

Characterization of Somatic Mutations in Malaysian Luminal Breast Cancer

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Abstract : Luminal breast cancer subtype (ER/PR+, HER2+/-) represents about two-thirds of all breast cancers, and better understanding of the genetic alterations underpinning the disease pathogenesis is desirable to develop improved treatment plans and to increase patients' survival. To date, other than hormonal and anti-HER2 therapies (e.g. tamoxifen, aromatase inhibitor, trastuzumab), no other targeted therapies have been approved by FDA for luminal breast cancer. Despite thousands of breast tumour samples have been sequenced, there is no data yet on Malaysian patients. Therefore, it is clinically important to identify actionable mutated genes or pathways implicated in our local breast cancer patients to establish a more defined framework for precision medicine and clinical trials. In this discovery study, a total of nine pairs of newly diagnosed luminal breast cancer cases (>80% tumour content) and their matched normal samples were subjected to exome sequencing. We detected a total of 491 somatic from nine pairs of breast tumour-normal samples. PIK3CA is the most frequently mutated gene in our discovery cohort of patients (n=4/9). Kinases and phosphatases were found as the most significantly enriched mutated genes (enrichment score = 3.12), with all nine luminal breast cancer samples harboring at least one non-synonymous mutation in this cluster of genes. This profile suggested that alteration in protein phosphorylation processes is among the key drivers in luminal breast cancer pathogenesis. Interestingly, genes involved in four key druggable cancer pathways, i.e. PI3K/AKT/mTOR, MAPK/ERK, NF- κ B and VEGF signalling pathways were found commonly mutated and require validation in a larger cohort. In conclusion, we have successfully profiled the somatic mutations in Malaysian luminal breast cancer and this is the first study conducted in Malaysian patients. These findings revealed the role of multiple gene testing in discovering luminal breast cancer mutational landscape.

Keywords: luminal breast cancer; exome sequencing; somatic mutation; actionable mutation; druggability

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BACKGROUND

Breast cancer is the most common malignancy among Malaysian women (National Cancer Registry Report 2007) and remains as the leading cause of cancer-related mortality in women worldwide^[1]. It is a heterogeneous disease and is broadly classified into three major subtypes based upon the expression of oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) status^[2]. Luminal breast cancer subtype represents about two-thirds of all breast cancers and is the most heterogeneous subtype comprising of tumours with six different profiles, namely, ER/PR/HER2 (ER+/

PR+/HER2+, ER+/PR-/HER2+, ER+/PR+/HER2-, ER-/PR+/HER2+, ER+/PR-/HER2-, ER-/PR+/HER2-). Even though ER positive breast cancer patients could be treated with anti-oestrogen (e.g. tamoxifen, fulvestrant) and ER/HER2 positive breast cancer patients with anti-oestrogen and/or anti-HER2 therapies (e.g. Nolvadex, Tamoxifen, Herceptin, Letrozole, Anastrozole, Exemestane), the lack of response in a subset of patients remains a major clinical issue. It has been reported that ER positive breast cancers exhibited de novo (~30%) and acquired resistance (~40%) to anti-oestrogen therapies^[3]. Also, nearly 20% of HER2 positive early breast cancers were resistant to anti-HER2 therapies, whilst 70% metastatic breast cancer cases were not responsive^[4]. Given that there is a significant number of patients who pre-

sented with de novo or acquired resistance to these therapies, it is therefore, imperative to obtain a better understanding of the molecular genetic changes underpinning the disease pathogenesis and to recommend additional treatment plans to improve patients' survival rates.

In the past decades, precision oncology with matching treatment plans based on the patient's tumour molecular profiles have yielded remarkable success in several malignancies. For instance, stratification of metastatic melanoma patients who harboured BRAF V600E to vemurafenib treatment has increased patient's survival rates^[5]. Additionally, stratification of lung cancer patients who harboured EGFR mutations to receive anti-EGFR therapies has achieved 70% response rate^[6]. Hence, incorporation of the mutational screening program to identify actionable therapeutic targets in cancer patients holds a great promise to transform clinical care and to allow implementation of personalised therapies. In recent years, the advent of next generation sequencing technologies have reshaped the paradigm of clinical trials from drug-centric to patient-centric^[7]. Accumulated evidence has shown that the individual tumour is unique and only harboured a low prevalence of common mutations across the different tumour types^[8]. In breast cancer, a comprehensive list of somatic mutations has been identified through several large-scale international sequencing projects^[9], and many somatic variants were found unique to individual patients. In fact, other than PIK3CA and TP53 which are found in 36% and 37% breast cancer respectively, the majority of the mutations were found in less than 5% patients^[10]. The lack of common mutations across cancer patients (prevalence of <5%) has increased the demand to implement genomic-driven clinical trials globally, and has strongly suggested that the concept "one drug fits all" may not be conducive anymore. Armed with the evidence that the individual tumour genetic pro-

file is distinct; it is believed that exome sequencing could be a more cost-effective approach to identify potential actionable candidate genes in an unbiased manner, whereby nearly 85% of the disease-causing variants were found in protein-coding regions^[11].

MATERIALS AND METHODS

Patient sample

A total of nine Malaysian female patients who were diagnosed with primary luminal breast cancer (grades II-IV) at the UKM Medical Centre in Kuala Lumpur were included into this study. Fresh frozen tissue samples and matched blood samples were collected from patients with written informed consent. This study has been approved by the Research Ethics Committee of the UKM Medical Centre (approval number: UKM 1.5.3.5/244/UMBI-2015-003). Approximately 6 ml of peripheral blood from the subject was collected into an EDTA blood tube, whereas the removed surgical tumour samples were immediately snap-frozen in liquid nitrogen prior to nucleic acid extraction. Three patients presented with metastasis (lung, liver or spinal) at the initial diagnosis whereas the other six patients did not have distant metastasis. All the patients were radiotherapy-naive and chemotherapy-naive at the time their samples were collected. Tumour samples were analyzed by immunohistochemistry for the status of oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) at the Histopathology Unit, Pathology Department. The summary of the patients' clinicopathological information is shown in Table 1.

DNA extraction

Sample	Age	Ethnicity	Richardson Grade	ER/PR/HER2	Distant Metastasis	Sample Type	On Target	Mean Depth (X)	20X coverage	Uniformity
C1	36	Malay	3	+/-/-	Lung, Liver, Spinal	Tumour	46.65%	51	82.82%	91.93%
						Normal	61.93%	52	86.87%	93.03%
C2	43	Chinese	3	+/-/-	Bone	Tumour	79.00%	62	85.38%	90.81%
						Normal	81.25%	55	85.82%	92.01%
C3	40	Malay	2	+ +/+	Spinal	Tumour	75.57%	82	88.94%	90.49%
						Normal	76.08%	71	89.12%	91.75%
C4	62	Malay	2	+ +/+	Absent	Tumour	75.64%	64	77.80%	82.87%
						Normal	74.37%	70	80.20%	84.02%
C5	58	Chinese	2	+ +/+	Absent	Tumour	75.89%	51	81.97%	89.60%
						Normal	70.45%	50	81.50%	89.58%
C6	47	Malay	2	+/-/+	Absent	Tumour	78.97%	58	85.12%	91.04%
						Normal	75.70%	50	81.23%	90.12%
C7	77	Chinese	3	+/-/+	Absent	Tumour	75.73%	60	85.24%	90.94%
						Normal	77.04%	53	86.84%	92.57%
C8	56	Malay	3	+/-/-	Absent	Tumour	78.96%	58	87.20%	92.67%
						Normal	75.39%	52	85.22%	92.50%
C9	76	Chinese	3	+ +/+	Absent	Tumour	73.17%	65	88.63%	92.46%
						Normal	81.95%	51	87.42%	93.52%
Average							74.71%	60X	85.20%	90.72%

Table 1. Patient clinicopathological information and sequencing metrics.

For tissue samples, a reference slide was taken from a 5 µm thickness cryosection, followed by staining with haematoxylin and eosin. The slides were then reviewed by a pathologist. Only samples with more than 80% tumour content were selected for DNA extraction using AllPrep DNA/RNA/miR-

NA Universal kit (Qiagen). For germline samples, DNA was extracted from EDTA-tube collected blood samples using salt precipitation protocol. The DNA purity was assessed by Nanodrop spectrophotometer (Thermo Scientific) whereas quantity was determined by Qubit dsDNA HS

Assay Kit (Thermo Scientific).

Preparation of libraries for TargetSeq exome capture

Briefly, 1 µg of total genomic DNA extracted from the tumour and matched blood samples (except for sample C1 which had matched normal tissue) was randomly sheared by focused acoustic shearing (Covaris). The sheared DNA was purified using the AMPure XP Reagent (Agencourt) with a target size peak of ~250 bp. The purified DNA was then subjected for library construction according to the Ion Xpress™ Fragment Library Kit manual (Thermo Scientific). The DNA was end repaired, ligated with the P1 adapter and barcoded (Ion Xpress™ Barcode Adapters Kit; Thermo Scientific), followed by size selection (~280 bp) using E-Gel® EX Agarose Gels, 2% (Thermo Scientific). The library was then amplified with Platinum PCR Supermix High Fidelity (Thermo Scientific), and purified with AMPure XP Reagent (Agencourt) prior to exome capture.

Exome capture and sequencing with Ion Proton sequencer

Pre-capture library DNA was hybridized to exome capture probes using the Ion TargetSeq Exome Enrichment Kit (Thermo Scientific) according to the manufacturer's specifications. The probe-hybridized library DNA was then captured with streptavidin-coated M-270 beads (Dyna) The probe-hybridized DNA was then subjected for 12 cycles of PCR amplification at 95°C for 15 s, 58°C for 15 s, and 72°C for 60 s. The amplified exome library was purified twice with a 1.5-fold volume of AMPure XP reagent, and eluted in 25 µL ddH₂O. The barcoded exome libraries were pooled and subjected to emulsion PCR using the OneTouch 2 instrument (Thermo Scientific) with an Ion PI Template OT2 200 Kit v2 following the manufacturer's instructions. The enrichment of template-positive Ion Sphere Particles (ISP) was performed using the Ion OneTouch ES enrichment system (Thermo Scientific). The enriched Ion Sphere™ particles were then loaded onto an Ion P1 Chip V2 and sequenced on the Ion Proton using Sequencing V3 kit.

Bioinformatics analysis

Briefly, the reads were aligned to the hg19 reference sequence, followed by removal of duplicates and variant detection by Torrent Variant Caller (TVC) version 4.4.2.1. The coverage of each amplicon was obtained by the Coverage Analysis Plugin software v4.4.0.20 (Thermo Scientific). Called variants with Phred quality scores of <30 were then filtered out using SnpSift, followed by annotation using ANNOVAR^[12]. The variants with the following criteria were included in further analysis: (a) variants in coding region and splice site; (b) a minimum coverage of 8X was achieved for both tumour and germline samples; and (c) a minimum of 2X variant reads in tumour samples and absent or with a variant threshold less than 1% in matched normal. Variants detected in the homopolymer region were excluded. The detected variants were then compared against public databases, including dbSNP142, 1000 Genomes project, and 6500 exomes of the National Heart, Lung, and Blood Institute Exome Sequencing Project to filter out polymorphisms with allele frequency >1%, ex-

cept for those variants reported in the COSMIC database (COSMIC 70). Somatic mutations were determined by subtracting against matched germline variants and manually verified by viewing through Integrative Genomics Viewer (IGV)^[13]. The deleterious effect of the mutations was predicted using SIFT^[14] and Polyphen2^[15]. The variants were annotated based on the Ensembl gene database for hg19. Furthermore, DAVID analysis (<http://david.abcc.ncifcrf.gov/>) was performed to investigate the gene ontology and enriched biological process or pathways implicated in the breast cancer pathogenesis.

Validation of mutations by Sanger sequencing

A total of 22 candidate druggable mutations were selected for validation by Sanger sequencing to confirm the somatic mutation status and to determine the accuracy of exome sequencing. Primers specific to the regions of interest were designed using Primer-BLAST (Additional File 1: Table S2). PCR amplification was conducted using AmpliTaq Gold® DNA Polymerase (Thermo Scientific) using the following cycling conditions: one cycle at 95 °C for 5 min, 35 cycles at 95 °C for 1 min, 56°C or 65°C for 1 min and 72 °C for 1 min, and one cycle at 72 °C for 5 min. Sequencing was performed with ABI BigDye Terminator v3.1 (Thermo Scientific). The sequence chromatograms were visually inspected with Bioedit software.

RESULTS

Breast cancer patients

This study involved nine Malaysian patients with luminal breast cancer with various combinations of oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) status. All the tumours expressed the ER marker. The median age of the patients was 56 years, with a range of 36-77 years (Table 1). There were five patients who presented with grade III tumours while the other four patients had grade II tumours. Three patients (C1, C2, and C3) presented with synchronous distant metastasis whereas the others (C4, C5, C6, C7, C8, and C9) were not diagnosed with distant metastasis.

Identification of somatic mutation by exome sequencing

Both tumour and matched normal samples were sequenced to a mean coverage of 56X (range, 51X–82X) and 61X (range, 50X–71X) respectively, with an average of 85% of targeted bases covered by at least 20X across the sample set (Table 1). By using the described filtration strategy (see Materials and Methods), we identified a total of 491 somatic mutations in the exonic and splice site regions. Of these mutations, 368 were protein-altering (288 missense, 50 indels, 17 nonsense, 12 splicing), whereas the remaining 124 were synonymous (Figure 1). Patients who were >50 years or with distant metastatic disease did not have significantly more mutations as compared to patients with age <50 years (*p*-value=0.38) or without distant metastatic disease (*p*-

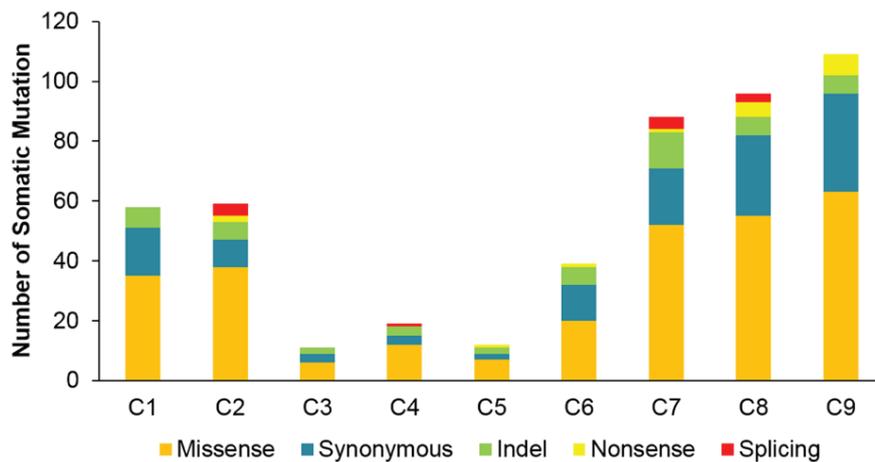


Figure 1. Number and type of somatic mutations identified in nine pairs of matched tumour-normal Malaysian luminal breast cancers. Our analysis identified a total of 491 somatic mutations, in which 367 were non-synonymous and 124 were synonymous.

value=0.47). Interestingly, patients with grade III tumour harbour (Figure 3) significantly more mutations (range 58-109) as compared to those with grade II tumour (range 11-39) (p -value = 0.002). To understand the impact of the mutations on gene functions further, we applied SIFT^[14] and Polyphen2^[15], and found that ~76% ($n=280/367$) of protein-altering mutations were likely to have functional consequences (damaging or probably damaging) according to at least one of the two methods (Additional File 1: Table

S1). In addition, consistent with previous data on breast cancer, analysis on the nucleotide substitution pattern in all our nine breast cancer samples confirmed that the C>T transitions were predominant (accounting for 50-60% of all substitutions) (Figure 2), a mutational signature associated with deamination of 5-methyl-cytosine to uracil by APOBEC enzyme^[16].

Recurrently mutated genes

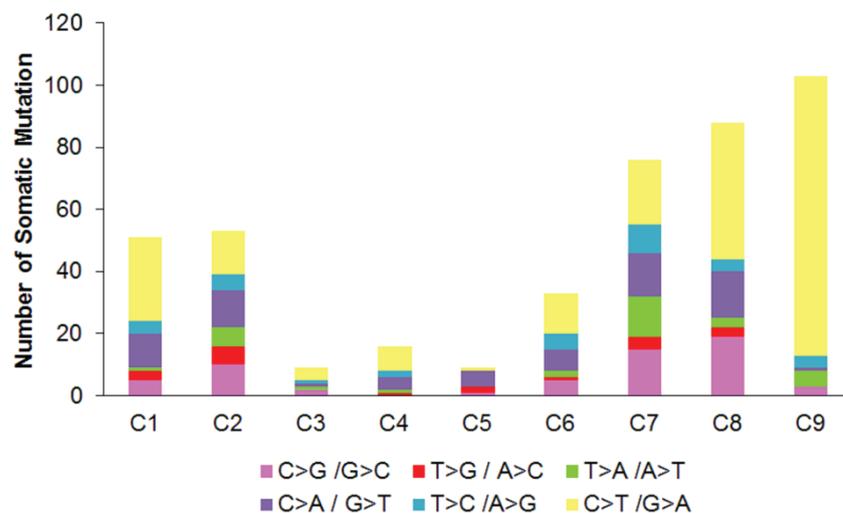


Figure 2. Mutation spectra of Malaysian luminal breast exomes. C>T transition was predominant, a mutation signature associated with APOBEC expression.

One way to identify driver mutations is to look for those that are recurrent across multiple samples. In our study, we detected a total of eight recurrently mutated genes, with PIK3CA as the most frequently mutated gene (p.E545K, $n=2$; p.H1047R, $n=1$; p.R38C, $n=1$), followed by KIF27 (p.R860X, p.G461A), MAP3K3 (p.I504N, p.R502W), SF1 (p.G572fs, p.G572R), SYNE1 (p.F7302fs, p.D4267Y), TCHH (p.A101D, p.E294X), UTP20 (p.E1986D, p.I1927V), and HPSE (c.1325+2T>A, p.K307M). Majority of the mutations were novel and not listed in COSMIC. Of these candidate mutations, PIK3CA mutations are hotspot mutations which have previously been classified as driver mutations in various types of cancer, including breast cancer^[9].

Enriched molecular functions and pathways

Functional enrichment analysis using DAVID (Database for Annotation, Visualization and Integrated Discovery) (<http://david.abcc.ncifcrf.gov>) revealed that genes involved in protein phosphorylation was the most significantly enriched (enrichment score =3.12, p -value=0.008; Additional File 1: Table S3), in which 21 were kinases (AAK1, AKT, CDC42BPA, SMG1, ADRBK2, CSK, CDKL5, DDR2, GALK2, IKKBK, IRAK2, IRAK3, KDR, LMTK2, MAST4, MAP3K3, OBSCN, PIK3CA, PRKAR1B, STK31, TTN) and 7 were phosphatases (ENPP3, INPPL1, PTEN, PTP4A2, PTPN11, PTPRO, SLC20A1) (Figure 3). Beyond that, our analysis also identified genes involved in cell adhesion processes (PPFIA2, TNXB, MAGI1, COL13A1, INPPL1, NELL1, LRRN2, FERMT3, TNC, NELL2, NLGN1, SDK1, PCDHGB6, VTN, DDR2, CDH5, PODXL2, NPHP4, FREM2,

LAMA5, DSG1, SUSD5, LAMC2, MUC5B) (enrichment score=1.7, p -value = 0.02; Additional File 1: Table S3), and chromatin modification (ING4, EPC1, CSRP2BP, SETDB2, HUWE1, C11ORF30, WHSC1L1, SMARCAL1, ACTL6B, CBX2, RB1, IRF4) (enrichment score = 1.4, p -value = 0.03; Additional File 1: Table S3) were commonly mutated, and may play significant roles in driving breast cancer pathogenesis.

Druggable alterations

Our analysis also identified the somatically mutated genes which are druggable, and for which approved drugs are available or in clinical trials. As depicted in Figure 4, among those pathways affected include the PI3K/Akt/mTOR signalling pathway (AGO3, AKK1, CREB5, COL4A4, DDR2, ESR1, GRAP, GNAZ, INPPL1, LAMA5, LMTK2, LAMC2, PDGFRB, PIK3CA, PPP2R2B, PTEN, PTPN11, RB1, TENC1, TNC, TNXB, TP53), MAPK/ERK signalling pathway (ADCY2, ATF7, CACNA2D3, COL13A1, FGF13, KSR1, MAP3K3, MAPK7, NF1, PRKAR1B), NF- κ B signalling pathway (IK β K β , IRAK2, IRAK3, IRF2, IRF4, MALT1, PAWR), and VEGF signalling pathway (CDH5, CSK, KDR, NELL1, NELL2, VTN). To evaluate the performance of exome sequencing on Ion semiconductor chemistry, we randomly selected 22 druggable alterations for Sanger validation. A total of 21/22 of the selected mutations were confirmed, gave rise to 95% specificity. However, one mutation in IK β K β gene (IK β K β g. 42162746C>T) was failed to be confirmed by Sanger Sequencing and identified as false positive.

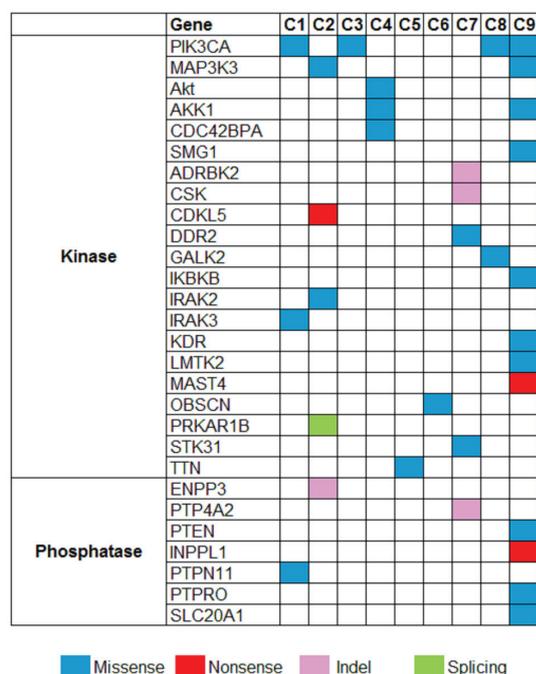


Figure 3. The distribution of mutated kinases and phosphatases. Our analysis found that dysregulation of protein phosphorylation processes is the key driver of luminal breast cancer pathogenesis.

DISCUSSION

It has been estimated that about 30–40% of women diagnosed with invasive breast cancer will eventually develop metastatic disease^[17], in which there is little or no cure. Even

though primary luminal breast tumour responds well to existing chemotherapy, anti-HER2 or hormonal therapy, the high recurrence rate indicates that current treatment strategies are not effective to eradicate residual disease which will later transform into a more aggressive and relatively resistant tumour subpopulation. Therefore, a broader range of therapeutic options is needed to allow the tumour cells to be eliminated through different druggable pathways during initial treatment. In this preliminary study, we sought to uncover the mutational landscape of Malaysian luminal breast cancers and to identify potentially actionable therapeutic targets for the disease. In agreement with many other studies^[9], our findings indicated that mutations are rarely shared across patients, and each tumour harboured a distinct set of mutated genes or mutations which affected multiple druggable pathways, and may be amenable to broader therapeutic options. Other than PIK3CA which was found to be recurrently mutated in 4/9 samples, other genes were mutated in only 2/9 samples (MAP3K3, KIF27, SF1, SYNE1, TCHH, UTP20, HPSE). In contrast to other findings which reported TP53 as the second most common mutated gene implicated in luminal breast cancer, we only detected one protein-altering mutation (p.M246V). The low prevalence of TP53 mutation in our local samples could be attributed to the small sample size and warrants further validation in additional samples sets. Despite individual breast tumour sample showing a distinct mutational profile, genes involved in protein phosphorylation processes appeared as the most significantly enriched biological process across all nine patients (Additional File 1: Table S3). We postulated that these non-recurrent mutated kinases and phosphatases may be interacting cooperatively leading to constitutive activation of signalling pathways essential in conferring growth advantage to breast cancer cells.

Our analysis found that at least four key druggable pathways were commonly mutated in luminal breast cancers (PI3K-Akt-mTOR, MAPK/ERK, NF- κ B, VEGF) with some cases harbouring numerous mutated genes involved in similar pathways (e.g. C9 had mutations in PIK3CA and PTEN which involve in PI3K-Akt-mTOR pathway; Figure 4), whereas others had several mutated genes affected multiple pathways (e.g. C2 had mutations in genes involved in PI3K-Akt-mTOR (CREB5, COL4A4, ESR1, GRAP, INPPL1, PPP2R2B), NF- κ B (IRAK2, PAWR), MAPK/ERK (ADCY2, MAP3K3, PRKAR1B) and VEGF (VTN); Figure 4). Genes involved in regulating the PI3K/Akt/mTOR pathway were found mutated in 7/9 of cases. Activation of PI3K-Akt-mTOR pathway plays pivotal roles in promoting cancer growth by mediating cell proliferation, angiogenesis, migration^[18], and associated with the development of resistance to hormonal treatment in breast cancer^[3]. Over the past decades, inhibition of the PI3K-Akt-mTOR pathway has increasingly gained interest as a potential treatment modality for breast cancer patients. Numerous clinical trials on PI3K inhibitors, mTOR inhibitors, and Akt inhibitors are actively ongoing, and preliminary findings supported that patients administered with combination therapies (aromatase inhibitor & PI3K/Akt/mTOR inhibitor) have a better outcome as compared to patients treated with aromatase inhibitor alone^[19]. Given

that somatic mutations in PIK3CA and PI3K/Akt/mTOR signalling pathways were observed in four and seven of our luminal breast tumour samples respectively, it is believed that a substantial number of our local breast patients could benefit from this class of targeted therapy.

It has been reported that genes regulating MAPK/ERK signalling pathway were recurrently mutated in nearly 2-10% of breast cancer^[10], and involved in promoting breast cancer proliferation, invasion, migration, and resistance to tamoxifen^[20]. In our study, mutations involving the MAPK signalling pathway were seen in five out nine cases (Figure 4). We also detected recurrent mutations in MAP3K3 (n=2/9). Constitutive activation of MAPK pathway is one of the key molecular alterations underpinning breast cancer oncogenesis, and increased MAPK activity was found to be associated with lymph nodes metastasis^[21], and shorter disease-free survival^[22]. The aggressiveness of the disease also has been attributed to the presence of cancer stem cells. Earlier study has shown that MEK inhibitors was effective in eliminating breast cancer stem cells and prevented lung metastasis^[23]. Moreover, the effectiveness of MEK inhibitor selumetinib in reversing resistance of ER-positive high grade serous ovarian cancer towards anti-oestrogen therapy^[24] also strengthened the fact that inhibition of MAPK/ERK pathway may be a potential therapeutic strategy for improving treatment outcome in breast cancer.

Genes involved in NF-κβ pathways were found mutated in 5/9 cases (Figure 4). Earlier studies have shown that constitutive activation of NF-κβ pathway is frequently observed in breast cancer^[25] and is associated with breast cancer oncogenesis and progression^[26]. Notably, NF-κβ activation has been reported to play an important role in ER-positive endocrine-resistant breast cancer and the acquisition of anti-oestrogen (specifically tamoxifen) resistance, which correlates with earlier relapse, metastasis and a reduced overall survival^[27]. Similarly, NF-κβ activation and their involvement in chemoresistance were also observed in breast tumour cells treated with paclitaxel^[28], cisplatin, 5-fluorouracil and gemcitabine^[29]. Earlier studies have shown the chemoresistance mechanism may be partially mediated by expansion of breast cancer stem cells which have self-renewal capacity and capable to drive tumour growth and recurrence^[30]. The efficacy of NF-κβ inhibition in selectively eliminate breast cancer stem cells^[31] shall be considered among the strategic therapeutic options to overcome chemoresistance in breast cancer treatment.

Beyond that, it has been well recognized that cancer pathways are highly complex and inhibition of a pathway may lead to augmentation of another pathway which promotes the disease progression. For instance, inhibition of PI3K signalling pathway alone led to augmented MEK/ERK signalling, and impaired anti-tumoural activity^[32]. Given that numerous evidence has shown deregulation of PI3K/Akt/mTOR, MAPK/ERK and NF-κβ pathways are associated with breast cancer pathogenesis and disease recurrence^[33], targeting these signalling pathways therefore represents among the most attractive and promising therapeutic option to overcome chemoresistance and to realizing personalized targeted therapies. Moreover, it is increasingly recognized that combination regimens are more effective than mono-agent therapy to eliminate tumour cells which

are genetically heterogeneous. For instance, a previous preclinical study showed that dual inhibition of PI3K/Akt/mTOR and MAPK/ERK pathways is more efficient than single pathway inhibition^[34]. Also, blockade of the ERK and NF-κβ pathways has been shown to actively suppress the EMT, migration, invasion, and stem-like features in breast cancer cells^[35]. Interestingly, clinical trials on the dual-targeting strategy involving PI3K/AKT/mTOR and RAS/MEK/ERK pathways were particularly effective in tumours with genetic alterations in both pathways^[36], and abrogated mechanisms of resistance in solid tumours, including lung cancer^[37] and melanoma^[38]. Our analysis demonstrated the prominent co-existence of mutations across PI3K/MAPK/NF-κβ pathways, hence suggested that a combination of drugs targeting oestrogen, PI3K, NF-κβ and MAPK pathways simultaneously or sequentially is a rational therapeutic strategy for breast cancer patients. The association of the mutational status and their actionability warrants further investigation.

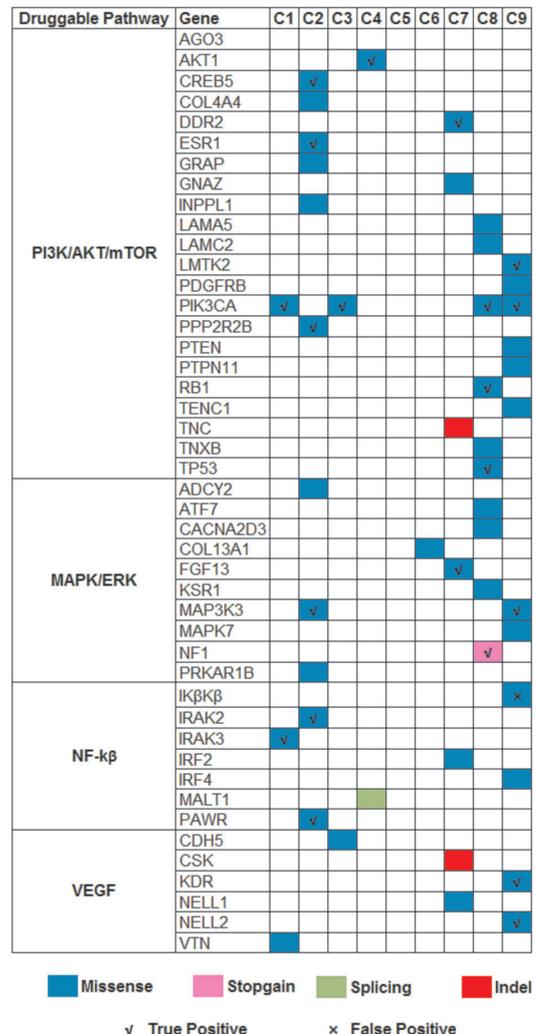


Figure 4. The landscape of potentially clinically actionable somatic alterations identified in 9 pairs of Malaysian luminal breast cancer.

CONCLUSION

In summary, PIK3CA appeared as the most frequently mutated gene in Malaysian luminal breast tumours. Our analysis revealed that each of the tumour harboured

distinct mutation profiles which affected numerous drug-gable pathways, notably PI3K, MAPK, NF- κ B, and VEGF signalling pathways. These findings highlight the role of multiple gene testing in discovering luminal breast cancer mutational landscape.

Notwithstanding breast cancer is genetically well-characterized in Caucasian populations, little is known about the mutational profile in Malaysian patients. Hence, this study was initiated to uncover the mutational landscape implicated in our Malaysian luminal breast cancer cases, and to identify potentially actionable therapeutic targets or pathways to establish a more define clinical trial framework for our local patients. In this study, a total of nine pairs of matched tumour-normal luminal breast cancer samples were subjected to whole exome sequencing using the benchtop Ion Proton Sequencer to an average coverage of 60-fold. Our findings from the discovery set of data, demonstrated that exome sequencing on Ion Proton platform is clinically useful to identify somatic mutations in an unbiased manner, with an accuracy of 95% validated by Sanger sequencing approach. However, further study in larger series of samples will be required to confirm the identified variants and how they implicated in treatment, especially in our local population.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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