Using Whole Exome Sequencing on Diagnosis of Pancreatic Ductal Adenocarcinoma

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O Uso do Sequenciamento Total do Exoma no Diagnóstico do Adenocarcinoma Ductal Pancreático

El uso de la Secuenciación del Exoma Total en el Diagnóstico del Adenocarcinoma Ductal Pancreático

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ABSTRACT

Introduction: The prevalence of pancreatic ductal adenocarcinoma (PDAC) in Brazil is around two percent of all neoplasms. It is an aggressive disease responsible for five percent of all deaths by cancer. The analysis of exome – part of the DNA encoding the proteins – allows the identification of tumor-specific variants and the patient polymorphism. This information is necessary to implement target therapy for PDAC, as it provides evidence to select, or exclude, PDAC treatments. **Objective:** Identify the somatic and germinative variants of clinical and pharmacological interest in the PDAC for four patients through the whole-exome sequencing technique (WES). **Method:** Public sequencing exome data published by Texas Cancer Research Biobank were utilized, from four tumor-normal samples pair of PDAC located in the pancreas head of Caucasian patients, T3N1M0 stage. To identify somatic and germinative variations, the GATK software was adopted. Furthermore, these variants were noted with their clinical and pharmacological information through the VEP software and its consequences were analyzed through the statistical software R. **Results:** Of the four tumors, one has a structural variant with duplication of the AKT2 gene; another, changes in the pathway of cyclins CDK14 and CDKN2C. Both findings alter the chemotherapy regimen; in the germline, one patient has variants in the XRCC1 gene, which suggests increased response to platinum. **Conclusion:** Although the pathology classifies all tumours as PDAC, each patient – as well as their respective tumor – shows specificities that affect the diagnosis and therapeutic possibilities. WES allows to identify them at a low cost, expanding the treatment possibilities of PDAC.

Key words: carcinoma, pancreatic ductal; whole exome sequencing; molecular targeted therapy.

Resumo

Introdução: O adenocarcinoma ductal pancreático (PDAC) é uma doença agressiva responsável no Brasil por 2% das neoplasias e 5% das mortes por câncer. A análise do exoma – parte do DNA que codifica as proteínas – permite identificar as variantes somáticas do tumor e as germinativas do paciente. Essa informação é necessária para implementar a terapia-alvo para o PDAC, pois fornece evidência para selecionar, ou excluir, tratamentos para a doença. **Objetivo:** Identificar as variantes de interesse clínico e farmacológico presentes no PDAC de quatro pacientes, por meio da técnica de sequenciamento total do exoma (WES). **Método:** Foram utilizados dados públicos de quatro amostras de pares tumor-normal de PDAC, localizados na cabeça do pâncreas de pacientes caucasianos, estádio T3N1M0, sequenciadas e publicizadas pelo *Texas Cancer Research Biobank*. Para identificar as variações somáticas e germinativas, utilizou-se o *software* GATK. As consequências clínicas e farmacológicas dessas variações foram anotadas por meio do *software* VEP e analisadas mediante o *software* estatístico R. **Resultados:** Dos quatro tumores, um possui variante estrutural com duplicação do gene AKT2; outro, variantes nos genes da via das ciclinas CDK14 e CDKN2C, o que altera o regime quimioterápico; na linhagem germinativa, um paciente tem variantes no gene XRCC1, que sugere aumento da resposta à platina. **Conclusão:** Embora a patologia classifique todos os tumores como PDAC, cada paciente – bem como o respectivo tumor – apresenta especificidades que afetam o diagnóstico e as possibilidades terapêuticas. O WES permite identificá-las a um custo baixo, o que amplia as possibilidades de tratamento do PDAC.

Palavras-chave: carcinoma ductal pancreático; sequenciamento completo do exoma; terapia de alvo molecular.

RESUMEN

Introducción: El adenocarcinoma ductal pancreático (PDAC) es una enfermedad agresiva que causa en Brasil 5% de las muertes por cáncer. El análisis del exoma – parte del ADN que codifica las proteínas – permite la identificación de mutaciones específicas del tumor, así como los polimorfismos del paciente. Esta información es necesaria para implementar la terapia dirigida para PDAC. **Objetivo:** Identificar las variaciones de interés clínico y farmacológico presentes en el PDAC de cuatro pacientes, mediante la técnica secuenciación del exoma completo (WES). **Método:** Se utilizaron datos públicos de cuatro muestras de pares de tumores normales (T-N) de PDAC, localizados en la cabeza del páncreas de pacientes caucásicos, estadio T3N1M0, secuenciadas y publicadas por *Texas Cancer Research Biobank*. Para identificar las variaciones somáticas y germinativas, se utilizó el *software* GATK. Se observaron las consecuencias clínicas y farmacológicas de estas variaciones a través del *software* VEP. Y analizadas sus consecuencias a través del *software* estadístico R. **Resultados:** De los cuatro tumores, uno tiene una variante estructural con duplicación del gen AKT2; otro, cambios en la vía de las ciclinas CDK14 y CDKN2C, que altera el régimen de quimioterapia; en el linaje germinal, un paciente tiene variantes en el gen XRCC1, lo que sugiere una mayor respuesta al platino. **Conclusión:** Aunque la patología clasifica todos los tumores como PDAC, cada paciente – así como el tumor respectivo – presenta especificidades que afectan el diagnóstico y las posibilidades terapéuticas. WES le permite identificarlos a un bajo costo, lo que amplía las posibilidades de tratamiento de PDAC.

Palabras clave: carcinoma ductal pancreático; secuenciación del exoma completo; terapia molecular dirigida.

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INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is aggressive and difficult to diagnose with high rate of mortality; in Brazil, it accounts for 2% of all neoplasms and 4% of deaths by cancer[1]. In USA, the 5-year survival rate is 9%[2]. Despite treatment advances, mortality rates continue to rise[2,3], suggesting the necessity of new approaches to treat this disease.

The macroscopic exam revealed that PDAC presents as a star-like greyish tumor mass with firm texture. Microscopically, it triggers an intense desmoplastic reaction with dense fibrotic stroma involving neoplastic cells[4]. Unspecified symptoms as anorexia, asthenia, abdominal pain and weight loss[5] are common in patients affected by PDAC.

Clinical staging determines the diagnosis through image and lab tests followed by pathological staging, with thoracic and pelvic computed tomography (CT) endoscopic ultrasound (US), magnetic resonance (MR for lesions undefined by CT), positrons emission tomography (PET-CT to identify metastasis) and endoscopic retrograde cholangiopancreatography (ERCP) for obstructed bile ducts and pancreatic ducts), liver function tests and cancer antigen 19-9 (CA19-9).

The staging locates the pancreas lesions (head or tail) and classifies them in resectable, borderline or irresectable. Resectable lesions are referred to surgery without malignancy confirmatory previous biopsies. For borderline and irresectable lesions, biopsies are required to initiate radiotherapy and chemotherapy[6].

The diagnosis proceeds with pathological sequencing of the specimens collected by surgical resection or biopsy. At that phase, TNM staging system is applied to evaluate the size (T), the lymph nodes involvement (N) and the presence or otherwise existence of metastasis spread (M) in addition to the primary tumor site, type and grade of histological differentiation[7], which allows to plan the prognosis and treatment.

Although widely adopted, this diagnostic approach has limitations due to the low specificity of clinical staging[8] and low post-therapy 5-year survival even in ideal conditions of margin-free resectable lesions (R0) at the pancreas (T1), whose patients have mean survival of 27 months[9]. This information reinforces the necessity of new approaches for the diagnostic and treatment of this disease.

Given that cancer is a genetic disease[10] caused by a spectrum of genomic alterations responsible for the phenotype found on the malignant cells, it is of the uttermost relevance to understand these mutations, including the somatic variants acquired in tumor cells and the inherited polymorphisms – or germinative variants[11] –, to expand the knowledge of pancreatic cancer potentially leading to different management and improving the prognosis.

Whole exome sequencing (WES) is one of the historically accepted molecular techniques[12] for sequencing only protein-coded regions, the exome[13], through which is possible to identify the somatic variants of the DNA of the tumor tissue and the germinative variants of the white blood cells (normal tissue) of the patient.

These variants have several types: polymorphisms or mutations of the nitrogenous base of the genome (SNP), small insertions or deletions of some nitrogenous base of the genome (INDEL) or great structural alterations of the genome involving thousands of nitrogenous bases (SV). The resulting alterations occur in protein structures and functions, that act on cells functioning, leading to the tumor phenoptype[14]. When identified, diagnosis and therapeutics possibilities may improve[15].

Genomic wide association studies (GWAS) involving PDAC showed few typical somatic variants in some oncogenes and tumor suppressor genes – KRAS, TP53, SMAD4 and CDKN2A – and diversity of less prevalent variants in other genes. This pattern warrants high specificity to each PDAC and suggests an explanation for the poor response to standard conventional therapies. These characteristics offer the possibility of approaching PDAC through more specific target-therapies. The studies identified variants associated to higher risk for PDAC in the germinative lineage: BRCA1, BRCA2, PALB2, STK11, CDKN2A, ATM, PRSS1, MLH1, MSH2, MSH6, PMS2, EPCAM and TP53[16].

They showed that tumor cells of PDAC with variants on the genes BRCA1, BRCA2 and PALB2 involved in DNA repair have structurally unstable genome[17] and have good response to platinum conjugated with poly (ADP-ribose) polymerase (PARP)[18] inhibitors. As prognostic indicator, of the patients with PDAC who express aberrant metastatic proteins S100A2 and S100A4, 50% died within a year of resection, which contraindicated surgery for these patients due to its high morbidity and modest benefit[19].

Systemic therapy is utilized in every stage of PDAC. The chemotherapy combination FOLFIRINOX[20] contains the drugs leucovorin calcium (folinic acid), fluorouracil, irinotecan hydrochloride and oxaliplatin or the combination of gemcitabine plus capecitabine as drugs of choice[6]. Aspects related to the pharmacodynamic of the substances utilized on the systemic treatment are affected by the germinative variants of the patient. Fluorouracil and capecitabine are affected by the polymorphisms of the gene dihydropyrimidine dehydrogenase (DPYD)[21] and irinotecan by polymorphisms of the gene UDP

glucuronosyltransferase 1 family, polypeptide A1 (UGT1A1)[22], both with maximum evidence level (1A) at the Pharmacogenomics Knowledge Base (PharmGBK)[23], indicating they need analysis of the germinative variants to be prescribed. For fluorouracil, patients who present polymorphisms that reduce the activity of the enzyme dipyrimidine dehydrogenase have risk of intoxication by FOLFIRINOX[21]. For irinotecan, the polymorphisms result in better efficacy and higher toxicity[24].

The objective of this article is to show the utilization of WES to identify somatic and germinative mutations of clinical and pharmacological interest present in PDAC of four patients sequenced with WES technique. The practical and objective approach bridges the current diagnosis and treatment with the recommendations of target-therapy to facilitate the understanding, dissemination and implementation by health teams and institutions. The patient benefits the most, increasing its odds in face of a disease with unchanged survival statistics for more than 40 years.

METHOD

Public data of sequencing of four samples of tumor-normal pairs (T-N) of PDAC were utilized to demonstrate the use of WES in the diagnosis and treatment of the disease located at the head of the pancreas of Caucasian patients, stage T3N1M0, sequenced in Illumina machines and available in digital files at the Internet by the Texas Cancer Research Biobank (TCRB)[25]. The individuals affected by PDAC whose exams became public by the TCRB were enrolled.

The identification of SNP and INDEL of somatic and germinative lineage involves successive steps of data processing. Initially, the sequences produced by Illumina are aligned to a reference genome and then, identify the existing somatic and germinative variants of the normal and tumor samples and ultimately, describe the clinical and phenotypical effects.

Each one of these steps requires different software executed in sequence so the output of one becomes the entry to the next; together, they form a pipeline whose results are the existing variants and mutations in that normal or tumor tissue annotated with the name of the gene, the protein altered, type of alteration and clinical consequences, among other information required for better understanding.

The software Burrows-Wheeler Aligner (BWA)[26] aligns the segments of 100 bases (reads) generated by the sequencers Illumina to a reference genome GRCh38, reconstructing the genome of the sample in the alignment phase. The digital files containing the data of the sequencing and the genome aligned are in the format FASTQ and BAM, respectively. At the end, the software FastQC[27] evaluates the quality of the files BAM generated.

Next, in the phase of identification of somatic variations, the software Mutec2[28] compares the files BAM containing the genomes of the patient's normal and tumor tissues samples in order to identify, classify, filter and list the somatic variants present in the tumor sample, saving them as VCF digital file. These variants are type SNP and INDEL present in tumor cells: insertions, deletions and base changes. Analogically, for the germinative variants, the software GATK[29] compares the genome of the normal tissue with the reference genome and identifies the SNP and INDEL present in it. The software R with the library CopywriteR[30] was utilized to identify the structural alterations. The quality of the germinative and somatic variants found is checked with the software Bcftools[31].

During the annotation of the somatic and germinative phase, the software Variant Effect Predictor (VEP)[32] analyzes the variants identified in the previous phase and notes the phenotypic consequences of these variants, for example, the gene where it occurred, type of variation and outcomes for the patient.

Eventually, the annotations made by VEP are searched in the literature to identify potential therapeutic targets of the disease.

The software were run in standard configurations; during the analyzes, the variants without the attribute "PASS" in the field filter of the file VCF were filtered and deleted. The attribute "PASS" is assigned to all the variants which meet cumulatively the quality standard conditions of the software utilized.

In compliance with Resolutions 466/2012[33] and 510/2016[34], the approval by the Institutional Review Board was waived because only public secondary deidentified data were utilized.

RESULTS

The clinical data of the patients investigated are shown in Table 1. The tumors are infiltrative ductal adenocarcinoma.

Tumors were located at the head of the pancreas, stage T3, indicating the tumor has grown beyond the pancreatic gland, N1, compromising the lymph nodes, M0, without metastasis, except TCRBOA5 for whom it was not possible to determine the presence or absence of metastasis.

The quality of WES of the normal and tumor sample was satisfactory as revealed by the software FastQC[27], all with more than 51 million sequences of 101 bases with content CG between 45% and 46% and without low

quality bases. Consequentially, BWA[26] was well aligned to the genome of reference, GRCh38 as shown in Table 2.

After the alignment, in the stage of identification of variants, the software GATK and Mutec2 found 123,174 variants, being 87,170 germinative and 36,004 somatic. The data per patient, sample and type of tissue are shown in Table 3.

Of the somatic variants SNP and INDEL found, 69 are of high quality as shown in Figure 1 grouped by patient. The gene KRAS is mutated in two of the four patients. The most prevalent are the missense mutations with mutation of the protein able or not to affect its function and the nonsense mutations where the

Table 1. Clinical data of the patients

Identification	Sex	Age range (years)	Ethnicity	Staging	Celularidade tumoral na amostra (%)	Localização do tumor	Grau do tumor
TNM	Tumor cellularity of the sample (%)	Tumor location	Tumor grade	T3N1M0	10	Cabeça do pâncreas	II
TCRBOA1	Male	51-60	White, Non-Hispanic or Latin	T3N1M0	10	Head of the pancreas	II
TCRBOA2	Female	61-70	White, Non-Hispanic or Latin	T3N1M0	60	Head of the pancreas	II
TCRBOA3	Male	51-60	White, Non-Hispanic or Latin	T3N1M0	20	Head of the pancreas	II
TCRBOA5	Male	51-60	White, Non-Hispanic or Latin	T3N1MX	5	Head of the pancreas	II

Caption: TCRBOA = Texas Cancer Research Biobank Open Access.

Note: The column tumor cellularity of the sample indicates the percentage of the sample tissue corresponding to the tumor. For the patient TCRBOA5 only 5% is tumor tissue

Table 2. Quality of the alignment per sample

Sample	Total quantity of reads	Quantity of aligned reads	Quantity of misaligned reads	Percent of aligned reads
TCRBOA1-N	81,258,689	80,777,836	480,853	99.41%
TCRBOA1-T	86,680,285	86,259,605	420,680	99.51%
TCRBOA2-N	97,446,346	96,650,302	796,044	99.18%
TCRBOA2-T	104,905,565	104,270,211	635,354	99.39%
TCRBOA3-N	107,054,252	106,520,977	533,275	99.50%
TCRBOA3-T	95,754,360	95,299,939	454,421	99.53%
TCRBOA5-N	87,775,785	87,464,673	311,112	99.65%
TCRBOA5-T	88,840,426	88,582,372	258,054	99.71%

Source: Processing log of software BWA[26].

Caption: TCRBOA = Texas Cancer Research Biobank Open Access.

Note: The software BWA aligns the reads of 100 pairs of bases to the genome of reference GRCh38. For each sample, the quantity of reads of 100 pairs of base produced by the sequencers Illumina (column total quantity of reads aligned), how many reads BWA has managed to align to the genome of reference (column quantity of reads aligned), how many it failed to align (columns quantity of reads non-aligned) and the percent of reads aligned. The indexes of alignment are higher than 99% for all the samples which indicate good quality. The denomination of the sample is formed by the identification of the patient followed by the termination T or N if the sample is from tumor tissue or normal, respectively. Thus, TCRBOA1-T identifies tumor sample of the patient TCRBOA1.

Table 3. Quantification of variants per sample and type

Identification of the patient	TCRBOA1		TCRBOA2		TCRBOA3		TCRBOA5		Total
Type of the sample tissue amostra	Normal	Tumoral	Normal	Tumoral	Normal	Tumoral	Normal	Tumoral	
Number of SNP	20,573	7,944	20,778	8,191	20,805	8,844	20,593	7,221	114,949
Number of INDEL	1,074	812	1,085	1,037	1,159	1,011	1,103	944	8,225
Total	21,647	8,756	21,863	9,228	21,964	9,855	21,696	8,165	123,174

Captions: TCRBOA = Texas Cancer Research Biobank Open Access; SNP = Single Nucleotide Polymorphism genome nitrogenous base ; INDEL = insertions/deletions of some genome nitrogenous base.

Note: Two tumoral samples of each patient were sequenced: one of the tumoral tissue (PDAC) and other of the healthy tissue (white blood cells). Once aligned to the reference genome GRCh38, the software GATK identified the variants present in the samples for each one of them. The table shows the SNP-type variants for each sample, where only one base is changed in the genome of the sample and the INDEL-type, where several bases were deleted or inserted.

alteration of the protein causes a probable loss of function. Additionally, among the somatic variants, three are of high impact (cause loss of function of the protein: genes AOPEP, DMD and DSCAM) and five pathogenic (genes KRAS, TP53, DMD and CYP27A1)[35]. Figure 1 portrays the somatic SNP and INDEL variants grouped by tumor, which shows didactically the diversity and specificity of the tumor at molecular level.

Of the structural variants (SV) a chromosomal duplication was found on region 19q13.2 with approximately 418 thousand bases (Figure 2). This duplication affected the genes MAP3K10, CNTD2, AKT2, C19orf47, PLD3, HIPK4, PRX, SERTAD1, BLVRB, SPTBN4[36].

Of the germinative variants, 14 were pathogenic, one associated with chronic pancreatitis (gene CFTR, variant rs113993960, deletion, patient TCRBOA3); 93 variants associated with risk factors for diseases as Von-Hippel-Lindau syndrome (gene CCND1, variant rs9344, genotype A/A, patient TCRBOA2) and hereditary pancreatitis (gene CTRC, variant rs121909239, genotype C/T, patient TCRBOA3)[35]. Finally, the variants associated with better response to the medications: for platinum compounds, the patient TCRBOA5 presents the variants rs1042522, gene TP53, genotype C/C (polymorphism of codon 72) and variant rs25487, gene XRCC1, genotype C/C[35].

It is feasible from these results to evaluate the possibilities of target-therapy for four patients according to the molecular characteristics of the cells contained in the tumor and normal samples.

DISCUSSION

As portrayed in Table 1, the clinical data of the patients suggest similar tumors and although no information about the condition of the patient for chemotherapy exist, the protocol determines analogous therapeutic approaches: surgery plus neoadjuvant chemotherapy and adjuvant with FOLFIRINOX[6]. According to the results obtained from WES, although histologically similar at molecular level, the tumors of the four patients investigated are quite distinct with important complications to choose the treatment.

The PDAC of the patient TCRBOA3 presents duplication of the segment 19q13.2 where the gene AKT2 is located. This oncogenesis belongs to the signaling path of the gene PI3K, associated with the growth, proliferation, survival and cellular invasion and chemotherapy resistance and dismal prognosis. Therefore, it is indicated the evaluation of target-therapies inhibiting the pathway PI3K/AKT/mTOR[37]. This patient, furthermore, has mutation of the gene KRAS (variant rs121913529, genotype C/T) that also promotes proliferation and cellular survival. Thus, inhibiting target-therapies of the pathway RAS[38] would be indicated as well.

In another therapeutic approach, due to the presence of the duplication of the segment 19q13.2, added to the elevated number of somatic mutations, it is suggested to treat a tumor with structural instability whose response to PARP inhibitors associated with platinum-based chemotherapics is satisfactory[3,18]. In addition, this patient has two polymorphisms associated with chronic pancreatitis (genes CFTR, variant rs113993960 and CTRC, variant rs121909239)[35], which indicates screening of PDAC of ascendants and descendants.

A different scenario occurred for the patient TCRBOA2. The analysis of somatic variants of the tumor reveals genes associated with the pathway of cyclins-dependent cyclins/kinases (CDK): CCNA1, CDK14 and CDKN2C that control the progression of the cellular cycle and whose

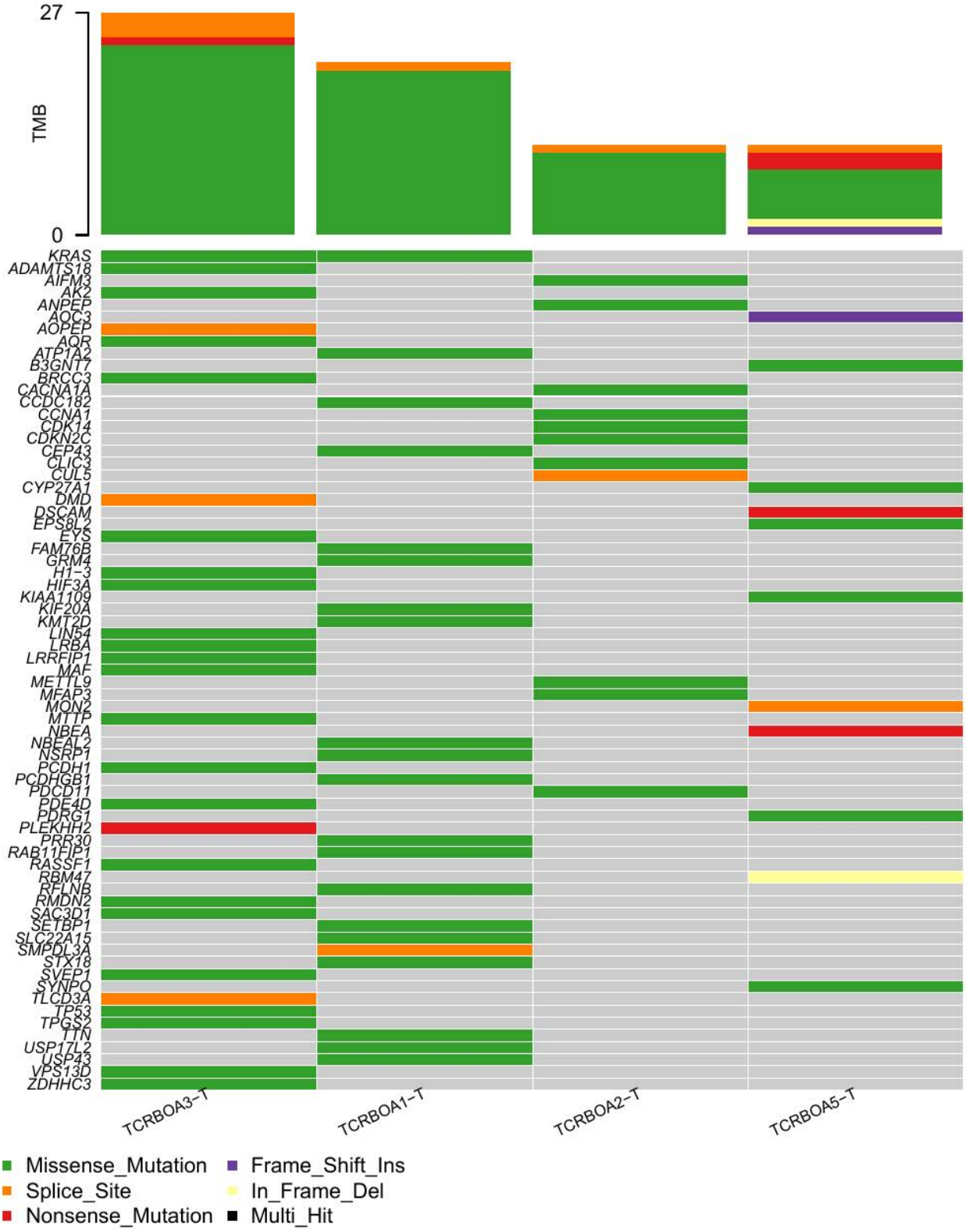

Figure 1. Somatic variants found in the patients' PDAC sample

Note: The left column lists the name of the gene with mutations. The patient is identified in the row. The color identified the type of mutation: missense mutations where the change of the base changes the codon of the amino acid, which can harm or not the function of the protein and are the most prevalent. The function of the protein in nonsense mutations is damaged by the mutation. The sample TCRBOA5-T has low tumor cellularity, which hampers the analysis.

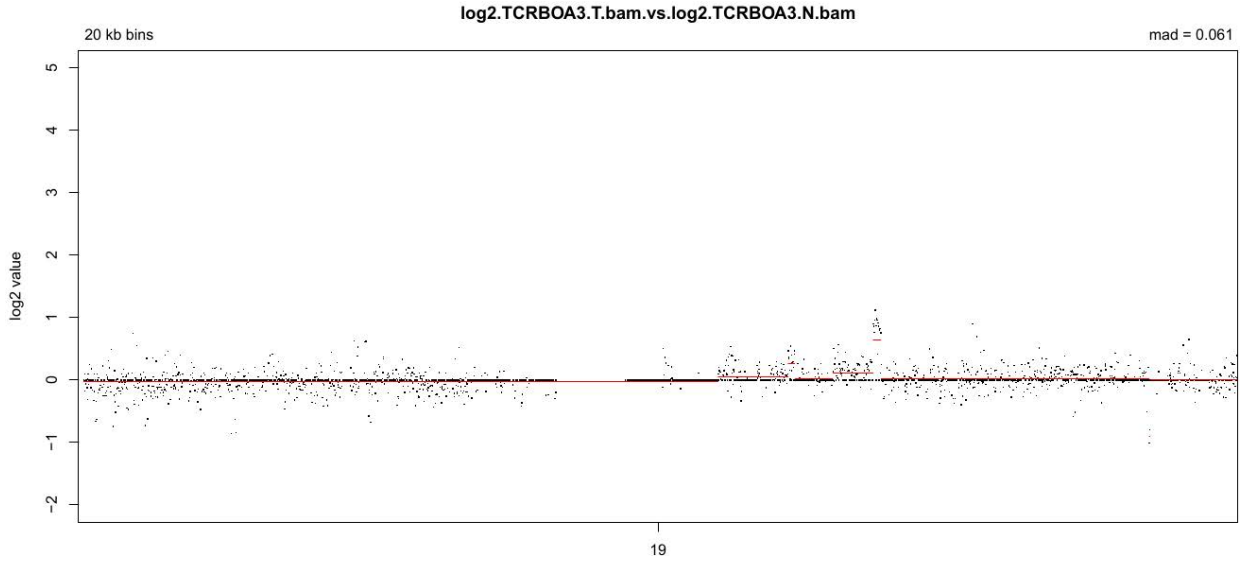

Figure 2. Structural alterations present in the chromosome 19 of the tumor sample of patient TCRBOA3

Note: The duplicate region appears as a horizontal parallel line, corresponding to segment 19q13.2 of the chromosome 19 and has approximately 418 thousand bases. The gene AKT2 is among the genes contained in this region, with evidence-based literature of association with more chromosomal instability of the tumor.

mutations result in uncontrolled cellular proliferation and formation of stem-cells, both mechanisms associated with an oncogenic process. It is possible to utilize target-therapy inhibiting the pathway of CDK[39] to block tumor growth.

This fact associated with few mutations and absence of relevant structural genomic alterations suggest genomic stability of the tumor, advising against the use of PARP inhibitors[3,18]. Not less important is the germinative variant associated with the Von-Hippel-Lindau syndrome (gene CCND1 variant rs9344)[35], which also indicates the screening of PDAC for the ascendants and descendants of this patient.

The somatic variants found in the tumor of patient TCRBOA5 had no association with those documented as PDAC inducers. The reason can be the low tumor cellularity present in the sequenced sample of only 5%. This analysis found nonsense mutations in genes NBEA and DSCAM, a deletion of gene RBM47, wild-type genes KRAS and TP53 and absence of relevant structural alterations. This patient has germinative variants associated with better response to platinum compounds and best survival (gene TP53, variant rs1042522, C/C and gene XRCC1, variant rs25487, C/C)[35].

Finally, the patient TCRBOA1 presented somatic missense mutations, among them the variant rs121913529, gene KRAS, genotype T whose meaning is pathogenic and associated with PDAC[35]. KRAS is an oncogene and patients with mutations have dismal prognosis and poor response to chemotherapy. Similar to the patient TCRBOA3, KRAS inhibiting target-therapies are indicated to treat this tumor. Another characteristic is the absence of structural instability of the genome, which contraindicates PARP inhibitors or immune therapy[3,18].

Based in the elements addressed previously, it was possible to identify molecular characteristics of the tumor and of the patient affecting the positive predictive value of the standard chemotherapeutic regimen. For the patient TCRBOA3, whose AKT2 is duplicate, the utilization of FOLFIRINOX without inhibiting this gene would hardly be effective; similar to patient TCRBOA2, whose CDK pathway is permanently activated.

In order to reduce the morbimortality of PDAC it is necessary to understand its molecular aspects to match the intervention to the tumor and to the patient. Therefore, WES is a satisfactory tool to meet this goal.

CONCLUSION

The present article presented all the required steps and tools to analyze the exome to identify the germinative and somatic variants and structural alterations of the PDAC genome. The methodology can be adopted for any other neoplasm and although performed *in silico*, not only reached the same previously identified tumor-inductor oncogenes in patients TCRBOA1, TCRBOA2, TCRBOA3 and TCRBOA5, but an unprecedent duplication in region 19q13.2 where oncogene AKT2 is located was identified in the tumor of the patient TCRBOA3.

It also showed that, despite the staging was able to classify all PDAC uniformly, each patient and the respective tumor has molecular specificities that affect

the diagnosis and clinical outcome. The technique WES allows to identify these specificities and widen the therapeutic possibilities from the knowledge of the somatic, germinative variants and structural alterations of the genome of the tumors. Considering the cost of approximately US$ 300 of WES, the cost-benefit is satisfactory because it allows to identify the somatic and germinative variants in a single exam. However, it is necessary to recognize that this technique requires the formation of specific laboratories and highly skilled personnel, including bioinformatics.

It is clear the necessity of divulging and expanding the use of the analysis of WES in oncologic services and train teams to perform and utilize it.

CONTRIBUTIONS

Both authors contributed substantially to the study design, acquisition, analysis and interpretation of the data, wording and critical review. They approved the final version to be published.

DECLARATION OF CONFLICT OF INTERESTS

There is no conflict of interests to declare.

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