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Original Research

Telomerase reverse transcriptase (TERT) promoter mutation is a rare event in ovarian clear cell carcinomas in the Japanese population

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Summary

Background: Telomerase reverse transcriptase (TERT) plays an important role in cancer cells. Recently, mutations in the promoter of the TERT gene were identified in several types of cancers, and have also been reported as indicators of poor outcome in some of these patients. The majority of reported mutations were located at two hotspots, -124C>T and -146C>T. The aim of this study was to examine the incidence of *TERT* promoter mutations in ovarian clear cell carcinomas (OCCCs) in the Japanese population. *Materials and Methods:* The authors screened for TERT promoter mutations (focusing on the two hotspots) in 40 patients with OCCCs: 11 patients with endometrioid carcinoma, and 10 patients with high-grade serous carcinoma. They further measured the effect of TERT promoter mutations in an OCCC cell line (ES-2) using the luciferase reporter assay. *Results:* The incidence of hotspot mutations was 2.5% (1/40) in the patients with OCCCs, but was 0% (0/11 and 0/10) in patients with endometrioid carcinoma and high-grade serous carcinoma. The luciferase assay confirmed that the identified hotspot mutation is a gain-of-function mutations in OCCC cells. *Conclusion:* TERT promoter mutations increased the activity of OCCC cells. However, the frequency of these mutations in OCCCs appears to be very rare in the Japanese population. The present data indicate that TERT promoter mutations do not play an important role in OCCCs in the Japanese population.

Key words: Ovarian clear cell carcinoma; Telomerase reverse transcriptase (TERT) promoter.

Introduction

Ovarian carcinoma is classified into four molecularly distinct histological types: serous, mucinous, endometrioid, and clear cell. Ovarian clear cell carcinomas (OCCCs) account for less than 10% of epithelial ovarian cancer cases in Europe, but approximately 25% of these cancers are in Japan. OCCCs show high chemoresistance and are associated with a very poor prognosis [1]. Several mechanisms for the chemoresistance of OCCCs have been reported [2], but the key contributing molecule remains unclear. Recently, telomerase reverse transcriptase (TERT) promoter mutations have been identified in various kinds of cancers such as melanoma, thyroid cancer, and glioblastoma [3-5]. Furthermore, TERT promoter mutations were identified as a prognostic factor in glioblastoma [6]. Telomeres are linked to cell senescence and can determine the cell lifespan. In somatic cells, the telomere length reduces at each cell division, and a shortened telomere restricts cell proliferation [7, 8]. However, 85% of cancer cells can elongate their telomeres through increased telomerase activity to avoid cell senescence. The telomerase complex consists of several components [9]. The most important subunits are the TERT catalytic subunit (TERT) and telomerase RNA component (TERC). The human TERT gene is located on chromosome 5, and its core promoter is 181-bp upstream of the start site [10, 11].

Two hotspots of TERT promoter mutations have been identified: -149C>T and -124 C>T. Both mutations create the same binding motif, CCGGAA/T. GGAA/T is the E-26 (ETS) motif, and CCGGAA/T is the ternary complex factor (TCF) motif. Bell *et al.* [12] confirmed that some members of the ETS family, ELF1, ETS1, and ETV4, bind to the mutated TERT promoter.

There have been several reports of a potential association of TERT promoter mutations in gynecological cancer [13]. However, to the present authors knowledge, there has been no report of the association of TERT promoter mutations with gynecological cancer in the Japanese population. In the present study, they aimed to clarify the frequency of TERT promoter mutations and their clinical significance in OCCCs in the Japanese population.

Materials and Methods

Samples were obtained from the Department of Obstetrics and Gynecology at the Shimane University Hospital and the Department of Obstetrics and Gynecology at Seirei Hamamatsu General Hospital. Diagnosis was based on the conventional morphologic examination of sections stained with hematoxylin and eosin

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Table 1. — *Prevalence of TERT promoter mutation in patients with three pathological types of ovarian cancer.*

Pathological type	Patients	Number of TERT promoter mutation	Type of TERT promoter mutation
Clear cell carcinoma	40	1 (2.5%)	-124 CC>TT
Endometorioid carcinoma	11	0	
High grade serous carcinoma	12	0	

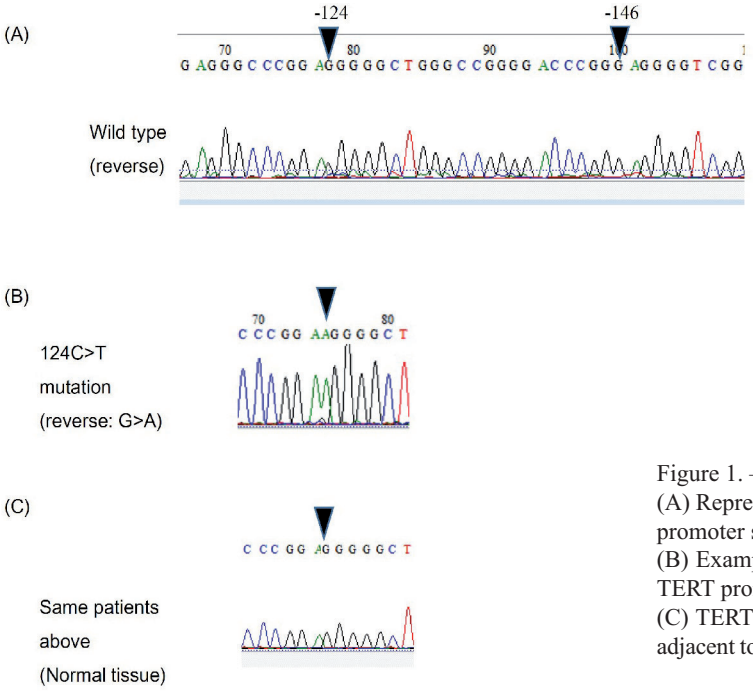

Figure 1. — TERT promoter mutations in Japanese OCCCs.
(A) Representative chromatograms showing the wild-type TERT promoter sequence.
(B) Example of an OCCC sequence showing one of the hotspot TERT promoter mutations.
(C) TERT promoter mutation did not occur in the normal tissue adjacent to the carcinoma portion with the mutant TERT promoter.

(H&E), and tumors were classified according to the World Health Organization (WHO) classification. Acquisition of tissue specimens and clinical information was approved by an institutional review board (Shimane University and Seirei Hamamatsu General Hospital) and written informed consent was obtained from all patients. The authors purified genomic DNA from 45 formalin-fixed and paraffin-embedded tissue samples and nine fresh tissue samples from patients with OCCCs. For comparison, they also purified genomic DNA from 21 fresh tissue samples collected from patients with endometrioid carcinoma, and from 15 fresh tissue sample from patients with high-grade serous carcinoma.

The TERT promoter region (targeting the two hotspot mutations) was amplified by nested-polymerase chain reaction (PCR). The primary primers were 5′-ACGAACGTGGCCAGCGGCAG-3′ and 5′-CTGGCGTCCCTGCACCCTGG-3′, and the nested-primers were 5′-M13 (GTAAAACGACGGCCAGT)-CAGCGCTGCCTGAAACTC-3′ and 5′-GTCCTGCCCCTTCACCTT-3′. The sequences were analyzed using the Lasergene program DNASTAR. PCR conditions and detail of sequencing methods were described in our previous report.

To determine the potential functional effect of TERT promoter mutations, the authors conducted a luciferase reporter assay in ES-2 cells, an OCCC cell line. ES2 (clear cell carcinoma) were obtained from the American Tissue Culture Center. The authors used the promoter reporter construct containing the core TERT promoter region, -181 bp (wild type), inserted upstream of the firefly luciferase reporter in the pGL3-basic vector. TERT promoter mutation constructs were generated using a mutagenesis basal kit. The authors used the pGL3-control luciferase reporter containing the SV40 promoter as a positive control. The wild-type and mutant constructs were transiently co-transfected into the ES-2 cell line with the pRL-TK *Renilla* luciferase reporter to normalize for transfection efficiency using lipofectamine 2000 reagent.

Luciferase outputs from both firefly and *Renilla* luciferase were measured step-wise by luminometry on a luminometer 48 hours post-transfection using a luciferase assay system.

Firefly luciferase values were normalized to the *Renilla* luciferase value for each data point, and then expressed as a percentage of the value obtained with the positive control vector (pGL3-control under the SV40 promoter).

Results

PCR and Sanger sequencing were successfully performed in the 40 OCCC, 11 endometrioid carcinoma, and 12 high-grade serous carcinoma samples (Table 1). A TERT promoter hotspot mutation (-124C>T) was detected in only one OCCC sample (Figure 1). The authors confirmed that this mutation is not a germline mutation by analyzing a normal tissue sample of this patient with a mutant TERT promoter in the carcinoma portion, which did not show the

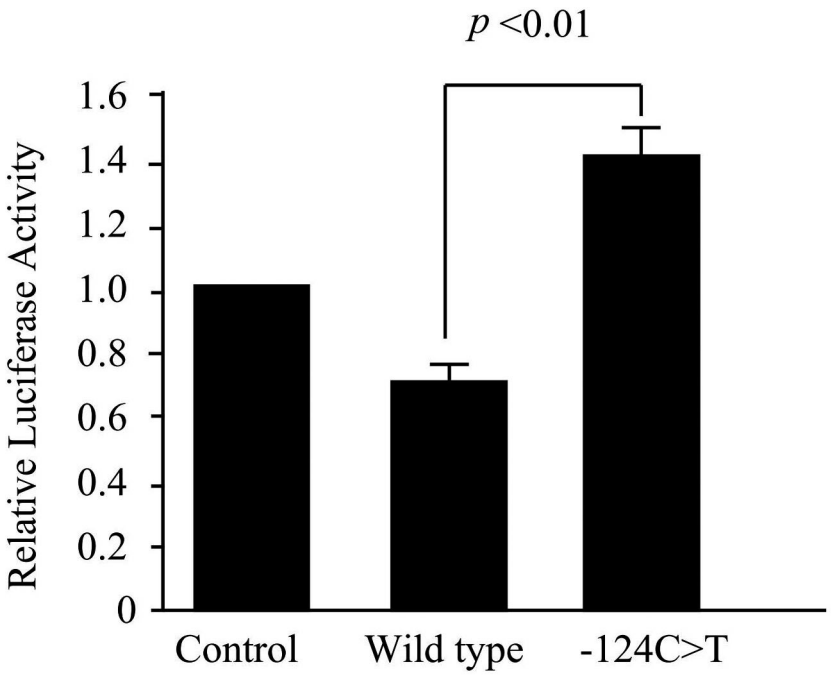

Figure 2. — Luciferase reporter assays in ES-2 OCCC cells. Cells with the -124C>T mutation in the TERT promoter showing increased transcriptional activity compared those with the wild-type TERT promoter.

mutation. No TERT promoter mutation was detected in any of the samples of endometrioid and high-grade serous ovarian carcinoma. Thus, the frequency of TERT promoter mutations was found to be 2.5% (1/40) in OCCCs and 0% (0/11 and 0/10) in endometrioid carcinoma and high-grade serous carcinoma. The authors also confirmed that the TERT promoter with the -124C>T mutation resulted in increased transcriptional activity compared to that of the wild-type TERT promoter in ES-2 OCCC cells using luciferase reporter assays (Figure 2).

Discussion

In this study, the authors found that TERT promoter mutations are very rare events in OCCCs in the Japanese population. Wu e*t al.* [13] reported that 16.5% of patients with OCCCs have TERT promoter mutations, although no significant difference in disease-specific overall survival was detected between the patients with and without TERT promoter mutations. In the current study, the prevalence of these mutations in OCCCs was only 2.5% (1/40). Taking the results of the current and previous studies together, the present authors hypothesize that this difference is prevalently due to a difference in the genetic background between the Japanese population and other ethnicities.

Approximately 85% of ovarian carcinomas are epithelial ovarian carcinomas. There are four major pathological types of epithelial carcinomas: serous, endometrioid, mucinous, and clear cell carcinoma. Among these histological types, unlike serous carcinoma, OCCC is associated with the development of chemoresistance and thus has a very poor prognosis [14]. OCCCs account for more than 20% of all epithelial ovarian carcinomas in Japan, but only account for 8–10% of such cases in the USA [14]. One of the possible reasons for the higher incidence of OCCCs in Japan is the much higher prevalence of endometriosis in Asia than in other regions [15]. Endometriosis is an estrogen-dependent disease characterized by an ectopic endometrial gland and endometrial stroma [16]. Endometriosis is considered to be a precursor lesion of OCCCs and endometrioid carcinomas, and is an important risk factor of these diseases. Even among Asian populations, the incidence of OCCCs is higher in Japan than in China [17].

Recently, Okamoto *et al.* [18] suggested that the copy number of potential oncogene *ZNF217* was greater in 62% of Japanese patients with OCCCs, which was a significantly higher frequency than that detected in patients in Korea (7%, $p = 0.001$) or Germany (25%, $p = 0.040$). Although the reasons for this difference among populations are not yet clear, this finding suggests a potential difference in the molecular carcinogenetic mechanism of OCCCs between patients from Japan and those of other areas.

There are some limitations of our study that should be noted. This study was underpowered for obtaining conclusive results of the association of the prognosis of OCCCs with TERT promoter mutations given the small sample size and low prevalence of mutations detected. Therefore, more cases should be analyzed to confirm this association. Indeed, the clinicopathological impact of TERT promoter mutations remains unclear. Wu *et al.* [12] reported that patients with the TERT promoter mutation did not have a sig-

nificantly different disease-specific overall survival as compared to those without the mutation. However, TERT promoter mutations have been reported as a poor prognostic factor in thyroid cancer [6, 19] . This difference may be due to organ-specific mechanisms of carcinogenesis and cancer progression. Based on the available evidence, the authors could hypothesize that TERT promoter mutations may not influence a signaling pathway involving the onocogenes of OCCCs, and therefore do not contribute to the prognosis of patients with OCCCs. In addition to its role in regulating telomeres, TERT is also involved in Wnt/β-catenin signaling [20], which up-regulates c-Myc and cyclin D expression to activate cell proliferation and carcinogenesis [21]. Therefore, to best understand a potential role of TERT in OCCCs, further detailed studies are needed to examine whether TERT promoter mutation upregulates these oncogenes of the Wnt/β-catenin signaling pathway in OCCC patient samples and/or cells.

In summary, the present preliminary analysis suggests that TERT promoter mutations may be very rare in Japanese patients with OCCC. Given the previous finding with the lack of a significant association between TERT promoter mutations and prognosis, the authors believe that the *TERT* promoter is not a relevant therapeutic target for OCCCs, at least not in the Japanese population.

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