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A novel BRCA2 mutation in prostate cancer sensitive to combined radiotherapy and androgen deprivation therapy

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ABSTRACT

Genetic factors contribute to more than 40% of prostate cancer risk, and mutations in *BRCA1* and *BRCA2* are well-established risk factors. By using target capture-based deep sequencing to identify potential pathogenic germline mutations, followed by Sanger sequencing to determine the loci of the mutations, we identified a novel pathogenic *BRCA2* mutation caused by a cytosine-to-guanine base substitution at position 4211, resulting in protein truncation (p.Ser1404Ter), which was confirmed by immunohistochemistry. Analysis of peripheral blood also identified benign polymorphisms in *BRCA2* (c.7397T>C, p.Val2466Ala) and *SRD5A2* (c.87G>C, p.Lys29Asn). Analysis of tumor tissues revealed seven somatic mutations in prostate tumor tissue and nine somatic mutations in esophageal squamous carcinoma tissue (single nucleotide polymorphisms, insertions, and deletions). Five-year follow-up results indicate that ADT combined with radiotherapy successfully treated the prostate cancer. To our knowledge, we are the first to report the germline *BRCA2* mutation c.4211C>G (p.Ser1404Ter) in prostate cancer. Combined ADT and radiotherapy may be effective in treating other patients with prostate cancer caused by this or similar mutations.

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BRCA2; prostate cancer; radiotherapy; androgen deprivation therapy

Introduction

Prostate cancer (PCa) is one of the most common cancers affecting men, especially in developed countries. For example, in the United States it is estimated that there will be 161,360 new PCa cases in 2017, and 26,730 men will die from PCa.[1] Other than advanced age, family history is the strongest risk factor for PCa,[2] with approximately 42% of the risk for this disease attributed to genetic factors.[3] Compared with PCa caused by somatic mutations, hereditary PCa has earlier onset and higher rates of metastasis and mortality.[4] Previous studies have identified more than 70 PCa susceptible loci, which account for approximately 30% of the familial PCa risk.[5] Mutations in the tumor suppressor genes *BRCA1* and *BRCA2* are well-known genetic risk factors for this cancer.

BRCA1 and *BRCA2* mutations are associated with an increased risk for many cancers including breast, ovarian, pancreatic, stomach, laryngeal, and fallopian tube cancer, as well as PCa.[6] The increased cancer risk in carriers of the *BRCA1*/*BRCA2* mutations is predominantly in breast cancer and ovarian cancer for women[7] and PCa for men.[8] Germline *BRCA2* and *BRCA1* mutations are present in 1.2% and 0.44% of PCa tumors, respectively.[9] Compared with the general population, the relative risk of PCa is 3.8 for carriers of *BRCA1* mutations up to 65 years of age[10] and 5 to 7 for carriers of *BRCA2* mutations.[11,12] Male *BRCA* mutation carriers with localized PCa are at substantially higher risk of dying from PCa than their non-mutation–carrying counterparts.[13] Moreover, *BRCA2* contributes to early onset, with 1.2% patients younger than 65 years old carrying germline *BRCA2* mutations.[9] *BRCA1/2* mutations are also associated with higher Gleason scores,[14] and germline *BRCA1*/2 mutations confer a more aggressive phenotype with a higher probability of nodal involvement, distant metastasis, and shorter survival.[15]

Tumors in *BRCA* mutation carriers that have defects in homologous recombination can be treated with radiotherapy, cisplatin, anthracyclines, or poly(ADP-ribose) polymerase inhibitors.[16,17] In addition, radical local therapy (e.g., radical prostatectomy or radiotherapy) can be effective when performed early for PCa with *BRCA2* mutations.[18] For metastatic castration-resistant PCa with biallelic inactivation of *BRCA2*, chemotherapy with platinum agents has been suggested.[19] In addition, Bryant et al.[16] reported that poly(ADP-ribose) polymerase inhibitors are efficacious in cancers with homologous recombination defects in tumors deficient in BRCA1 and BRCA2 but not in tumors with functional BRCA1 or BRCA2 proteins. However, optimal treatment strategies for specific mutations are unclear. Here we report a patient with locally advanced PCa carrying a novel germline *BRCA2* mutation. The patient was treated with androgen deprivation therapy (ADT) combined with radiotherapy, and serum

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prostate-specific antigen (PSA) levels were within the normal range for almost 4 years, even after stopping ADT.

Results

Case presentation

In May 2011, a 46-year-old Chinese man with dysuria lasting for 6 months was referred to Daping Hospital of the Third Military Medical University. Results of digital rectal examination revealed a hard and enlarged prostate with irregularities. Laboratory test results showed elevated serum levels of total PSA (56.39 ng/ml) and free PSA (10.30 ng/ml). Pelvic magnetic resonance imaging (MRI) scans showed lesions in the peripheral zone of the prostate with low-intensity signal (3.4 × 4.6 cm) and multiple enlarged pelvic lymph nodes with high-intensity signal (largest node, approximately 1.5 × 2.2 cm) on T2-weighted imaging (Fig. 1A). Ultrasound-guided transrectal needle biopsies obtained June 23, 2011 confirmed prostate adenocarcinoma (Gleason score 5 + 4). Immunohistochemistry results showed positive staining for Ki-67, p504S, and PSA, and negative results for p63 and 34βE2. Results of positron emission tomography (PET)-computed tomography (CT) showed a high level of asymmetrical ^{18}F fluorodeoxyglucose (FDG) uptake in the prostate along the left side, confirming metastasis in pelvic lymph nodes. The patient had no relevant family history, and his parents had died of unknown causes.

To treat this locally advanced PCa, ADT was initiated immediately with goserelin injections and oral bicalutamide, and testosterone and total/free PSA levels were monitored during and after treatment (Fig. 2). Serum PSA levels were maintained within the normal range for approximately 17 months, but total PSA (9.36 ng/ml) was found to be elevated on November 9, 2012. Results of MRI performed on November 26, 2012 (Fig. 1B) showed that the pelvic lymph nodes and tumor had shrunk (2.1 × 2.7 cm), presumably as a result of ADT. Local radiotherapy (66 Gy/30 F or 2.2 Gy/F) was carried out November 28, 2012. MRI results on January 4, 2013 showed the tumor had shrunk further (1.6 × 2.4 cm) (Fig. 1C). To treat lymph nodes, pelvic radiotherapy (13.2 Gy/6 F or 2.2 Gy/F) was continued until January 23, 2013. Because total and free PSA levels were still within the normal range, ADT was discontinued November 13, 2013, and on follow-up MRI on February 3, 2016, the prostate tumor was barely detectable (Fig. 1D). The prostate tumor appeared to be under control for 4 years without further intervention (Fig. 1E). In addition, serum testosterone level had returned to normal, and the patient's libido and sexual activity recovered completely.

In May 2015, the patient was again referred to our hospital because of dysphagia lasting for a month. Gastroscopy revealed an esophageal mass (29 × 34 cm) far from the incisors, and biopsy results suggested esophageal squamous carcinoma. PET-CT images showed a high level of asymmetrical FDG uptake in the

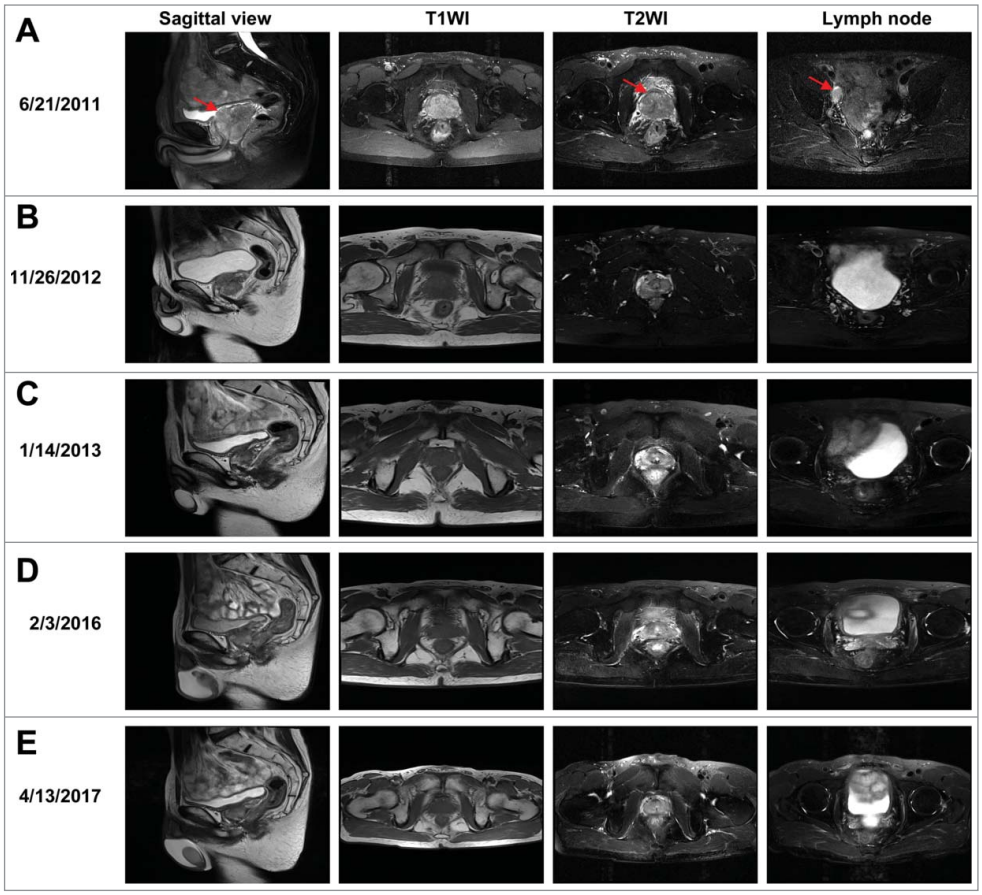

Figure 1. MRI scans of the patient before and after treatment for prostate cancer. (A–E) T1-weighted image (T1WI) and T2-weighted image (T2WI), as well as the sagittal view and lymph node images from T2WI from June 21, 2011 to April 13, 2017. Red arrows indicate the tumor in the prostate and lymph node metastasis.

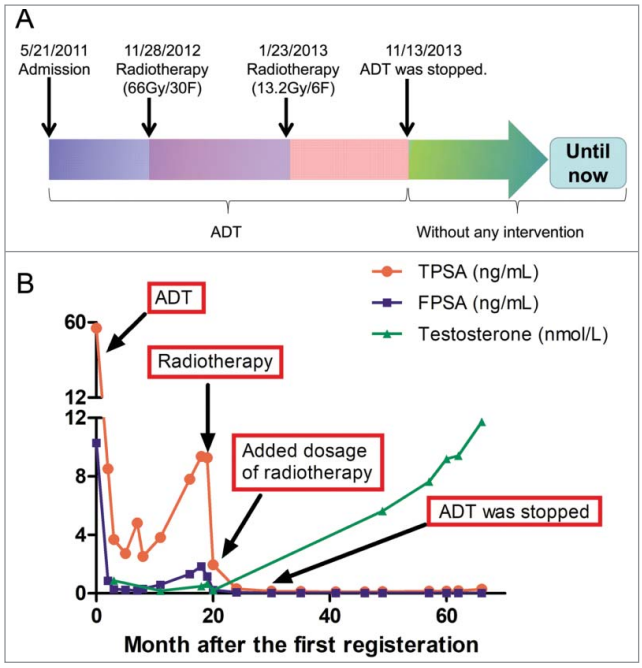

Figure 2. Treatment regimen and laboratory test results during and after treatment for prostate cancer. (A) Therapeutic schedule. (B) Serum levels of TPSA, FPSA, and testosterone during and after treatment. FPSA, free prostate-specific antigen; TPSA, total prostate-specific antigen. ADT: Androgen deprivation therapy.

esophagus only. On May 11, 2015, the patient underwent esophageal resection and thoracoscopic lymph node resection.

Identification of pathogenic mutation

To identify potential pathogenic mutations, we screened a panel of seven genes (*BRCA2, CHEK2, ELAC2, HSD17B3, HSD3B2, RNASEL*, and *SRD5A2*) using the patient's peripheral blood and identified a novel germline *BRCA2* mutation: Ser1404Ter caused by a C>G point mutation at position 4211 (Fig. 3A). We also identified two germline mutations in *BRCA2* (c.7397T>C, p.Val2466Ala) and *SRD5A2* (c.87G>C, p.Lys29Asn) that resulted in benign polymorphisms (data not shown). In addition, somatic mutations were identified by whole exome sequencing. The following seven somatic single nucleotide polymorphism/indel mutations were identified in PCa tumor tissue: *TP53* (c.1049T>C, p.L350P), *PIK3CB* (c.2527G>C, p.A843P), *MLL* (c.2806T>A, p.S936T), *PTCH1* (c.2075>A, p.V692E), and *TERT* (c.-58-u5148C>A; c.-58-u3620G>A; c.-58-u1324T>C). The following nine somatic single nucleotide polymorphism/indel mutations were identified in esophageal squamous carcinoma tissue: *TP53* (c.743G>A, p.R248Q; c.713G>C, p.C238S), *PIK3CA* (c.1636C>A, p.Q546K), *PTPRD* (c.5083G>A, p.E1695K), *MLL3* (c.4093-2A>G; c.10249C>A, p.Q3417K), *OR4C6* (c.541C>A, p.Q181K), *MSH6* (c.124C>G, p.P42A), and *TMPRSS2* (c.589G>A, p.V197M).

Expression and subcellular location of the truncated BRCA2 protein

To determine whether the patient's *BRCA2* mutation resulted in a truncated protein, we analyzed prostate and esophageal tumor tissues by immunohistochemistry. Using the BRCA2 C-terminal antibody, fewer BRCA2-positive cells were detected in the patient's prostate and esophagus tumor tissues compared with control tissues (Fig. 4A). However, using the BRCA2 N-terminal antibody, the number of BRCA2-positive cells in the tumor tissues was comparable between the patient and control, confirming the presence of a truncated protein (Fig. 3B). Levels of full-length BRCA2 in the patient's tumor tissues were considerably lower than those of control tissues, presumably due to the heterozygosity of the *BRCA2* mutation expressing the truncated protein. Truncated BRCA2 protein was detected primarily in the cytoplasm, whereas full-length BRCA2 protein was detected primarily in the nucleus (Fig. 4A). Furthermore, by using western blot, we identified the truncated BRCA2 protein at around 170 kDa (Fig. 4B).

Discussion

To the best of our knowledge, this is the first description of PCa caused by a germline *BRCA2* mutation in a Chinese patient,

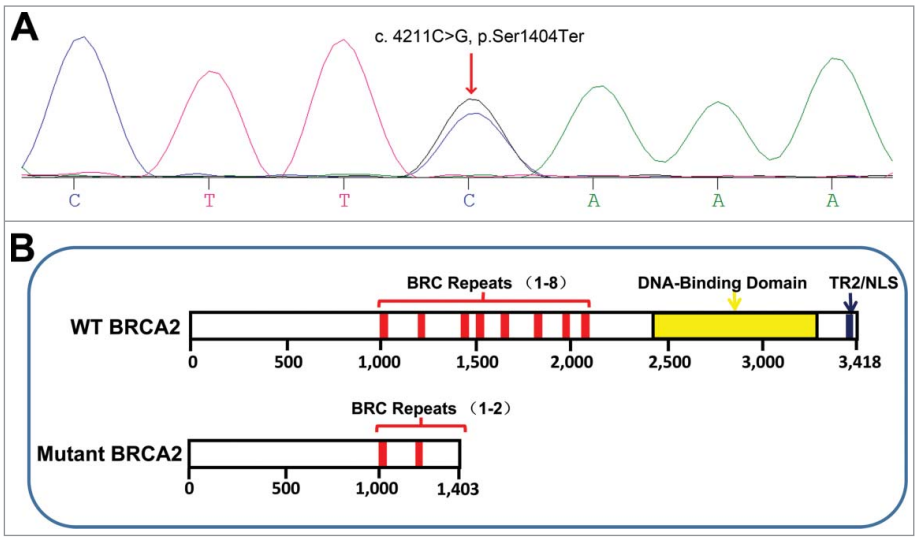

Figure 3. (A) *BRCA2* mutation in patient with prostate cancer and esophageal squamous carcinoma. (B) Diagram of wild type (WT) (ref. 37) and mutant BRCA2 proteins, as predicted from cDNA and genomic sequencing. NLS, nuclear localization signal; TR2, RAD51-binding domain.

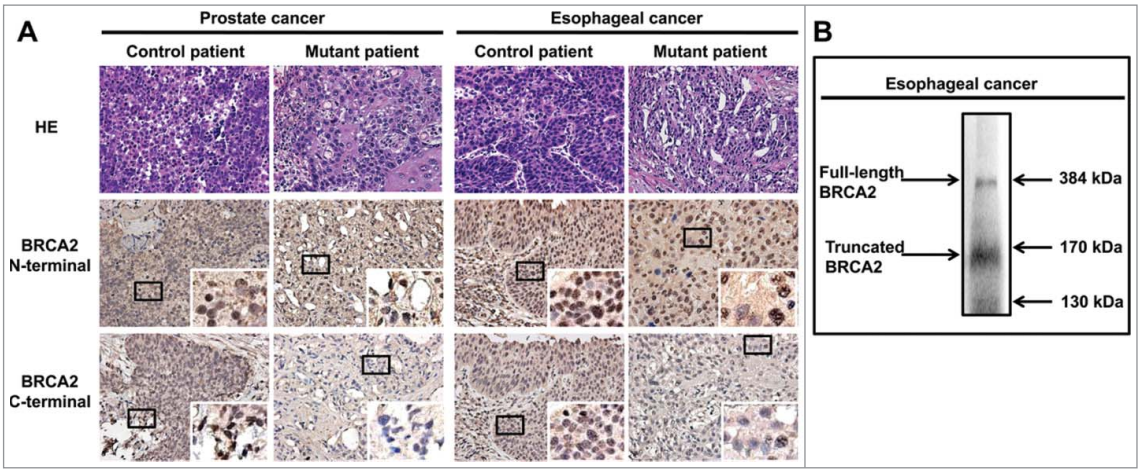

Figure 4. Immunohistochemistry and western blot analysis of tumor tissues. (A) The patient's prostate and esophageal tumor tissues were stained with antibodies against BRCA (C-terminus and N-terminus) and compared with the corresponding control tumor tissues. HE, hematoxylin and eosin. (B) The patient's esophageal tumor tissues kept in liquid nitrogen were collected, lysed and then analyzed with western blot assays with specific antibodies against BRCA2 N-terminus.

and the first report of the *BRCA* mutation c.4211C>G, which results in a truncated protein (p.Ser1404Ter). We also demonstrated that PCa associated with this mutation is sensitive to ADT combined with radiotherapy.

BRCA2 encodes a 3418-amino acid protein containing eight BRC repeats, a DNA-binding domain, and a nuclear localization signal.[20] As a component of the double-strand break (DSB) repair machinery, BRCA2 interacts with RAD51 through the BRC repeats and the RAD51-binding domain at its C-terminus (residues 3196–3232).[21,22] The mutation identified in this report introduces a premature stop codon, resulting in a truncated 1403-amino acid protein (Fig. 3B). Analysis of previously reported *BRCA2* mutations suggests that truncation producing a protein smaller than 3308 amino acids severely affects protein function.[23] The truncated BRAC2 identified in our patient contains only two of the eight BRC repeats and lacks the essential C-terminal domain. Loss of the nuclear localization signal (Fig. 3B) may account for cytoplasmic localization of the truncated BRAC2. Based on these observations, we conclude that this mutation leads to loss of function.

Table 1. The levels of TPSA, FPSA and testosterone of the patient during the treatment.

Time	TPSA (ng/ml)	FPSA (ng/ml)	Testosterone (nmol/L)
2011/5/21	56.39	10.3	
2011/7/27	8.51	0.88	
2011/8/31	3.69	0.28	
2011/9/1			0.868
2011/10/26	2.72	0.25	
2011/12/14	4.81	0.19	
2012/1/18	2.52	0.31	
2012/4/18	3.83	0.6	0.174
2012/9/16	7.81	1.32	
2012/11/9	9.36	1.84	0.486
2012/11/26	9.28	1.17	0.66
2013/1/13	1.96	0.24	0.208
2013/5/2	0.31	0.07	
2013/11/13	0.15	0.03	
2014/4/23	0.13	0.03	
2014/11/12	0.1	0.02	
2015/3/25	0.1	0.02	
2015/6/10	0.11	0.03	5.625
2016/2/3	0.14	0.02	7.639
2016/5/18	0.14	0.02	9.201
2016/7/20	0.17	0.02	9.41
2016/11/9	0.29	0.02	11.736

Previous studies have described *BRCA2* mutations associated with esophagus cancer, including the mutations c.203G>A,[24] c.10462A>G (p.Ile3412Val), c.8415G>T (p.Lys2729Asn),[25] and c.10204A>T (p.Lys3326Ter).[26,27] The mutation identified in this case (C.4211C>G, p.Ser1404Ter) also appears increase the risk of esophageal cancer, suggesting that special attention should be paid to patients with germline mutations of *BRCA2* during examination of the upper aero-digestive tract. This mutation may also increase the risk of other cancers, given the important role of BRCA2 in DNA repair.

According to the two-hit hypothesis,[28] multiple mutations are necessary to cause cancer. Thus, accumulation of acquired and uncorrected somatic mutations is expected in individuals with germline *BRCA2* mutations. Indeed, whole exome sequencing revealed multiple somatic mutations in our patient's prostate and esophageal tumor tissues. Misrepair or inefficient repair of DSBs can lead to genetic instability and ultimately carcinogenesis.[29,30] Indeed, BRCA2 has been identified as one of the most common mutations among the 63 pathogenic germline mutations (PPGMs) in cancers.[31] In our patient, haploinsufficiency of BRCA2 and inefficient DSB repair is likely to be the pathologic mechanism underlying the development of cancer. Consistent with the finding that most cancer-causing somatic mutations are associated with chromatin remodeling and DNA repair,[32] we found that both tumors in our patient had mutations in the tumor protein p53 (TP53), phosphatidylinositol-4,5-bisphosphate 3-kinase (PIK3C), and mixed lineage leukemia (MLL) pathways. However, we do not have direct evidence linking these somatic mutations to the BRCA2 truncation.

Most PCa patients carrying *BRCA2* mutations are treated with radical local therapy with or without adjuvant ADT.[32] Here we assessed the effect of initial ADT and subsequent radiotherapy on PCa associated with a *BRCA2* mutation. The initial ADT appeared to be effective, as evidenced by decreased PSA level and reduced tumor size,

suggesting an ADT-sensitive tumor. However, the tumor become ADT-resistant, as evidenced by an increase in total PSA level (9.36 ng/ml); therefore, radiotherapy was administered.

Radiation therapy, which is used to treat many solid tumors, directly induces DSBs and indirectly induces other types of DNA damage, in part by producing reactive oxygen species. Signals from the damaged DNA trigger cell cycle arrest[33] and activate the DNA repair machinery. Although unrepaired DNA damage in normal cells can lead to tumorigenesis, irreparable DNA damage in cancer cells leads to apoptosis.[34] Because of its role in DNA repair, BRCA2 plays pivotal roles in both tumorigenesis and radiotherapy. BRCA2 haploinsufficiency increases sensitivity to DNA-damaging agents, as evidenced by multiple somatic mutations in the patient's prostate and esophagus tumors. On the other hand, cancer cells expressing truncated BRCA2 are expected to be more responsive to radiotherapy. Polymorphisms in genes such as *XRCC3* and *RAD51* are also associated with radiosensitivity,[29] but whether the *SRD5A2* mutation (c.87G>C, p.Lys29Asn) identified in our patient plays a role in radiosensitivity is unclear. Nevertheless, the treatment outcome of our patient indicates that ADT combined with radiotherapy was effective for PCa caused by this *BRCA2* germline mutation.

In conclusion, we believe we are the first to describe this germline mutation of *BRCA2* (c.4211C>G, p.Ser1404Ter) in a patient with PCa, which was effectively treated with ADT and radiotherapy.

Materials and methods

All procedures involving human participants were carried out in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Daping Hospital of Third Military Medical University waived institutional review board approval for the study; however, written informed consent for the use of medical records and related images was obtained from the patient.

Identification of patient mutations

Germline DNA was extracted from the patient's leukocytes, and tumor DNA was extracted from PCa and esophageal squamous carcinoma tissues using the QIAamp DNA Micro Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. In addition, total DNA purified from the patient's peripheral blood was analyzed by target capture-based deep sequencing (BGI Health, China) to identify potential mutations in the following genes: *BRCA2, CHEK2, ELAC2, HSD17B3, HSD3B2, RNASEL*, and *SRD5A2*. The potential mutations were analyzed by Sanger sequencing to determine the loci and then compared with reference sequences in GenBank (http://www.ncbi.nlm.nih.gov/genbank/) using Mutation Surveyor version 2.51 (SoftGenetics LLC). Whole exome sequencing of the tumor DNA was performed by Geneplus-Beijing Institute (Beijing, China).

Immunohistochemistry

Tissue biopsies (greatest dimension, 1.5 mm) were taken from clinical specimens of the patient and two controls lacking the identified mutation (another patient with PCa and a patient with esophagus cancer). As previously described,[35,36] the paraffin-embedded tissues were sectioned (4 mm thick), mounted on glass slides, and baked at 60°C for 6 h. After deparaffinization with xylene, the sections were rehydrated in graded ethanol and 3% hydrogen peroxide to block endogenous peroxidase activity. Goat serum (ZSGB-BIO, China) was used to block nonspecific interactions before incubating the sections at 4°C with specific primary antibodies against the BRCA2 C-terminus (amino acids 2587–2601, Abcam ab53887, Cambridge, MA, USA) and BRCA2 N-terminus (amino acids 100–150 amino acids, Proteintech 19791-1-ap, Rosemont, IL, USA). After incubation with secondary antibody conjugated to streptavidin-biotin-horseradish peroxidase complex, the tissue sections were counterstained with hematoxylin and eosin, dehydrated, and covered with coverslips. Images of the slides were obtained with an Olympus CCD camera (Tokyo, Japan) connected to a Nikon Eclipse Ti-S inverted microscope (Tokyo, Japan) and captured with NIS-Elements F3.2 imaging software. The images were assessed by two urological pathologists.

Western blot

The esophageal squamous carcinoma tissues of the patient kept in liquid nitrogen were collected and lysed in a RIPA buffer containing a protease inhibitor cocktail tablet and phosphotase inhibitor cocktail (KeyGEN BioTECH, NanJing, China). 80 ug of tissue protein were separated by SDS-PAGE and transferred onto PVDF membrane (PALL, NY). After 1 h of incubation in blocking solution (5% BSA in PBS), primary antibody: BRCA2 N-terminus (amino acids 100–150 amino acids, Proteintech 19791-1-ap, Rosemont, IL, USA) was used to dected the truncated protein.

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Disclosure of potential conflicts of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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