

# ALTERATIONS OF *P53*, *PTEN* AND *EGFR* GENES IN GLIOBLASTOMA MULTIFORME

Jasna Bankovic<sup>1</sup>, Vedrana Milinkovic<sup>1</sup>, Miodrag Rakic<sup>2</sup>, Milos Jokovic<sup>2</sup>, Tijana Andjelkovic<sup>1</sup>, Ana Podolski-Renic<sup>1</sup>, Nikola Tanic<sup>1</sup>, Sabera Ruzdijic<sup>1</sup>  
<sup>1</sup>University of Belgrade, Institute for Biological Research, Department of Neurobiology, Bulevar Despota Stefana 142, 11060 Belgrade, Serbia  
<sup>2</sup>University of Belgrade, Institute for neurosurgery, Clinical Centre of Serbia, Visegradska 26, 11000 Belgrade, Republic of Serbia

## INTRODUCTION:

- **Glioblastomas** are the most frequent and the most aggressive human brain tumors.
- Oncogene *EGFR* and tumor suppressor genes *p53*, *PTEN* are involved in the evolution of glioblastomas.
- **EGFR** (epidermal growth factor receptor) is a cell-surface receptor, that, upon activation by its growth factor ligands, initiates several signal transduction cascades leading to DNA synthesis and cell proliferation.
- **p53** is a tumor suppressor protein that, among many anticancer mechanisms, plays important roll in apoptosis, genetic stability and inhibition of angiogenesis.
- **PTEN** (phosphatase and tensin homolog) is part of PI3K/AKT pathway that regulates signaling of multiple biological processes such as apoptosis, metabolism, cell proliferation and cell growth.

## AIM OF STUDY:

- To investigate alterations of *EGFR*, *PTEN* and *p53* genes in 35 glioma specimen:
- *EGFR* amplification
- *p53* mutational status (exons 5-9)
- LOH of *p53*
- LOH of *PTEN*
- To correlate the alterations with clinicopathological parameters and evaluate their role in glioblastoma progression.

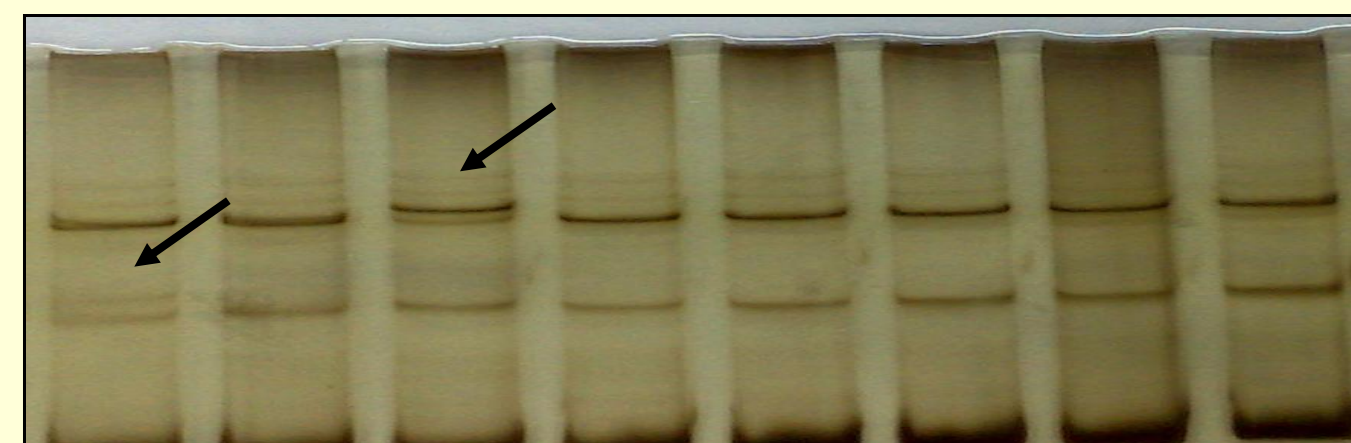
## MATERIAL AND METHODS:

- Genomic DNA isolated from 35 samples of human brain tumor tissue and blood
- Differential PCR for analysis of *EGFR* amplification status
- PCR-SSCP (Single strand conformation polymorphism) and sequencing for determination of *p53* mutational status
- Fragment analyses of 4 microsatellite loci (D17S786, D17S1537, TP53pentanucleotide, TP53 dinucleotide) for detection of *p53* LOH
- Fragment analyses of 5 microsatellite loci (D10S579, D10S215, D10S1765, D10S541, AFMa086wg9) for detection of *PTEN* LOH

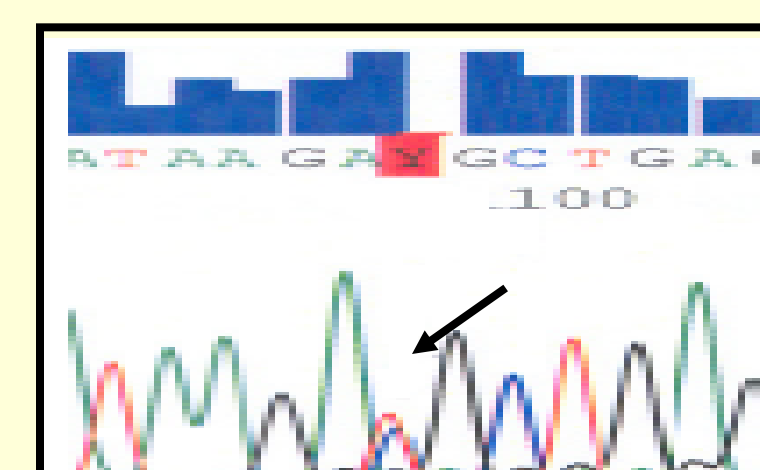
## RESULTS:

### Mutational status of *p53*

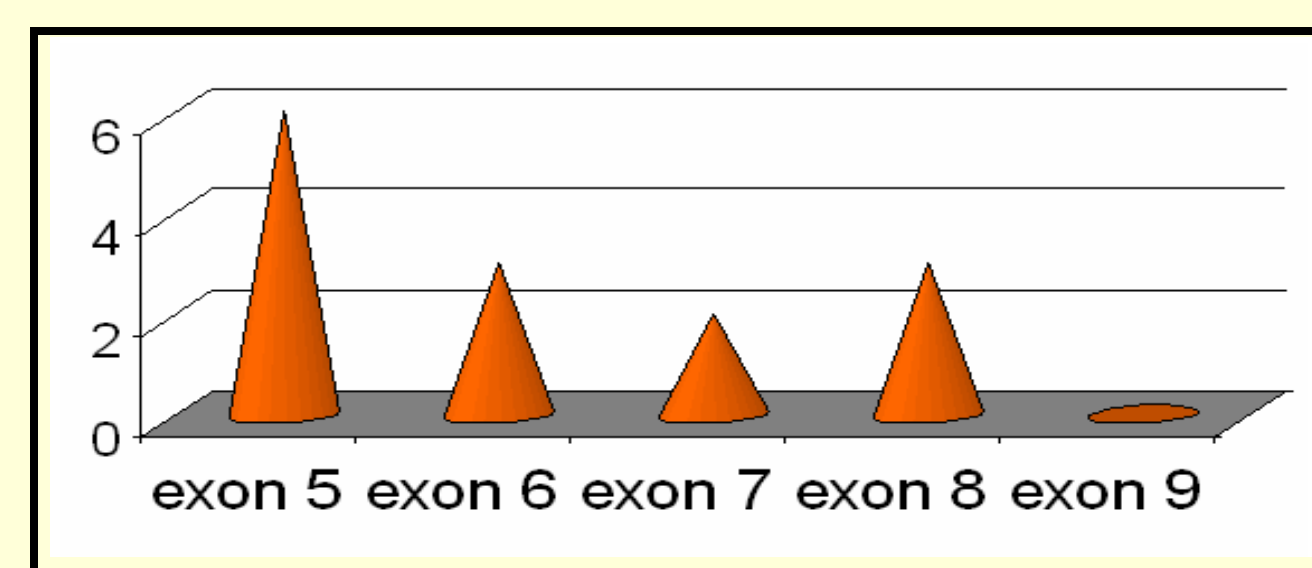
40 41 42 50 54 88 89 Control



PCR-SSCP results of exon 6 of *p53*; arrows indicate mutated single strand conformers in patients #40 and #42



Part of reverse sequence of exon 6 of *p53* gene from patient #40; arrow indicates nucleotide change (A>G) in codon 193

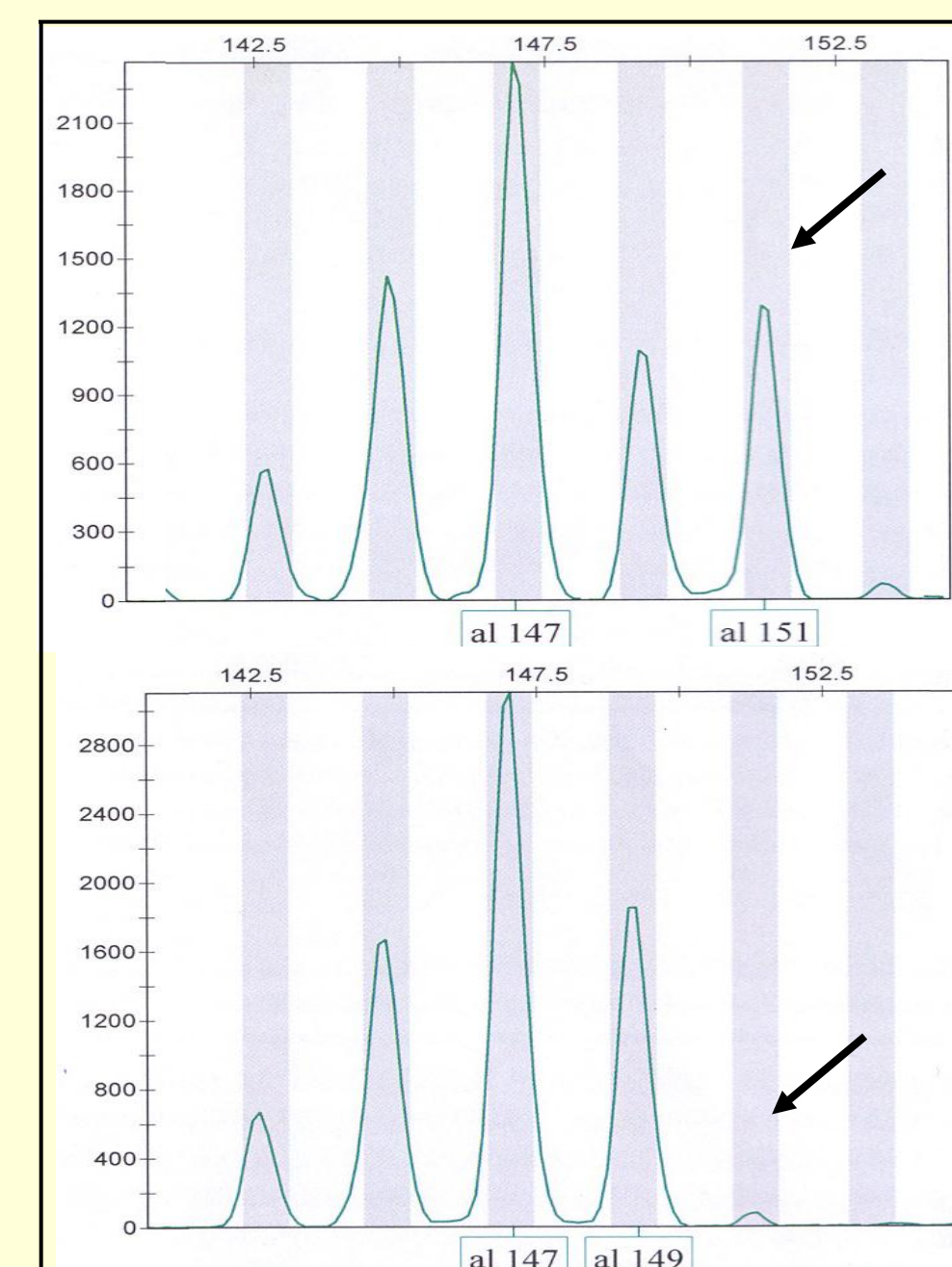


Distribution of confirmed mutations in exons 5-9 of *p53*

Patient Number	Exon number	Codon number	Nucleotide change	Predicted effect
1	Exon 5	144	CAG→CAC	Gln→His
22	Exon 5	158	CGC→CAC	Arg→His
	Exon 8	267	CGG→TGG	Arg→Trp
	Exon 8	286	GAA→TAA	Glu→Stop
24	Exon 5	158	CGC→CAC	Arg→His
25	Exon 5	144	CAG→CAC	Gln→His
27	Exon 5	144	CAG→CAC	Gln→His
30	Exon 5	144	CAG→CAC	Gln→His
32	Intron 9	-14766	T→C	polymorphism
40	Exon 6	193	CAT→CGT	His→Arg
	Exon 6	208	GAC→GGC	Asp→Gly
42	Exon 6	195	ATC→ACC	Ile→Thr
	Exon 7	231	Add T	frame shift
88	Exon 7	234	Del 14028-14050	frame shift
89	Exon 8	289	Add T	frame shift

List of mutations of *p53* gene in glioma samples identified by DNA sequencing; boxes indicate novel mutations that haven't been associated with brain tumor before

### Loss of heterozygosity (LOH) of *p53* and *PTEN*



Example of fragment analysis of *p53* with D17S786 microsatellite locus in patient 22; tumor sample exhibits extensive loss of heterozygosity in allele 151

<i>PTEN</i>		<i>P53</i>	
Low instability (%)	High instability (%)	Low instability (%)	High instability (%)
11 (31,4)	11 (31,4)	6 (17,2)	1 (2,8)
22 (62,9)		7 (20,0)	

Presence of loss of heterozygosity (LOH) of *p53* an *PTEN* in glioma samples; low instability – LOH of only one marker, high instability – LOH of two or more markers

### Patient and tumor characteristics

Patient Characteristic	
Age	Mean 54 (range 6-75)
Sex	15 F, 20 M
Resection	35 of 35
Reccurence	3 of 35
Tumor Characteristic	
Grade II	2
Grade III	9
Grade IV	24
Molecular Event	
<i>EGFR</i> amplification	14 of 35 (40%)
<i>p53</i> <i>p53</i> mutation	10 of 35 (28,6%)
<i>p53</i> <i>p53</i> LOH	7 of 35 (20%)
<i>PTEN</i> LOH	22 of 35 (62,9%)

### Co-presence of genetic alterations

	<i>p53</i> mutation	<i>p53</i> LOH	<i>EGFR</i> amplification
<i>PTEN</i> LOH	6	3	11
<i>p53</i> mutation		3	4
<i>p53</i> LOH			2

### Correlation analyses

Parameter	Total NP	<i>PTEN</i> LOH		<i>p53</i>				<i>EGFR</i> amplification		
		NP (%)	P value	LOH NP (%)	LOH P value	Mutation NP (%)	Mutation P value	total NP (%)	total P value	
Histological grade	35	22 (62,9)		7 (20,0)		10 (28,6)		14 (40,0)		
	II	2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
	III	9	3 (33,3)	0,03 <sup>b</sup>	3 (33,3)	0,36	5 (55,6)	0,09	7 (77,8)	0,02
	IV	24	19 (79,2)	0,06 <sup>c</sup>	4 (16,7)	1,00	5 (20,8)	1,00	7 (29,2)	1,00
Age	<20	2	0 (0)	1,00 <sup>d</sup>	1 (50,0)	0,38	1 (50,0)	1,00	1 (50,0)	1,00
	20-50	8	2 (25,0)	0,008 <sup>e</sup>	1 (12,5)	1,00	3 (37,5)	0,65	4 (50,0)	0,68
	>50	25	20 (80,0)	0,06 <sup>f</sup>	5 (20,0)	0,40	6 (24,0)	0,46	9 (36,0)	1,00

NP, number of patients per group;  
a-grade II compared with grade III;  
b-grade III compared with grade IV;  
c-grade II compared with grade IV;  
d-age<20 compared with age20-50;  
e-age20-50 compared with age>50;  
f-age>50 compared with age<20;  
uc – unique change present in genome

## CONCLUSIONS:

- Loss of heterozygosity (LOH) of *PTEN* is the most frequent alteration in this set of samples, followed by *EGFR* amplification, *p53* gene mutation and *p53* LOH
- 5 novel mutations of *p53* gene, that haven't been associated with brain tumors before have been identified
- Exon 5 of *p53* is the most commonly mutated one
- *p53* alterations were significantly associated with younger age (<50) and lower grade in contrast to *PTEN* and *EGFR*
- Concomitant *PTEN* inactivation and *EGFR* amplification appeared more often in higher grade tumors
- LOH of *PTEN* and *EGFR* amplification may lead to tumor progression
- Alteration of *p53* could be an early event in tumor development