

Impact of Ethnicity on Somatic Mutation Rates of Pancreatic Adenocarcinoma

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Abstract. *Background/Aim:* Ethnicity has an effect on survival in patients with pancreatic adenocarcinoma (PDAC), which may be reflected in the rate of somatic driver mutations. The Brazilian population represents an extensive interethnic admixture and little is known about the spectrum and rates of somatic driver mutations in Brazilian PDAC cases. *Materials and Methods:* Direct sequencing of six genes in 23 PDAC cases was performed and the ancestry of patients was determined using a validated panel of ancestry-informative insertion/deletion DNA polymorphisms. *Results:* KRAS proto-oncogene (KRAS) was the most commonly mutated gene (60%). A novel putatively pathogenic mutation in phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) (c.2948T>A; p.M983K) was identified. Mutations in epidermal growth factor receptor (EGFR) (4%), PIK3CA (4%), cyclin-dependent kinase inhibitor 2A (CDKN2A) (4%) and TP53 (8%) were noted, in rates that are less frequent than those reported for other populations. Mutations of B-Raf proto-oncogene, serine/threonine kinase (BRAF) were not present. All individuals with high African ancestral component (allelic frequency, >0.45) exhibited KRAS mutations. *Conclusion:* Our results highlight the importance of the effect of ethnicity on somatic mutations in Brazilian patients with PDAC.

Pancreatic ductal adenocarcinoma (PDAC) is the most common cancer of the pancreas, and a leading cause of

cancer-related death (1). The long-term prognosis for patients with PDAC remains dismal with 5-year survival rates ranging from 15-25%. Surgical resection offers the best chance for a cure and remains the mainstay of therapy but only 10-20% of patients present with resectable disease (2, 3).

Pancreatic intraepithelial neoplasm (PanIN) is the most common non-invasive precursor lesion of PDAC (4). Mutations in KRAS proto-oncogene (KRAS) gene occur early in PDAC development and are reportedly detected in 75-100% of PanIN cases (5), inactivation of cyclin-dependent kinase inhibitor 2A (CDKN2A) is an intermediate step in PDAC carcinogenesis and is detected in 30-55% of PanIN and 92% of early-stage PDAC (6) and inactivation of TP53 and deleted in pancreatic carcinoma 4/SMAD family member 4 (DPC4/SMAD4) are relatively late-stage molecular events that have rarely been described in PanIN and in 30% of advanced stage PDAC (4). In addition to KRAS-activating mutations, other genes along the RAS pathway have also been associated with pancreatic cancer pathogenesis such as EGFR and BRAF at rates ~2% (7-9).

Like other tumor types, PDAC incidence and disease course are, in part, dependent on ethnicity (10). The survival rate of US African-American patients with PDAC is 20% worse compared to US Caucasians and this difference was not attributable to different modes of treatment (11). Likewise, US African-American colorectal cancer cases have higher mortality rates compared with US Caucasians, differences that are not accounted for by socioeconomics, comorbidities, or treatment modalities (12).

The Brazilian population is one of the most genetically heterogeneous populations worldwide as a result of five centuries of admixture between three ethnic groups: Amerindians, Europeans and Africans. It has been demonstrated that skin color in Brazilian individuals is a poor predictor of ethnicity hence the importance of using genetic markers to define population ancestry structure, where ethnicity may affect disease rates or clinical course (13).

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The aim of the present study was to assess the rates of somatic mutations in several key driver pathogenic genes involved in PDAC tumorigenesis and the effect of ancestry analysis on mutational spectrum in patients with PDAC from Southeastern Brazil.

Materials and Methods

The study cohort encompassed all consenting consecutive patients with PDAC who attended Hospital das Clínicas (Belo Horizonte) and Hospital das Clínicas (Ribeirão Preto), Brazil from June 2011 to May 2016. This study was approved by the University Ethics Committee (#CAAE 09135912.6.0000.5149). Only participants aged 18 or older were enrolled in this study after signing an informed consent form. Controls (germline DNA) were 96 healthy individuals older than 55 years of age with no previous personal or family history of cancer, randomly recruited from the outpatient clinics in the same medical centers in Belo Horizonte during the same time, using an ethically approved protocol. This group of healthy Brazilian individuals are representative of the ethnic makeup of Southeastern part of Brazil and were used as controls for genomic ancestry.

Tumor samples of PDAC were obtained during surgical procedures and immediately stored at -80°C . Before DNA extraction, the samples were microdissected to ensure the highest fraction of tumor cells in the analyzed samples. Genomic DNA was isolated according to a proteinase K-based standard protocol. Exons 2 and 3 of *KRAS*, exon 15 of *BRAF*, exons 18 to 21 of *EGFR*, exon 9 and 20 of *PIK3CA*, exons 1 and 2 of *CDKN2A* and exons 4 to 9 of *TP53* were amplified by polymerase chain reaction (PCR) with specific primers for each region (primer sequences and PCR conditions available on request). PCR products were purified using Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, São Paulo, Brazil) following the manufacturer's protocol and visualized on a silver-stained 6.5% polyacrylamide gel. Sequences were obtained on ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Bi-directional sequence data was analyzed using Sequencer 4.9 software (Gene Codes Corporation, Ann Arbor, MI, USA).

Genomic DNA of all pancreatic samples and controls were genotyped with a set of 40 biallelic short insertion/deletion polymorphisms (InDels) (14). Amplicons were sized fractioned using an ABI 3130 DNA sequencer (Applied Biosystems) and analyzed using the GeneMapper® Software, version 3.7 (ThermoFisher Scientific, São Paulo, Brazil). The Structure program, version 2.3, (<http://pritch.bsd.uchicago.edu/structure.html>) was used to estimate the proportion of European, African and Amerindian bio-geographical ancestry for each individual.

The proportion of European, African and Amerindian bio-geographical ancestry of each individual was considered for statistical analysis. Two-tailed Mann-Whitney *U*-test was used for statistical comparisons between cases and controls. Single-marker allelic and genotypic association tests were performed using the Unphased software package version 3.0.12 (www.mrcbsu.cam.ac.uk/personal/frank/software/unphased/). *p*-Values of 0.05 or less were considered statistically significant.

In order to analyze the deleteriousness of the novel *PIK3CA* mutation (see Results), a homology model of *PIK3CA* from its translated protein sequence was obtained. Using psiBLAST

(ProteinDataBank) and Swiss-Model Platform, templates with 99% and 99.81% identity to human canonical p110 alpha, PDB 2RDO_A and PDB 3HHM_A, respectively, were obtained allowing us to see how this mutation in exon 20 affected the *PIK3CA* structure. To correlate this point mutation with a change in the protein's behavior, a model using Modeller (<https://salilab.org/modeller/>) was built. Pymol software (The PyMOL Molecular Graphics System, Version 1.5.0.4; Educational Pymol; Schördinger, LLC, Mannheim, Germany) was used to generate structural images (15, 16). Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>), SIFT (<http://sift.bii.a-star.edu.sg/>) and Mutation Taster (<http://www.mutationtaster.org/>) software were used to predict the functional effect of amino acid substitution.

Results

Overall, 23 patients with seemingly operable PDAC were recruited: there were 14 women (61%) and nine men (39%) with a mean age of 58 years (range=32-90 years; SD=12.1 years). Clinical staging was determined according to the American Joint Committee on Cancer (<https://cancerstaging.org/Pages/default.aspx>), with stage IIB being the most prevalent (62%), followed by stage IV (14%), stages III and IIB (9.5%) and IA (5%).

Codon 12 *KRAS* mutations were detected in 14 samples (60%): Gly12Val (43%), Gly12Arg (29%), Gly12Asp (21%) and Gly12Ser (7%). There were no mutations detected in codons 13 and 61 of *KRAS*.

A P753S missense mutation in exon 19 of *EGFR* was found in one patient only. In exon 20, the polymorphism rs1050171 [NM_005228.4(*EGFR*):c.2361G>A; p.Gln787] was identified in 17 samples (74%). There were no mutations or polymorphisms in exon 15 of *BRAF* or in *CDKN2A*. No sequence alterations were demonstrated in exons 18 and 21 of *EGFR*.

In exon 20 of *PIK3CA*, a novel heterozygous mutation (g.85992T>A; c.2948T>A; p.M983K) was found. To confirm this novel mutation, exon 20 of *PIK3CA* was sequenced in 96 healthy control individuals and this alteration was not present in any of the controls. *In silico* analysis of the M983K mutation was performed using Modeller software (<https://salilab.org/modeller/>). Prediction of the functional effect of this point mutation indicated its deleteriousness (PolyPhen-2 score=0.999; SIFT score=0.05; Mutation Taster score = 95), all scores were associated with protein instability (Figure 1).

There were two different mutations in exon 8 of the *TP53* gene: R273C and R306*. No pathogenic sequence alterations were found in any other *TP53* exons. In exon 4 of *TP53*, the rs1042522 polymorphism was present in 18 samples (78%).

There were no differences in the proportions of genomic ancestry in Africans, Europeans and Amerindians between patients with PDAC and controls (Figure 2). All four patients with high African ancestral component (allelic frequency>0.45) had mutations in *KRAS*.

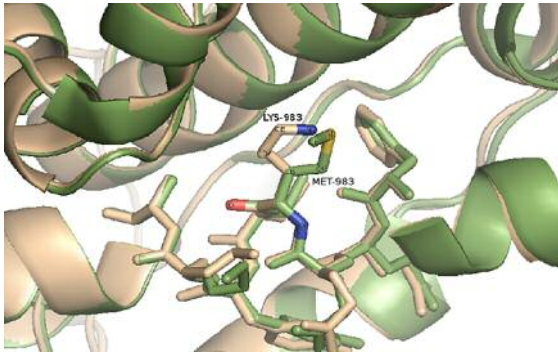


Figure 1. Close-up view of the interface of the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) kinase domain, showing the exchange between the mutated residues, in beige, and native residues, in green. The residues MET-983 and LYS-983, which may affect the protein conformation are shown with stick representation.

Discussion

Somatic activating mutations in *KRAS*, and inactivating *TP53* and *CDKN2A* mutations are frequently reported in the transition from PanIN to invasive PDAC (4). The rates of these mutations vary by population and are, in part, determined by ethnicity. In the present study, the rate of mutations in these pivotal PDAC driver genes differ from those of other previously studied populations (2, 17). Mutations in codon 12 of *KRAS* have been reported as the most prevalent genetic alteration in PDAC, ranging from 80-100% in Caucasians (2, 17, 18). The rate of *KRAS* mutation in the current study sample (60%) was similar to the rate reported in Korean cases (52.2%) (19). These data are consistent with the notion that the rate of mutation in codon 12 of *KRAS* vary by ethnicity and that this may in fact partially affect the clinical outcome. Despite the low rate of mutations of *EGFR* in PDAC (20; Cosmic: Catalogue of Somatic Mutations in Cancer, <https://cancer.sanger.ac.uk/cosmic>, accessed 10th September 2018), this receptor is a therapeutic target, as somatic overexpression of the gene product makes the tumor amenable to treatment with monoclonal antibody. In other cancer types (*e.g.* breast cancer) such treatment was associated with a significant improvement in clinical outcome (20). This is not yet established in PDAC and in fact a recent study failed to show any survival benefit in this tumor type of EGFR-targeted monoclonal antibody (21). Determining EGFR expression and mutational status is still pursued in many cancer types, including PDAC. The rates of *EGFR* mutations in our cohort as well as mutations in *BRAF* are consistent with other populations (22, 23).

Another mutated gene described herein is *PIK3CA* (24). Its activation is an important event in cell growth and

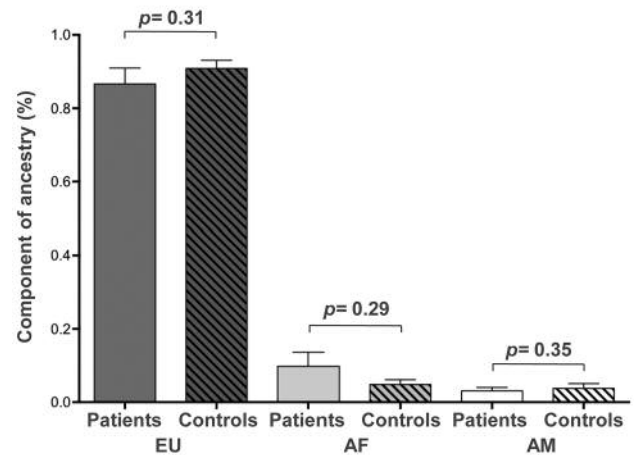


Figure 2. Analysis of the genomic ancestry of patients with pancreatic cancer of this study. EU: European; AF: African; AM: Amerindian; CTRL: control.

survival of several types of cancer (25). In agreement with the rarity of this mutation (less than 4%) in PDAC cases in previous studies (23, 24, 26), only one mutation was detected. As far as we are aware, this novel missense mutation (p.M983K) in *PIK3CA* has not been previously described in PDAC. The *in silico* analysis indicated that this point mutation is associated with altered protein stability using PolyPhen-2, SIFT and Mutation taster software.

Loss of function or loss of expression of *CDKN2A* is reportedly present in 80% to 95% of sporadic PDAC (17). Loss of function or expression of this gene and its product may be mediated by biallelic inactivation (heralded by allelic loss) or methylation in the promoter region (27). Interestingly, no somatic mutations were observed in *CDKN2A* in our study, a finding in contrast with other reports showing rates of 17% to 34% (2, 28). These differences in specific gene-related mutations between ethnically diverse populations may in part account for the differences in age at diagnosis and disease course and outcome.

The *rs1042522* polymorphism of the *TP53* gene has been associated with increased risk for developing pancreatic cancer (29) and was present in 78% of our samples. However, despite findings that *TP53* mutations are present in pancreatic cancer (2, 30), only two mutations (9%), R273C and R306*, were found in our samples.

Conclusion

Our results highlight the possible impact of ethnic heterogeneity on somatic genetic mutations in PDAC, and that this, in turn, may affect ethnic-related differences may impact outcome and therapeutic response.

Conflicts of Interest

The Authors declare that they have no conflicts of interest regarding this study.

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