Exon 8-9 Mutations of DNA Polymerase β in Ovarian Carcinomas in Haldia, India

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Abstract

Background: Ovarian cancer is the number one killer among all the gynecological cancers. We undertook association study to identify potential alterations in the genomic DNA of a DNA repair gene, DNA polymerase beta (polβ), involved in base excision repair (BER), in ovarian carcinomas of patients from Haldia, India. Mutations, splice variants have been reported earlier in different tumors other than ovarian tumors. Aim: In this study we explored the possibility of association of any mutation of polβ (Exon 8) with prognosis in 152 ovarian cancer samples. Results: Alteration in the exon 8 region (Exon 8:468, A→C; 15.1%) was noted among fifty seven polymorphism positive samples. Alteration in the intervening sequence 8 (IVS8, -25, A→C; 3.9%) was also noted. All alterations are heterozygous in nature. Conclusion: We found no significant association among the samples from serous type, stage IV, and the polβ mutations (P>0.01). Only a slight tendency of association was evident between IVS8, -25, A to C; and stage III. Further analysis with a larger number of samples is needed.

Keywords: DNA polymerase beta - DNA repair - somatic mutation - polymorphism - DNA-SSCP - Haldia - India

Introduction

It is estimated that 22,280 women will be diagnosed with ovarian cancer in 2012 out of which 15,500 patients may decease (ACS, 2012). Mortality due to ovarian cancer this year may exceed 69% in women. A number of diagnostic tools are available now such as the serum CA-125 level, ploidy level, ultrasound etc (Canevari et al., 2006). The present study was undertaken to identify potential association of alterations in the genomic DNA of DNA polymerase beta (polβ), with ovarian carcinoma. Polβ is an essential enzyme for gap filling synthesis in short-patch and long-patch base excision repair pathway (Frosina et al., 1996; Wood, 1996; Klungland and Lindahl, 1997). Polβ is located at 8p12, a region which is frequently lost in colorectal, prostrate, stomach, breast, lung, kidney, and bladder carcinoma (Emi et al., 1992; Lundgren et al., 1992; Yaremko et al., 1995; Muleris et al., 1986; Ochi et al., 1986; Kovacs et al., 1987; Knowles et al., 1993; Starcevic et al., 2004). So far, 189 tumor samples along with 124 normal samples have been studied for the possible mutation within polβ gene and thirty five percent of this tumor samples have been identified with polβ mutations (Starcevic et al., 2004). None of these mutations were noticed in normal samples, thereby indicating a relationship between cancer and the polβ mutation (Starcevic et al., 2004).

Recently, two laboratories have studied 286 human colon and ovarian cancer samples and found more than 56% samples showed mutation within polβ gene (Katherine et al., 2012, Khanra et al., 2012a, b). In addition, an 87-bp deletion has been found in primary colorectal, lung, and breast cancer (Bhattacharyya and Banerjee, 1997; Bhattacharyya et al., 1999a; 1999b; Bhattacharyya and Banerjee, 2001), is a dominant negative mutant and inhibits the function of WT protein in human cell line. Therefore, there may be a chance that other mutant(s), if found in the tumor samples of ovarian tumors, may have a similar biological significance. Thus, in this report, our goal is to determine the association between the polβ mutation and ovarian carcinoma.

Materials and Methods

Ovarian carcinomas

One hundred seventy five tumor samples were collected from Haldia Seba Sadan and different Hospitals or nursing homes at Haldia. The samples were snap frozen in liquid N2 immediately after collection and were given a number for identification and to protect the identity of the patients. Institutional Review Board approval for this study was received from the IRB of the Haldia Institute of Technology. A board-
DNA Isolation
DNA samples were isolated from tumors according to the standard protocol by phenol/chloroform preceding proteinase K treatment (Strauss, 2001). In brief, genomic DNA was isolated from samples powdered in liquid N2 and then digesting at 56°C for 10-12 hrs with DNA lysis buffer (100mM NaCl; 100 mM Tris-HCl pH 8.0; 20mM EDTA pH-8.0; 05% SDS; 0.1mg/ml proteinase K). The aqueous solutions were separated with Phenol/ Chloroform. The supernatant was added with ½ volume of 7.5 M ammonium acetate and 2 volume of chilled ethanol to precipitate the DNA. The purity and the quantity of the nucleic acids were measured by a spectrophotometer.

DNA PCR-SSCP- Sequencing
A sensitive “cold” PCR-SSCP was used to screen for mutations in DNA [19]. The sequences of forward and reverse primers used for the amplification of genomic DNA were FPE8&9: GCTGGTATGGCACGGACAA; RPE8&9: AACCAGAGTATGAAGTATG. The PCR reaction was performed in a volume of 20 μl containing 500 nM unlabeled primers and 2 units of pfu DNA polymerase (Fermentas). After an initial 3 min denaturation at 95°C, PCR was run for 30 cycles of 15 sec denaturation at 95°C followed by a 5 min annealing extension at 72°C. PCR products were denatured in 95% formamide containing 500 mM sodium acetate, ethanol and heated at 95°C for 10-12 hrs with DNA lysis buffer (100mM NaCl; 100 mM Tris-HCl pH 8.0; 20mM EDTA pH-8.0; 05% SDS; 0.1mg/ml proteinase K). The aqueous solutions were separated with Phenol/ Chloroform. The supernatant was added with ½ volume of 7.5 M ammonium acetate and 2 volume of chilled ethanol to precipitate the DNA. The purity and the quantity of the nucleic acids were measured by a spectrophotometer.

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the results are shown in Table 1. Association between the
Exon 8:468 and the different stages revealed that stages
can not be used as risk factor. Although in case of stage
IV, the odd ratio value is 2.1 (95% CI value ranges from
0.43 to 5.16) and the p value is 0.177 suggesting that
there is slight tendency of risk. On the other hand, the
association studies between Exon 8:468 and the types of
tissue indicate negative relation. The association study
between IVS8, -25 and the stages indicate that there is
a significant relationship between the stage III
samples and the mutation. In this case the likelihood
ratio is 2.82 and OR is 4.67 (95% CI value ranges from 0.8 to 26.98)
and the p value is 0.06. There is no relation between other
stages and the mutation. None of the types of tissues show
any relationship with the IVS8, -25 mutations.

The correlation study indicates that association
between types of tissues and the mutations are not
statistically significant. The correlation between stages
and the Exon8 and IVS 8 mutations is also statistically
significant. On the other hand there is a slight tendency
towards correlation between stage III and IVS8 mutation
where the Pearson correlation values is 0.24 with p value
of 0.06. Age is not associated significantly with the
mutation types.

Variant impact prediction, the mutation is predicted
to be benign with a score of 0.00 (sensitivity:1.00;
specificity:0.00)

Discussion

Five-year survival rate of the sporadic ovarian cancer
patient diagnosed at an advanced stage is estimated to be
less than 30% whereas for patients diagnosed with stage
I disease, the 5 year survival is reported to be in excess
of 90% (Jacobs et al., 2004). DNA polymerase beta is a
highly conserved DNA repair protein that is essential for
base excision repair (BER) function. Studies on human
subjects suggest that the frequently detected somatic polβ
mutation is linked to cancer (Starcevic et al., 2004).

A recent review showed that 84% of the ovarian
cancers having BRCA1 and BRCA2 mutations were
serous carcinomas (Henry et al., 2009). Similarly, in
Creighton registry, 25% of the serous carcinomas carried
mismatch repair gene mutation as compared to 84%
serous carcinomas contains BRCA1 and BRCA2 mutation
(Gieseking et al., 2011). In this study, we excluded those
samples having hereditary ovarian cancer history thereby
eliminating the involvement of the BRCA1/2 mutation.
Besides these clinico-pathological parameters, it has also
been noticed that all these patients resides in the urban
to village region. They never consumed alcohol, 90% of
the patients are non-vegetarian, and 10% of the patients
consumed some kind of tobacco product.

To detect polβ gene mutations in the ovarian cancer
patients, genomic DNA was used to amplify polβ
gene. Only exon 8 and 9 and their flanking sequences
were amplified by using pairs of primers. Twenty three
samples exhibited heterozygous alteration in Exon 8 at
nucleotide position 468 changing the amino acid from
leucine to phenylalanine. The impact of this mutation
using PolyPhen 2v2.2.2r398 suggests that this mutation
is benign which corroborates our statistical analysis data.
Although the PolyPhen prediction algorithms to assess the
effect of the mutation on protein function is not absolute as
shown by Katherine et al. 2012. Hence, missense mutation
in Exon 8 where A has been changed to C, may have effect
on the fidelity of the enzyme

In conclusion, our findings did not demonstrate any
significant association of the polymorphism of polβ in
Exon 8 and 9 region and ovarian cancer in Indian patients.
Although there is a slight tendency of association between
stage III and this IVS 8 mutation, further studies with
larger size of the samples are needed to assess and confirm
this finding.

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