CANCER GENOMICS & PROTEOMICS 7: 143-146 (2010)

Genomic Analysis of the 55 kDa Subunit of DNA Polymerase ε in Human Intracranial Neoplasms

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Abstract. *Background: Defects of some DNA polymerases have shown cancer associations, but there are only limited data on DNA polymerase (Pol) ε. Materials and Methods: We examined 26 human brain neoplasm DNA samples and 8 control blood samples (from Poland) for possible mutations in the entire coding region of the 55 kDa small subunit of human DNA Pol ε gene using polymerase chain reaction–single-strand conformation polymorphism (PCR-SSCP) analysis, and sequence analysis of DNA. Results: One single base intronic transition in intron 14 was found. The AATT deletion previously found in some breast and colorectal tumors was not found in samples from brain neoplasms or controls, but it was found in 1/100 normal blood samples from South-West Finland. Conclusion: We found no evidence that potential mutations in the 55 kDa subunit of DNA Pol ε are a contributing factor in the development of the tested cases of human intracranial tumors.*

DNA polymerase (Pol) ε has been implicated in numerous aspects of DNA metabolism, including replication, repair, recombination, and coordination of mitosis with the completion of S phase (1, 2). Human Pol ε, is composed of four subunits, a large catalytic subunit of 261 kDa and three associated subunits of 55, 17 and 12 kDa. The 55 kDa subunit located on chromosome 14 (2). An antibody that inhibits Pol ε activity reduced DNA synthesis when injected into human fibroblasts (3), and immunodepletion of Pol ε impaired the elongation step of DNA replication in *Xenopus* egg extracts (4). A mouse model with a proofreading-deficient variant of Pol ε exhibits increased mutation rate, and produces a unique spectrum of spontaneous cancers, mainly intestinal adenomas and adenocarcinomas, as well as histiocytic sarcomas (5). Like human Pol ε, DNA Pol ε from *Saccharomyces cerevisiae* is composed of four subunits: Pol2p, Dpb2p, Dpb3p, and Dpb4p (6, 7), and it participates in leading-strand DNA replication (8). Strains carrying a temperature-sensitive mutation in the *dpb2* gene (the gene of the 55 kDa subunit of Pol ε) suggest that *dpb2* is also essential for DNA replication (9, 10). At the restrictive temperature, dpb2 mutated cells carrying the mutated dpb2-1 variant are dumbbell shaped, which is characteristic of a DNA replication defect (9). dpb2p is phosphorylated by cdc28p, a cyclin-dependent protein kinase, in a cell-cycle-dependent manner (11). dpb2p contributes to the fidelity of DNA replication in yeast and dpb2 mutants impaired in the interaction with the catalytic subunit pol2p exhibit much higher mutation rates (9, 12).

As a step towards better understanding of the potential role of Pol ε in carcinogenesis, the gene coding for the exon regions of the 55 kDa subunit of human DNA Pol ε was screened for variations in brain cancer samples.

Materials and Methods

Studied samples. Twenty-six human brain tumor samples, and 8 control samples (normal blood DNA from the same patient), all collected at the Laboratory of Neurosurgical Pathology at the Department of Pathology of the Poznan University of Medical Sciences in Poland, one placenta control sample from the Turku University Central Hospital, and 100 population control samples from South-West Finland were used in this study. The histological diagnosis by a neuropathologist was available in all cases. The diagnosis was based on the revised WHO (World Health Organization) Classification of Tumours of the Nervous System (13).

Abbreviations: PCR-SSCP, polymerase chain reaction-single strand conformation polymorphism; Pol, polymerase, Pols polymerases.

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Key Words: Brain neoplasms, DNA polymerase epsilon (ε), genomic changes, metastasis, glioblastoma, astrocytoma, atypical meningioma.

Neoplastic DNA samples were available from the following: i) Ten metastatic neoplasms (7 adenocarcinomas (1 breast, 1 renal cell, 1 clear cell, 4 not otherwise specified (NOS) adenocarcinomas), 2 non-differentiated carcinomas, 1 large cell anaplastic carcinoma of the lung). There were 4 female and 6 male patients. Ages ranged from 51 to 70 years. All metastatic tumors were atypical, corresponding to histological grade III carcinomas. ii) Eight glioblastomas, grade IV. Ages ranged from 14 to 70 years. There were 7 female, and 1 male patients. iii) Five anaplastic astrocytomas, grade III. Ages ranged from 36 to 47 years. There were 1 female, and 4 male patients. iv) Three atypical meningiomas, grade II, all females, aged 22, 59 and 68 years. v) Eight blood control samples from 8 different patients listed among the former samples. These control samples could be compared with the neoplastic DNA samples from the same patients and as a group basicly presented samples of nonneoplastic DNA of the population treated at the Poznan University Hospital.

Neoplastic samples were collected from the surgical material remaining after frozen section diagnosis. The surgical material was first fresh by frozen at –130˚C and cryostat cut for diagnosis. The frozen section was stained with H&E. A part of the remaining material was fixed in 10% buffered formalin and embedded in paraffin after at least 1 day of fixation. DNA was isolated from the other part after thawing.

The researchers of the genomic features at no phase of the study had any information available on the identity of the persons whose DNA and/or neoplastic DNA was being studied. The DNA samples were collected and studied in agreement with the policies of the local Ethical Committees in Finland and Poland.

PCR amplification. The 19 sets of intronic primers listed in two earlier studies (14) were designed to amplify the entire coding region of the 55 kDa subunit of human Pol ε (15). The SP6 promoter sequence, GACACTATAGAATAC, and T7 promoter sequence, CGACTCACTATAGGG, were attached to the 5' end of each upstream and downstream primer, respectively. The 35 cycles of polymerase chain reaction (PCR), each consisting of denaturing for 1 min at 94˚C, except with exon 1, 11 and 12 at 95˚C, annealing at different temperatures (14) for 1 min, and extension for 1 min at 72˚C, were performed in a Perkin Elmer Cetus DNA Thermal Cycler 480 (Perkin Elmer, USA). The second pair of Cy^5-labelled primers (SP6 5': TTTAGGTGACACTATAGAATAC and T7 5': GTAATACGACTCACTATAGGG) were used for subsequent secondary PCR and to produce Cy^5-labelled PCR products. The 30 cycles included 30 s denaturation at 94˚C , except exon 1 at 96˚C, 30 s annealing at 55˚C, and 30 s extension at 72˚C. The PCR products were electrophoresed on 2.5% agarose gel.

Single-strand comformation polymorphism (SSCP) analysis. The Cy^5-labelled PCR products were mixed with an equal volume (4.5 μl) of denaturing solution containing 100% deionized formamide, and 0.05% bromophenol. The mixture was heated at 95˚C for 4 min, and thereafter chilled on ice. Two undenatured samples (one from tumors, one from controls, no heating at 95˚C) were added to each run. Subsequently 2.5 μl of the mixture was loaded on a 6% polyacrylamide gel (acrylamide: bisacrylamide, 99:1) in an ALFexpress II with Cool kit (Pharmacia Biotech, Sweden). The running conditions for the gel electrophoresis were 35 W for 10 h at 12˚C with external cooling. The SSCP data were collected by ALFwin software and analyzed by fragment analyser 1.02 (Pharmacia Biotech, Sweden).

Sequence analysis. Samples displaying variant bands on SSCP were additionally analyzed by sequencing. DNA samples were amplified by PCR as described above. The amplification products were purified with a GFX™ PCR DNA and Gel Band Purification Kit (Amersham Biosciences, Sweden). ABI PRISM BigDye® Terminator v3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, USA) was used for standard sequencing reactions and an ABI PRISM 3130xl® Genetic Analyzer (Applied Biosystems) was used for sequencing. The sequencing results were compared with chromosome 14 genome (Gene *POLE2* (ENSG00000100479) – Ensembl release 57), and the described changes refer to the *POLE2* coding strand, which is the reverse strand of the chromosome 14 assembly.

Results

Genomic DNA from 26 brain tumors and 9 control samples was screened by SSCP for mobility shifts in exons 1-19, and all abnormalities were sequenced. A polymorphic DNA alteration was detected in the samples by the primer set 14 (exon 14). Sequencing results showed this to be a G-A transition in intron 14 at position 14:50120858 of the current Ensembl release 57 (Table I), corresponding to the previously described variation rs3212323. Because of overlap, a corresponding change was also present when primer pair 15 was used. This change was present in 8/26 brain neoplasms, and in 1/8 controls. This change was present in 2/10 metastases, 3/8 of grade IV glioblastomas, 2/5 grade III astrocytomas, and 1/3 meningiomas. Glioblastomas and anaplastic astrocytomas combined (AA/GBM group) had this change in 5/13 cases.

In previous studies, we found an intronic AATT deletion (Ensembl 14:50110476-50110479) in breast and colorectal cancer (14, 16); this AATT deletion was also found in corresponding normal tissues in one case of colorectal cancer (at a distance of 2-5 cm from the tumor). Out of 100 normal DNA samples from South-West Finland, one sample contained the AATT deletion. In contrast, we were not able to find this AATT deletion in brain cancer samples, nor in control samples from Poland. In these controls, the G-A transition at position 14:50120858 was present in 6/15 cases.

Discussion

In this study, we tested for the presence mutations in the 55 kDa subunit of Pol ε in brain neoplasms. No new mutations or polymorphisms were found. The DNA G-A transition at position 14:50120858 seems to be a single nucleotide polymorphism (SNP) and was detected previously in breast cancer and colorectal cancer and control samples in the Finnish population (14, 16). This change does not seem to increase in frequency in intracranial neoplasms. This change is also listed as an SNP in the Ensembl database.

The SSCP methodology can detect 88-90% of mutations (17) and therefore we cannot fully exclude the presence of

Table I. *Nucleotide sequence variation found in the 55 kDa small subunit of Pol ε as compared with reference (Gene POLE2 ENSG00000100479 – Ensembl release 57) in 26 brain cancer samples (number 1-26) and 8 control samples from normal blood DNA from the same patients (numbers 27-34) and 1 control placental DNA (number 35).*

Sample number	Polymorphism
1	No change
2	No change
3	No change
4	G-A transition in intron 14, at position 14:50120858
5	No change
6	No change
7	No change
8	No change
9	No change
10	No change
11	No change
12	No change
13	G-A transition in intron 14, at position 14:50120858
14	No change
15	No change
16	No change
17	G-A transition in intron 14, at position 14:50120858
18	No change
19	G-A transition in intron 14, at position 14:50120858
20	No change
21	G-A transition in intron 14, at position 14:50120858
22	G-A transition in intron 14, at position 14:50120858
23	No change
24	G-A transition in intron 14, at position 14:50120858
25	G-A transition in intron 14, at position 14:50120858
26	No change
27	No change
28	G-A transition in intron 14, at position 14:50120858
29	No change
30	No change
31	No change
32	No change
33	No change
34	No change
35	No change

mutations within the analyzed gene in these individuals. The SSCP method is sensitive for the detection of point mutations and small deletions, but it may possibly miss large deletions. The SSCP method is relative simple and of low cost, and is widely used as a screening method. It has been suggested that temperature modulated high-performance liquid chromatography has higher sensitivity than SSCP (18).

As to other changes, we earlier reported the AATT deletion of 55 kDa subunit of DNA Pol ε in a similar location in breast and colorectal cancer (14, 16). Irrespective of the character of the change, mutational or less dramatically polymorphic in character, this genomic change may be associated with the origin or progression of cancer. The fact that no AATT deletion was found in brain neoplasms or control samples at and around Poznan, Poland, suggests the possibility that the change is a subtle evolutionary genetic difference between two human populations. Continued investigation of other types of cancer is required to further understand the potential for tumorigenesis involving the 55 kDa subunit of DNA Pol ε.

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Received March 19, 2010
Revised April 27, 2010
Accepted April 28, 2010