Pathology & Oncology Research (2019) 25:811–814
https://doi.org/10.1007/s12253-018-0407-7

LETTER TO THE EDITOR

Genetic Analysis of Brazilian Patients with Gallbladder Cancer

Cristina Sábato[1] · Luciana Bastos-Rodrigues[2] · Debora Chaves Moraes[1] · Eitan Friedman[3,4] · Luiz De Marco[1] · Vivian Resende[1]

Received: 6 January 2017 / Accepted: 7 March 2018 / Published online: 19 March 2018

To the Editor,

Gallbladder cancer (GBC) is a rare neoplasm with poor prognosis and overall survival [1]. Its underlying molecular pathogenesis remains largely elusive and the accumulation of multiple somatic genetic alterations as well as chronic inflammation associated with gallstone formation promotes epithelial dysplasia and progression to adenocarcinoma postulated [2–4].

It has been shown that some indigenous people and ethnic groups have higher incidence and mortality rates, with differences within the same country and some populations identified as high-risk groups for GBC development. The highest incidence rates are found in populations living west of the Andes, northern India and populations of American and Mexican Indians [1].

The potential association in genetically heterogeneous Brazilian GBC patients has not been previously reported. Based in a comprehensive study [4] and COSMIC database (http://cancer.sanger.ac.uk/cosmic) we investigated the contribution of *TP53, KRAS, CDKN2A, PIK3CA* and *BRAF* and ancestry component to the pathogenesis of GBC in a subset of Brazilian patients.

All GBC patients who underwent surgical procedure in our Hospital (August 2011–December 2012) were eligible and signed an informed consent, a study approved by the local Ethics Committee. Clinical data were collected by a structured questionnaire. Tumor related data such as histologic type and staging were also obtained (Table 1).

Genomic DNA from peripheral blood and tumor DNA were extraced using standard protocols. Tumor samples were immediately placed in tubes containing RNAlater® and stored at −80 °C. Subsequently, tumors were micro-dissected to ensure enrichment. To enable a specific amplification of all relevant exons of *BRAF, KRAS, P53, CDKN2A* and *PIK3CA* genes, primers were designed (sequences available on request). The PCR reaction followed protocol parameters of AmpliTaq Gold® PCR Master Mix (Thermofisher Scientific, São Paulo, Brazil). Bi-directional sequencing reactions were performed and analyzed.

DNA from tumor tissue of GBC cases and peripheral blood DNA from 75 healthy controls were genotyped independently employing biallelic indels as described [5]. The Structure software version 2.3, (http://pritch.bsd.uchicago.edu/structure.html) estimated the biogeographical ancestry for each individual. As parental populations, we used individuals of European, African, and Amerindian origin (http://www.cephb.fr/HGDP-CEPH-Panel). Mann-Whitney test was performed to compare groups. PolyPhen-2 software evaluated the impact of the amino acid substitution in protein structure (http://genetics.bwh.harvard.mutations edu/pph2/) and PROVEAN software (http://provean.jcvi.org/index.php) indicated whether the change was deleterious.

Overall 12 cases were recruited and tumor characteristics, relevant clinical and ancestry data are shown (Table 1); control group included 75 healthy individuals.

Candidate genes genotyping and ancestry component are shown in Table 2. One tumor had more than one mutation. In three tumors no somatic mutations were found. Five tumors displayed *CDKN2A* mutations, three harbored known *KRAS* mutations and two presented *TP53* mutations. Both patients with somatic *TP53* deletions exhibited a second anatomically distinct tumor: an intrahepatic cholangiocarcinoma and a melanoma of the nasal mucosa. Mutations in *BRAF* and *PIK3CA* were not present.

✉ Luiz De Marco
ldemarco@ufmg.br; luiz.demarco@gmail.com

[1] Department of Surgery, Universidade Federal de Minas Gerais, Av. Alfredo Balena 190, room 114, Belo Horizonte 30130-100, Brazil

[2] Department of Nutrition, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

[3] The Susanne Levy Gertner Oncogenetics Unit, Chaim Sheba Medical Center, Tel-Hashomer, Israel

[4] Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel

Table 1 Clinical and ancestry characteristics of patients with gallbladder cancer

Variables			N	%
Age	Median	65		
	Variation	48–75		
Gender	Male		3	25
	Female		9	75
Histology	Adenocarcinoma		12	100
Smoking	Yes		1	8,33
	No		9	75
	Ex-smoker		1	8,33
	unknown		1	8,33
Alcoholism	Yes		–	–
	No		10	83,33
	Ex		1	8,33
	unknown		1	8,33
Cholelithiasis	Yes		9	75
	No		2	16,66
	unknown		1	8,33
Positive family history of cancer	Yes		4	33,33
	No		2	16,66
	unknown		6	50
Tumor Staging (AJCC)	I		1	8
	II		2	17
	III		2	17
	IV		7	58
Ancestry	European >0.80		9	75
	European <0.80		3	25
	African >0.2		2	16.7
	African <0.2		10	83.3
	Amerindian >0.2		0	0
	Amerindian <0.2		12	100

AJCC, american joint committee on cancer classification

Table 2 Genotyping and ancestry component of patients with gallbladder cancer

Patient	*TP53*	*CDKN2A*	*PIK3CA*	*KRAS*	*BRAF*	Component of Ancestry		
						European	African	Amerindian
1	–	–	–	–	–	0,975	0,013	0,013
2	–	–	–	–	–	0,914	0,039	0,048
3	–	–	–	p.Gly12Val	–	0,795	0,192	0,013
4	–	p.Ala127Ser	–	–	–	0,926	0,038	0,036
5	–	–	–	–	–	0,948	0,033	0,019
6	c.345_373del	p.Ala148Thr	–	–	–	0,837	0,14	0,023
7	–	–	–	p.Gly13Asp	–	0,585	0,394	0,021
8	–	–	–	p.Gly12Arg/ p.Ile55Thr	–	0,969	0,013	0,018
9	–	p.Trp15X	–	–	–	0,905	0,091	0,004
10	c.909_993 + 14 del	–	–	–	–	0,796	0,1	0,104
11	–	p.Ala127Ser	–	–	–	0,875	0,119	0,005
12	–	p.Ala148Thr	–	–	–	0,877	0,021	0,101

There was no difference in genomic ancestry among Brazilian GBC patients and healthy control individuals and the strong imprint of European genomic ancestry was confirmed (Table 2).

The clinical features were similar to previous reports [1] with the majority being females, having current or past gallstones and diagnosed at an advanced stage.

Two GBCs showed *TP53* mutations, an important and early event in gallbladder tumorigenesis [6]. Indeed, somatic *TP53* mutations range from 27% to 70% in ethnically diverse populations [4–7] rates that are higher than the one reported herein (16.7%) which could be explained by ethnic differences and our small sample size.

KRAS is the most studied oncogene in GBC with a reported mutation rate ranging from 2.7% to 100% [8] In Western countries, these mutations are uncommonly found (0–10%) whereas in Japan can reach 100% [9], a discrepancy attributed to ethnic and geographical variations. In the present study, 33% of samples carried *KRAS* mutations probably reflecting the ethnic makeup of our population. One interesting finding was a novel missense mutation in codon 55 (p.Ile55Thr) that localizes to a conserved protein region, being deleterious to prediction algorithms. Further studies evaluating the rate and functional effect of this mutation are clearly warranted.

Mutations in the *CDKN2A* gene have been reported in ~ 50% of GBC cases [1]. In the present series two sequence variants (p.Ala127Ser, p.Ala148Thr) were detected. The A148T mutation is a rare polymorphism reported in approximately 2% of the European population. Yet, this variant has been associated with an increased risk to melanoma [10]. The second variant (A127S) is a predicted damaging mutation, has not been reported in GBC and was previously reported as a germline mutation in skin neoplasms [11]. Yet, no consistent conclusion as to the putative role of A127S has been reached.

Studies evaluating the expression, deletion and methylation of *CDKN2A* gene clearly show its importance in GBC pathogenesis. We detected a bona fide mutation (W15X) in a single GBC, a mutation previously described in melanoma, pancreatic cancer, hepatic cancer and aero digestive tract carcinoma (COSMIC database). Given that only a few studies have looked for *CDKN2A* somatic mutations in GBC, the current study emphasizes the need to expand the number of GBC genotyped mutations in this gene.

The fact that we could not show any mutations in relevant regions in the *PIK3CA* and *BRAF* genes may reflect the small number of tested individuals or the ethnic makeup of our population.

Some indigenous people and ethnic groups have higher incidence and mortality rates of GBC [1]. Brazil is home of genetically heterogeneous people, the product of admixture between Amerindians, Europeans, and Africans. We used a set of indel polymorphisms to properly characterize of our studied samples [6]. Despite these heterogeneous ancestral roots we did not find differences in genomic ancestry between groups indicating that, at least in Brazilian GBC patients, genomic ancestry does not seem to have a role. However, larger sample of patients with GBC need to be investigated.

In conclusion some analyzed GBC did not exhibit mutations in the tested genes; however, about 75% had mutations in a gene related to cancer. This finding emphasizes the need to expand the panel of genes tested to better understand the molecular mechanisms of these rare neoplasms.

Acknowledgements This work was partially funded by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq # 405053/2013-4) and Fundação de Amparo a Pesquisa de Minas Gerais (FAPEMIG # APQ-00220-14).

Compliance with Ethical Standards

This work was approved by the University Ethics Committee (CAAE-09135912.6.0000.5149).

Disclosure Statement Nothing to disclose.

References

1. Bal MM, Ramadwar M, Deodhar K, Shrikhande S (2015) Pathology of gallbladder carcinoma: current understanding and new perspectives. Pathol. Oncol. Res. 21:509–525
2. Jain K, Mohapatra T, Das P, Misra MC, Gupta SD, Ghosh M, Kabra M, Bansal VK, Kumar S, Sreenivas V, Garg PK (2014) Sequential occurrence of preneoplastic lesions and accumulation of loss of heterozygosity in patients with gallbladder stones suggest causal association with gallbladder cancer. Ann. Surg. 260:1073–1080
3. Espinoza JA, Bizama C, García P, Ferreccio C, Javle M, Miquel JF, Koshiol J, Roa JC (2016) The inflammatory inception of gallbladder cancer. Biochim. Biophys. Acta 1865:245–254
4. Nakamura H, Arai Y, Totoki Y, Shirota T, Elzawahry A, Kato M, Hama N, Hosoda F, Urushidate T, Ohashi S, Hiraoka N, Ojima H, Shimada K, Okusaka T, Kosuge T, Miyagawa S, Shibata T (2015) Genomic spectra of biliary tract cancer. Nat. Genet. 47:1003–1010
5. Bastos-Rodrigues L, Pimenta JR, Pena SD (2006) The genetic structure of human populations studied through short insertion-deletion polymorphisms. Ann. Hum. Genet. 70:658–665
6. Serra S (2014) Precursor neoplastic lesions of the biliary tract. J. Clin. Pathol. 67:875–882
7. Nagahashi M, Ajioka Y, Lang I, Szentirmay Z, Kasler M, Nakadaira H, Yokoyama N, Watanabe G, Nishikura K, Wakai T, Shirai Y, Hatakeyama K, Yamamoto M (2008) Genetic changes of p53, K-ras, and microsatellite instability in gallbladder carcinoma in high-incidence areas of Japan and Hungary. World J. Gastroenterol. 14:70–75
8. Saetta AA (2006) K-ras, p53 mutations, and microsatellite instability (MSI) in gallbladder cancer. J. Surg. Oncol. 93:644–649
9. Hanada K, Tsuchida A, Iwao T, Eguchi N, Sasaki T, Morinaka K, Matsubara K, Kawasaki Y, Yamamoto S, Kajiyama G (1999) Gene mutations of K-ras in gallbladder mucosae and gallbladder carcinoma with an anomalous junction of the pancreaticobiliary duct. Am. J. Gastroenterol. 94:1638–1642

10. Bakos RM, Besch R, Zoratto GG, Godinho JM, Mazzotti NG, Ruzicka T, Bakos L, Santos SE, Ashton-Prolla P, Berking C, Giugliani R (2011) The CDKN2A p.A148T variant is associated with cutaneous melanoma in southern Brazil. Exp. Dermatol. 20: 890–893

11. Soufir N, Ribojad M, Magnaldo T, Thibaudeau O, Delestaing G, Daya-Grosjean L, Rivet J, Sarasin A, Basset-Seguin N (2002) Germline and somatic mutations of the INK4a-ARF gene in a xeroderma pigmentosum group C patient. J Invest Dermatol 119: 1355–1360