Absence of Hotspot *TERT* Promoter Mutations in Brazilian Patients with Breast Cancer

Rhafaela Lima Causin[1,*], Sara de Freitas Abrão[2,*], Adriana Cruvinel-Carloni[1], Adriane Feijó Evangelista[1], Cristiano de Pádua Souza[3], Ana Caroline Neuber[1], René Aloisio da Costa Vieira[3], Rozany Mucha Dufloth[4], Rui Manuel Reis[1,5,6], Márcia Maria Chiquitelli Marques[1,2]

[1]Molecular Oncology Research Center, Barretos Cancer Hospital, Barretos, SP, Brazil

[2]Barretos School of Health Sciences, Barretos, SP, Brazil

[3]Department of Clinical Oncology, Barretos Cancer Hospital, Barretos, SP, Brazil

[4]Department of Pathology, Barretos Cancer Hospital, SP, Brazil

[5]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal

[6]ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal

*These authors have contributed equally to this work

RESUMO

Introdução: O câncer de mama é a doença maligna mais comum entre as mulheres, com taxas crescentes em todo o mundo. Os carcinomas mamários são considerados uma doença complexa heterogênea e, apesar de vários estudos sobre seus aspectos moleculares, alguns genes de alto risco não foram relatados em determinadas populações. Mutações pontuais hotspot (c.-124 pb G> A e c.-146 pb G> A) no promoter da transcriptase reversa da telomerase (*TERT*) em vários tipos de tumores sólidos foram recentemente descritas. **Objetivo:** Neste estudo, objetivamos determinar a frequência das mutações dos hotspots da região promotora do gene *TERT* usando o sequenciamento Sanger em uma série de 98 pacientes brasileiras com câncer de mama. **Resultados:** A análise mutacional mostrou a ausência de mutações pontuais hotspot no promoter *TERT*, mas revelou uma certa frequência do alelo menor rs2853669. **Conclusão:** Podemos concluir diante dos resultados identificados no presente estudo que, as mutações somáticas do hotspot *TERT* podem não ser um fator contribuinte na carcinogênese do câncer de mama na população brasileira.

Palavras-chave: Carcinomas mamários, detecção precoce, mutações do promotor de *TERT*.

ABSTRACT

Introduction: Breast cancer is the most common malignancy in women, with increasing rates worldwide. Breast carcinomas are considered a heterogeneous complex disease and despite several studies regarding its molecular aspects, some high-risk genes have not been reported in certain populations. Hotspot mutations (c.-124 bp G>A and c.-146 bp G>A) in the telomerase reverse transcriptase (*TERT*) promoter in several types of solid tumors has been recently described. **Aim:** In this study, we aimed to determine the frequency of hotspot *TERT* promoter mutations using Sanger sequencing in a series of 98 Brazilian patients with breast cancer **Results:** The mutation analysis showed the absence of mutations in the hotspots of the *TERT* promoter but revealed a certain frequency of the rs2853669 minor allele. **Conclusion:** We can conclude from the results identified in this study that TERT somatic hotspot mutations may not be a contributing factor in breast cancer carcinogenesis in the Brazilian population.

Keywords: Breast carcinomas, early detection, *TERT* promoter mutations.

INTRODUCTION

Breast cancer (BC) is the second most frequent type of carcinoma and the most common malignant tumor affecting women worldwide. BC survival rates vary globally depending on several risk factors that affect the pathogenesis of the disease and the mortality after diagnosis. Overall, it is estimated to be the fifth leading cause of cancer-related deaths[1] .

Breast carcinomas are considered a heterogeneous complex disease having distinct biological features and behavior with a consequent varying response to treatment modalities and clinical outcomes[2]. Traditional classification systems of this cancer are based on morphological features, clinical characteristics, and the status of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2)[3]. Studies involving gene expression analysis using high-throughput technologies have characterized the BC heterogeneity and identified several BC subgroups, including the widely accepted basal-like, human epidermal growth factor receptor 2–positive ($HER2^+$/ER^+), luminal A, luminal B, and triple negative subtypes[4,5].

Recently, The Cancer Genome Analysis (TCGA), the International Cancer Genome Consortium (ICGC), the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC), and other projects provided genomic and epigenomic information regarding BC carcinogenesis and pathway interactions[6–9]. These findings revealed that BC is more complex than previously thought and showed a higher frequency of mutations in several genes that can vary according to a molecular subtype and subgroups of tumors with similarities in copy number aberrations (CNA)[9,10]. Furthermore, regarding the distribution of mutations in high-risk genes by BC subtypes, the *TERT* gene could be an interesting high-risk candidate for further evaluation.

The human telomerase reverse transcriptase (*TERT*) gene encodes a catalytic subunit of telomerase that is associated with telomere genomic integrity[11,12]. The *TERT* recurrent somatic mutations described in the promoter region are mainly located in two hotspots (c.-124 bp G>A and c.-146 bp G>A) creating de novo Ets/TCF-binding sites as a novel mechanism of telomerase expression in cancer[11,12]. This gene has been found to be frequently mutated in skin melanomas in conjunction with BRAF mutations, in central-nervous-system tumors (especially gliomas), in bladder tumors, and in follicular-cell–derived thyroid cancers[11–15]. Our group has explored *TERT* somatic mutations in several tumors, including BC[16], gastrointestinal stromal tumors (GIST)[17], soft tissue sarcomas[18], testicular germ cell tumors[19], acral lentiginous melanomas[20], and colorectal precursor lesions and cancer[21]. In BC, a higher frequency of *TERT* hotspot mutations has been reported in the phyllodes subtype, a group of rare fibroepithelial neoplasms[16,22,23], and lower frequencies in the other most common types of BC were described (0.9% of 319 cases)[24]. In the present study, we investigated the frequency of *TERT* mutations in Brazilian patients with BC.

MATERIAL AND METHODS

Study population

We included 98 Brazilian patients from the Barretos Cancer Hospital, with approval of the local ethics committee. The clinical and molecular features of the patients are reported in Table 1. All included patients had an average age of 54.6 years (ranging from 22 to 79). Patients with clinical stage I, II, and III, the absence of previous treatment, and availability of the material at the Barretos Cancer Hospital's biobank were included. All cases were reviewed by an expert pathologist and categorized according to the World Health Organization classification[25].

DNA extraction

Breast cancer frozen tissue samples were undergoing genomic DNA extraction using the QIASymphony SP (QIAGEN, Hilden, Germany) automated system based on magnetic-bead technology (QIAGEN, Hilden, Germany) according to the manufacturer's protocol (QIAsymphony DNA Mini Kit). Quantification and DNA quality assessment were performed using a Nanodrop 2000 spectrophotometer (NanoDrop Products, Wilmington, DE, USA) and

stored at -20º C until further genetic analysis.

Identification of mutations by genomic DNA sequencing

Identification of the c.-146bp G > A and c.-124bp G > A hotspot *TERT* mutation on tumor genomic DNA was accomplished by amplifying exon 16 of the TERT gene using the primers and Polymerase Chain Reaction (PCR) conditions that we established previously[14,26]. Briefly, a 235-bp region of the *TERT* promoter containing the hotspots of C228T and C250T mutations was PCR-amplified using primers Fw: 50 -CAGCGCTGCCTGAAACTC-30 and Rw: 50 - GTCCTGCCCCTTCACCTT-30 resulting in a PCR product of 235 bp, which contained the sites chr5.hg19:g.1295228 C > T and chr5. hg19:g.1295250C > T, corresponding to the c.-124bp G > A and c.-146bp G > A mutations, respectively. PCR amplification of the genomic DNA (25–100 ng) was performed using the Qiagen Multiplex PCR kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol, using Q solution. Sequencing reactions were performed with the ABI Prism BigDye Terminator Kit (Thermo Scientific, Waltham) and the fragments were run in an ABI prism 3100 and 3500 xL Genetic Analyzer (Thermo Scientific, Waltham). The sequencing reaction was performed in forward direction. When a mutation was identified, an independent PCR amplification/ sequencing, both in forward and reverse directions, was performed to confirm the result.

Ethics statement

This study was approved by the Research Ethics Committee of the Barretos Cancer Hospital under Protocol No. 1212/2016. All information that could be used to identify the study participants was kept confidential and encrypted in the database to ensure the confidentiality of the data and the anonymity of the participants. Each research participant provided written informed consent for the publication of any data associated with the present study.

RESULTS

Clinical and pathological features

Clinical and histopathological data of the 88 breast cancer patients enrolled in this study are presented in Table 1. All patients included in this cohort were female (100%, n= 98) with ages ranging from 22 to 79 years (median = 52.6 years). Tumor histology was subdivided into Adenocarcinoma (2%, n= 2), Invasive ductal carcinoma (86.7%, n= 85), and Lobular carcinoma (11.1%, n= 11). Tumor histological grade were as follows: 21.4% (n= 21) in stage I, 41.8% (n= 41) in stage II, 31.6% (n= 31) in the stage III, and 6% (n= 6) of patients showed no conclusive results. Regarding of hormonal receptors of these patients 81.6% (n= 80) were positive estrogen receptor and 72.4% (n= 71) were positive progesterone receptor. We identified that 13.3% (n= 13) of these patients had hyperexpression of human epidermal growth factor receptor 2 (HER-2). Clinical stage was T1 (37.7) in 37 cases, T2 was (50%) in 49 cases and T3 (8.1%) in 8 cases; 37 (37.8%) of the cases had clinically positive lymph nodes (N1/N2) and 51 cases (58.1%) were N0; 94 (95.9%) had not metastasis.

Hotspot *TERT* Promoter Mutations in Brazilian Patients with Breast Cancer

In the present study, the promoter SNP rs2853669 (-245A>G) was analyzed in 98 samples. To determine whether breast cancer had mutations in Brazilian Patients, the genomic DNA from breast cancer was examined for mutation by direct Sanger Sequencing. We obtained a failure rate of 0%, which means that of the 98 samples tested by PCR 98/98 presented conclusive results in the Sanger sequencing. The results of sequencing analysis showed the absence of mutation in the exon 16 of the *TERT* gene in all of the 98 different cases of primary breast cancer.

We identified a single nucleotide polymorphism (SNP), corresponding to the minor allele of rs2853669 was detected in the *TERT* gene in 39 (39,8%) cases of the 98 patients examined (Figure 1 and Table S1).

Table 1. Clinicopathological features of all patients.

Variables		N	%
Age	54.6 y mean	98	-
	(range 22- 79)		
Gender	Female	98	100.0
Histology	Adenocarcinoma	2	2.0
	Invasive ductal carcinoma	85	86,7
	Lobular carcinoma	11	11.1
	N/C	7	7.1
Histological grade	I	21	21.4
	II	41	41.8
	III	31	31.6
	N/C	6	6.2
ER	Positive	80	81.6
	Negative	14	14.3
	N/A	5	5.1
PR	Positive	71	72.4
	Negative	23	23.5
	N/A	5	5.1
HER2	Positive	13	13.3
	Negative	81	83.5
	N/A	5	5.1
T	T1	37	37.7
	T2	49	50.0
	T3	8	8.1
	N/A	5	5.1
N	N0	57	58.1
	N1	26	26.5
	N2	11	11.2
	N/A	5	5.1
M	M0	94	95.9
	N/A	5	5.1

N/A, Not available; N/C, Not conclusive; ER, estrogen receptor; PR, progesterone receptor.
with the mean values.
“Conc.= concentration”; “sd= standard deviation”.

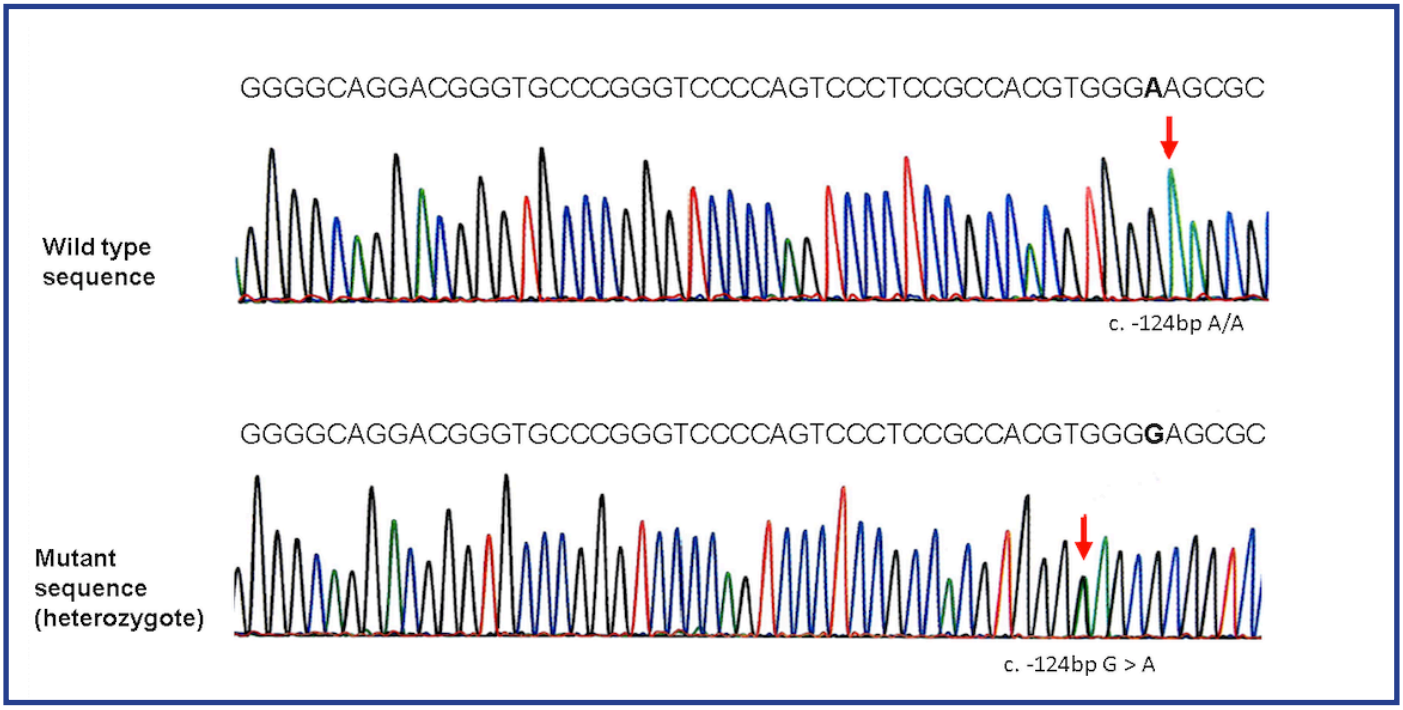

Figure 1. Representation and electropherogram of TERT showing the wild-type sequence for both mutation hotspots and the minor allele of the rs2853669 polymorphism. The sites analyzed (-124 bp, -146 bp and -245 bp) are highlighted.

DISCUSSION

Telomerase expression plays a role in cellular senescence, as it is normally repressed in postnatal somatic cells resulting in progressive shortening of telomeres[27]. The many studies reported that telomerase is reactivated in the majority of human cancers denotes that it plays a crucial role in tumor development and/or progression[26]. *TERT* has been found to be overexpressed in 90% of human cancer[28]. The identification of *TERT* mutations has provided a genetic cause for telomerase reactivation in a subset of human cancers, namely in gliomas[26)], GIST[17], melanomas[20] and colorectal precursor lesions and cancer[21], where *TERT* mutations are particularly frequent.

The *TERT* promoter SNP rs2853669 and its association with cancer risk has been reported in various cancer types in different populations[29–31]. Our results are in agreement with findings of a report that showed a low frequency of *TERT* promoter mutation in the most common types of BC[24]. Interestingly, a meta-analysis uncovered an increased risk of BC associated with the rs2853669 minor allele[32]. Further case-control studies are warranted to determine whether the minor allele of rs2853669 also constitute a risk factor in the Brazilian population. Moreover, this is the first study to analyze the *TERT* mutation status of Brazilian BC patients. In this sense, Shimoi et al identified that given the rarity of *TERT* promoter mutations, further studies are needed to confirm their prognostic significance in breast cancer cases[24]. In another study, the authors found definitive evidence for the genetic control of telomere length from the evaluation of common genetic variants at the *TERT* locus and reported multiple associations with breast cancer risk in both the general population and in BRCA1 mutation carriers[33].

In this study, we did not evaluate the limit of detection (LoD) of the Sanger sequencing technique, since this is a gold standard method. According to Tsiatis et al.[34] Sanger sequencing can provide a LoD of 15 to 20% [34], while other techniques can present a very different LoD. For example, the Pirosequencing technique generally provides a LoD of 5% (34), while NGS targeted resequencing provides a limit of detection down to 1% (35,36). Although, several

studies have shown that Sanger sequencing does not have the sensitivity required to detect mosaic variants consistently[37,38], in this study we can observed that the sequencing performed well among the analyzed samples.

The major limitation of this study lies in the absence of a control group, through which we could observe the influences of SNPs in different groups. Another limitation is based on the fact that the detection limit of Sanger sequencing was not evaluated. However, frozen tissue samples were used, and the extracted genomic DNA showed excellent quality, which made the assay quite efficient. We believe that other studies could be carried out with a larger court in which it was possible to obtain a well-defined control group.

CONCLUSION

In conclusion, the analysis of TERT promoter mutations in BC of Brazilian patients is described for the first time. The results showed the absence of TERT promoter mutations in the hotspots, suggesting that these alterations are not involved directly in BC carcinogenesis.

ABBREVIATIONS

TERT – Telomerase Reverse Transcriptase
BC – Breast Cancer
ER – Estrogen Receptor
PR – Progesterone Receptor
HER 2 – Human Epidermal Growth Factor Receptor 2
TCGA – The Cancer Genome Analysis
ICGC – International Cancer Genome Consortium
METABRIC – Molecular Taxonomy of Breast Cancer International Consortium
CNA – Copy Number Aberrations
PCR – Polymerase Chain Reaction

ACKNOWLEDGMENTS

The authors would like to thank the Barretos Cancer Hospital's Biobank for sample provision and RNA extraction.

FUNDING

The present study was supported by the Public Ministry of Work.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA: a cancer journal for clinicians. 2019;69(1):7–34.
2. Yersal O, Barutca S. Biological subtypes of breast cancer: Prognostic and therapeutic implications. World J Clin Oncol. 10 de agosto de 2014;5(3):412–24.
3. Hanby AM, walker C. Tavassoli FA, Devilee P: Pathology and Genetics: Tumours of the Breast and Female Genital Organs. WHO Classification of Tumours series - volume IV. Lyon, France: IARC Press. Breast Cancer Research. 31 de março de 2004;6(3):133.
4. Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. Mol Oncol. fevereiro de 2011;5(1):5–23.
5. Bandyopadhyay S, Bluth MH, Ali-Fehmi R. Breast Carcinoma: Updates in Molecular Profiling 2018. Clin Lab Med. 2018;38(2):401–20.
6. Koboldt DC, Fulton RS, McLellan MD, Schmidt H, Kalicki-Veizer J, McMichael JF, et al. Comprehensive molecular portraits of human breast tumours. Nature. outubro de 2012;490(7418):61–70.
7. Nik-Zainal S, Davies H, Staaf J, Ramakrishna M, Glodzik D, Zou X, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. Nature. junho de 2016;534(7605):47–54.
8. Bertucci F, Chaffanet M, Birnbaum D. An ICGC major achievement in breast cancer: a comprehensive catalog of mutations and mutational signatures. Chin Clin Oncol. 2017;6(1):4.
9. Russnes HG, Lingjærde OC, Børresen-Dale A-L, Caldas C. Breast Cancer Molecular Stratification: From Intrinsic Subtypes to Integrative Clusters. Am J Pathol. outubro de 2017;187(10):2152–62.
10. Russnes HG, Vollan HKM, Lingjærde OC, Krasnitz A, Lundin P, Naume B, et al. Genomic architecture characterizes tumor progression paths and fate in breast cancer patients. Sci Transl Med. 30 de junho de 2010;2(38):38ra47.
11. Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, et al. TERT promoter mutations in familial and sporadic melanoma. Science. 22 de fevereiro de 2013;339(6122):959–61.
12. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. Science. 22 de fevereiro de 2013;339(6122):957–9.
13. Killela PJ, Reitman ZJ, Jiao Y, Bettegowda C, Agrawal N, Diaz LA, et al. TERT promoter mutations occur frequently in

gliomas and a subset of tumors derived from cells with low rates of self-renewal. Proc Natl Acad Sci USA. 9 de abril de 2013;110(15):6021–6.
14. Vinagre J, Almeida A, Pópulo H, Batista R, Lyra J, Pinto V, et al. Frequency of TERT promoter mutations in human cancers. Nature Communications. 2013;4:2185.
15. Heidenreich B, Rachakonda PS, Hemminki K, Kumar R. TERT promoter mutations in cancer development. Current Opinion in Genetics & Development. fevereiro de 2014;24:30–7.
16. de Souza Rodrigues K, Nunes de Matos Neto J, Haddad R, Madureira de Oliveira D. Clinical relevance of telomerase polymorphism for breast cancer: A systematic review. J BUON. dezembro de 2017;22(6):1494–9.
17. Campanella NC, Celestino R, Pestana A, Scapulatempo-Neto C, de Oliveira AT, Brito MJ, et al. Low frequency of TERT promoter mutations in gastrointestinal stromal tumors (GISTs). Eur J Hum Genet. junho de 2015;23(6):877–9.
18. Campanella NC, Penna V, Abrahão-Machado LF, Cruvinel-Carloni A, Ribeiro G, Soares P, et al. TERT promoter mutations in soft tissue sarcomas. Int J Biol Markers. 28 de fevereiro de 2016;31(1):e62-67.
19. Cárcano FM, Vidal DO, van Helvoort Lengert A, Neto CS, Queiroz L, Marques H, et al. Hotspot TERT promoter mutations are rare events in testicular germ cell tumors. Tumour Biol. abril de 2016;37(4):4901–7.
20. Vazquez V de L, Vicente AL, Carloni A, Berardinelli G, Soares P, Scapulatempo C, et al. Molecular profiling, including TERT promoter mutations, of acral lentiginous melanomas. Melanoma Research. abril de 2016;26(2):93–9.
21. Cruvinel-Carloni A, Yamane L, Scapulatempo-Neto C, Guimarães D, Reis RM. Absence of TERT promoter mutations in colorectal precursor lesions and cancer. Genet Mol Biol. março de 2018;41(1):82–4.
22. Yoshida M, Ogawa R, Yoshida H, Maeshima A, Kanai Y, Kinoshita T, et al. TERT promoter mutations are frequent and show association with MED12 mutations in phyllodes tumors of the breast. British Journal of Cancer. 20 de outubro de 2015;113(8):1244–8.
23. Piscuoglio S, Ng CK, Murray M, Burke KA, Edelweiss M, Geyer FC, et al. Massively parallel sequencing of phyllodes tumours of the breast reveals actionable mutations, and TERT promoter hotspot mutations and TERT gene amplification as likely drivers of progression. J Pathol. março de 2016;238(4):508–18.
24. Shimoi T, Yoshida M, Kitamura Y, Yoshino T, Kawachi A, Shimomura A, et al. TERT promoter hotspot mutations in breast cancer. Breast Cancer. maio de 2018;25(3):292–6.
25. WHO C of T. WHO Classification of Tumours [Internet]. [citado 9 de outubro de 2020]. Disponível em: https://whobluebooks.iarc.fr/
26. Batista R, Cruvinel-Carloni A, Vinagre J, Peixoto J, Catarino TA, Campanella NC, et al. The prognostic impact of TERT promoter mutations in glioblastomas is modified by the rs2853669 single nucleotide polymorphism. Int J Cancer. 15 de 2016;139(2):414–23.
27. NCBI G. TERT telomerase reverse transcriptase [Homo sapiens (human)] - Gene - NCBI [Internet]. [citado 9 de outubro de 2020]. Disponível em: https://www.ncbi.nlm.nih.gov/gene/7015
28. Jiang Y, Chen C, Chen S-M, Wang Y-Q, Xu Y, Wang Y, et al. Telomerase reverse transcriptase promotes the proliferation of human laryngeal carcinoma cells through activation of the activator protein 1. Oncology Letters. 1o de julho de 2013;6(1):75–80.
29. Park C-K, Lee S-H, Kim JY, Kim JE, Kim TM, Lee S-T, et al. Expression level of hTERT is regulated by somatic mutation and common single nucleotide polymorphism at promoter region in glioblastoma. Oncotarget. 30 de maio de 2014;5(10):3399–407.
30. Spiegl-Kreinecker S, Lötsch D, Ghanim B, Pirker C, Mohr T, Laaber M, et al. Prognostic quality of activating TERT promoter mutations in glioblastoma: interaction with the rs2853669 polymorphism and patient age at diagnosis. Neuro-Oncology. setembro de 2015;17(9):1231–40.
31. Mosrati MA, Malmström A, Lysiak M, Krysztofiak A, Hallbeck M, Milos P, et al. TERT promoter mutations and polymorphisms as prognostic factors in primary glioblastoma. Oncotarget. 30 de junho de 2015;6(18):16663–73.
32. Li Z-Y, Dong Y-L, Feng Y, Zhang Z, Cao X-Z. Polymorphisms in the telomerase reverse transcriptase promoter are associated with risk of breast cancer: A meta-analysis. J Cancer Res Ther. junho de 2016;12(2):1040–4.
33. Bojesen SE, Pooley KA, Johnatty SE, Beesley J, Michailidou K, Tyrer JP, et al. Multiple independent variants at the TERT locus are associated with telomere length and risks of breast and ovarian cancer. Nat Genet. abril de 2013;45(4):371-384e2.
34. Tsiatis AC, Norris-Kirby A, Rich RG, Hafez MJ, Gocke CD, Eshleman JR, et al. Comparison of Sanger Sequencing, Pyrosequencing, and Melting Curve Analysis for the Detection of KRAS Mutations. J Mol Diagn. julho de 2010;12(4):425–32.
35. Schuster SC. Next-generation sequencing transforms today's biology. Nat Methods. janeiro de 2008;5(1):16–8.
36. Shendure J, Ji H. Next-generation DNA sequencing. Nat Biotechnol. outubro de 2008;26(10):1135–45.
37. Jamuar SS, Lam A-TN, Kircher M, D'Gama AM, Wang J, Barry BJ, et al. Somatic mutations in cerebral cortical malformations. N Engl J Med. 21 de agosto de 2014;371(8):733–43.
38. Lindhurst MJ, Sapp JC, Teer JK, Johnston JJ, Finn EM, Peters K, et al. A mosaic activating mutation in AKT1 associated with the Proteus syndrome. N Engl J Med. 18 de agosto de 2011;365(7):611–9.

AUTOR DE CORRESPONDÊNCIA
Márcia Maria Chiquitelli Marques Silveira
mmcmsilveira@gmail.com

Molecular Oncology Research Center, Barretos Cancer Hospital, Rua Antenor Duarte Villela, Barretos, 1331, São Paulo, 14784-400, Brazil.

Supplementary material

Table S1: Clinical and molecular features of patients with breast cancer.

Patient nº	Age (years)	Gender	Histology	Histological grade	TERT SNP rs2853669 (-245A>G)
1	78	Female	Invasive ductal carcinoma	II	A/G
2	51	Female	Invasive ductal carcinoma	II	A/G
3	41	Female	Invasive ductal carcinoma	II	A/G
4	37	Female	Invasive ductal carcinoma	II	A/G
5	45	Female	Invasive ductal carcinoma	II	A/G
6	53	Female	Invasive ductal carcinoma	I	A/G
7	58	Female	Invasive ductal carcinoma	II	G/G
8	67	Female	Invasive ductal carcinoma	I	A/G
9	48	Female	Invasive ductal carcinoma	II	A/G
10	71	Female	Invasive ductal carcinoma	II	A/G
11	54	Female	Invasive ductal carcinoma	III	A/G
12	68	Female	Invasive ductal carcinoma	II	A/G
13	65	Female	Invasive ductal carcinoma	II	G/G
14	57	Female	Invasive ductal carcinoma	I	A/G
15	49	Female	Lobular carcinoma	II	A/G
16	59	Female	Adenocarcinoma	I	A/G
17	73	Female	Lobular carcinoma	II	A/G
18	67	Female	Adenocarcinoma	I	G/G
19	77	Female	Invasive ductal carcinoma	II	A/G
20	44	Female	Invasive ductal carcinoma	II	A/G
21	45	Female	Invasive ductal carcinoma	II	A/G
22	46	Female	Invasive ductal carcinoma	III	A/G
23	43	Female	Invasive ductal carcinoma	II	A/G
24	47	Female	Invasive ductal carcinoma	II	A/G
25	45	Female	Invasive ductal carcinoma	III	A/G
26	59	Female	Invasive ductal carcinoma	I	A/G
27	47	Female	Invasive ductal carcinoma	II	A/G
28	63	Female	Invasive ductal carcinoma	II	A/G
29	60	Female	Invasive ductal carcinoma	II	A/G
30	53	Female	Invasive ductal carcinoma	I	A/G
31	42	Female	Invasive ductal carcinoma	II	A/G
32	61	Female	Invasive ductal carcinoma	I	A/G
33	41	Female	Lobular carcinoma	I	A/G
34	55	Female	Invasive ductal carcinoma	II	A/G
35	58	Female	Lobular carcinoma	I	A/G
36	42	Female	Invasive ductal carcinoma	I	A/G
37	69	Female	Invasive ductal carcinoma	II	A/G
38	39	Female	Invasive ductal carcinoma	III	A/G

39	58	Female	Invasive ductal carcinoma	II	A/G
40	51	Female	Invasive ductal carcinoma	III	A/A
41	28	Female	Lobular carcinoma	III	A/A
42	59	Female	Invasive ductal carcinoma	II	A/A
43	60	Female	Invasive ductal carcinoma	I	A/A
44	50	Female	Invasive ductal carcinoma	II	A/A
45	48	Female	Invasive ductal carcinoma	III	A/A
46	43	Female	Invasive ductal carcinoma	II	A/A
47	62	Female	Invasive ductal carcinoma	III	A/A
48	70	Female	Invasive ductal carcinoma	II	A/A
49	63	Female	Lobular carcinoma	II	A/A
50	22	Female	Invasive ductal carcinoma	III	A/A
51	40	Female	Invasive ductal carcinoma	III	A/A
52	62	Female	Invasive ductal carcinoma	III	A/A
53	44	Female	Invasive ductal carcinoma	II	A/A
54	44	Female	Invasive ductal carcinoma	III	A/A
55	37	Female	Invasive ductal carcinoma	III	A/A
56	55	Female	Invasive ductal carcinoma	III	A/A
57	61	Female	Lobular carcinoma	II	A/A
58	36	Female	Invasive ductal carcinoma	III	A/A
59	71	Female	Invasive ductal carcinoma	III	A/A
60	69	Female	Invasive ductal carcinoma	III	A/A
61	68	Female	Lobular carcinoma	I	A/A
62	55	Female	Invasive ductal carcinoma	III	A/A
63	50	Female	Invasive ductal carcinoma	II	A/A
64	71	Female	Invasive ductal carcinoma	III	A/A
65	79	Female	Invasive ductal carcinoma	I	A/A
66	70	Female	Invasive ductal carcinoma	II	A/A
67	69	Female	Lobular carcinoma	II	A/A
68	54	Female	Invasive ductal carcinoma	III	A/A
69	61	Female	Invasive ductal carcinoma	II	A/A
70	43	Female	Invasive ductal carcinoma	III	A/A
71	68	Female	Invasive ductal carcinoma	I	A/A
72	46	Female	Invasive ductal carcinoma	I	A/A
73	37	Female	Invasive ductal carcinoma	I	A/A
74	70	Female	Invasive ductal carcinoma	II	A/A
75	37	Female	Lobular carcinoma	I	A/A
76	56	Female	Invasive ductal carcinoma	III	A/A
77	47	Female	Invasive ductal carcinoma	III	A/A
78	45	Female	Invasive ductal carcinoma	II	A/A
79	54	Female	Invasive ductal carcinoma	II	A/A
80	52	Female	Invasive ductal carcinoma	III	A/A
81	58	Female	Invasive ductal carcinoma	III	A/A
82	46	Female	Invasive ductal carcinoma	III	A/A

83	45	Female	Lobular carcinoma	I	A/A
84	71	Female	Invasive ductal carcinoma	II	A/A
85	67	Female	Invasive ductal carcinoma	II	A/A
86	49	Female	Invasive ductal carcinoma	III	A/A
87	22	Female	Invasive ductal carcinoma	III	A/A
88	57	Female	Invasive ductal carcinoma	I	A/A
89	35	Female	Invasive ductal carcinoma	III	A/A
90	36	Female	Invasive ductal carcinoma	III	A/A
91	55	Female	Invasive ductal carcinoma	I	A/A
92	50	Female	Invasive ductal carcinoma	III	A/A
93	45	Female	Invasive ductal carcinoma	III	A/A
94	60	Female	Invasive ductal carcinoma	II	A/A
95	73	Female	Invasive ductal carcinoma	I	A/A
96	59	Female	Invasive ductal carcinoma	II	A/A
97	39	Female	Invasive ductal carcinoma	II	A/A
98	59	Female	Invasive ductal carcinoma	II	A/A