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Genetic changes of CDH1, APC and CTNNB1 found in human brain tumors

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Running title: CDH1, APC and CTNNB1 in human brain tumors

Key words: adenomatous polyposis coli gene (APC), beta-catenin gene (CTNNB1), E-cadherin gene (CDH1), tumors of the CNS, wnt signaling pathway

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Abstract

Research carried out in this paper focused on changes of genes, E-cadherin (CDH1), adenomatous polyposis coli (APC) and beta-catenin (CTNNB1) in a palette of 50 central nervous system tumors. All gene products are components of adherens junctions, but are also involved in Wnt signalling. The results of our analysis showed LOH of CDH1 gene in 31% of meningiomas examined (correlation significant at 0.002 level). One LOH was found in a single case of germinoma, while other tumor types did not demonstrate changes of the CDH1. Fourteen samples (29.2%) with changes of APC gene were observed. The changes were distributed to: 33.3% of glioblastomas, 27% of meningiomas, 1 LOH in five informative astocytomas (20%), and 1 in six informative neurinomas (17%). One oligoastrocytoma showed LOH at exon 11 and one medulloblastoma had allelic imbalance at both exons. Five samples (10%) showed heteroduplexes in β-catenin's exon 3. Potential mutations were confined to two meningiomas, an astrocytoma, a glioblastoma, and a germinoma.

Our results suggest that genetic changes of wnt components are involved in brain tumor genesis. Changes of E-cadherin are involved in meningiomas, while changes of APC gene are distributed among different tumor types, with glioblastomas showing the highest percentage.

Key words: adenomatous polyposis coli gene (APC), beta-catenin gene (CTNNB1), E-cadherin gene (CDH1), tumors of the CNS, wnt signaling pathway

Introduction

In our study, we tried to analyze genetic changes in a palette of central nervous system (CNS) tumors regarding the roles of three genes, E-cadherin (CDH1), adenomatous polyposis coli (APC) and beta-catenin (CTNNB1). All gene products are components of the adherens junction, where E-cadherin is bound to β-catenin, which in turn binds to the central part of the *APC* protein (15, 22). Besides roles in cellular architecture all molecules have roles in wnt signaling, where beta-catenin is the main signaling molecule, and E-cadherin, as currently understood, is indirectly involved in the modulation of the signal (17). APC protein acts as a negative regulator of the wnt pathway and is a critical component of the beta-catenin destruction machinery heading to the proteasome (6, 19). In response to wnt signaling, or under the circumstances of mutated APC, beta-catenin is stabilized, accumulates in the cytoplasm and enters the nucleus, where it finds a partner, a member of the DNA binding protein family LEF/TCF (16). Together they activate new gene expression programs, among others, c-myc and cyclin D1 (9).

It has been well documented that wnt genes, together with other components of wnt signaling pathway, are implicated in tumorigenesis and lately also in brain tumorigenesis (5, 10). Our interest in genes of the wnt pathway stemmed principally from several findings. First, classical cadherins such as E-type and N-type are involved in forming both adherens and synaptic junctions in the nervous system. Moreover, Shimamura and Takeichi (24) found that E-cadherin is transiently expressed in restricted regions of the mouse embryonic and adult brain. New knowledge on wnt signaling shows that wnt proteins regulate critical developmental processes of normal brain development (7, 14) Mutations of beta-catenin gene have been reported in sporadic medulloblastoma (28) and in 2003. beta-catenin was identified as a critical factor for dendritic morphogenesis (29).

APC has been thought of primarily as a colon-specific tumor suppressor gene, but its critical involvement in particular syndromes, like the Tourcot syndrome, which includes the development of primary brain tumors (8), and APC's high expression in the CNS suggests that it performs important functions in these tissues also (1).

Genetic background of specific histopathological type of brain tumor still needs to be elucidated. In this paper we offer three new candidates to fill in the puzzle of genetic basis of human brain tumors.

Materials and methods

Tumor specimen

Samples of 50 central nervous system tumors together with 50 autologous blood tissues were collected from the Department of Neurosurgery, University Hospital "Sisters of Charity", Zagreb, Croatia. Using the magnetic resonance imaging (MRI) tumor lesions were found in different cerebral regions (predominantly temporal and parietal region), with the surrounding zone of perifocal oedema (table 1). During the operative procedure the tumor was removed using a microneurosurgical technique. The patients had no family history of brain tumors and did not undergo chemotherapy or radiotherapy prior to surgery. Collected tumor tissues were frozen in liquid nitrogen and transported to the laboratory, where they were immediately transferred at -75°C. The peripheral blood samples were collected in EDTA and processed immediately. All tumors were studied by pathologists and classified according to the WHO criteria. There were 36% of glioblastomas (18/50); 30% meningiomas (16/50); 12% astrocytomas (6/50); 12% neurinomas (Schwannomas) (6/50); one germinoma (2%), one oligoastrocytoma, one ganglioma and one medulloblastoma. The glioblastomas we considered primary because the diagnosis of glioblastoma was made at the first biopsy, without clinical or histopathologic evidence of a less malignant precursor lesion.

Thirty patients were female (60%), and twenty male (40%). The age of patients varied from 13 to 77 (mean age=51.8). The mean ages at diagnosis for both sexes were similar (M = 52.6; F = 51.3).

The local Ethical Committee approved our study and patients gave their informed consent.

DNA extraction.

Tumor sample for DNA isolation was the part of obvious tumor mass evaluated by the neurosurgeon and based on macroscopic appearance and tissue color, density, and consistency on gross section. The sample was also evaluated for the percentage of tumor cells by pathologist and consisted of more than 85% of tumor cells. Genomic DNA was isolated from unfixed frozen tumor samples and peripheral blood leucocytes by standard methods using proteinase K and phenol chloroform.

Polymerase chain reaction

The D16 S752 (GATA51G03) polymorphic region linked to the E-cadherin gene was amplified in a total volume of 25 μl, (each primer 5'-AATTGACGGTATATCTATCTGTCTG-3'; and 5'-GATTGGAGGAGGGTGATTCT-3') 5

pmol, 200 ng DNA, 2.5 µl 10X buffer II, 1.5 mM $MgCl_2$, 2.5 mM of each dNTP, 0.5 U Taq polymerase (Eppendorf, Germany). PCR conditions: initial denaturation, 3 min/96°C; denaturation, 30 sec/96°C; annealing, 35 sec/55°C; extension, 30+1 sec/72°C; final extension, 72°C/10 min; 35 cycles.

The optimal reaction mixture (25 µl) for APC's exon 11 and 15 amplification as well as PCR conditions were described previously (18).

The reaction mixture (25 µl) for CTNNB1's exon 3 amplification was: 10 pmol of each primer (5´- CCA ATC TAC TAA TGC TAA TAC TG-3´ and 5´- CTG CAT TCT GAC TTT CAG TAA GG -3´), 200–400 ng template DNA, 2.5 µl PCR buffer, 2.5 mM $MgCl_2$, 2.5 mM of each dNTP, 0.5 U Taq polymerase (Eppendorf, Germany). PCR conditions were the same as already described for CDH1 gene.

All PCR products were analyzed on 2% agarose gels.

Loss of heterozygosity

To discover LOH of the E-cadherin gene, a polymorphic marker D16S752, was chosen from the Genome DataBase. Heterozygous samples were visualized on 15% polyacrylamide gels, stained with silver and on Spreadex EL 300 gels (Elchrom scientific, Switzerland), stained with SyberGold (Molecular Probes, Netherlands). Absence or significant decrease in the intensity of one of D16S752 alleles in tumor compared to the autologous blood sample was considered as LOH of CDH1 gene.

LOH of the APC gene was detected on the basis of restriction fragment length polymorphism (RFLP) of the PCR products. Two different polymorphisms were investigated. One is an Rsa I polymorphic site in exon 11, and the other is an Msp I polymorphic site in exon 15. PCR amplification of exon 11 generated a 133- bp fragment that is cleaved to 85- and 48- bp fragments by Rsa I restriction if the polymorphic site is present, and remains uncleaved if the site is absent. The amplified fragment of exon 15 is 550 bp long and is cleaved with the Msp I endonuclease to two 250 bp fragments if the restriction site is present. LOH/Rsa I was demonstrated when the tumor DNA showed loss of either the single uncut band (133 bp) or of the two cut bands (85+48 bp) compared to autologous blood. For Msp I polymorphism heterozygous patients demonstrated two bands (550+250 bp), while LOH was shown when either band was missing in comparison to the autologous blood. Samples that demonstrated quantitatively weaker allelic band in tumor tissue than in normal blood DNA were described as samples with allelic imbalance (AI).

PCR aliquots (10-15 µl) were digested with 6 U Rsa I (Gibco, USA; 12 h at 37°C) and with 6 U Msp I (Gibco, USA, overnight at 37°C) and were electrophoresed on Spreadex gels EL 300 in the SEA 2000 submarine electrophoresis apparatus (Elchrom scientific, Switzerland) at 120V. Temperature of the running buffer was kept constant at 55°C. The samples with LOHs were additionally electrophoresed on 15% polyacrylamide gels stained with silver.

Heteroduplex analysis

Exon 3 of the CTNNB1 gene was screened for mutations. Heteroduplexes were formed by heating 3 µl of PCR products (tumor mixed with normal DNA) at 95 ^{o}C for 3 min, followed by incubation on ice for 20 min. About 3 µl of each sample was mixed with 7 µl of mixture of formamide and 10 mM NaOH (1:100) prior to loading to a gel. The electrophoresis was performed on the GMA gels in the SEA 2000 submarine electrophoresis apparatus (Elchrom scientific, Switzerland). The temperature of the running buffer was kept constant at 9^{o} C.

Statistical Analysis

All statistical evaluations were performed according to the SPSS statistical package (SPSS Inc., Chicago, IL, USA).

Results

The pathohistological diagnosis of the analyzed sample, tumor localization and the duration of symptoms are shown in table 1.

The polymorphic marker for E-cadherin gene, D16S752, was highly informative 42/50 (84%), which means that high percent of patients were heterozygous for this polymorphism. D16S752 is a polymorphic GATA tetranucleotide repeat that could show 7 different allelic variants in Croatian population.

The results of our analysis showed 6 samples with LOH of the CDH1 gene out of 42 heterozygous patients (14.3%) when tumor DNA was compared to autologous constitutive DNA. LOHs of the CDH1 gene were confined to meningiomas. Thirty one % (5/16) of total meningioma sample examined showed LOH of the CDH1 gene, which is shown in Figure 1. The correlation between meningiomas and changes of the CDH1 was according to Spearman's test significant at the 0.002 level.

One LOH was also found in a single case of germinoma, while other tumor types did not demonstrate changes of the CDH1 gene.

From 50 brain tumor samples analysed 48 were heterozygous when analyzed with both APC gene markers (96%). The total number of changes that both markers revealed is 14 samples with changes of the APC gene (9 LOHs and 5 allelic imbalances) out of 48 heterozygous patients (29.2%).

When specifying changes to distinct gene regions there were 12 alterations in exon 11 (28.6%) and 7 in exon 15 (17.5%). Five brain tumor samples had both LOHs at exon 11 and 15.

LOHs of the APC gene that both markers revealed are shown in Figure 2 A (exon 11) and B (exon 15). When distributing APC's genetic changes to a specific tumor type, we observed 6 allelic changes in 18 informative glioblastomas (33.3%), 4 LOHs in 15 informative meningiomas (27%), 1 allelic imbalance in five informative astocytomas (20%), and 1 LOH in six informative neurinomas (17%). One oligoastrocytoma showed LOH at exon 11 and one medulloblastoma had allelic imbalance at both exons.

In heteroduplex analysis, the conformational properties of the double stranded molecules are used to distinguish different base pairing (i.e. mutations). Annealing of mutant DNA to wild type probe gives duplexes with one or more mismatched bases (heteroduplexes). Mismatching causes the double helix to take on a conformation which retards its mobility during electrophoresis. The results of heteroduplex analysis of beta-catenin's exon 3 showed 5 tumor samples with additional bands (10%, 5/50), when the tumor and normal DNA samples were mixed, suggesting that those brain tumors harbor mutations of the CTNN1 gene. Two meningiomas, one astrocytoma, one glioblastoma and one germinoma demonstrated heteroduplexes, which is shown in Figure 3.

In samples with E-cadherin or APC'S LOHs we did not detect genetic changes of the CTNNB1 gene, except in the case of germinoma whose analysis demonstrated both LOH of the E-cadherin gene and heteroduplex of the CTNNB1 gene. Only two meningiomas demonstrated gross deletions of both CDH1 and APC gene.

In all the samples analyzed genetic changes were not correlated with the age or sex of patients (Spearman's test).

The result of this study demonstrates that changes of E-cadherin are involved in fibrous and angiomatous meningioma formation, while changes of APC gene are more commonly distributed among different tumor types, with glioblastomas showing the highest percentage of changes. Genomic changes of all three genes investigated and the polymorphic status for all markers used are summarized in table 1.

Discussion

The formation of brain tumors is the result of multiple consecutive genetic changes that represent a critical factor in tumor evolution (2). The etiology and pathogenesis of tumors of the central nervous system are still inadequately explained. Therefore, identification of new genes that will improve understanding of the basis of tumor development and progression is very important. With this in mind, we investigated new candidate genes, CDH1, APC and CTNNB1, in different brain tumor types.

Changes of CDH1 gene were found almost exclusively in meningiomas with correlation significant at 0.002 level. Thirty one percent of total meningiomas examined showed LOH of CDH1gene. Other tumor types did not demonstrate genetic alterations of E-cadherin gene, except for a single germinoma sample. CDH1 allelic losses were observed in fibrous and angiomatous meningiomas. This finding is intriguing since fibrous and angiomatous meningiomas are usually considered benign lesions with low metastatic potential. However, novel revisions of meningioma classification (12) recommend caution on benign meningioma prognosis, proposing their proliferative activity and brain invasion as the hallmarks that should be considered. The loss of E-cadherin is a well known prerequisite for tumor cell invasion and metastasis formation. Our observations are in agreement with other authors working with E-cadherin's expression patterns in meningiomas. Schwechheimer and co-workers (23) found that E-cadherin's expression was absent from the majority of morphologically malignant meningiomas and that the loss of its expression was correlated with tumor dedifferentiation. Utsuki et al. (26) also reported on negative E-cadherin immunostaining in all of the fibrous meningiomas they examined.

It is long known that meningiomas exhibit desmosomes (23), the epithelial type of cell contact, so the presence of E-cadherin in meningiomas is not unusual. We may speculate that meningiomas, in which we identified E-cadherin losses, would later on exhibit aggressive behavior due to reduction or loss of protein product of this suppressor gene. Meningiomas need not exhibit morphological signs of malignancy, and loss of E-cadherin gene may change the situation at the tumor-brain interface and thus initiate the mechanisms of future expansion.

One of the most important characteristics of malignant gliomas is their invasive behavior. The culprit of the highly invasive phenotype of human gliomas is thought to be associated to the cadherin group of adhesion molecules. Although E-cadherin is a well-known and almost universal suppressor of invasion, little is known on the role of cell-cell adhesion in astrocytes and its alteration in migrating and invasive glioblastomas. In our study the glial branch of brain tumors never showed a single LOH of E-cadherin gene even though some of

the tumors showed aggressive characteristics. This result is interesting and in accordance to Perego et al. (20). In 2002. they demonstrated that instability and disorganization of cadherin mediated junctions rather than reduced expression of cadherin-catenin system components are required to promote migration and invasiveness in glioblastoma cell lines.

Our next finding on APC tumor suppressor gene allelic losses in 29.2% of total brain tumor sample, should be discussed when APC changes are distributed to a specific histopathological type. Thirty-three percent of glioblastomas demonstrated allelic changes of the APC gene which indicated that gross deletions of APC are part of genetic profile of these tumors. One oligoastrocytoma demonstrated LOH and one astrocytoma diffusum allelic imbalance of the APC gene.

Somatic mutations of the APC gene were reported predominantly in sporadic medulloblastoma (13), and are much less frequent in glioblastoma. Nevertheless, Steigerwald and co-workers (25) found base change mutations in APC gene in two of 23 sporadic glioblastomas examined and a heterozygous G to A transition in exon 0.3 in cell line SW 1088 from a human astrocytoma. Microarray technology has been applied to the genome of glioblastoma by Roversi et al. (21). In this extensive study the authors have found among many other candidate chromosomal regions, recurrent losses of 5q22.2-q23.3 region in 10 glioblastoma cell lines (IV WHO grade) they analysed, which is in accordance to our results on glioblastoma.

Behaviorally, gliomas can be viewed as consisting of two discreet subpopulations of cells, the proliferative cells at the tumor core, and cells invading the brain parenchyma. Thus, the gene expression profile of the tumor core may not necessarily depict the profile of genes active in the invading rim. Demuth and Berens (3) identified genes differentially expressed in invasive glioma cells and illustrated the differing biology of the invasive cells in contrast to the tumor cells at the core. Findings on different glioblastoma cellular components could also explain for the portion of glial tumors that did not show instabilities of APC gene. The considerable number of allelic losses of the APC gene in our glioma branch may be attributed to random variation in tumors, but the observed frequency led us to conclude that gross deletion of the APC gene are an important event in the mechanisms of glial tumorigenesis. Since they are more frequent in glioblastomas, and scarcer in astrocytomas, it seems that those changes do not represent an initiation event but rather come along the path of astrocytic progression.

A considerable number of changes (27%) of the APC gene was also found in meningiomas, showing that yet another wnt component is involved in their genesis. Wrobel

and co-workers (27) reported on increased expression of beta-catenin and cyclin D1 in meningiomas they examined by microarray, but unfortunately did not study APC expression profile.

One LOH of APC gene was found in six informative neurinomas (17%). This is to our knowledge the first report on changes of this gene in neurinoma, but it is difficult to assess the biologic impact of this change.

The observed genetic changes of the APC gene are dispersed among different tumor types, indicating once again that APC is not the first event in the formation of specific brain tumor.

The results of heteroduplex analysis of beta-catenin's exon 3 showed 10% of tumor samples with additional bands suggesting that those brain tumors harbour mutations of the CTNN1 gene. We targeted exon 3 of CTNNB1 gene since it has been reported as mutational hot spot. As for beta-catenins changes, they were not very frequent nor were they exclusive to specific tumor type probably due to a small number of tumors in some of the subsets we investigated. Although beta-catenin is frequently mutated in medulloblastoma and was recently proposed a prognostic factor for medulloblastoma (4), our medulloblastoma sample did not demonstrate mutations of beta-catenin, but showed allelic imbalance at both APC's exons. This finding is not unusual since many investigations (11, 13, 28) collectively demonstrated that approximately 15% of medulloblastomas harbor mutations in APC, beta-catenin or Axin. These mutations are mutually exclusive which is supported with our result.

The results reported in this paper indicated that changes of E-cadherin are involved in meningiomas, while changes of APC gene are more commonly distributed among different tumor types, with glioblastomas showing the highest percentage of changes. The functional consequences of the changes we found at the genetic level, would need to be confirmed in future studies at the protein level.

Our findings may contribute to better understanding of brain tumors genetic profile and could be used as prognostic marker of disease evolution and progression.

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Table 1. Pathohistological diagnosis, genomic changes of all genes and the polymorphic status for markers used, localization of the tumor and the duration of symptoms.

No.	*Diagnosis*	*CDH1*	*APC 11*	*APC 15*	*CTNNB1*	*Localization*	*Symptoms*
1	Meningothelial meningioma	HETERO	HETERO	HETERO	-	FP left	15
2	Meningothelial meningioma	HETERO	HETERO	HETERO	**HD**	Spinal ThIV-ThV	8
3	Meningothelial meningioma	HOMO	HETERO	HETERO	**HD**	PCC	14
4	Meningothelial meningioma	HETERO	HETERO	HETERO	-	P right	9
5	Meningothelial meningioma	HETERO	HETERO	HETERO	-	T left	4
6	Meningothelial meningioma	HOMO	HETERO	HETERO	-	PO left	84
7	Meningothelial meningioma	HETERO	**LOH**	HETERO	-	P right	16
8	Meningothelial meningioma	HETERO	HETERO	HETERO	-	T right	36
9	Meningothelial meningioma	HETERO	HOMO	HOMO	-	P left	72
10	Angiomatous meningioma	**LOH**	HETERO	HETERO	-	PCA	12
11	Angiomatous meningioma	**LOH**	**LOH**	**LOH**	-	PCA	5
12	Angiomatous meningioma	**LOH**	HETERO	HOMO	-	Spinal CI, CII	12
13	Fibrous meningioma	**LOH**	HOMO	HETERO	-	P left	16
14	Fibrous meningioma	**LOH**	**LOH**	HOMO	-	FT right	48
15	Fibrous meningioma	HETERO	HETERO	**LOH**	-	PCA	18
16	Fibrous meningioma	HETERO	HETERO	HOMO	-	PCC	12
17	Pilocytic astrocytoma	HETERO	HETERO	HETERO	-	Cerebellum	5
18	Pilocytic astrocytoma	HOMO	HETERO	HOMO	-	FP right	10
19	Pilocytic astrocytoma	HETERO	HOMO	HOMO	-	T left	3
20	Diffuse astrocytoma	HETERO	HETERO	**AI**	-	FTP left	6
21	Anaplastic astrocytoma	HOMO	HOMO	HETERO	**HD**	T right	4
22	Anaplastic astrocytoma	HETERO	HETERO	HETERO	-	F right	0,5
23	Oligoastrocytoma	HETERO	**LOH**	HETERO	-	FP left	14
24	Glioblastoma	HETERO	HOMO	HETERO	-	P right	2
25	Glioblastoma	HETERO	HETERO	HETERO	-	FTP right	0,5

26	Glioblastoma	HETERO	HOMO	HETERO	-	TO right	2
27	Glioblastoma	HETERO	HOMO	HETERO	-	FPO right	3
28	Glioblastoma	HETERO	**AI**	HETERO	-	TP right	7
29	Glioblastoma	HETERO	HOMO	HETERO	-	FTP left	2
30	Glioblastoma	HETERO	HETERO	HETERO	-	F right	5
31	Glioblastoma	HETERO	**LOH**	**LOH**	-	T left	3
32	Glioblastoma	HETERO	HETERO	HETERO	**HD**	FTP left	0,5
33	Glioblastoma	HETERO	**AI**	HETERO	-	P left	1
34	Glioblastoma	HETERO	**LOH**	**LOH**	-	FT left	2
35	Glioblastoma	HOMO	HETERO	HOMO	-	T right	18
36	Glioblastoma	HETERO	HETERO	HETERO	-	FT left	0,5
37	Glioblastoma	HETERO	**LOH**	HETERO	-	O right	1
38	Glioblastoma	HETERO	HETERO	HOMO	-	P right	9
39	Glioblastoma	HETERO	HETERO	HETERO	-	FT left	0,5
40	Glioblastoma	HOMO	HETERO	HETERO	-	F right	1
41	Glioblastoma	HETERO	**AI**	HETERO	-	T right	0,5
42	Neurinoma	HETERO	**LOH**	**LOH**	-	PCA	72
43	Neurinoma	HETERO	HETERO	HETERO	-	Spinal LI	3
44	Neurinoma	HETERO	HETERO	HETERO	-	PCA	42
45	Neurinoma	HOMO	HETERO	HOMO	-	PCA	5
46	Neurinoma	HETERO	HETERO	HETERO	-	PCA	48
47	Neurinoma	HETERO	HETERO	HETERO	-	PCA	36
48	Ganglioglioma	HETERO	HETERO	HETERO	-	T right	4
49	Germinoma	**LOH**	HETERO	HOMO	**HD**	Pineal	3
50	Medulloblastoma	HOMO	**AI**	**AI**	-	PCC	1

LOH=loss of heterozygosity; AI=allelic imbalance ;HD=heteroduplex; *Symptoms duration/ months; FP=frontoparietal region; PCC=posterior cranial cavity; P=parietal region; T=temporal region; PO=parietooccipital region; PCA=ponto-cerebral angle; FT=frontotemporal region; FTP=frontotemporoparietal region; TO=temporooccipital region; FPO=frontoparietooccipital region; TP=temporoparietal region.

Figure captions

Figure 1. Loss of heterozygosity of the E-cadherin gene in three meningioma samples on Spreadex gels (Elchrom Scientific) stained with Sybergold (Molecular Probes). Polymorphic marker D16S752 is shown. Lane 1- M3 standard; lanes 3, 4, 6, 8,–corresponding blood samples; lanes 2, 5, 7, 9, - LOHs of the E-cadherin gene.

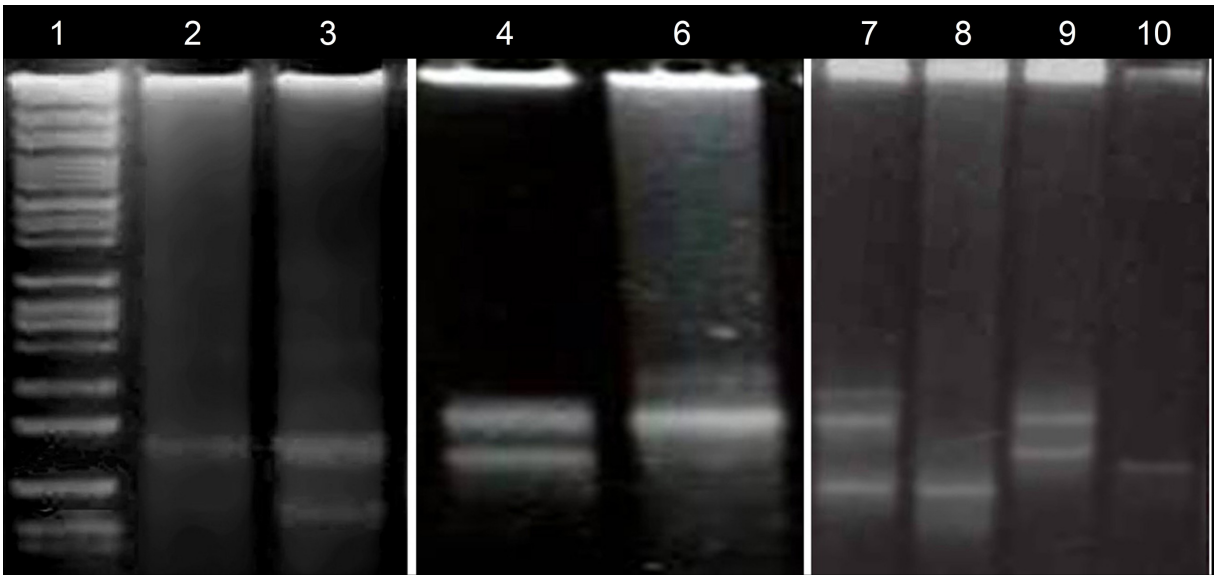
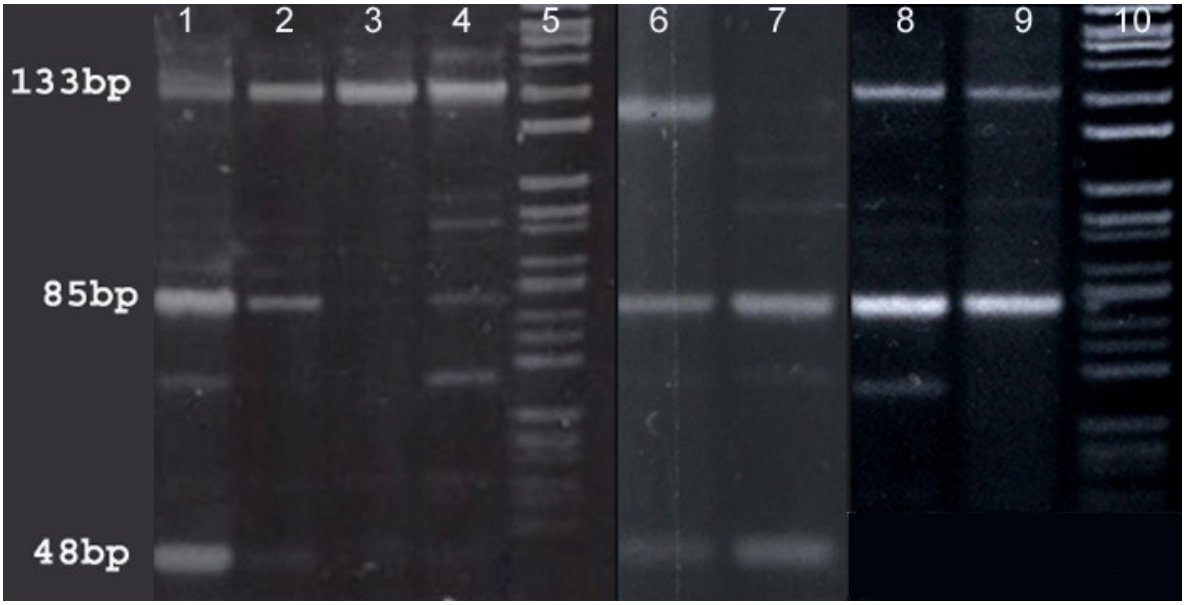
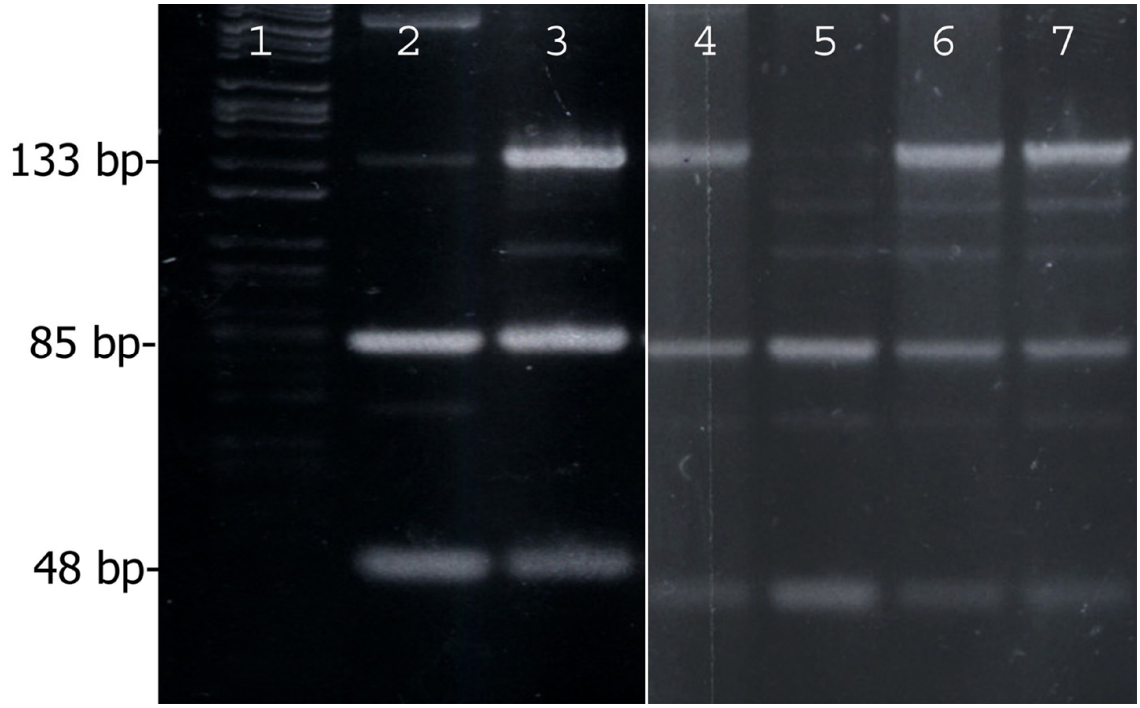


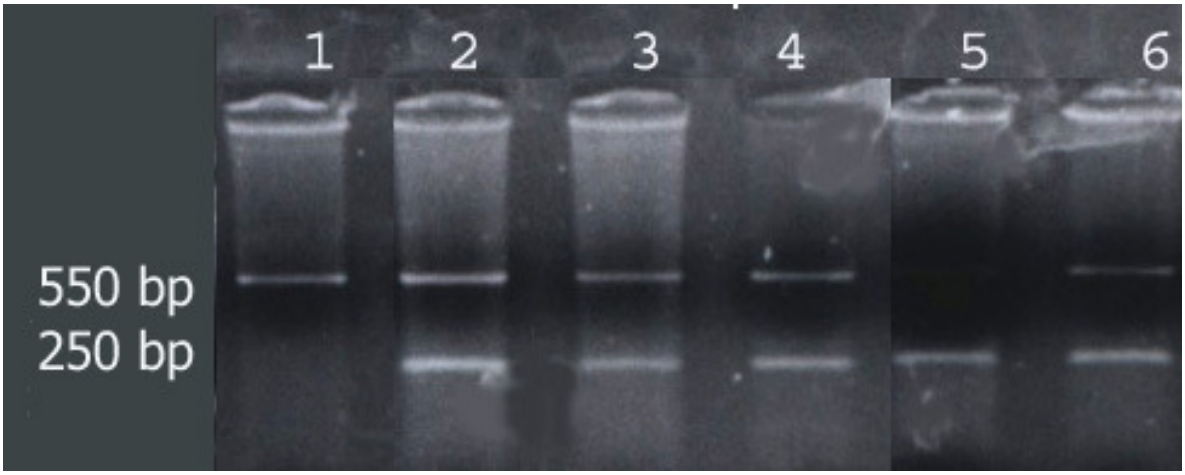
Figure 2. A. Loss of heterozygosity of APC gene in two glioblastoma samples. Exon 11/RsaI/RFLP is demonstrated. Lanes 1, 2- heterozygous sample (tumor and blood); lane 3- LOH in glioblastoma patient (the digested/cut allele is missing); lane 4- informative blood sample of the same patient; lanes 5, 10 - standard M3 (Elchrom scientific); lane 6- informative blood sample; lane 7- LOH in the corresponding glioblastoma (uncut allele is missing); lanes 8 –informative blood sample; lane 9- allelic imbalance in the corresponding glioblastoma (uncut allele is weaker, 48 bp fragments are not shown). **B.** Lane 1- standard M3 (Elchrom scientific); 2- LOH in a meningioma sample (uncut allele is missing); 3- corresponding blood sample; lane 4- informative blood sample of the neurinoma patient; lane 5 - LOH in the corresponding neurinoma (uncut allele is missing); lanes 6, 7 - heterozygous samples. **C.** Loss of heterozygosity of APC gene in 2 patients with glioblastoma. Exon 15/MspI/RFLP is demonstrated. Lanes 1, 5 - LOHs in glioblastoma samples; lanes 2, 6 – corresponding heterozygous blood samples; lanes 3, 4 - heterozygous samples, both alleles, cut and uncut, are visible.



A



B



C

Figure 3. Heteroduplex analysis of the CNS tumor samples. Exon 3 of the CTNNB1 gene was screened for mutations on GMA gels (Elchrom Scientific). Lane 1 - M3 standard; lanes 3, 5, 8, 9, 12 -additional bands showing heteroduplexes when tumor and normal samples are mixed; lanes 2, 4, 7, 10, 11- corresponding blood DNA samples; lane 6 -tumor DNA sample.