patient received both adjuvant chemotherapy and chest wall radiotherapy with only minimal response.

Nayak *et al.* analysed the prognostic features and reported age over 60 years, tumour non- keratinisation and presence of a spindle cell component as adverse prognostic factors. Lymph node metastasis and high pathological stage were significantly associated with poor OS of patients.[5]

To conclude, breast SqCC is a rare and aggressive disease associated with frequent local and distant metastasis and death. Current adjuvant treatment options are limited because of lack of expression of hormone receptors and HER2 over-expression. These tumours are genetically distinct from other triple negative breast cancers and genomic profiling can be of help in the management of some of these tumours. New therapeutic regimens including EGFR tyrosine kinase/mTOR pathway inhibitors and cisplatin based adjuvant chemotherapy may play a role in treatment of these aggressive tumours.[5]

Acknowledgements: The authors would like to acknowledge Dr Stephen Hayes and Dr William Mackie for providing clinical information and tissue for diagnosis.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

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DOI: https://doi.org/10.1016/j.pathol.2019.03.003

The utility of a targeted gene mutation panel in refining the diagnosis of breast phyllodes tumours

Sir,

Phyllodes tumours (PTs) of the breast are uncommon biphasic fibroepithelial neoplasms that comprise 0.3–1.0% of all primary breast tumours. Morphologically resembling intracanalicular fibroadenomas, PTs are characterised by a leaf-like architecture consisting of bilayered epithelium with hypercellular stroma. On the basis of assessment of five histological features (stromal cellularity, stromal atypia, stromal overgrowth, tumour borders, and mitotic activity), PTs are graded as benign, borderline, and malignant.[1]

Accurate diagnosis of PTs can be challenging. Despite seemingly straightforward guidelines for PT grading, the actual application can be problematic. The manner in which assessment of the various histological parameters is amalgamated to establish the final grade can also be subjective. Furthermore, differential diagnoses may arise during diagnostic evaluation. At the benign end of the histological spectrum, it may be difficult to differentiate some benign PTs from fibroadenomas, while at the malignant end, a high grade spindle cell lesion raises histological considerations of malignant PT, spindle cell metaplastic breast carcinoma (SCMBC) or primary/secondary breast sarcoma.[2]

Previously, we studied the genomic alterations of fibroepithelial tumours and found recurrent mutations in *MED12* and *RARA* in both fibroadenomas and PTs, with additional aberrations observed in the latter, especially in the borderline and malignant grades where derangements in cancer driver genes are seen.[3] From this study, 16 of the most frequently mutated genes in fibroepithelial tumours (*MED12, TERT, SETD2, KMT2D, RARA, FLNA, NF1, PIK3CA, EGFR, RB1, PTEN, ERBB4, BCOR, TP53, IGF1R*, and *MAP3K1*) that were compiled into a customised panel, were found to be helpful as diagnostic adjuncts when applied to two challenging cases of PTs.

In Case 1, a 35-year-old Caucasian female was diagnosed with malignant PT of the breast on excision biopsy of a right breast lump. She sought a second opinion at our institution, where a diagnosis of borderline PT was rendered based on histology. The tumour was composed of epithelium-lined fronds with myxoid and variably cellular stroma. Stromal cellularity ranged from mild to moderate, with foci of relatively densely packed fascicles of spindle cells that were reminiscent of fibrosarcoma. Mitoses averaged about 3 per 10 high-power fields (HPF) (field diameter 0.6 mm), although in one section, mitoses that numbered up to 12 per HPF were observed amongst cellular stroma with inflammatory cells.

Stromal atypia was mild to moderate. Neither stromal overgrowth nor malignant heterologous elements were identified. In some sections, tumour borders were irregular and permeative, reaching the inked surgical margins in several places (Fig. 1a–d). Immunohistochemistry (IHC) showed about 10% overall of stromal nuclear staining for Ki-67, and diffuse expression of CD34 among stromal cells, with patchy areas of slightly diminished reactivity (Fig. 1e,f). Overall, pathological features favoured a borderline grade of PT. As the initial and reviewed grades differed that impacted on management, with mastectomy recommended by the surgical oncologist for a malignant diagnosis, the customised panel was applied to determine if it could assist in refining grade assignment.

For the second case, a 45-year-old Chinese female was discovered on mammogram to have an irregular hypoechoeic mass (3 cm) in her left breast, which had grown in size from her previous mammograms. An ultrasound was also performed. The mass was excised and histology slides (H&E and IHC stains) and three formalin-fixed, paraffin-embedded (FFPE) blocks were submitted to our institution for consultation. The tumour was composed of spindle and epithelioid cells, showing moderate to marked nuclear pleomorphism and brisk mitoses of about 34 per 10 HPF. The tumour infiltrated into surrounding tissue. In one section, an enclosed hyalinised fibroadenoma was seen in the midst of the tumour. Figure 2a–c shows H&E sections of Case 2 that were submitted for sequencing.

Submitted IHC slides were negative for AE1/3, CK5/6, CK14, CK7, EMA, p63, ER, PR, and HER2. A weak blush was observed in occasional spindle cells for HMB45 and S100, interpreted as negative. Faint cytoplasmic blush for MNF116 was seen in rare tumour cells. CD31 was negative in tumour cells, but demonstrated some staining in small vessels within the tumour. E-cadherin was positive in a significant proportion of tumour cells (Fig. 2d). Additional IHC stains performed on the submitted FFPE blocks revealed negative results for 34βE12, CK5/6, CK14, and S100. Patchy but positive staining in tumour cells was observed for p63. CD34 and F8 both showed some staining in intra-tumoural vessels, but CD34 was negative in tumour cells, with F8 demonstrating strong background staining and cytoplasmic reactivity in some tumour cells.

The final diagnosis was a malignant high-grade spindle cell tumour, which in light of the absence of a malignant epithelial component and lack of epithelial markers on IHC, favoured a sarcoma. The distinction between malignant spindle cell appearances of a metaplastic carcinoma from a malignant PT or sarcoma is crucial with regard to the divergent management approach.

This study was approved by the SingHealth Centralised Institutional Review Board (CIRB Ref: 2018/2581). Informed consent was obtained from both patients for further analysis using our customised gene panel. Tumour areas were marked out on H&E slides and matched to FFPE samples for macro-dissection. DNA was extracted with the QIAamp

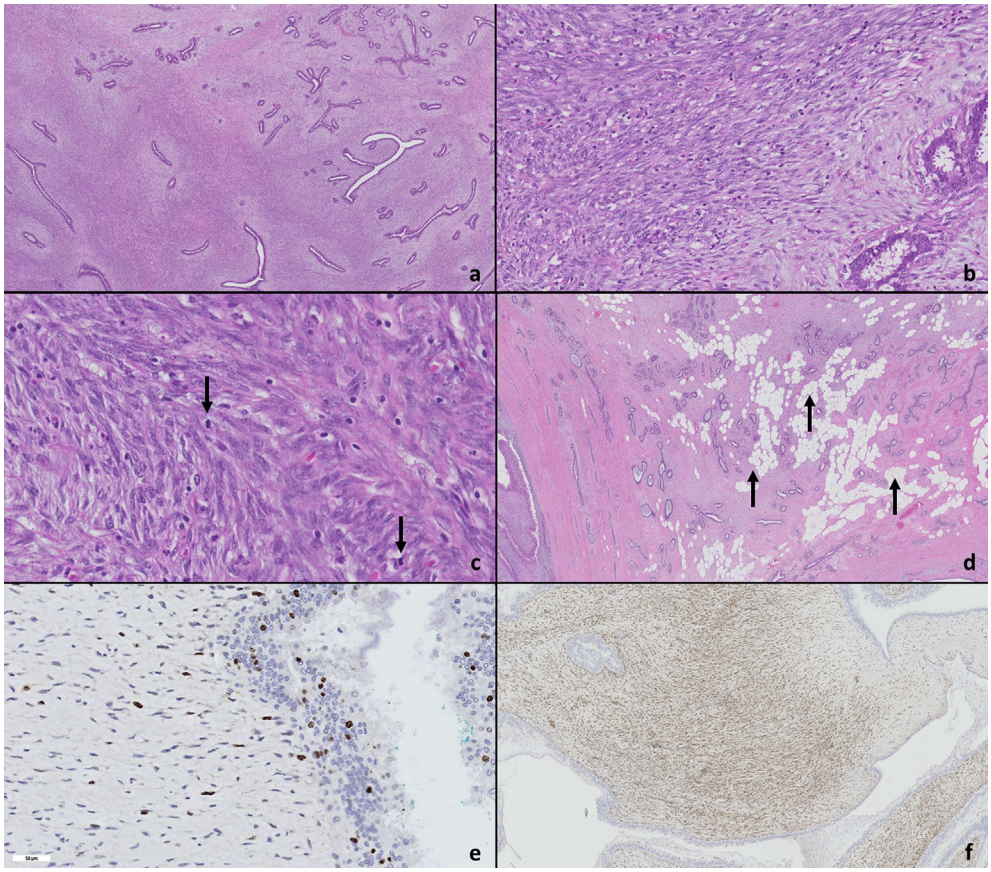

Fig. 1 Light microscopy images of sections from case 1 show (a) increased stromal cellularity in section 2 at low magnification, (b) increased stromal cellularity at medium magnification in section 12, (c) frequent mitoses (arrows) in section 2 at high magnification, and (d) permeative borders (arrows) in section 9 at low magnification. (e) IHC for Ki-67 shows scattered nuclear staining in both stromal and epithelial cells at medium magnification. (f) IHC for CD34 shows diffuse reactivity in the stromal cells of the PT (low magnification).

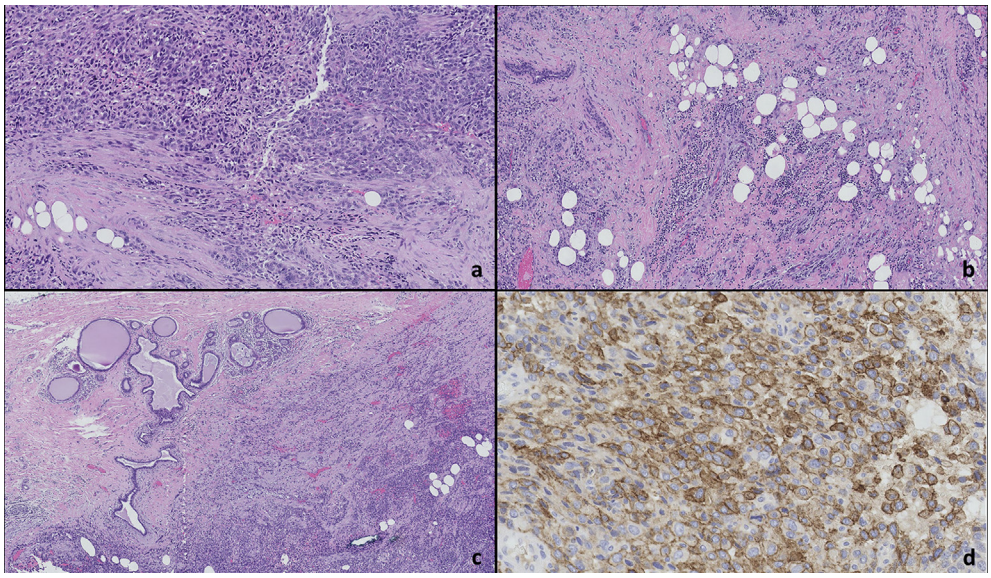

Fig. 2 (a–c) Light microscopy images from Case 2, with a–c corresponding to sections 1–3 (low magnification). (d) Positive expression of E-cadherin in tumour cells (medium magnification).

DNA FFPE extraction kit (Qiagen, Germany) from FFPE samples, which were assessed for quality and quantity through Nanodrop and PicoGreen dsDNA quantitation assay (ThermoFisher, USA). The samples that met the minimum requirement of 50 ng were enriched using an amplicon-based 16-gene custom QIAseq targeted DNA Panel (Qiagen) and sequenced to a minimum depth of 100× using the Hiseq 4000 sequencer (Illumina, USA) to generate 150 base pairs paired end results. FreeBayes (v1.1.0-4-gb6041c6, settings: -m 30 -q 30 -F 0.01 -u) was used in variant calling for single nucleotide variant (SNV) and indels, and all reported variants were manually curated on the IGV genome browser.[4]

Case 1 showed mutations for *TERT, RARA* and *MED12*, while Case 2 demonstrated mutations for *MED12* and *TP53*. The variant allele frequencies (percentage of mutant gene expressed) for these mutations are shown in Table 1.

Previously, we found that higher grade PTs are more likely to possess cancer-associated mutations such as *NF1, RB1, TP53, PIK3CA, ERBB4*, and *EGFR*.[3] The finding of only *TERT, RARA*, and *MED12* mutations in Case 1 leaned away from a malignant PT and supported the reviewed diagnosis of a borderline PT.

The marked differences in variant allele frequencies for *RARA* observed in Case 1, sections 2, 9, 10, 12, 13, could be attributed to tumour heterogeneity. Although only tumour regions were macro-dissected and submitted for sequencing, there could be genomic heterogeneity within the tumour. Sections 2, 12 and 13 showed increased stromal cellularity, with section 2 also displaying frequent mitoses numbering about 12 per 10 high power fields (Fig. 1a–c). Permeative borders were also seen in sections 9 and 10 (Fig. 1d).

RARA is a retinoic acid receptor that has been previously reported to be associated with oestrogen-related activities.[5,6] Our previous study[3] showed that the co-occurrence of *RARA* and *MED12* mutations happened at rates that are higher than expected by chance alone (permutation test p=0.0046,

Table 1 Variant allele frequencies (%) of mutations found in Cases 1 and 2

Case and section no.	TERT chr5:1295228	RARA chr17:38510601-38510603	MED12 chrX:70,339,215	
Case 1				
Section 1	20.4	13.6	19.8	–
Section 2	30.7	<1	27.4	–
Section 3	21.5	16.1	23.5	–
Section 4	27.2	14.7	24.5	–
Section 5	22.7	17.4	26.1	–
Section 6	24.9	19.3	25.0	–
Section 7	17.2	16.4	19.4	–
Section 8	26.0	15.4	21.8	–
Section 9	20.6	7.7	21.8	–
Section 10	12.5	<1	10.8	–
Section 11	22.3	22.4	28.7	–
Section 12	19.7	<1	28.5	–
Section 13	26.6	<1	17.7	–
			MED12 chrX:70339253 G>C	TP53 chr17:7577550 C>T
Case 2				
Section 1	–	–	40.7	61.4
Section 2	–	–	12.8	13.1
Section 3	–	–	19.2	22.6

100,000 trials). *MED12* is also involved in oestrogen signalling and its mutations have been proposed to comprise the driver genetic event of the more benign end of the fibroepithelial tumour spectrum.[7] This suggests that *RARA* mutations might also represent early events in formation of PTs,[3] possibly giving rise to the low variant allele frequencies observed in sections 2, 9, 10, 12 and 13, which displayed more aggressive histology.

As mentioned above, a malignant spindle cell tumour on histology observed in Case 2 gives rise to differential diagnoses of malignant PT, SCMBC or breast sarcoma. Studies that have sequenced SCMBC are few,[8–10] and none of the mutations detected corresponded to our panel except for *PIK3CA*, *TP53*, *PTEN* and *TERT* promoter. Alterations in *PIK3CA*, *TP53* and *PTEN* are common in breast cancer and thus not particularly helpful in distinguishing a PT from SCMBC. Interestingly, in Krings and Chen's study, no mutations in *TP53* were observed for SCMBC compared to other metaplastic breast carcinoma subtypes. Furthermore, to our knowledge, no alterations in *MED12* exon 2 have been identified in SCMBC, with only *MED12* G44D mutation seen in a metaplastic breast carcinoma of squamous subtype by Ross and team.[8] The discovery of only *MED12* exon 2 and *TP53* mutations in Case 2 helped to lean the diagnosis towards PT rather than SCMBC. Although the morphological diagnosis was a malignant spindle cell tumour favouring a sarcoma, the presence of *MED12* exon 2 mutation suggests an underlying PT. Studies have shown that malignant PTs and breast sarcomas appear to be closely related, with similar clinicopathological features and clinical outcomes.[11,12]

In summary, we report two histologically challenging cases: one where grading of the PT was in question, while the second case was a diagnostic dilemma posed by a malignant spindle cell tumour. Application of the 16-gene mutation panel helped support the revised grading of an originally diagnosed malignant PT to borderline, and was also useful in differentiating between malignant PT and SCMBC. Therefore, this mutation panel shows potential use as an adjunctive tool in diagnostic pathology, and validation in larger cohorts is ongoing.

Acknowledgements: We would like to thank Dr Aye Aye Thike and Dr Mihir Gudi for pathology contributions, and Dr Jinnie Pang and Dr Ong Kong Wee who were the managing surgeons for their clinical contributions. We are grateful to Ms Diyana Nasir, Ms Vikneswari Rajasegaran and Mr Guan Peiyong for their assistance in sample preparation and genomic data analysis, and Associate Professor George Yip for his input on the manuscript. V. C. Y. Koh is a recipient of the SingHealth TDF Award (Research).

Conflicts of interest and sources of funding: This study was supported by the SingHealth and Duke-NUS Research Collaborative Grant (SDC/021/2015) awarded to Dr P. H. Tan. The authors state that there are no conflicts of interest to disclose.

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DOI: https://doi.org/10.1016/j.pathol.2019.04.005

Primary adenosquamous carcinoma in a patient with Lynch syndrome

Sir,

Adenosquamous colorectal carcinoma (ASCC) is rare and reported to represent between 0.06% and 0.20% of colorectal carcinoma (CRC).[1] Microsatellite instability-high (MSI-H) CRC caused by DNA mismatch repair (MMR) deficiency is detected in 15% of CRC, of which 12% are sporadic. These are caused by hypermethylation of MLH1 gene promoter, whilst the remaining 3% are inherited and associated with Lynch syndrome (LS).[2] Recently, ASCC has been reported in association with MSI-H and LS, raising the possibility of MSI in the molecular pathogenesis and whether it may represent another histological subtype of MSI-H CRC. The molecular pathogenesis of ASCC remains poorly studied to date. Herein, we report the first case of ASCC with detailed molecular analysis by next-generation sequencing (NGS) of the glandular and squamous components, highlighting