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Absence of loss of heterozygosity of *BRCA1* in a renal tumor from a *BRCA1* germline mutation carrier

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Abstract

BRCA1 functions as a tumor suppressor gene and germline and somatic mutations in this gene have been shown to be associated with many types of cancer. We report the first tumor study of renal cell carcinoma in a carrier of the deleterious *BRCA1* mutation-c.68_69delAG.

Introduction

Mutations in *BRCA1* are associated with 60-90% lifetime risk of developing breast cancer and 50% for ovarian cancer [1]. *BRCA1* is a tumor suppressor [2], and is moderately expressed in kidney tissue and in renal cell carcinoma [3]. Thus, it is plausible that germline *BRCA1* mutations could contribute to the development of renal cell carcinoma. To investigate this hypothesis, we examined the renal tumor removed from a patient who carried a deleterious *BRCA1* germline mutation.

Case identification and history

To identify germline *BRCA1* and *BRCA2* mutation carriers, who had been diagnosed with renal cancer, we reviewed the charts of 1260 *BRCA1* or *BRCA2* mutation-positive patients counselled by the Clinical Genetics Service at Memorial Sloan-Kettering Cancer Center (43% of these are of Ashkenazi Jewish Ancestry). Of this cohort, only three patients had a history of renal cancer (not significantly different from number of patients with kidney cancer in non-carriers in our *BRCA1/2* tested cohort, $P=0.1$). All three patients were females, of Ashkenazi Jewish descent, and carried the *BRCA1* Ashkenazi Jewish founder

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mutation, c.68_89delAG. Each had been diagnosed with unilateral kidney cancer at 36, 57, and 59 years of age, respectively. Clinical data and tumor tissue were available for one patient (59 years old at diagnosis) who presented with right flank pain of one month duration, and was then found to have a 6.3 cm right renal mass on magnetic resonance imaging done for the back pain work up that was suspicious for renal cell carcinoma. She also had multiple pulmonary nodules that were suspicious of metastasis. Nephrectomy was performed and pathologic examination confirmed the diagnosis of renal cell carcinoma - clear cell type (nuclear grad III/IV). Given her cancer diagnosis and family history of breast cancer related to *BRCA1* mutations (c.68_69delAG), she was tested and was found to be carrier of c.68_69delAG as well. Her family history was significant for breast cancer, but not for kidney cancer.

Experiment and results

To investigate the potential mechanisms of tumorigenesis, we analyzed the renal tumor from the case of interest for loss of heterozygosity (LOH) for the *BRCA1* wild-type allele and looked for altered *BRCA1* protein expression. Frozen tumour tissue was obtained through IRB-approved protocols. A frozen section of the sample was cut, and stained with hematoxylin-eosin. A pathologist experienced in the evaluation of urologic tumors examined the section and mapped areas containing >90% viable tumor cells. These areas were dissected from the frozen tissue sample, and DNA was extracted from this tissue using DNAeasy Blood and Tissue kit (cat# 69504. QIAGEN Inc., Valencia CA). Sanger sequencing of the *BRCA1* c.68_69delAG mutation was performed to test for LOH, and Immunohistochemistry (IHC) was performed to test for BRCA1 protein, using a BRCA1 antibody (clone MS110, Cat # OP92. EMD Millipore Corp., Billerica MA), expression. The results of the Sanger sequencing negated our hypothesis of LOH of *BRCA1* in the tumor tissue as the tumor tissue was heterozygous for mutant and wild type allele (Fig. 1A). IHC showed expression of *BRCA1* in the tissue decreasing the likelihood that other mechanisms of loss of expression of *BRCA1* could be involved in tumor development in our patient (Fig. 1B).

Discussion

Kidney cancer has well known hereditary components and is characterized by inactivation of the *VHL* gene (encoding the von Hippel-Lindau protein). *VHL* is located on chromosome 3p25, and is a two-hit tumor suppressor gene. In clear cell kidney cancer, one allele of *VHL* is typically inactivated through a point mutation (or indel), and the other is inactivated through a large deletion[4]. Also, it was recently reported that LOH in *BAP1*, another tumor suppressor gene, characterized a new subtype of clear cell tumors and was associated with high grade tumors [5]. *BRCA1* is a tumor suppressor gene that was associated with multiple malignancies including breast, ovarian, and prostate tumors [1, 6]. Using the model of *VHL* and *BAP1*, it is possible that *BRCA1* mutations could contribute to the development of kidney tumors, or be associated with adverse pathologic characteristics. There are two previous reports of germline mutations of *BRCA1* in kidney cancer patients, but neither reported testing of tumor tissue [7, 8]. In fact, in the study by Thompson et al, the hazard ratio estimates predicted a tendency towards protective effect of *BRCA1* mutations against

kidney cancer (Confidence interval included 1). However, The comparison group in that study was the expected number of kidney cancer cases from large cancer registries and the hazard ratio estimates may have changed if the comparison group was test confirmed *BRCA1* non-carriers [8]. Also, previous literature indicated an absence of LOH of *BRCA1* in kidney cancer cell lines[9], but to our knowledge, this is the first report on the status of *BRCA1* mutations in a patient's renal tumor tissue. We did not find LOH of *BRCA1* suggesting that aberrations of *BRCA1* may not be causally associated with renal tumors. This finding is consistent with the lack of reported mutations in the COSMIC mutation database. The lack of somatic evidence suggestive of a germline mechanism of tumor susceptibility could also be explained by the small sample size and the dominance of sporadic renal tumors, the other types of possible “second-hits” in *BRCA1* and their different biologic effects, and intratumor heterogeneity. Finally, these finding have therapeutic implications. *BRCA1* is necessary for the homologous repair pathway that corrects DNA breaks encountered during cell replication [10]. Cells deficient in *BRCA1* were shown to be highly sensitive to *PARP1* inhibition. *PARP1* functions in the same repair pathway as *BRCA1*, and inhibition of *PARP1* leads to accumulation of DNA breaks and apoptosis[11]. Although such agents could be potentially used in treating other cancers in patients with *BRCA1* mutations, these findings suggest that these targeted therapeutic strategies may not be relevant for kidney cancer.

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Figure 1.

A) Histologic features of renal tumor - typical appearances of clear cell renal cell carcinoma, characterized by nests of epithelial cells with clear cytoplasm separated by a delicate vascular network (hematoxylin and eosin, $\times 100$). Inset - immunohistochemistry for BRCA1 shows nuclear staining in tumor cells ($\times 100$). B) 1- Results of Sanger sequencing of the kidney cancer tissue from BRCA1 mutation carrier - electropherogram shows persistence of frameshift indicating no loss of the wild type allele. 2- Results of Sanger sequencing of germline DNA from the same BRCA1 mutation carrier - electropherogram confirming the presence of *BRCA1 c.68-69delAG*