RESEARCH ARTICLE

Exon 8-9 Mutations of DNA Polymerase β in Ovarian Carcinoma Patients from Haldia, India

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Abstract

    <u>Background</u>: 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. <u>Aim</u>: In this study we explored the possibility of association of any mutation of polβ (Exon 8) with prognosis in 152 ovarian cancer samples. <u>Results</u>: 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. <u>Conclusion</u>: 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 polb gene and thirty five percent of this tumor samples have been identified with polb 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. In addition, signed written consent was collected from each patient before collection of the samples. Institutional Review Board approval for this study was received from the IRB of the Haldia Institute of Technology. A board-

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certified pathologist (RS) reviewed H&E stained section from each block of ovarian tissue and graded the tumors according to WHO classification and nomenclature of ovarian neoplasm. Twenty three samples having family history of ovarian cancer were excluded from this study. We analyzed the remaining one hundred fifty two cases with the diagnosis of ovarian cancers including tumors of Grade I to Grade IV. The distant normal ovarian epithelial tissue from the same patient was used as normal.

DNA Isolation

DNAs 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: AACCCAAGATTAGGAAGTATG. 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℃, PCR was run for 30 cycles of 95℃ for 30 sec, the annealing temperature was 50°C and 72℃ for 30 ses followed by a 5 minute final extension at 72℃. PCR products were denatured in 95% formamide with 10 mM NaOH, and heated at 95℃ for 5 minutes and then cooled at ice and run in 14% non-denaturing polyacrylamide gel at 7.5W for 6 hrs using 1XTBE buffer at 4℃. Double stranded DNA is completely denatured to single stranded DNA by alkaline and heating condition, each single stranded DNA form secondary structure in rapid cooling. Single stranded DNA molecules fold into complex 3-D structure as a results intra-strand base pairing. So any changes of nucleotide bases results different conformation therefore vary in electrophoretic mobility. Patterns of resultant single stranded DNA mobility were visualized by staining with SYBR® Gold Nucleic Acid gel stain (Molecular Probes). Tumor sample PCR product showing extra one or more band than normal sample PCR product were purified and sequenced directly in sequencer.

Statistical analysis

Bivariate analysis using the different variables was performed with the aid of SPSS 10.0 (IBM-SPSS). Pearson's χ^2 test or Fisher's exact test was used for this purpose. Correlation analysis was performed to identify any predictors for association of polβ mutation with stage, type or age. Odd ratio (OR) and the 95% confidence interval (CIs) were also determined.

Variant impact prediction

The impact of any mutation where the amino acid has been changed was assessed using Polyphen (Adzhubei et al., 2010).

Results

Descriptive Data

The histopathology results show that 19 samples (12.5%) are of either Clear Cell Type, or Endometrioid type each. Whereas 96 samples (63.2%) were serous carcinomas. Eighteen samples (11.8%) were mucinous. It has also been found that 15 (9.9%), 44 (28.9%), 40 (26.3%), and 53 (34.9%) samples were from stage I, II, III, and IV respectively. Seventeen patients (11.2%), 48 patients (31.6%), forty eight patients (31.6%), thirty two patients (21.1%), and 7 patients (4.6%) belong to the age group between 21-30, 31-40, 41-50, 51-60, and 61-70 years of age respectively. The average age of the patients is 42.95 yrs with minimum age is 24 yrs and the maximum age is 69 yrs with standard deviation of 10.07.

Clinical features associated with polβ mutation

Somatic mutation in the Exons, a specific somatic mutation was detected in the exon region of polβ of ovarian carcinomas. Change of the nucleotide from A to C occurred in exon 8 at nucleotide position 468 of the polβ gene changing the amino acid leucine (TTA) to phenylalanine (TTC) of 23 patients (15.1%) showing in Figure 1a.

Somatic mutations in the intervening sequence, another alteration was also observed in the intron regions of the polβ gene of ovarian carcinomas. Heterozygous alteration was noticed in the IVS 8 at -25 where A has been changed to C (Figure 1b) in six samples (3.9%).

Association between clinical features and polβ mutations, Polβ mutations were found in 29 (19.7%) out of 152 samples. Association between clinical features and polβ mutations were studied using univariate analysis,
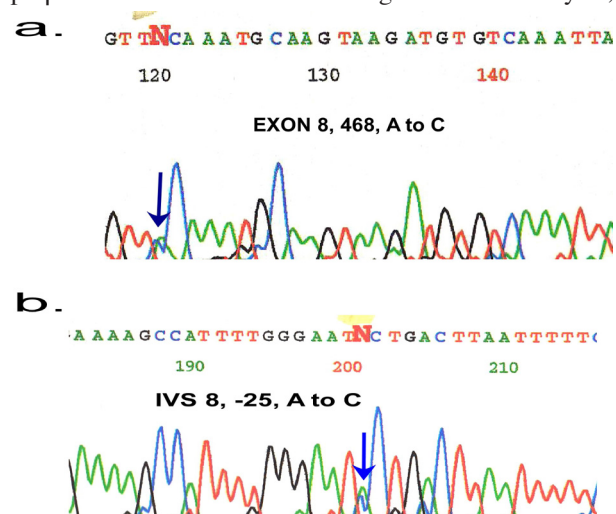
Figure 1. Two Types of Somatic Alterations Polb Gene within Exon 8 and 9 Region of Ovarian Carcinomas are being Shown. a. depicts a heterozygous mutation at exon 8 at nucleotide position 468 where A has been changed to C; b.

Table 1. Association of Polymerase Beta Variants with Either Stage III/IV or Serous Type of Tumor

Alterations of polß/stage or type	Fisher Exact Test (p) 2-sided /1-sided value	Pearson Chi-square value	p value	Likelihood ratio	OR 95% CI value (Lower-Higher)
Exon 8,468,A to C/serous	0.55/0.37	0.41	0.51	0.42	1.5	0.43- 5.16
Exon 8,468,A to C/Stage IV	0.27/0.14	1.82	0.17	1.84	2.1	0.70- 6.27
IVS8,-25,A to C/Stage III	0.10/0.10	3.38	0.06	2.82	4.67	0.80-26.98

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 2 v2.2.2r398 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.

Acknowledgements

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