Homologous recombination repair gene mutations in Malaysian prostate cancer patients

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ABSTRACT

Genetic alterations in the homologous recombination repair (HRR) genes are associated with an increased risk of prostate cancer development, and patients harboring these mutations can benefit from targeted therapy. The main aim of this study is to identify genetic alterations in HRR genes as a potential target for targeted treatment. In this study, targeted next generation sequencing (NGS) is used to analyze mutations in the protein-coding regions of the 27 genes involved in HRR and mutations in hotspots of 5 cancer-associated genes in four FFPE samples and three blood samples from prostate cancer patients. We identified two mutations in TP53 and KRAS. We also identified four conflicting interpretations of pathogenicity variants in BRCA2, STK11 genes and one variant of uncertain significance in the RAD51B gene. In addition, we detected one drug response variant in TP53, and two novel variants in CDK12 and ATM. Our results revealed some actionable pathogenic and potential pathogenic variants that may be associated with response to the Poly (ADP-ribose) polymerase (PARP) inhibitor treatment. More studies in a larger cohort are needed to evaluate and determine the association of HRR mutations with prostate cancer.

Introduction

Prostate cancer (PC) is the second most common cancer and the fifth leading cause of cancer death among males worldwide. In 2020, 1.4 million new cases and 375,000 death were reported globally (1). In the US, PC is the most frequently diagnosed cancer after lung cancer and the second cause of death in men (2). Major PC risk factors are age, race and family history of prostate cancer (3). Based on a twin study, 57% of PC risk is attributable to inherited factors (4). The most common mutations in hereditary prostate cancer (HPC) occur in the HOXB13 gene, mismatch repair (MMR) genes and HRR genes (5).

HRR genes play an essential role in maintaining genomic integrity and cancer prevention in humans (6). Homologous recombination function is required for repairing DNA lesions including DNA double-strand breaks, meiosis (7), and restart of stalled forks (8). It is vital to measure homologous recombination deficiency (HRD) because cells with HRD are sensitive to PARP inhibition (9). HRD is mostly caused by biallelic alterations of BRCA1, BRCA2, PALB2 and RAD51C. HRD has been identified in different types of cancer including ovarian and breast cancer, pancreatic and prostate cancer (10). HRR gene mutations have been detected in more than 20% of metastatic castration-resistant prostate cancer (mCRPC) (11). The most frequently identified gene mutations among the HRR-related genes in PC patients have been reported in BRCA2 and ATM genes (12, 13, 14, 15, 16). Mutations in these HRR genes (17, 18), as well as some of the other HRR genes such as CHEK2 (19), PALB2 (20), CDK12 (21), BRCA 1 (22), are associated with an increased risk of PC development in some populations.

In this study, targeted NGS was used to analyze mutations in the protein-coding regions of the 27 homologous recombination genes and mutations in hotspots of 5 cancer-associated genes in four FFPE samples and three blood samples from prostate cancer patients.

Materials and Methods

Samples

This study was approved by National Medical Research (NMRR). Formalin-fixed paraffin-embedded (FFPE) tissue blocks and blood samples were obtained from the Serdang Hospital. Hematoxylin and eosin (H&E) stained tissue sections of tumor biopsy samples were reviewed by a pathologist to ensure that all FFPE tumor samples have at least 30% tumor cells. The tumor area with <30% tumor cells were circled in H&E slides and used as a guide for the macrodissection. Tumor tissues were macrodissected and guided by H&E sections. The blood samples were collected from patients who had been referred to the hospital for the treatment of PC. The young subjects ≤18 years old

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and patients with post-prostatectomy duration of less than six months were excluded from this study.

**DNA extraction**

The DNA was extracted from two 10-μm section of FFPE tumor samples and 0.5 mL of peripheral blood samples by the GeneRead FFPE DNA Kit (Qiagen, Hilden, Germany) and the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany), respectively following manufacturer’s instructions. The quality of extracted DNA was assessed by QIAxcel Advanced capillary electrophoresis system (Qiagen, Hilden, Germany), and DNA concentration was measured by the Qubit fluorometer (Thermo Fisher Scientific, Paisley, UK). Samples with a concentration of less than 14 ng/μL and total DNA of less than 100 ng were excluded from this study.

**Library preparation**

DNA libraries were prepared using the HRR handle panel (32 genes, Amoy dx) according to the manufacturer’s instructions. The quality of the libraries was checked out by QIAxcel Advanced capillary electrophoresis system (Qiagen, Hilden, Germany). The prepared DNA libraries were sequenced on MiSeq System (Illumina, San Diego,) with 2x150 bp paired-end reads and a median coverage depth of 1297X.

**Bioinformatical analysis**

The read data were aligned to the human HG19 reference genome. Sequence variants were annotated using an Annotator. Samples with a sequencing depth of less than 500X and allelic frequency < 5% were not reported. Natural germline variants were filtered by the 1000 Genomes Project. For variant analysis, 1000 Genome Frequency and Ingenuity Knowledge Base databases(https://www.internationalgenome.org/1000-genomes-browsers), Catalogue of Somatic Mutations in Cancer (COSMIC) (https://cancer.sanger.ac.uk/cosmic.), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar) were used. Sequence variants were classified as pathogenic, likely pathogenic, Variant of Uncertain Significance (VUS), and likely benign or benign based on ClinVar. A variant was considered as a novel if it was absent in the COSMIC database, 1000 Genome, LOVD, and dbSNP. In silico predictor tools Mutation Taster (23) was used to predict the potential impact of amino acid substitution on protein function.

**Results**

Table 1 shows the clinical characteristics of 7 Malaysian PC patients. The mean age at first PC diagnosis was 56 years (range: 52–58 years) and all samples were at stage 4 of PC. Twenty-seven HRR genes were fully covered in the coding sequence (CDS), and the UTRs (Table 2), while SNVs/Indels variants in the hotspot region of five other genes including BRAF, ERBB2, KRAS, NRAS, and PIK3CA were covered. The sequencing coverage for all samples was 100% and the average depth was 1297X with a minimum depth of 885X.

Of seven prostate cancer patients, 6 had at least one pathogenic, VUS, conflicting interpretations of pathogenicity (CIP) or drug response variant. Pathogenic mutation was detected in TP53 (c.818G>A; p. (R273H)), KRAS (c.34G>C; p. G12R) genes while conflicting interpretations of pathogenicity (CIP) variants were detected in BRCA2 (c.6325G>A; p. V2109I; c.9875C>T; p. P3292L, c.943T>A; p. C315S), STK11 (c.1062C>G; p. F354L), and VUS was identified in RAD51B (c.619G>T; p. V207L). Moreover, one drug response variant in TP53 (c.215C>G p. P72R) gene was identified in 6 samples, and two novel variants including c.2447del; p. (M816Fs*5) and c.3648del; p. (L1217Fs*6) were identified in CDK12 and ATM, respectively. The most frequently mutated genes were TP53 (4/7), followed by BRCA2 (2/7), STK11 (2/7), RAD51B (1/7), and KRAS (1/7). The table 3 shows the detailed information on genetic alterations.

**Discussion**

In the present study, targeted NGS of protein-coding regions of the 27 homologous recombination genes and mutations in hotspots of 5 cancer-associated genes, identified pathogenic and potential pathogenic variants in TP53, BRCA2, STK11, KRAS, RAD51B, CDK12, and ATM. DNA libraries were prepared using AmoyDx® HANDLE HRR NGS Panel. This kit is based on Defer-Ligation Enrichment system (HANLE system) technology and Halo-Shape Annealing. Each individual DNA molecule is tagged during the library preparation with a unique molecular index (UMI) at both ends to eliminate any library amplification and sequencing bias that results in high sensitivity in variant detection. The Limit of Detection (LoD) for this kit is 5% allele frequency for somatic variant, with an accuracy of 100%, specificity of 100% and Precision of 100% (24).

In our study, TP53 variants including c.215C>G p. (P72R) and c.818G>A p. (R273H) were identified in 4 FFPE and 2 blood samples. 4 FFPE and 2 blood samples had drug response c.215C>G; p. (P72R) variant, while one FFPE sample had pathogenic c.818G>A; p. (R273H) variant. TP53 is the most frequently mutated gene in human cancer (25), and its mutations are associated with different types of human cancer (26). While some studies have shown an association between p53 P72R polymorphism

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age</th>
<th>TNM</th>
<th>Stage</th>
<th>Sample types</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58</td>
<td>Malay</td>
<td>4</td>
<td>Blood</td>
</tr>
<tr>
<td>2</td>
<td>58</td>
<td>Malay</td>
<td>4</td>
<td>FFPE</td>
</tr>
<tr>
<td>3</td>
<td>52</td>
<td>Chinese</td>
<td>4</td>
<td>FFPE</td>
</tr>
<tr>
<td>4</td>
<td>54</td>
<td>Chinese</td>
<td>4</td>
<td>FFPE</td>
</tr>
<tr>
<td>5</td>
<td>56</td>
<td>Malay</td>
<td>4</td>
<td>Blood</td>
</tr>
<tr>
<td>6</td>
<td>56</td>
<td>Malay</td>
<td>4</td>
<td>FFPE</td>
</tr>
<tr>
<td>7</td>
<td>58</td>
<td>Chinese</td>
<td>4</td>
<td>Blood</td>
</tr>
</tbody>
</table>

**Table 1. Clinicopathological features of the 7 patients with PC.**

<table>
<thead>
<tr>
<th>AmoyDx®</th>
<th>HANDLE</th>
<th>HRR</th>
<th>NGS</th>
<th>Panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR</td>
<td>ATM</td>
<td>ATR</td>
<td>100%</td>
<td>BRD1</td>
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<tr>
<td>BRCA2</td>
<td>BRIPI</td>
<td>CDH1</td>
<td>CDK12</td>
<td>CHEK1</td>
</tr>
<tr>
<td>CHEK2</td>
<td>ESR1</td>
<td>FANCA</td>
<td>FANCL</td>
<td>HDAC2</td>
</tr>
<tr>
<td>HOXB13</td>
<td>MRE11</td>
<td>NBN</td>
<td>PALB2</td>
<td>PPP2R2A</td>
</tr>
<tr>
<td>PTE1</td>
<td>RAD51B</td>
<td>RAD51C</td>
<td>RAD51D</td>
<td></td>
</tr>
<tr>
<td>RAD54L</td>
<td>STK11</td>
<td>TP53</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Included homologous recombination genes in AmoyDx NGS Panel.**
and increased risk of prostate cancer (27, 28), other studies have not found any significant association (29, 30). TP53 c.818G>A; p.(R273H) variant has already been identified in prostate cancer patients (31). It has been suggested that Mutant p.35-R273H is associated with increased cancer cell survivability, anoikis resistance (32), cell migration and tumor metastasis (33).

Among the homologous recombination genes, BRCA2 is the most mutated gene (12). BRCA2 plays a role in homologous recombination repair by RAD51 regulation (34). We detected three variants including c.9875C>T; P3292L, c.943T>A; p.(C315S) and 6325G>A  V2109I in BRCA2 gene. c.9875C>T; P3292L variant that was detected in one of our FFPE samples, has already been found in prostate cancer (31). It has also been reported in BC patients (35), and pancreatic ductal adenocarcinoma (36). In a study by Tram et al, the P3292L variant was suggested as potentially clinically significant rather than VUS (37). Another variant detected in BRCA2, was c.943T>A; p.(C315S). This variant that was identified in one of our FFPE samples, has been found in both healthy and breast cancer patients in the Malaysian population with a frequency of 1.2% and 0.9%, respectively, and has been suggested as a likely benign variant (38). To our knowledge, this variant has not been reported in prostate cancer. We also identified 6325G>A  V2109I variant in two FFPE samples. This variant has already been found in Japanese Families with prostate cancer (39), familial esophageal cancer (40), and breast and/or ovarian cancer patients (41).

Kirsten Rat Sarcoma virus (KRAS) is an oncogene that was first discovered in 1982 (42). It has been reported that mutant KRAS induces bone metastasis and prostate cancer stemness (43). c.34G>C; p.(G12R) variant from the KRAS gene was detected in one of our FFPE samples. This variant has already been reported in metastatic prostate cancer (44), colorectal cancer (45), pancreatic cancers (46), non-small-cell lung cancers (47) and adenoid cystic carcinoma (48). KRAS mutations are commonly linked to resistance to targeted therapies and poor outcomes in cancer patients, and no targeted therapy has been approved for patients harboring KRAS mutations. However, there has been promising progress in targeting KRAS mutations for cancer treatment as the phase 1 trial of sotorasib has been encouraging in the treatment of patients with advanced solid tumors that harbor the KRAS p.G12C mutation (49).

STK11 c.1062C>G; p.(C315S) variant that was detected in two of our FFPE samples, has already been reported in prostate cancer (31), and colorectal tumors (50). The STK11 gene (also called LKB1) is a tumor suppressor gene that plays a role in cell cycle arrest (51). Loss of STK11 expression has been reported during PC progression (52).

RAD51B is a RAD51 homolog that participates in recombinational repair and meiotic recombination (53). In our study, RAD51B c.619G>T; p.(Val207Leu) VUS variant that was detected in two of our FFPE samples, has already been reported in prostate cancer (31), and colorectal tumors (50). The STK11 gene (also called LKB1) is a tumor suppressor gene that plays a role in cell cycle arrest (51). To our knowledge, this variant has not been reported in prostate cancer patients. The association between RAD51B alterations with prostate cancer has not been well studied. However, RAD51B up-regulation has been linked to poor prognostic in gastric cancer and suggested as a biomarker for GC diagnosis (55).

The strength of our study was the high depth of coverage (>×1000) that provides a highly accurate interpretation of data. However, the most limitation of the current study is the limitation of the case number.

Conclusion

In our study, targeted NGS sequencing revealed some variants with potential pathogenicity their association with prostate cancer is unknown. More researches in a larger population are needed to reveal the association of homologous recombination genes with prostate cancer which may result in benefiting more patients from targeted therapy.

Acknowledgments

Table 3. Somatic and germline mutations detected in Malaysian PC patients.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Allele Change</th>
<th>AA* mutation</th>
<th>Type of mutation</th>
<th>Mutation Taster</th>
<th>Sample ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>c.215C&gt;G</td>
<td>p.(P72R)</td>
<td>NS</td>
<td>DR</td>
<td>1, 2, 3, 4, 5, 6</td>
</tr>
<tr>
<td>BRCA2</td>
<td>c.6325G&gt;A</td>
<td>p.(V2109I)</td>
<td>NS</td>
<td>CIP</td>
<td>3, 2</td>
</tr>
<tr>
<td>TP53</td>
<td>c.818G&gt;A</td>
<td>p.(R273H)</td>
<td>NS</td>
<td>P/LP</td>
<td>3</td>
</tr>
<tr>
<td>STK11</td>
<td>c.1062C&gt;G</td>
<td>p.(F354L)</td>
<td>NS</td>
<td>CIP</td>
<td>3, 4</td>
</tr>
<tr>
<td>KRAS</td>
<td>c.34G&gt;C</td>
<td>p.(G12R)</td>
<td>NS</td>
<td>P</td>
<td>3</td>
</tr>
<tr>
<td>BRCA2</td>
<td>c.9875C&gt;T</td>
<td>p.(P3292L)</td>
<td>NS</td>
<td>CIP</td>
<td>3</td>
</tr>
<tr>
<td>BRCA2</td>
<td>c.943T&gt;A</td>
<td>p.(C315S)</td>
<td>NS</td>
<td>CIP</td>
<td>4</td>
</tr>
<tr>
<td>RAD51B</td>
<td>c.619G&gt;T</td>
<td>p.(V207L)</td>
<td>NS</td>
<td>VUS</td>
<td>5</td>
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<tr>
<td>CDK12</td>
<td>c.2447del</td>
<td>p.(M816Rfs*5)</td>
<td>FS</td>
<td>N/A</td>
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<tr>
<td>ATM</td>
<td>c.3648del</td>
<td>p.(L1217Wfs*6)</td>
<td>FS</td>
<td>N/A</td>
<td>7</td>
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</tbody>
</table>

* Amino Acid. Abbreviations: NS, nonsynonymous; P, pathogenic; P/LP, pathogenic/likely pathogenic; FS, frameshift; VUS, Variants of uncertain clinical significance; CIP, Conflicting interpretations of pathogenicity; DR, Drug response.
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Interest conflict
The authors have no conflicts of interest to declare.

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