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ARTICLE

Corrected: Correction

TERT promoter mutation in adult granulosa cell tumor of the ovary

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Abstract

The telomerase reverse transcriptase (*TERT*) gene is highly expressed in stem cells and silenced upon differentiation. Cancer cells can attain immortality by activating *TERT* to maintain telomere length and telomerase activity, which is a crucial step of tumorigenesis. Two somatic mutations in the *TERT* promoter (C228T; C250T) have been identified as gain-of-function mutations that promote transcriptional activation of *TERT* in multiple cancers, such as melanoma and glioblastoma. A recent study investigating *TERT* promoter mutations in ovarian carcinomas found C228T and C250T mutations in 15.9% of clear cell carcinomas. However, it is unknown whether these mutations are frequent in other ovarian cancer subtypes, in particular, sex cord-stromal tumors including adult granulosa cell tumors. We performed whole-genome sequencing on ten adult granulosa cell tumors with matched normal blood and identified a *TERT* C228T promoter mutation in 50% of tumors. We found that adult granulosa cell tumors with mutated *TERT* promoter have increased expression of *TERT* mRNA and exhibited significantly longer telomeres compared to those with wild-type *TERT* promoter. Extension cohort analysis using allelic discrimination revealed the *TERT* C228T mutation in 51 of 229 primary adult granulosa cell tumors (22%), 24 of 58 recurrent adult granulosa cell tumors (41%), and 1 of 22 other sex cord-stromal tumors (5%). There was a significant difference in overall survival between patients with *TERT* C228T promoter mutation in the primary tumors and those without it ($p = 0.00253$, log-rank test). In seven adult granulosa cell tumors, we found the *TERT* C228T mutation present in recurrent tumors and absent in the corresponding primary tumor. Our data suggest that *TERT* C228T promoter mutations may have an important role in progression of adult granulosa cell tumors.

Telomeres are conserved, repetitive (TTAGGG) DNA–protein complexes that are added to the ends of chromosomes by the enzyme telomerase to prevent DNA damage and maintain replicative potential [1]. Telomere attrition during DNA replication induces genomic instability that can result in tumorigenesis [2]. Telomerase consists of a catalytic protein subunit known as telomerase reverse transcriptase (*TERT*) and a functional RNA called telomerase RNA component (*TERC*) [3]. *TERT* is highly expressed in stem cells and is silenced upon differentiation in somatic cells [4]. Most cancer cells attain proliferative immortality by upregulating the *TERT* gene to maintain telomere length and telomerase activity [5]. The known mechanisms of telomerase activation include mutations in the *TERT* promoter, *TERT* gene amplification, CpG methylation at the *TERT* promoter, changes in alternative splicing of *TERT* pre-mRNA and upregulation of transcriptional activators [6]. Approximately 90% of cancers express *TERT*, while the remaining 10–15% of cancers maintain their telomere length through a telomerase-independent method called alternative lengthening of telomeres [7].

TERT promoter mutations were first reported in familial melanoma and subsequently in sporadic melanoma [8, 9]. There are two hot-spot *TERT* promoter mutations, C228T and C250T, each generates an identical 11 base-pair sequence containing a consensus binding motif for ETS transcription factors, and functions as either a transcriptional activator or repressor to regulate telomerase expression [8, 9]. These two mutations are implicated in

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the activation of telomerase in other malignancies such as central nervous system tumors, hepatocellular carcinomas, bladder cancers, and thyroid cancers [10, 11]. A recent study on *TERT* promoter mutations in gynecological malignancies, including ovarian and uterine carcinomas, reported *TERT* hot-spot mutations in 15.9% of ovarian clear cell carcinomas [12]. However, it is unknown whether *TERT* promoter mutations are frequent in sex cord-stromal tumors, including adult granulosa cell tumors. In this study, we evaluated the biological and clinical significance of *TERT* promoter mutations, specifically C228T, in total of 251 primary ovarian sex cord-stromal tumors.

Materials and methods

Patient cohort description

Adult granulosa cell tumors ($n = 303$) were collected from Helsinki University Hospital (Helsinki, Finland; $n = 142$), Tübingen University Hospital (Tübingen, Germany; $n = 49$), Massachusetts General Hospital (Boston, USA; $n = 41$), Netherlands Cancer Institute (Amsterdam, Netherlands; $n = 30$), OvCaRe Gynecological Tumor Bank, (Vancouver, Canada; $n = 20$), the Jikei University School of Medicine (Tokyo, Japan; $n = 14$), and Referral Center for Gynecopathology (Mannheim, Germany; $n = 7$). The other sex cord-stromal tumors were collected from Vancouver General Hospital (Vancouver, Canada; $n = 22$). The respective institutional research ethics board approved the waiver for patient consent. The British Columbia Cancer Agency and the University of British Columbia Research Ethics Boards approved the overall project methods. All cases were reviewed by at least one of the following expert pathologists (CBG, FK, HMH, ANK, RB, HL-C, BTC, and LH) to confirm the diagnosis of the sex cord-stromal tumor. A *FOXL2* C402G allelic discrimination assay was used to validate adult granulosa cell tumor diagnosis. Of the 229 primary adult granulosa cell tumors, 223 harbored a heterozygous *FOXL2* C402G mutation and 6 harbored a homozygous, and were considered molecularly defined adult granulosa cell tumor as previously described [13]. For the adult granulosa cell tumor sequencing cohort, tumors were reviewed from frozen material, by at least two expert gynecological pathologists (HMH, ANK, HL-C, and CBG), and CBG approved the final selected cohort. All adult granulosa cell tumors sequenced were primary tumor samples. Each respective institution for Helsinki, Boston, Tübingen, Amsterdam, Mannheim, and Tokyo cases collected clinical follow-up data.

Whole-genome sequencing analysis of adult granulosa cell tumors

A total of ten fresh-frozen adult granulosa cell tumor tissues with >80% tumor cellularity (based on frozen hematoxylin and eosin slide review) were selected for cryosectioning and DNA extraction. Patient tumor and normal blood samples were collected at diagnosis during standard-of-care debulking surgery. DNA was extracted from matched normal (peripheral blood buffy coat) and tumor (frozen tissue) samples using QIAmp Blood and Tissue DNA (Qiagen, Mississauga, ON) and quantified using the Qubit Fluorometer with the dsDNA High Sensitivity Assay Kit (ThermoFisher Scientific, Canada). Whole-genome sequencing was performed using Illumina HiSeq 2500 v4 chemistry (Illumina Inc., Hayward, CA) with the PCR-free protocol to eliminate PCR-induced bias and improve coverage across the genome. Whole-genome sequencing analysis was performed to identify somatic alterations in each case, including single-nucleotide variants, small indels, copy number alterations, and structural variants. For a full description of library construction and sequencing methods, please refer to Wang et al. [14].

Direct sequencing of TERT promoter in adult granulosa cell tumors

TERT promoter (including positions 228 and 250) was PCR amplified from DNA extracted from all ten adult granulosa cell tumors and the recurrent adult granulosa cell tumor-derived KGN cell line using the following primers: 5′-CAGCGCTGCCTGAAACTC-3′ (forward) and 5′-GTC CTGCCCCTTCACCTT-3′ (reverse). DNA was denatured at 94 °C for 2 min, then DNA was amplified over 35 cycles (94 °C for 30 s, 62 °C for 30 s, 68 °C for 30 s), followed by a final extension at 68 °C for 5 min using an MJ Research Tetrad (Ramsey, MN). PCR products were bi-directionally sequenced using an ABI BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) and an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Screening TERT C228T promoter mutation in extension cohort

Allelic discrimination for *TERT* C228T promoter mutation in 229 primary adult granulosa cell tumors, 58 recurrent adult granulosa cell tumors and 22 other sex cord-stromal tumors was determined using a Custom TaqMan SNP Genotyping assay (Applied Biosystems, Foster City, CA). The sequences of the primers were as follows: 5′-CTGCCCCTTCACCTTCCA-3′ (forward) and 5′-GGG

CCGCGGAAAGGAA-3′ (reverse). The wild-type-specific probe (5′-VIC dye-CCCAGCCCCCTCCGG-NFQ (non-fluorescent quencher)) and mutant-specific probe (5′-FAM dye-CCAGCCCCTTCCGG-NFQ) were included in the TaqMan Genotyping Master Mix (2×) and reactions were performed in the QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems, Foster City, CA) using 20 ng of DNA. After denaturation at 95 °C for 10 min, DNA was amplified over 50 cycles (95 °C for 15 s, 60 °C for 90 s).

TERT mRNA expression quantification in adult granulosa cell tumors

RNA was extracted using the miRNAeasy tissue kit (Qiagen, Mississauga, ON) and quantified using the NanoDrop ND1000 Fluorospectrometer (ThermoFisher Scientific, Canada). cDNA synthesis was performed using the SuperScript IV First-Strand Synthesis System on total RNA with 50 µM random hexamers (Invitrogen, Carlsbad, CA). *TERT* mRNA transcript expression was determined from the cDNA by real-time PCR in the QuantStudio 6 Flex Real-Time PCR System using the *TERT* TaqMan probe spanning exons 3 and 4 (Hs00972650_m1) with TaqMan Fast Advanced Master Mix (2×). The 18S (Hs03928990_g1) housekeeping gene was used for normalization (Applied Biosystems, Foster City, CA). The relative levels were calculated using the ΔΔCT method [15].

Telomere length evaluation in adult granulosa cell tumors

Telomere length was determined using XpYp single telomere length analysis (STELA) as previously described [16, 17]. Briefly, extracted DNA from all ten adult granulosa cell tumors was quantified by Hoechst 33258 fluorometry (Bio-Rad, Hercules, CA, USA) before dilution to 10 ng/µl in 10 mmol/l Tris-HCl, pH 7.5. A total of 10 ng of DNA was further diluted to 250 pg/µl in a volume of 40 µl containing 1 µmol/l Telorette2 linker and 1 mmol/l Tris-HCl, pH 7.5. Multiple polymerase chain reactions (PCRs; typically 6 reactions per sample) were carried out for each test DNA in 10-µl volume with 250 pg of DNA, 0.5 µmol/l of the telomere-adjacent and Teltail primers, 75 mmol/l Tris-HCl, pH 8.8, 20 mmol/l $(NH_4)_2SO_4$, 0.01% Tween-20, 1.5 mmol/l $MgCl_2$, and 0.5 units of a 10:1 mixture of Taq (ABGene, Thermo Scientific) and Pwo polymerase (Roche Molecular Biochemicals, Burgess Hill, West Sussex, UK). The reactions were cycled with an MJ PTC-225 thermocycler (MJ Research, Bio-Rad). The DNA fragments were resolved by 0.5% Tris acetate EDTA agarose gel electrophoresis, and detected by Southern blot hybridization with random-primed α-33P–labeled (Perkin Elmer, Waltham, Massachusetts, USA) TTAGGG repeat probe together with probes to detect the 1-kb (Stratagene, Agilent Technologies, Santa Clara, CA, USA) and 2.5-kb (Bio-Rad) molecular weight markers. The hybridized fragments were detected by phosphorimaging with a Typhoon FLA 9500 phosphorimager (GE Healthcare). The molecular weights of the DNA fragments were calculated using the Phoretix 1D quantifier (Nonlinear Dynamics, Newcastle upon Tyne, UK).

Statistical analysis

Two-tailed Fisher exact tests were performed to compare the frequencies of *TERT* C228T mutation in adult granulosa cell tumors and other sex cord-stromal tumors, and in primary versus recurrent adult granulosa cell tumors. The frequency of C228T mutation in adult granulosa cell tumors collected from different institutions was evaluated using a two-tailed Fisher's exact test. The *TERT* mRNA expression levels and telomere lengths of *TERT* mutant and *TERT* wild-type tumors were compared using a Mann–Whitney *U* test to determine whether *TERT* C228T promoter mutation was associated with increased *TERT* mRNA expression and longer telomeres, respectively. Overall survival, disease-specific survival and disease-free survival of adult granulosa cell tumors with and without *TERT* C228T promoter mutation were compared using the Kaplan-Meier method, followed by the log-rank test to determine significance.

Results

TERT C228T promoter mutation is frequent in adult granulosa cell tumors

Whole-genome sequencing analysis was performed on ten adult granulosa cell tumors and identified a *TERT* C228T mutation in 50% of tumors (Fig. 1a). We previously performed whole-genome sequencing analysis on ovarian surface epithelial, including clear cell ovarian carcinomas, endometrioid ovarian carcinomas, and high-grade serous ovarian carcinomas. The frequency of *TERT* promoter mutations is summarized in Table 1. We found *TERT* promoter mutations in 2 of 35 clear cell ovarian carcinomas (5.7%), 1 of 29 endometrioid ovarian carcinomas (3.4%) and 0 of 59 high-grade serous ovarian carcinomas (0%) [14]. We did not identify any *TERT* C250T promoter mutations in adult granulosa cell tumors, thus our allelic discrimination analysis was performed for *TERT* C228T mutation in the extension cohort. The frequency of *TERT* C228T promoter mutation in the extension cohort of adult granulosa cell tumors and other sex cord-stromal tumors is summarized in Table 2. The C228T change was detected in

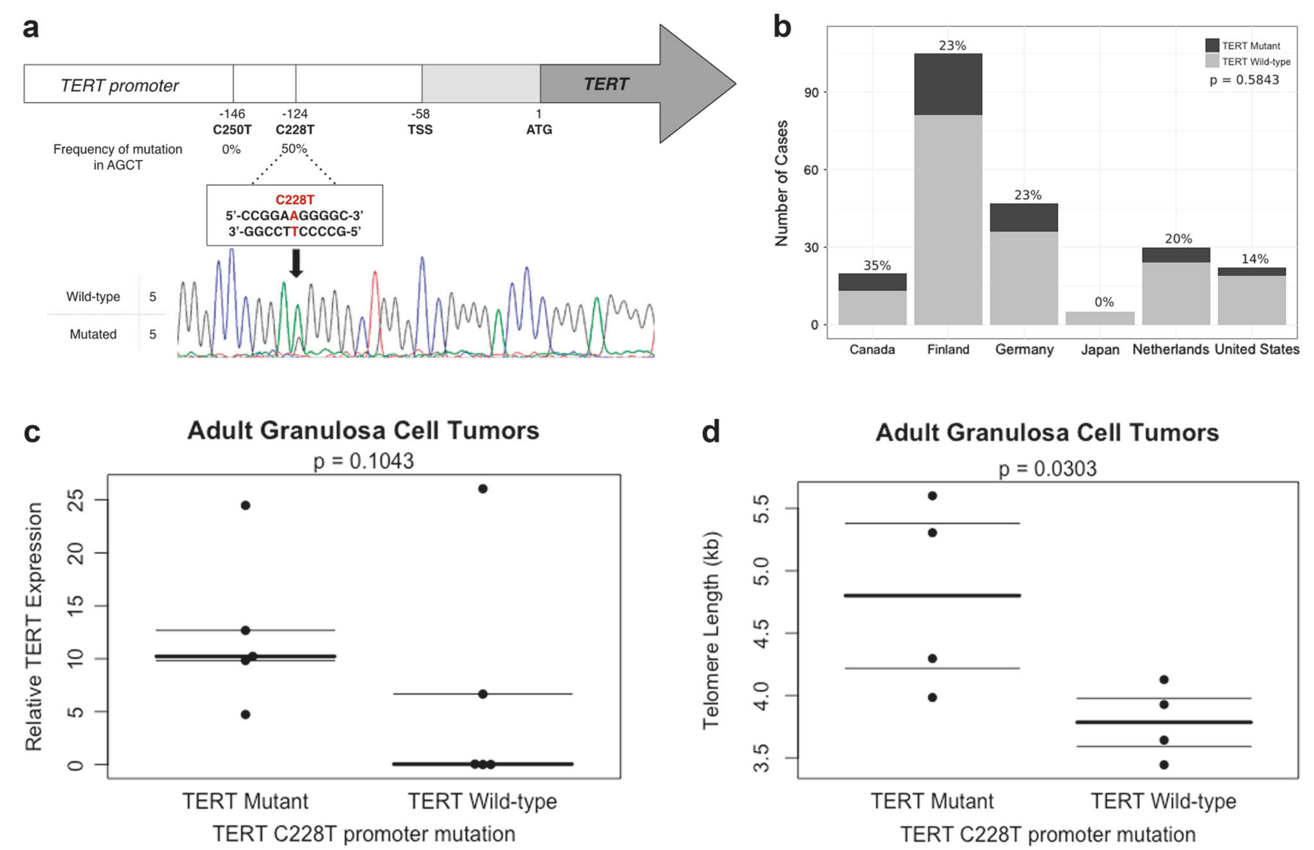

Fig. 1 **a** *TERT* promoter mutation in adult granulosa cell tumors. Shown is the schematic diagram of the *TERT* promoter region, representative chromatograms of the two hot-spot mutations (C228T; C250T). **b** Mutation status of *TERT* C228T promoter in adult granulosa cell tumors separated by the country in which they were collected from: dark gray, *TERT* C228T mutated promoter; light gray, *TERT* wild-type promoter. **c** Adult granulosa cell tumors with mutated *TERT* promoter have increased expression of *TERT* mRNA; black horizontal bar, mean. **d** Comparison of XpYp STELA telomere length mean between adult granulosa cell tumors with and without *TERT* promoter mutation; black horizontal bar, group mean

52 of 251 primary tumor samples (21%), with the majority occurring in adult granulosa cell tumors. The mutation was present in 51 of 229 primary adult granulosa cell tumors (22%) and 24 of 58 recurrent adult granulosa cell tumors (41%), while only in 1 of 22 other sex cord-stromal tumors (5%). The mutation frequency between primary adult granulosa cell tumors and other sex cord-stromal tumors was not significant ($p = 0.05448$, two-tailed Fisher's exact test). The C228T mutation was also present in the KGN cell line, which was derived from a patient with a recurrent adult granulosa cell tumor. There was no significant difference in *TERT* C228T mutation frequency among adult granulosa cell tumors collected from different countries ($p = 0.5843$, two-tailed Fisher's exact test, Fig. 1b).

Table 1 Prevalence of *TERT* C228T and C250T promoter mutations in ovarian cancer

	C228T	C250T	Mutated/ Total	%
Ovarian cancer				
Adult granulosa cell tumor	5	0	5/10	50
Clear cell ovarian carcinoma	1	1	2/35	5.7
Endometrioid ovarian carcinoma	1	0	1/29	3.4
High-grade serous ovarian carcinoma	0	0	0/59	0

TERT C228T promoter mutation is associated with elevated mRNA expression in adult granulosa cell tumors

Among the ten adult granulosa cell tumors, we found that the five cases with a *TERT* C228T mutation showed a 10.8-fold increase of *TERT* mRNA expression as compared to the five tumors without a *TERT* promoter mutation. The majority of adult granulosa cell tumors harboring a *TERT* C228T mutation expressed higher levels of *TERT* mRNA compared to those with a wild-type *TERT* promoter (Fig. 1c). However, the difference was not significant ($p = 0.1043$, Mann–Whitney U test). Interestingly,

Table 2 Prevalence of *TERT* C228T promoter mutation in ovarian sex cord-stromal tumors

	Mutated/total	%
Ovarian sex cord-stromal tumor		
Primary adult granulosa cell tumor	51/229	22
Recurrent adult granulosa cell tumor	24/58	41
Adult granulosa cell tumor KGN cell line	1/1	100
Sertoli–Leydig cell tumor	1/5	20
Fibroma/Thecoma	0/14	0
Gynandroblastoma	0/1	0
Unclassified	0/2	0

we found one wild-type *TERT* adult granulosa cell tumor expressed a high level of *TERT* mRNA due to a copy number gain ($n = 3$). When this wild-type *TERT* adult granulosa cell tumor was removed from the group, *TERT* promoter mutants expressed significantly higher levels of *TERT* mRNA compared to those with *TERT* wild-type promoter ($p = 0.01587$, Mann–Whitney *U* test). We excluded this wild-type *TERT* adult granulosa cell tumor with the *TERT* copy number gain (VOA1405b) from subsequent analysis.

Adult granulosa cell tumors with TERT C228T promoter mutation tend to have longer telomeres

To evaluate the relationship between *TERT* C228T promoter mutation and telomere length, we assessed the XpYp telomere length in ten adult granulosa cell tumors (Fig. 1d). Some of the tumors exhibited more heterogeneous telomere length distributions, for example VOA985c, VOA1173b, VOA1405b, and VOA1744b (Supplementary Figure 1A-B). Other tumors exhibited more homogenous telomere length distributions along with longer telomeres (Supplementary Figure 1A-B). *TERT* C228T promoter mutant adult granulosa cell tumors had significantly longer telomeres compared to those with wild-type *TERT* promoter ($p = 0.0303$, Mann–Whitney *U* test). Three of four tumors (VOA1617a; VOA3082b; VOA4047b) with a *TERT* mutation yield homogenous telomere length profiles that are typical of clonal growth as seen in the HT1080 Clone 2 control cell line and is most likely mediated by telomerase (Supplementary Figure 1A). The *TERT* promoter wild-type tumors (VOA985c; VOA1405b) that express *TERT* mRNA exhibit a wide distribution of telomere lengths and is similar to what has been previously described in alternative lengthening of telomeres cell lines, which use a recombination telomere maintenance process independent of telomerase (Supplementary Figure 1B). Two of the remaining three adult granulosa cell tumors with no *TERT* mRNA expression yield heterogeneous telomere lengths and resemble tumors that use the alternative lengthening of telomeres process. However, we interrogated the whole-genome sequencing data and did not find any mutations in *ATRX* or *DAXX*, the two commonly mutated genes described in tumors using the alternative lengthening of telomeres method [18, 19].

TERT C228T promoter mutation is present in recurrent adult granulosa cell tumors but absent in corresponding primary tumors

Despite the numerous studies on telomerase and *TERT* expression in multiple cancers, it is poorly defined at which stage telomerase is activated in tumorigenesis. Recent reports identified *TERT* promoter mutations in early stages of melanoma [20], precursor lesions of hepatocellular carcinomas [21], and benign urothelial lesions [22], suggesting that *TERT* promoter mutation is an early genetic event in oncogenesis. To determine whether *TERT* C228T promoter mutation is an early mutation event in adult granulosa cell tumors, we compared the frequency of C228T mutation in primary (22%) and recurrent (41%) tumors and found the difference to be significant ($p = 0.003334$, two-tailed Fisher's exact test, Fig. 2a). We found seven adult granulosa cell tumors with no *TERT* C228T mutation in the primary tumor but with the mutation in either the first or second recurrent tumor (Fig. 2b). The remaining five adult granulosa cell tumors with primary and recurrent pairs were *TERT* promoter wild type.

Adult granulosa cell tumor patients with TERT C228T promoter mutation tend to have worse survival

In 186 primary adult granulosa cell tumor patients with available follow-up data, there was a significant difference in overall survival between patients with *TERT* C228T promoter mutation and those with *TERT* wild-type promoter ($p = 0.00253$, log-rank test, Fig. 3a). There was no significant difference in disease-specific survival ($p = 0.0754$, log-rank test, Fig. 3b) or disease-free survival ($p = 0.705$, log rank test, Fig. 3c).

Discussion

In this study, we found that adult granulosa cell tumors have the highest frequency of *TERT* C228T promoter mutation among tumors that have been studied in the ovary including surface epithelial carcinomas and sex cord-stromal tumors. Adult granulosa cell tumors account for ~90% of sex cord-stromal tumors and are characterized by their slow, indolent

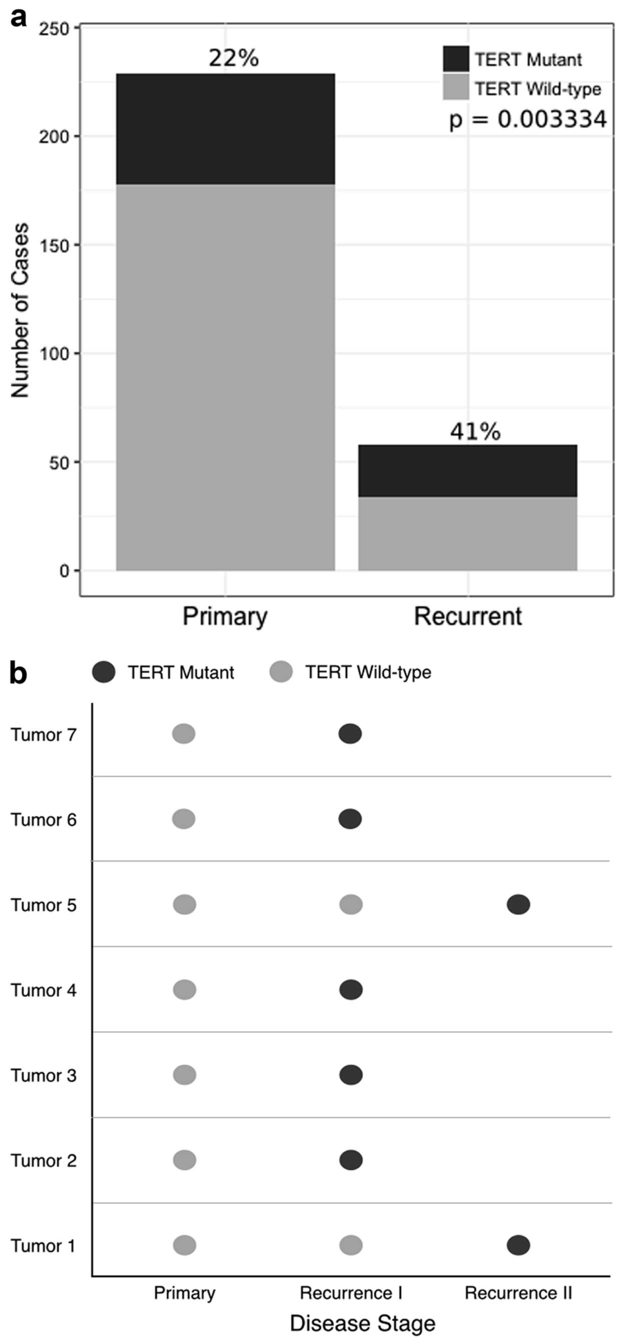

Fig. 2 **a** Mutation status of *TERT* C228T promoter in primary and recurrent adult granulosa cell tumors: dark gray, *TERT* C228T mutated promoter; light gray, *TERT* wild-type promoter. **b** An example of acquired *TERT* C228T promoter mutations in seven adult granulosa cell tumors with primary and recurrent tumor pairs: dark gray, *TERT* C228T mutated promoter; light gray, *TERT* wild-type promoter

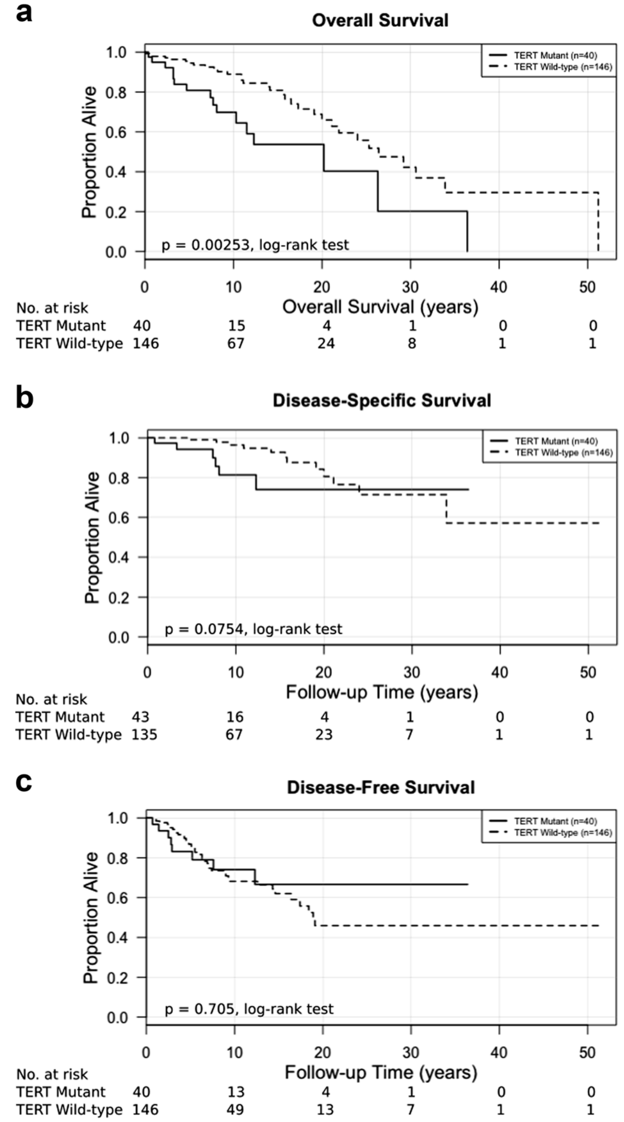

Fig. 3 Kaplan–Meier survival curves of adult granulosa cell tumor patients. **a** Overall survival in patients with *TERT* C228T promoter mutation is worse than patients with wild-type *TERT* promoter ($p = 0.00253$, log-rank test). **b** Disease-specific survival in patients with *TERT* C228T promoter mutation is similar to patients with wild-type *TERT* promoter ($p = 0.0754$, log-rank test). **c** Disease-free survival in patients with *TERT* C228T promoter mutation is similar to patients with wild-type *TERT* promoter ($p = 0.705$, log-rank test)

growth [23]. They are thought to arise from proliferating granulosa cells of the pre-ovulatory stage, as the share several morphological and hormonal features, including estrogen and inhibin synthesis [24]. A single somatic missense mutation in *FOXL2* (c.402C > G; pC134W) identified by Shah et al. [25] was reported to be present in 86 of 89 morphologically defined adult granulosa cell tumors (97%). Subsequent validation analysis from independent research groups reported the high frequency and specificity of *FOXL2* C402G mutation for adult granulosa cell tumors [26–28]. This mutation was also found in small number of other malignant ovarian sex cord-stromal tumors, but not in benign ovarian sex cord-stromal tumors, ovarian surface epithelial tumors or unrelated tumors [25, 29, 30]. This suggests *FOXL2* C402G is pathognomonic of adult granulosa cell tumors and can aid in resolving diagnosis for histologically challenging cases [31]. *FOXL2* is a member of the forkhead box family of transcription factors and plays a fundamental role in ovarian folliculogenesis [32]. The majority of adult granulosa cell tumors are diagnosed at Stage I and therefore cured with surgery. However, late tumor recurrence is common and over 80% of patients with advanced-stage or recurrent tumors succumb to their disease [33]. As the *FOXL2* C402G mutation is present in essentially all adult granulosa cell tumors, it is likely that additional mutations drive tumor progression [25]. Our study is the first to use whole-genome sequencing to provide a comprehensive catalog of coding and non-coding genetic events in adult granulosa cell tumors, the most common among all sex cord-stromal tumors of the ovary. We compared the genomes of ten adult granulosa cell tumors with their matched normal genomes with the objective to identify recurrent mutations that will aid in understanding and improving prognosis. The high frequency of *TERT* C228T promoter mutation in recurrent adult granulosa cell tumors compared to primary adult granulosa cell tumors highlights a potential mechanism for clonal expansion and recurrence.

Several studies have shown the two hot-spot *TERT* promoter mutations (C228T; C250T) generate a binding motif for ETS transcription factors and increase transcriptional activity of the *TERT* promoter [11]. Similarly, we observed higher *TERT* mRNA expression in adult granulosa cell tumors harboring a *TERT* C228T promoter mutation, and *TERT* mutated tumors had significantly longer telomeres compared to *TERT* wild-type tumors. One *TERT* wild-type promoter adult granulosa cell tumor with an identified copy number gain expressed high levels of *TERT*. This result highlights the importance of determining both the genotype and gene expression to identify copy number gains or epigenetic regulation. During ovarian follicle development, telomerase activity is tightly regulated in a temporal and spatial pattern, where *TERT* expression is dependent on cell type and folliculogenesis stage [34]. Studies have reported that *TERT* expression correlates with the length of telomeres in primary granulosa cells and that small pre-ovulatory follicles have the highest telomerase activity with the longest telomeres [35]. As the granulosa cells undergo differentiation into luteinized granulosa cells and the follicle develops into the corpus luteum, there is a large decline in telomerase activity [36, 37]. This suggests that telomerase plays an essential role in granulosa cell apoptosis and follicular atresia [36, 38]. Thus, persistent telomerase expression in granulosa cells would result in continued survival beyond the necessary cell divisions and could lead to the development of an adult granulosa cell tumor. Our findings are consistent with a revised model recently proposed by Chiba et al. [39] whereby cells harboring activating *TERT* promoter mutations extend their proliferative capacity. Our results highlight the importance of telomerase activity in tumor development, where *TERT* mRNA expression is seen in seven of ten cases, five of which harbor the *TERT* C228T promoter mutation.

Allelic discrimination analysis of primary and recurrent adult granulosa cell tumors indicates that *TERT* C228T promoter mutation are already present in some primary tumors, but may be late events which occur during adult granulosa cell tumor progression. Studies have reported *TERT* promoter mutations as an early genetic event in malignant transformation of multiple cancers [20–22]. In contrast, we found that *TERT* C228T mutations were present in a significantly higher proportion of recurrent compared to primary adult granulosa cell tumors. We observed *TERT* mutations in recurrent tumors where no mutations were detected in the corresponding primary tumor (Fig. 2b), suggesting that *TERT* promoter mutations were acquired during their progression. However, it may also be possible that *TERT* C228T mutation was present in the primary tumors at a very low frequency that was undetectable. This discordance of *TERT* mutation in paired primary and recurrent adult granulosa cell tumors suggests that these tumors exhibit intratumoral genetic heterogeneity. Future studies using targeted deep sequencing at the *TERT* promoter would elucidate whether *TERT* mutations are present at low frequencies in primary tumors. It is notable that we found the *TERT* C228T promoter mutation in the adult granulosa cell tumor-derived cell line KGN. Similarly, addition of *TERT* expression was required for immortalization of the SVOG cell line that was derived from primary granulosa cells, suggesting the need for telomerase activity in adult granulosa cell tumors. Previous studies have associated *TERT* promoter mutations with a worse prognosis in melanoma [40], glioblastoma, and bladder cancer patients [41]. In contrast, previous analysis of disease-specific survival in clear cell ovarian carcinoma comparing mutated *TERT* promoter and wild-type *TERT* promoter was not significant ($p = 0.9592$, log-rank test) [12]. Although in our analysis there was a trend of worse disease-specific survival for *TERT* C228T mutant primary adult granulosa cell tumors, it was not significant ($p = 0.0754$, log-rank test). Additional studies investigating all *TERT* promoter mutations, including the less common ones C250T, C242T, and C243T [42], would be required to accurately compare the prognosis of *TERT* promoter mutant and wild-type adult granulosa cell tumor patients.

Overall, we found that *TERT* C228T promoter mutation was most common in adult granulosa cell tumors among ovarian carcinomas and sex cord-stromal tumors. Our data confirm the activation of telomerase in adult granulosa cell tumors via *TERT* C228T promoter mutation, although alternative telomerase activation methods in adult granulosa cell tumors may exist. Our results suggest that *TERT* activation may play a role in adult granulosa cell tumor recurrence. As such, telomere biology may be important for the progression of adult granulosa cell tumors.

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Compilance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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