ORIGINAL ARTICLES

Methylation: A second hit in the two-hit hypothesis

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Summary

This case illustrates that methylation of one BRCA1 allele may serve as the second hit in a patient with a diploid locus and missense mutation on the other allele.

Key words: BRCA1; Methylation; Mutation; DNA silencing.

Introduction

Knudson first described his two-hit hypothesis of tumorigenesis in 1971 [1]. In his original model, each allele would have to be affected for a tumor to evolve. Most commonly, the two hits, at least in BRCA1, involve loss of heterozygosity (LOH) and a somatic mutation of the other allele [2]. Lallas *et al.* demonstrated the value of single strand conformation polymorphism analysis (SSCP) in screening for BRCA1 mutations in ovarian cancer patients [3, 4]. More recently, we have previously described the importance of methylation in the silencing of BRCA1 when coupled with LOH[2].

Case Report

The patient involved had a Stage IA clear cell carcinoma of the ovary. Her tumor was studied for the presence of a BRCA1 mutation. First, three intragenic markers (D17S855, D17S1322, D17S1323) were utilized and demonstrated no loss of heterozygosity (LOH) in the tumor at these BRCA1 loci. Using SSCP as previously described, the patient's ovarian carcinoma was examined for the presence of a BRCA1 mutation [3]. SSCP revealed an abnormal migration in an early fragment of exon 11. A protein truncation test (PTT) did not reveal the presence of a nonsense or frameshift mutation [5].

cDNA-based sequencing (directed by SSCP) revealed the presence of a missense mutation (E565D). A DNA-based confirmation reaction confirmed the mutation but also demonstrated the heterozygosity of this locus with strong G and T bands being present (i.e., a mutated strand and a non-mutated strand). Blood-based sequencing did not reveal the mutation to be present in the germline.

Methylation specific PCR (MSPCR) was undertaken to determine whether silencing of the BRCA1 promoter was present in one of the alleles accounting for the difference in DNA and cDNA sequence results [2]. MSPCR demonstrated that one allele was silenced.

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Discussion

We have described a tumor in which Knudson's two-hit hypothesis was fulfilled in a novel way. Since cDNA sequencing revealed the presence of a missense mutation, but DNA sequencing revealed a heterozygous locus, we hypothesized that the other allele must have been silenced by some genetic or epigenetic phenomenon [2, 6, 7]. Performance of MSPCR revealed the hypothesis to be correct, the silencing of the second allele was caused by epigenetic methylation. Thus, although LOH and a mutation, often somatic, are the usual causes leading to a tumor phenotype, it is possible in BRCA1 for the two hits to include a somatic mutation in one allele and DNA silencing by methylation in the other allele.

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