driver mutations, only 4.7% (6/128) of tumors had their original diagnoses revised, with three fibroadenomas reclassified as benign phyllodes tumors, two benign phyllodes tumors reclassified as borderline, and one fibroadenoma reclassified as a borderline/malignant phyllodes tumor. When we reviewed benign phyllodes tumors with mutations in more than two non-cancer driving genes, there were no tumors that were reclassified. While histological assessment remains reliable in classifying these lesions, the possibility of morphological heterogeneity underpinned by the molecular alterations has to be considered. While central review of all histological sections may be useful, these cases were appraised and diagnosed by international pathologist collaborators based on current histological criteria, with findings from this study according additional genomic insights that can help refine light microscopic interpretation in participating institutions.

Pareja et al. proposed that fibroepithelial tumor progression from normal mammary stroma toward phyllodes tumors, occurs either through the *MED12*-mutant pathway or the *MED12*-wild-type pathway. Fibroadenomas are the first step in the *MED12*-mutant pathway, after the stroma acquires mutations in *MED12* exon 2 [84]. Our study, like others, confirm that *MED12* mutations are not universally present in all breast fibroepithelial lesions [5, 6, 11, 12, 58, 61, 63, 65]. This provides corroborating evidence that phyllodes tumors can indeed develop through the *MED12*-wild-type pathway by acquisition of alterations in cancer driver genes.

In a study of 23 fibroadenomas using comparative genomic hybridization analysis, Ojopi et al. found that the most frequently overrepresented segments were 5p14, 5q34-qter, 13q32-qter, 10q25-qter, and 18q22 [85]. Amiel et al. detected genetic aberrations in chromosomes 4–6, 8–13, 16, 18, 19, 20, and 22 [86]; while Cavalli et al. and Ried et al. reported no alterations in the DNA copy number of 20 fibroadenomas and 13 fibroadenomas, respectively [87, 88]. Additionally, Xie et al. observed through whole exome sequencing that most of the 27 recurrent somatic CNVs were clustered in chromosomes 1, 4, 9, 11, 13, 15, 19, and were deletions [60].

As for benign phyllodes tumors, Lv et al. noted that chromosomal regions prevalently involved in copy number gains were 1p12-q21 (4/12), 18p11.2-q11.2 (3/12) and losses were most frequently found on chromosome regions 9q34 (3/12), 10q26 (3/12), 17q25 (3/12), 19q13.3 (3/12) [89]. Laé et al. reported losses in 6q (4/9), 10p (1/9), 16q (1/9), and 22q (1/9), and gains in 1q (1/9) [90]. We also observed losses in 19q11-q13.11 (14/15), 10p15.3-p14 (14/15), and 22q11.22-q12.1 (8/15), although our data showed gains in 9q33.3-q34.3 (7/15) instead.

These studies used different experimental techniques (comparative genomic hybridization and whole exome sequencing) compared to our low-pass WGS, thus the data are not entirely comparable. Our assessment of CNV did not show significant differences between the three tumor types (see Supplementary material, Table S1 and Fig. S1) in terms of chromosomal alterations too, hence somatic DNA profiling may be a better tool to differentiate them.

The strength of our study includes the large number of benign phyllodes tumors with representation from various countries. This helps to eliminate bias based on ethnic or geographic differences and adds confidence to our results. The limitations include the restricted panel of 16 genes that are studied which may not fully reveal the genetic differences between fibroadenomas and phyllodes tumors. Approaches such as WGS may help us gain greater insights into the full molecular spectrum of breast fibroepithelial tumors. WGS has already been performed in uterine leiomyomas, which share certain clinical and mutational characteristics with fibroadenomas [91]. In addition, the number of cellular fibroadenomas are disproportionately smaller than the number of benign phyllodes tumors and our results for cellular fibroadenomas may therefore be susceptible to bias. There is also a proportion of cases that did not have available H&E sections for histological review.

In summary, next generation sequencing using a targeted 16 gene panel revealed that benign phyllodes tumors have a higher mean number of mutations compared to fibroadenomas. Cellular fibroadenomas are genetically similar to conventional fibroadenomas. Most of the cancer driver genes studied showed no significant difference in mutation frequencies when comparing benign phyllodes tumors and fibroadenomas, but *RB1* and *EGFR* were notably mutated in a higher proportion of benign phyllodes tumors than fibroadenomas. Benign phyllodes tumors possessed a significantly higher percentage of *TERT* promoter mutations compared to cellular fibroadenomas and this may be a useful adjunct when evaluating challenging cellular fibroepithelial tumors. Histologic criteria remain reliable in the classification of benign fibroepithelial tumors with only a small percentage of tumors being reclassified with the help of molecular findings. Further study of the genetics of benign fibroepithelial tumors beyond the scope of the 16 gene panel is required to help us better chart the genomic landscape of these tumors.

Data availability

The dataset generated is available in the NCBI Sequence Read Archive (SRA) under accession code PRJNA516727.

Acknowledgements We extend our gratitude to the International Fibroepithelial Consortium for their contribution of samples and clinical data.

International Fibroepithelial Consortium Norraha Abd Rahman[11], S. M. Khodeza Nahar Begum[12], Phaik Leng Cheah[13], Chih Jung Chen[14], Emmanuel Dela Fuente[15], Aaron Han[16], Oi Harada[17], Naoki Kanomata[18], Cheok Soon Lee[19], Jonathan Yu Han Lee[20], Mohammed Kamal[21], Rieko Nishimura[22], Yasuyo Ohi[23], Elinor J. Sawyer[24], Kean Hooi Teoh[13], Alex Koon Ho Tsang[25], Julia Yuen-Shan Tsang[26], Gary M. K. Tse[26], Rin Yamaguchi[27]

[11]Pantai Hospital Kuala Lumpur, Kuala Lumpur, Malaysia; [12]Bangladesh Specialized Hospital LTD, Dhaka, Bangladesh; [13]University of Malaya, Kuala Lumpur, Malaysia; [14]School of Medicine, Chung Shan Medical University, Taichung, Taiwan; [15]Makati Medical Center, Makati, Philippines; [16]Mohammed Bin Rashid University, Dubai, UAE; [17]Showa Medical School Hospital, Tokyo, Japan; [18]St. Lukes International Hospital, Tokyo, Japan; [19]School of Medicine, Western Sydney University, Sydney, NSW, Australia; [20]School of Science and Health, Western Sydney University, Sydney, NSW, Australia; [21]Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh; [22]Department of Pathology, Nagoya Medical Center, Nagoya, Japan; [23]Hakuaikai Sagara Hospital, Kagoshima, Japan; [24]Guy's Cancer Centre, Guy's Hospital, King's College London, London, UK; [25]Yan Chai Hospital, Tsuen Wan, Hong Kong; [26]The Chinese University of Hong Kong, Shatin, Hong Kong; [27]Kurume University Medical Center, Fukuoka, Japan

Author contributions PHT, BTT, PT, GWCY, and BHB were responsible for study conceptualization and design, and project supervision. CCYN, NDMN, BNL, VR, WL, and JYL performed the experiments. KTEC, MAG, PM, BKTT, VKMT, CYW, WSY, GHH, KWO, and IFC provided resources and clinical data. CCYN, NDMN, BNL, VR, WL, JYL, PG, AHL, AAT, and TKYT collected, analyzed, and interpreted the data. CCYN, NDMN, BNL, PG, and AHL wrote the manuscript. All the authors revised and provided input to the manuscript.

Funding This study was funded by a SingHealth Duke-NUS Research Collaborative Grant (SDC/021/2015) and an SHF-Foundation Research Grant (SHF/PRISM018/2017), as awarded by the SingHealth Foundation and Duke-NUS; as well as a grant from the Singapore Ministry of Health's National Medical Research Council (MOH-STAR18NOV-0001).

Compliance with ethical standards

Conflict of interest PT, BTT, and PHT jointly hold patent applications for PCT/SG2015/050107 (Breast fibroadenoma susceptibility mutations and use thereof) and PCT/SG2015/050368 (Method and kit for pathologic grading of breast neoplasms). Other authors declare no competing interests.

Ethical approval This study was approved by the SingHealth Centralized Institutional Review Board (CIRB Ref: 2018/2581), with a waiver of informed consent granted.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Tan PH, Sahin AA. Atlas of differential diagnosis in breast pathology. New York: Springer Science, Business Media LLC; 2017.
2. WHO Classification of Tumours Editorial Board. WHO classification of tumours of the breast. 5th ed. Lyon: IARC Press; 2019.
3. Krings G, Bean GR, Chen YY. Fibroepithelial lesions; the WHO spectrum. Semin Diagn Pathol. 2017;34:438–52.
4. Loke BN, Md Nasir ND, Thike AA, Lee JYH, Lee CS, Teh BT, et al. Genetics and genomics of breast fibroadenomas. J Clin Pathol. 2018;71:381–7.
5. Lim WK, Ong CK, Tan J, Thike AA, Ng CC, Rajasegaran V, et al. Exome sequencing identifies highly recurrent MED12 somatic mutations in breast fibroadenoma. Nat Genet. 2014;46:877–80.
6. Tan J, Ong CK, Lim WK, Ng CC, Thike AA, Ng LM, et al. Genomic landscapes of breast fibroepithelial tumors. Nat Genet. 2015;47:1341–5.
7. Cani AK, Hovelson DH, McDaniel AS, Sadis S, Haller MJ, Yadati V, et al. Next-Gen sequencing exposes frequent MED12 mutations and actionable therapeutic targets in phyllodes tumors. Mol Cancer Res. 2015;13:613–9.
8. Piscuoglio S, Ng CK, Murray M, Burke KA, Edelweiss M, Geyer FC, et al. Massively parallel sequencing of phyllodes tumours of the breast reveals actionable mutations, and TERT promoter hotspot mutations and TERT gene amplification as likely drivers of progression. J Pathol. 2016;238:508–18.
9. Liu SY, Joseph NM, Ravindranathan A, Stohr BA, Greenland NY, Vohra P, et al. Genomic profiling of malignant phyllodes tumors reveals aberrations in FGFR1 and PI-3 kinase/RAS signaling pathways and provides insights into intratumoral heterogeneity. Mod Pathol. 2016;29:1012–27.
10. Nozad S, Sheehan CE, Gay LM, Elvin JA, Vergilio JA, Suh J, et al. Comprehensive genomic profiling of malignant phyllodes tumors of the breast. Breast Cancer Res Treat. 2017;162:597–602.
11. Piscuoglio S, Murray M, Fusco N, Marchiò C, Loo FL, Martelotto LG, et al. MED12 somatic mutations in fibroadenomas and phyllodes tumors of the breast. Histopathology. 2015;67:719–29.
12. Mishima C, Kagara N, Tanei T, Naoi Y, Shimoda M, Shimomura A, et al. Mutational analysis of MED12 in fibroadenomas and phyllodes tumors of the breast by means of targeted next-generation sequencing. Breast Cancer Res Treat. 2015;152: 305–12.
13. Piscuoglio S, Geyer FC, Burke KA, Murray MP, Ng CK, Mota A, et al. Massively parallel sequencing analysis of synchronous fibroepithelial lesions supports the concept of progression from fibroadenoma to phyllodes tumor. NPJ Breast Cancer. 2016;2: 16035.
14. Tan PH. Fibroepithelial lesions revisited: implications for diagnosis and management. Mod Pathol. 2021;34:15–37.
15. Tan BY, Acs G, Apple SK, Badve S, Bleiweiss IJ, Brogi E, et al. Phyllodes tumours of the breast: a consensus review. Histopathology. 2016;68:5–21.
16. Karim RZ, Gerega SK, Yang YH, Spillane A, Carmalt H, Scolyer RA, et al. Phyllodes tumours of the breast: a clinicopathological analysis of 65 cases from a single institution. Breast. 2009;18: 165–70.
17. Kim S, Kim JY, Kim DH, Jung WH, Koo JS. Analysis of phyllodes tumor recurrence according to the histologic grade. Breast Cancer Res Treat. 2013;141:353–63.
18. Gordon PB, Gagnon FA, Lazkowsky L. Solid breast masses diagnosed as fibroadenoma at fine-needle aspiration biopsy: acceptable rates of growth at long-term follow-up. Radiology. 2003;229:233–8.
19. Liberman L, Bonaccio E, Hamele-Bena D, Abramson AF, Cohen MA, Dershaw DD. Benign and malignant phyllodes tumors: mammographic and sonographic findings. Radiology. 1996;198: 121–4.
20. Kalambo M, Adrada BE, Adeyefa MM, Krishnamurthy S, Hess K, Carkaci S, et al. Phyllodes tumor of the breast: ultrasound-pathology correlation. Am J Roentgenol. 2018;210:W173–9.

21. Chao TC, Lo YF, Chen SC, Chen MF. Sonographic features of phyllodes tumors of the breast. Ultrasound Obstet Gynecol. 2002;20:64–71.
22. Wurdinger S, Herzog AB, Fischer DR, Max C, Raabe G, Schneider A, et al. Differentiation of phyllodes breast tumors from fibroadenomas on MRI. Am J Roentgenol. 2005;185:1317–21.
23. Lawton TJ, Acs G, Argani P, Farshid G, Gilcrease M, Goldstein N, et al. Interobserver variability by pathologists in the distinction between cellular fibroadenomas and phyllodes tumors. Int J Surg Pathol. 2014;22:695–8.
24. Yasir S, Gamez R, Jenkins S, Visscher DW, Nassar A. Significant histologic features differentiating cellular fibroadenoma from phyllodes tumor on core needle biopsy specimens. Am J Clin Pathol. 2014;142:362–9.
25. Lee AH, Hodi Z, Ellis IO, Elston CW. Histological features useful in the distinction of phyllodes tumour and fibroadenoma on needle core biopsy of the breast. Histopathology. 2007;51:336–44.
26. Tsang AKH, Chan SK, Lam CCF, Lui PCW, Chau HHL, Tan PH, et al. Phyllodes tumours of the breast—differentiating features in core needle biopsy. Histopathology. 2011;59:600–8.
27. Jara-Lazaro AR, Akhilesh M, Thike AA, Lui PCW, Tse GMK, Tan PH. Predictors of phyllodes tumours on core biopsy specimens of fibroepithelial neoplasms. Histopathology. 2010;57: 220–32.
28. Tan WJ, Cima I, Choudhury Y, Wei X, Lim JCT, Thike AA, et al. A five-gene reverse transcription-PCR assay for pre-operative classification of breast fibroepithelial lesions. Breast Cancer Res. 2016;18:31.
29. Md Nasir ND, Ng CCY, Rajasegaran V, Wong SF, Liu W, Ng GXP, et al. Genomic characterisation of breast fibroepithelial lesions in an international cohort. J Pathol. 2019;249:447–60.
30. 1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, et al. A global reference for human genetic variation. Nature. 2015;526:68–74.
31. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25:1754–60.
32. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence alignment/map format and SAMtools. Bioinformatics. 2009;25:2078–9.
33. Garrison E, Marth G. Haplotype-based variant detection from short-read sequencing. Preprint at https://arxiv.org/abs/1207.3907 (2012).
34. Yang H, Wang K. Genomic variant annotation and prioritization with ANNOVAR and wANNOVAR. Nat Protoc. 2015;10: 1556–66.
35. Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, et al. dbSNP: the NCBI database of genetic variation. Nucleic Acids Res. 2001;29:308–11.
36. Forbes SA, Beare D, Gunasekaran P, Leung K, Bindal N, Boutselakis H, et al. COSMIC: exploring the world's knowledge of somatic mutations in human cancer. Nucleic Acids Res. 2015;43: D805–11.
37. Landrum MJ, Lee JM, Benson M, Brown G, Chao C, Chitipiralla S, et al. ClinVar: public archive of interpretations of clinically relevant variants. Nucleic Acids Res. 2016;44:D862–8.
38. Landrum MJ, Lee JM, Riley GR, Jang W, Rubinstein WS, Church DM, et al. ClinVar: public archive of relationships among sequence variation and human phenotype. Nucleic Acids Res. 2014;42:D980–5.
39. Thorvaldsdottir H, Robinson JT, Mesirov JP. Integrative genomics viewer (IGP): high-performance genomics data visualization and exploration. Brief Bioinform. 2013;14:178–92.
40. Ross DS, Giri DD, Akram MM, Catalano JP, Olcese C, Zee KJV, et al. Fibroepithelial lesions in the breast of adolescent females: a clinicopathological study of 54 cases. Breast J. 2017;23:182–92.
41. Jacobs TW, Chen YY, Guinee DG Jr, Holden JA, Cha I, Bauermeister DE, et al. Fibroepithelial lesions with cellular stroma on breast core needle biopsy: are there predictors of outcome on surgical excision? Am J Clin Pathol. 2005;124:342–54.
42. Kim S, Xu X, Hecht A, Boyer TG. Mediator is a transducer of Wnt/beta-catenin signaling. J Biol Chem. 2006;281:14066–75.
43. Pérot G, Croce S, Ribeiro A, Lagarde P, Velasco V, Neuville A, et al. MED12 alterations in both human benign and malignant uterine soft tissue tumors. PLoS ONE. 2012;7:e40015.
44. Kang YK, Guermah M, Yuan CX, Roeder RG. The TRAP/Mediator coactivator complex interacts directly with estrogen receptors alpha and beta through the TRAP220 subunit and directly enhances estrogen receptor function in vitro. Proc Natl Acad Sci USA. 2002;99:2642–7.
45. Toska E, Osmanbeyoglu HU, Castel P, Chan C, Hendrickson RC, Elkabets M, et al. PI3K pathway regulates ER-dependent transcription in breast cancer through the epigenetic regulator KMT2D. Science. 2017;355:1324–30.
46. Ross-Innes CS, Stark R, Holmes KA, Schmidt D, Spyrou C, Russell R, et al. Cooperative interaction between retinoic acid receptor-alpha and estrogen receptor in breast cancer. Genes Dev. 2010;24:171–82.
47. Ding X, Li L, Tang C, Meng C, Xu W, Wei X, et al. Cytoplasmic expression of estrogen receptor β may predict poor outcome of EGFR-TKI therapy in metastatic lung adenocarcinoma. Oncol Lett. 2018;16:2382–90.
48. Tse GMK, Lee CS, Kung FYL, Scolyer RA, Law BKB, Lau T, et al. Hormonal receptors expression in epithelial cells of mammary phyllodes tumors correlates with pathologic grade of the tumor. Am J Clin Pathol. 2002;118:522–6.
49. Tan WJ, Chan JY, Thike AA, Lim JCT, Md Nasir ND, Tan JSY, et al. MED12 protein expression in breast fibroepithelial lesions: correlation with mutation status and oestrogen receptor expression. J Clin Pathol. 2016;69:858–65.
50. Luo XL, Deng CC, Su XD, Wang F, Chen Z, Wu XP, et al. Loss of MED12 induces tumor dormancy in human epithelial ovarian cancer via downregulation of EGFR. Cancer Res. 2018;78: 3532–43.
51. Nagaria TS, Shi C, Leduc C, Hoskin V, Sikdar S, Sangrar W, et al. Combined targeting of Raf and Mek synergistically inhibits tumorigenesis in triple negative breast cancer model systems. Oncotarget. 2017;8:80804–19.
52. Bemanian V, Sauer T, Touma J, Lindstedt BA, Chen Y, Ødegård HP, et al. The epidermal growth factor receptor (EGFR/HER-1) gatekeeper mutation T790M is present in European patients with early breast cancer. PLoS ONE. 2015;10:e0134398.
53. Bethune G, Bethune D, Ridgway N, Xu Z. Epidermal growth factor receptor (EGFR) in lung cancer: an overview and update. J Thorac Dis. 2010;2:48–51.
54. Kim N, Cho D, Kim H, Kim S, Cha YJ, Greulich H, et al. Colorectal adenocarcinoma-derived EGFR mutants are oncogenic and sensitive to EGFR-targeted monoclonal antibodies, cetuximab and panitumumab. Int J Cancer. 2020;146:2194–200.
55. Yun CH, Mengwasser KE, Toms AV, Woo MS, Greulich H, Wong KK, et al. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. Proc Natl Acad Sci USA. 2008;105:2070–5.
56. Cimino-Mathews A, Hicks JL, Sharma R, Vang R, Illei PB, De Marzo A, et al. A subset of malignant phyllodes tumors harbors alterations in the Rb/p16 pathway. Hum Pathol. 2013;44: 2494–500.
57. Yoshida M, Ogawa R, Yoshida H, Maeshima A, Kanai Y, Kinoshita T. et al. TERT promoter mutations are frequent and show association with MED12 mutations in phyllodes tumors of the breast. Br J Cancer. 2015;113:1244–8.

58. Garcia-Dios DA, Levi D, Shah V, Gillett C, Simpson MA, Hanby A, et al. MED12, TERT promoter and RBM15 mutations in primary and recurrent phyllodes tumours. Br J Cancer. 2018;118: 277–84.
59. Tay TKY, Guan P, Loke BN, Md Nasir ND, Rajasegaran V, Thike AA, et al. Molecular insights into paediatric breast fibroepithelial tumours. Histopathology. 2018;73:809–18.
60. Xie SN, Cai YJ, Ma B, Xu Y, Qian P, Zhou JD, et al. The genomic mutation spectrums of breast fibroadenomas in Chinese population by whole exome sequencing analysis. Cancer Med. 2019;8:2372–9.
61. Yoshida M, Sekine S, Ogawa R, Yoshida H, Maeshima A, Kanai Y, et al. Frequent MED12 mutations in phyllodes tumours of the breast. Br J Cancer. 2015;112:1703–8.
62. Nagasawa S, Maeda I, Fukuda T, Wu W, Hayami R, Kojima Y, et al. MED12 exon 2 mutations in phyllodes tumors of the breast. Cancer Med. 2015;4:1117–21.
63. Pfarr N, Kriegsmann M, Sinn P, Klauschen F, Endris V, Herpel E, et al. Distribution of MED12 mutations in fibroadenomas and phyllodes tumors of the breast—implications for tumor biology and pathological diagnosis. Genes Chromosomes Cancer. 2015;54:444–52.
64. Ng CC, Tan J, Ong CK, Lim WK, Rajasegaran V, Nasir ND, et al. MED12 is frequently mutated in breast phyllodes tumours: a study of 112 cases. J Clin Pathol. 2015;68:685–91.
65. Lien HC, Huang CS, Yang YW, Jeng YM. Mutational analysis of MED12 exon 2 in a spectrum of fibroepithelial tumours of the breast: implications for pathogenesis and histogenesis. Histopathology. 2016;68:433–41.
66. Yoon N, Bae GE, Kang SY, Choi MS, Hwang HW, Kim SW, et al. Frequency of MED12 mutations in phyllodes tumors: inverse correlation with histologic grade. Genes Chromosomes Cancer. 2016;55:495–504.
67. Lae M, Gardrat S, Rondeau S, Richardot C, Caly M, Chemlali W, et al. MED12 mutations in breast phyllodes tumors: evidence of temporal tumoral heterogeneity and identification of associated critical signaling pathways. Oncotarget. 2016;7:84428–38.
68. Darooei M, Khan F, Rehan M, Zubeda S, Jeyashanker E, Annapurna S, et al. MED12 somatic mutations encompassing exon 2 associated with benign breast fibroadenomas and not breast carcinoma in Indian women. J Cell Biochem. 2019;120:182–91.
69. Pareja F, Da Cruz Paula A, Murray MP, Hoang T, Gularte-Merida R, Brown D, et al. Recurrent MED12 exon 2 mutations in benign breast fibroepithelial lesions in adolescents and young adults. J Clin Pathol. 2019;72:258–62.
70. Kim JY, Yu JH, Nam SJ, Kim SW, Lee SK, Park WY, et al. Genetic and clinical characteristics of phyllodes tumors of the breast. Transl Oncol. 2018;11:18–23.
71. Sim Y, Ng GXP, Ng CCY, Rajasegaran V, Wong SF, Liu W, et al. A novel genomic panel as an adjunctive diagnostic tool for the characterization and profiling of breast Fibroepithelial lesions. BMC Med Genomics. 2019;12:142.
72. Millikan R, Hulka B, Thor A, Zhang Y, Edgerton S, Zhang X, et al. p53 mutations in benign breast tissue. J Clin Oncol. 1995;13:2293–300.
73. Vorkas PA, Poumpouridou N, Agelaki S, Kroupis C, Georgoulias V, Lianidou ES. PIK3CA hotspot mutation scanning by a novel and highly sensitive high-resolution small amplicon melting analysis method. J Mol Diagn. 2010;12:697–704.
74. Tan BY, Md Nasir ND, Chang HY, Ng CCY, Guan P, Nagarajan S, et al. Morphologic and genetic heterogeneity in breast fibroepithelial lesions-a comprehensive mapping study. Mod Pathol. 2020;33:1732–45.
75. Tan PH, Thike AA, Tan WJ, Thu MM, Busmanis I, Li H, et al. Predicting clinical behaviour of breast phyllodes tumours: a nomogram based on histological criteria and surgical margins. J Clin Pathol. 2012;65:69–76.
76. Jones AM, Mitter R, Springall R, Graham T, Winter E, Gillett C, et al. A comprehensive genetic profile of phyllodes tumours of the breast detects important mutations, intra-tumoral genetic heterogeneity and new genetic changes on recurrence. J Pathol. 2008;214:533–44.
77. Mitus J, Adamczyk A, Majchrzyk K, Kowalik A, Ryś J, Niemiec J. Comparison of mutation profile between primary phyllodes tumors of the breast and their paired local recurrences. Pol J Pathol. 2020;71:7–12.
78. Koh VCY, Thike AA, Tan PH. Distant metastases in phyllodes tumours of the breast: an overview. Appl Cancer Res. 2017;37:15.
79. Reinfuss M, Mituś J, Duda K, Stelmach A, Ryś J, Smolak K. The treatment and prognosis of patients with phyllodes tumor of the breast: an analysis of 170 cases. Cancer. 1996;77:910–6.
80. Mangi AA, Smith BL, Gadd MA, Tanabe KK, Ott MJ, Souba WW. Surgical management of phyllodes tumors. Arch Surg. 1999;134:487–92.
81. Chaney AW, Pollack A, McNeese MD, Zagars GK, Pisters PW, Pollock RE, et al. Primary treatment of cystosarcoma phyllodes of the breast. Cancer. 2000;89:1502–11.
82. Asoglu O, Ugurlu MM, Blanchard K, Grant CS, Reynolds C, Cha SS, et al. Risk factors for recurrence and death after primary surgical treatment of malignant phyllodes tumors. Ann Surg Oncol. 2004;11:1011–7.
83. Abdalla HM, Sakr MA. Predictive factors of local recurrence and survival following primary surgical treatment of phyllodes tumors of the breast. J Egypt Natl Canc Inst. 2006;18:125–33.
84. Pareja F, Geyer FC, Kumar R, Selenica P, Piscuoglio S, Ng CKY, et al. Phyllodes tumors with and without fibroadenoma-like areas display distinct genomic features and may evolve through distinct pathways. NPJ Breast Cancer. 2017;3:40.
85. Ojopi EP, Rogatto SR, Caldeira JR, Barbiéri-Neto J, Squire JA. Comparative genomic hybridization detects novel amplifications in fibroadenomas of the breast. Genes Chromosomes Cancer. 2001;30:25–31.
86. Amiel A, Kaufman Z, Goldstein E, Bruchim RB, Kidron D, Gaber E, et al. Application of comparative genomic hybridization in search for genetic aberrations in fibroadenomas of the breast. Cancer Genet Cytogenet. 2003;142:145–8.
87. Cavalli LR, Cornelio DA, Lima RS, Urban CA, Rone JD, Cavalli IJ, et al. Lack of DNA copy number alterations revealed with comparative genomic hybridization in fibroadenomas of the breast. Cancer Genet Cytogenet. 2004;153:173–6.
88. Ried T, Just KE, Holtgreve-Grez H, du Manoir S, Speicher MR, Schröck E, et al. Comparative genomic hybridization of formalin-fixed, paraffin-embedded breast tumors reveals different patterns of chromosomal gains and losses in fibroadenomas and diploid and aneuploid carcinomas. Cancer Res. 1995;55:5415–23.
89. Lv S, Niu Y, Wei L, Liu Q, Wang X, Chen Y. Chromosomal aberrations and genetic relations in benign, borderline and malignant phyllodes tumors of the breast: a comparative genomic hybridization study. Breast Cancer Res Treat. 2008;112:411–8.
90. Laé M, Vincent-Salomon A, Savignoni A, Huon I, Fréneaux P, Sigal-Zafrani B, et al. Phyllodes tumors of the breast segregate in two groups according to genetic criteria. Mod Pathol. 2007;20:435–44.
91. Mehine M, Kaasinen E, Makinen N, Katainen R, Kampjarvi K, Pitkanen E, et al. Characterization of uterine leiomyomas by whole-genome sequencing. N Engl J Med. 2013;369:43–53.