

## ASSOCIATION OF OVEREXPRESSED MYC GENE WITH ALTERED PHACTR3 AND E2F4 GENES CONTRIBUTES TO NON-SMALL CELL LUNG CARCINOMA PATHOGENESIS

POVEZANOST POVIŠENE EKSPRESIJE MYC GENA SA IZMENJENIM PHACTR3 I E2F4 GENIMA DOPRINOSI PATOGENEZI NESITNOĆELIJSKOG KARCINOMA PLUĆA

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### Summary

**Background:** C-Myc is one of the major cellular oncogenes overexpressed in non-small cell lung carcinoma (NSCLC). Its deregulated expression is necessary but not sufficient for malignant transformation. We evaluated expression of MYC gene in NSCLC patients and its association with alterations in the genes previously identified to be related to NSCLC pathogenesis, *PHACTR3* and *E2F4*.

**Methods:** We analyzed MYC gene expression by qRT-PCR in 30 NSCLC patients' samples and paired normal lung tissue. MYC expression was further statistically evaluated in relation to histopathological parameters, *PHACTR3* and *E2F4* gene alterations and survival. Alterations in aforementioned genes were previously detected and identified based on AP-PCR profiles of paired normal and tumor DNA samples, selection of DNA bands with altered mobility in tumor samples and their characterization by the reamplification, cloning and sequencing.

**Results:** MYC expression was significantly increased in NSCLC samples and its overexpression significantly associated with squamous cell carcinoma subtype. Most importantly, MYC overexpression significantly coincided with mutations in *PHACTR3* and *E2F4* genes, in group of all patients and in squamous cell carcinoma subtype. Moreover, patients with jointly overexpressed MYC and altered *PHACTR3* or *E2F4* showed trend of shorter survival.

### Kratak sadržaj

**Uvod:** C-Myc je jedan od glavnih ćelijskih onkogeni koji je prekomerno eksprimiran kod nesitnoćelijskog karcinoma pluća (NSCLC). Promene u ekspresiji ovog onkogeni iako neophodne nisu dovoljne za malignu transformaciju. U ovoj studiji smo proučavali promene ekspresije MYC gena kod pacijenata sa NSCLC kao i međupovezanost sa promenama u genima *PHACTR3* i *E2F4*, za koje je ranije pokazano da su povezani sa patogenezi NSCLC.

**Metode:** Ekspresija gena MYC je analizirana qRT-PCR metodom u uzorcima tumorskog i odgovarajućeg normalnog plućnog tkiva 30 pacijenata obolelih od NSCLC. Promene MYC ekspresije su dalje analizirane u odnosu na histopatološke parametre, promene u genima *PHACTR3* i *E2F4*, kao i u odnosu na preživljavanje pacijenata. Mutacije u pomenutim genima su prethodno otkrivene i identifikovane na osnovu uporedne analize AP-PCR profila parova normalnih i tumorskih DNK uzoraka, odabira DNK traka sa izmenjenom pokretljivošću u tumorskim uzorcima, i zatim njihovom karakterizacijom pomoću reamplifikacije, kloniranja i sekvenciranja.

**Rezultati:** Ekspresija gena MYC je povećana u uzorcima NSCLC i predstavlja značajnu karakteristiku karcinoma skvamoznih ćelija. Prekomerna ekspresija ovog gena asociira sa prisustvom mutacija u genima *PHACTR3* i *E2F4* u celoj grupi pacijenata, kao i u podgrupi karcinoma skvamoznih ćelija. Takođe, pacijenti sa povećanom ekspresijom

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List of abbreviations: NSCLC, non-small cell lung carcinoma; qRT-PCR, quantitative real time polymerase chain reaction; AP-PCR, arbitrarily primed polymerase chain reaction; ACTB, beta-actin; PHACTR3, phosphatase and actin regulator 3/ scaffold-associated PP1 inhibiting protein.

**Conclusions:** Overall, *MYC* is frequently overexpressed in NSCLC and it is associated with mutated *PHACTR3* gene, as well as mutated *E2F4* gene. These joint gene alterations could be considered as potential molecular markers of NSCLC and its specific subtypes.

**Keywords:** *E2F4*, *MYC*, Non-small cell lung carcinoma, *PHACTR3*

## Introduction

For more than 3 decades lung cancer has been the most common type of human malignancies, representing approximately 12–13% of all newly diagnosed cancer cases worldwide (1, 2). The estimated number of lung cancer related deaths has been constantly rising during this period (2). Although there have been improvements in lung cancer patient survival, it is still very poor with less than 18% 5-year survival rate (3). Therefore, scientists are persistently looking for novel and reliable molecular markers to improve diagnosis of lung cancer, optimize treatments and enable monitoring disease progression and response to therapy. Molecules that regulate cellular processes essential for maintaining malignant phenotype, such as *c-Myc* oncogene, stand out as promising candidates to serve as molecular markers. This nuclear phosphoprotein functions as transcriptional regulator of multiple genes and controls cell proliferation, induces apoptosis, and regulates stemness, senescence, metabolism and genome stability (4). Its expression is deregulated in number of human cancers including breast cancer, Burkitt's lymphoma, medulloblastoma, neuroblastoma and lung cancer (5). In most studies on non-small cell lung carcinoma (NSCLC), a predominant clinical form of lung cancer, *c-Myc* protein is overexpressed in 40–75% of cases (6–9). Even though it is highly expressed in NSCLC, it does not associate with histopathological parameters and its prognostic value for this type of lung cancer is not clear (7, 9, 10). These data implicate that *c-Myc* is important for NSCLC pathogenesis but not sufficient to individually drive this process. Accordingly, we evaluated expression of *MYC* gene in 30 NSCLC patients' samples and analyzed its association with histopathological parameters and patients survival. Our main focus was to investigate the possible association of *MYC* expression with alterations in the genes previously identified to be related with NSCLC pathogenesis, *PHACTR3* and *E2F4* (11). The aim was to determine their mutual importance for NSCLC pathogenesis and potential to serve as molecular markers of this disease. Association analyses of *MYC* overexpression and aforementioned gene alterations were performed on whole set of patients' samples and samples stratified according to histopathological parameters. To evaluate their joint prognostic significance we analyzed their effects on patients' survival.

*MYC* gena i istovremeno mutiranim *PHACTR3* ili *E2F4* genima su pokazali trend kraćeg preživljavanja.

**Zaključak:** Povećana ekspresija *MYC* gena je prisutna sa visokom učestalošću kod NSCLC i povezana je sa mutiranim *PHACTR3* genom, kao i mutiranim *E2F4* genom. Ove združene genske promene se mogu razmatrati kao potencijalni molekularni markeri NSCLC i njegovih specifičnih podtipova.

**Ključne reči:** *E2F4*, *MYC*, nesitnoćelijski karcinom pluća, *PHACTR3*

## Materials and Methods

### *Tissue samples*

We analyzed paired tumor samples and adjacent normal lung tissue from 30 patients with NSCLC who underwent surgery at the Clinic for Thoracic Surgery (Belgrade, Serbia). The specimens were frozen in liquid nitrogen, where they were kept until DNA and RNA extraction. The samples were collected and used after obtaining patients' informed consents and approval from the Ethics Committee, in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Diagnosis of NSCLC and its classification according to histological subtype, grade stage, and lymph node invasion were established by histological analyses of the surgical specimens. Distribution of all specimens by relevant histopathological parameters was presented previously (11). Overall, 9 adenocarcinomas and 21 squamous cell carcinomas were included in the study. 26 NSCLC samples were well to moderately differentiated (grade 1/2), while 4 were poorly differentiated (grade 3). 16 samples were in early pathological stages (stages I/II), and 14 cases were in late stage (stage III). Lymph node invasion was observed in 25 samples. Neither pre-surgical chemotherapy nor radiotherapy had been administered.

### *RNA extraction and Reverse Transcription*

Total RNA was isolated from tumor and corresponding normal tissues samples of all 30 NSCLC patients. The isolation was carried out using Trizol® reagent (Invitrogen Life Technologies, USA) according to the manufacturer's instructions. RNA was quantified by spectrophotometry and quality was verified by agarose gel electrophoresis. Subsequently, reverse transcription reactions were performed using 2 µg of total RNA, with a High-capacity cDNA reverse transcription kit (Applied Biosystems), following the manufacturer's protocol.

### *Quantitative real-time PCR*

Quantitative real time PCR (qRT-PCR) was performed to evaluate the mRNA expression level of *MYC* gene. PCR reactions with 100 ng of cDNA were

performed using pair of primers specific for MYC transcripts (12). For each sample ACTB was used as a reference gene for the normalization of target gene expression (13). The primer sequences were as follows: 5'-CAA GAG GCG AAC ACA CAA CGT CT-3' (sense) and 5'-AAC TGT TCT CGT CGT TTC CGC AA-3' (antisense) for MYC, and 5-TGG ACA TCC GCAAAG ACC TGT AC-3 (sense) and 5-TCA GGA GGA GCA ATG ATC TTG A-3 (antisense) for the ACTB. PCR reactions were performed using a Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, USA) on a QuantStudio 3 Real-Time PCR system (Thermo Fisher Scientific, USA) according to the manufacturer's recommendations. Each sample was tested in triplicate and relative gene expression levels were analyzed by the  $2^{-\Delta\Delta Ct}$  method (14).

#### Identification of NSCLC associated genes

In our preceding study we identified several genes not previously related to NSCLC pathogenesis (11). Overall procedure of identifying those genes included: DNA isolation, generation and comparison of AP-PCR profiles of paired normal and tumor DNA samples, selection of DNA bands with altered mobility in tumor versus matching normal samples and their further characterization through the reamplification, cloning and sequencing.

#### DNA extraction

DNA was extracted using the phenol/chloroform/isoamyl alcohol method (15). The quality of the DNA was verified by electrophoresis on 0.8% agarose gel and staining with ethidium bromide. The DNA concentration was established spectrophotometrically.

#### AP-PCR DNA fingerprinting

AP-PCR (Arbitrarily Primed Polymerase Chain Reaction) was used to generate DNA fingerprint profiles of paired DNA samples, normal and tumor, of the same patient (16). In short, six different, randomly chosen, primers were used to amplify DNA, initially under low-stringency conditions and then at high-stringency conditions, to generate informative fingerprints of moderate complexity that could distinguish tumor from normal tissue. The reactions were performed in a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA, USA) using PCR reaction kits supplied by Fermentas Life Sciences (Lithuania). Each reaction consisted of an initial denaturation step (95 °C for 5 min), 4 cycles at low-stringency conditions (specific for each primer), 35 cycles at high-stringency conditions (specific for each primer), and a final extension (72 °C for 7 min). Five out of six tested primers (p53 A, MDR A, E5A p53, GAPDH A, and GAPDH S) produced informative sequence alterations differentiating normal from tumor tissue. Primer sequences, AP-PCR amplification profiles and

**Table I** AP-PCR primer sequences, amplification profiles and reaction conditions.

Primers	Sequences	Amplification profiles		Reaction conditions
		Low-stringency conditions	High-stringency conditions	
p53A	5'-TTG GGC AGT GCT CGC TTA GT-3'	95 °C 30"; 40 °C 2'; 72 °C 1'	95 °C 30"; 60 °C 2'; 72 °C 1'	0.2 mmol/L each dNTP 3.5 mmol/L MgCl <sub>2</sub> 5 μmol/L primer 1U Taq DNA Polymerase
MDR A	5'-GTT CAA ACT TCT GCT CCT GA-3'	95 °C 30"; 40 °C 2'; 72 °C 1'	95 °C 30"; 58 °C 2'; 72 °C 1'	0.4 mmol/L each dNTP 2.5 mmol/L MgCl <sub>2</sub> 5 μmol/L primer 1U Taq DNA Polymerase
E5A p53	5'-CAG CCC TGT CGT CTC TCC AG-3'	95 °C 30"; 40 °C 2'; 72 °C 1'	95 °C 30"; 55 °C 2'; 72 °C 1'	0.6 mmol/L each dNTP 3.5 mmol/L MgCl <sub>2</sub> 3 μmol/L primer 1U Taq DNA Polymerase
GAPDH A	5'-AGC CTT CTC CAT GGT GGT GAA GAC-3'	95 °C 30"; 50 °C 2'; 72 °C 1'	95 °C 30"; 72 °C 2'; 72 °C 1'	0.2 mmol/L each dNTP 2.5 mmol/L MgCl <sub>2</sub> 3 μmol/L primer 1U Taq DNA Polymerase
GAPDH S	5'-CGG AGT CAA CGG ATT TGG TCG TAT-3'	95 °C 30"; 50 °C 2'; 72 °C 1'	95 °C 30"; 70 °C 2'; 72 °C 1'	0.4 mmol/L each dNTP 2.5 mmol/L MgCl <sub>2</sub> 5 μmol/L primer 1U Taq DNA polymerase

reaction conditions are described in our previous publications (16, 11) and summarized in *Table I*. The AP-PCR products were separated on 6–8% non-denaturing polyacrylamide gels and visualized by silver staining.

#### *Isolation, cloning and sequencing of altered bands*

Some of the DNA bands with altered mobility, in tumor compared to normal samples, were selected for further characterization. Briefly, PCR amplicons of chosen bands were scrapped from silver stained gels with a hypodermic 22-gauge needle previously soaked in the PCR master mix solution. The needle was dipped in the PCR master mix for 2 min and then discarded. Further reamplification of the PCR products was performed with the same primers used for AP-PCR reactions at high-stringency conditions specific for each particular primer. The obtained products were cloned and sequenced as described (11). In short, the reamplified material was administrated on 1.5% agarose gels, than purified using DNA Extraction Kit (Fermentas Life Sciences, Lithuania) and cloned with GeneJet™ PCR Cloning Kit (Fermentas Life Sciences, Lithuania) according to manufacturers' instructions. After cloning procedure, plasmids were purified using GeneJet™ Plasmid Miniprep Kit (Fermentas Life Sciences, Lithuania). Further, sequencing was performed in both directions on several clones for each selected DNA band on an ABI Prism 3130 Genetic Analyzer automated sequencer (Applied Biosystems, Foster City, CA, USA) using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The obtained sequences were analyzed using BLAST software in the NCBI GenBank and EBI (Sanger Institute) database to identify selected alterations.

#### *Statistical analysis*

Significant differences between the data sets were determined by GraphPad Prism 6 software (La Jolla, CA USA) and statistical package R (version 3.1.3 (2015-03-09) Copyright (C) 2015 The R Foundation for Statistical Computing). Paired t-test was used to assess statistical differences in expression level of MYC gene between paired tumor and normal sample of each patient. On the other hand, difference in MYC relative expression level between total tumor and normal samples was evaluated using unpaired t-test. Fisher exact test was used to examine the relationship between histopathological parameters (histological subtype, grade stage, and lymph node invasion) and MYC overexpression. The same statistical analysis was used to study the possible associations between frequencies of MYC overexpression and alterations in *PHACTR3* and *E2F4*. Survival analyses were performed by Kaplan & Meier product-

limit method. The log rank test was used to evaluate the significance of the difference between pairs of survival probabilities. Overall survival rate was calculated from day one after surgery to the last follow-up examination or death of the patient. The total follow up period was 117 months. Statistical differences were considered significant when  $p$  value was  $< 0.05$ .

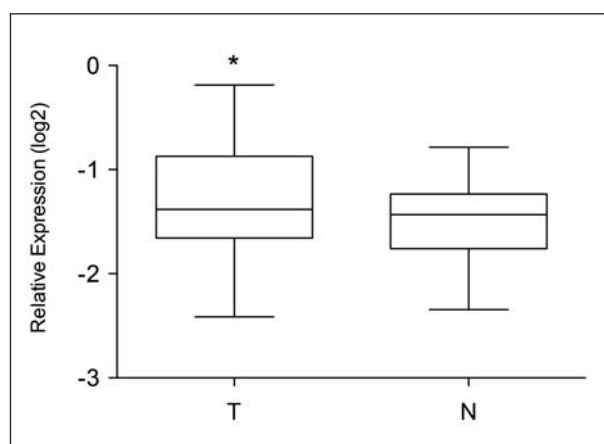
## Results

### *MYC gene expression analysis*

Alterations in MYC gene expression between tumor and matching normal samples were analyzed by qRT-PCR. MYC gene expression was significantly higher in NSCLC samples ( $p=0.018$ ) compared with normal lung samples (*Figure 1*). Specifically, MYC gene was overexpressed in 11 out of 30 tumor samples (37%).

### *Increased MYC gene expression in relation to histopathological parameters*

Significance of MYC overexpression was initially evaluated in relation to major histopathological parameters: NSCLC subtype, histological grade, stage and lymph node invasion (*Table II*). Statistical analyses revealed that MYC overexpression was significantly associated with histological subtype ( $p=0.01$ ). In adenocarcinoma increased MYC expression was completely absent (0/9), while in squamous cell carcinoma MYC overexpression was more frequently present (11/21, 52.4%).



**Figure 1** MYC gene expression. Box plot analysis of MYC expression level in primary tumor (T) and normal (N) samples. Results are presented as log<sub>2</sub> of MYC gene expression relative to *ACTB* gene level. \* indicates  $p < 0.05$  as significant gene overexpression in tumor samples compared to normal samples.

**Table II** Association between MYC overexpression and histopathological parameters.

Parameter	Total NP <sup>b</sup>	MYC overexpression		
		Yes NP (%)	No NP (%)	p value
Total	30	11	19	
NSCLC subtype				
Adenocarcinoma	9	0 (0.0)	9 (100.0)	<b>0.01<sup>c</sup></b>
Squamous cell carcinoma	21	11 (52.4)	10 (47.6)	
Histological grade <sup>a</sup>				
g1/2	26	11 (42.3)	15 (57.7)	0.36
g3	4	0 (0.0)	4 (100.0)	
Stage				
I/II	16	6 (37.5)	10 (62.5)	1
III	14	5 (35.7)	9 (64.3)	
Lymph node invasion				
Positive	25	9 (36.0)	16 (64.0)	1
Negative	5	2 (40.0)	3 (60.0)	

<sup>a</sup>g1/2, well to moderately differentiated; g3, poorly differentiated; <sup>b</sup>NP, number of patients per group; <sup>c</sup>bold indicates statistically significant value,  $p < 0.05$ .

### Increased MYC gene expression in relation to gene alterations

We further examined MYC expression in relation to the presence of alterations in *PHACTR3* and *E2F4*. In previous study (11), mutations in these two genes were identified according to sequence data and BLAST search results. Specifically, multiple nucleotide substitutions were detected in *PHACTR3* and *E2F4*.

### Association between MYC overexpression and altered PHACTR3 gene

Patients with altered *PHACTR3* gene had more frequently increased MYC gene expression (Table III;  $p = 0.015$ ). Among six patients with altered *PHACTR3* gene, 5 (83.3%) had increased MYC mRNA level, while in 1 patient (16.7%) MYC was not overexpressed. Similar association was present in subgroup of patients with squamous cell carcinoma (Table III;  $p = 0.035$ ). In this group all patients with *PHACTR3* alterations had overexpressed MYC.

### Association between MYC overexpression and altered E2F4 gene

MYC overexpression in the presence of *E2F4* gene alterations showed the same pattern of conjunction as with the incidence of altered *PHACTR3* gene (Table III). Namely, 83.3% of patients with abnormal *E2F4* had overexpressed MYC compared to 16.7% of patients that did not show overexpression of this oncogene ( $p = 0.015$ ). In squamous cell carcinoma subtype altered *E2F4* exclusively coincided with MYC overexpression (100%) while unchanged *E2F4* was predominantly associated with non-overexpressed MYC (62.5% versus 37.5%;  $p = 0.035$ ).

**Table III** Association of overexpressed MYC oncogene with altered PHACTR3 and E2F4 genes.

		MYC overexpression		Squamous cell carcinoma	
		MYC overexpression		MYC overexpression	
		Yes	No	Yes	No
		NP <sup>a</sup> (%)	NP (%)	NP <sup>a</sup> (%)	NP (%)
PHACTR3 alteration	Yes	5 (83.3)	1 (16.7)	5 (100.0)	0 (0.0)
	No	6 (25.0)	18 (75.0)	6 (37.5)	10 (62.5)
p value		<b>0.015<sup>b</sup></b>		<b>0.035</b>	
E2F4 alteration	Yes	5 (83.3)	1 (16.7)	5 (100.0)	0 (0.0)
	No	6 (25.0)	18 (75.0)	6 (37.5)	10 (62.5)
p value		<b>0.015<sup>b</sup></b>		<b>0.035</b>	

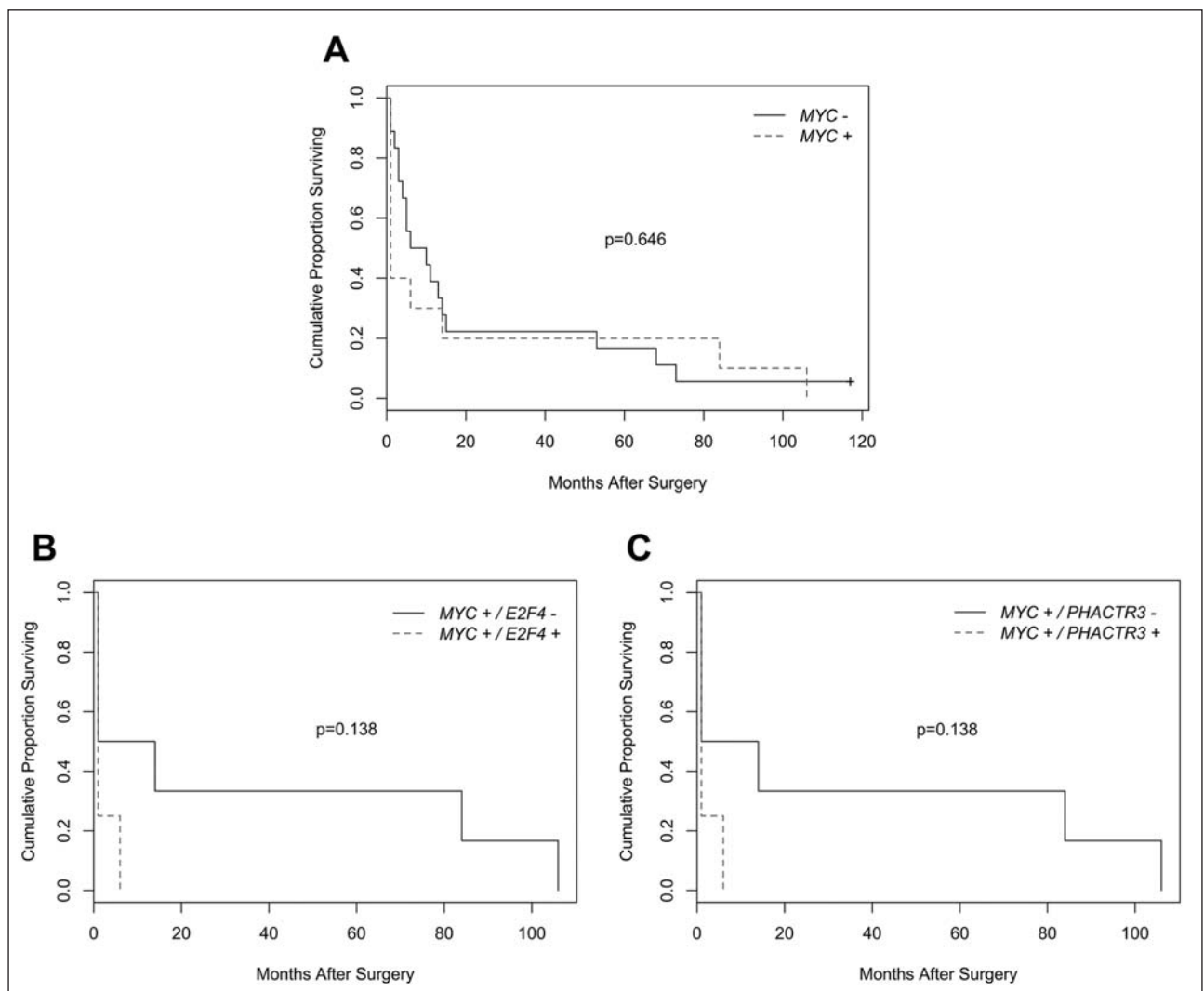
<sup>a</sup>NP, number of patients per group; <sup>b</sup>bold indicates statistically significant values,  $p < 0.05$ .

### Effects of gene alterations and MYC overexpression on survival rate

Kaplan-Meier survival curves were analyzed to evaluate the effect of MYC gene overexpression in the presence of aforementioned gene alterations on NSCLC patients' survival. When individually examined, MYC overexpression did not significantly affect the survival rate (Figure 2A;  $p=0.646$ ). However, when considered in combination with altered *PHACTR3* and *E2F4*, MYC overexpression showed tendency towards shorter survival although did not reach statistical significance. The median survival time was 1 month for patients with overexpressed MYC and altered *PHACTR3* or *E2F4* compared to 7.5 months for those with overexpressed MYC only (Figure 2B and C;  $p=0.138$ ).

### Discussion

C-Myc is one of the most important cellular oncogenes necessary for malignant transformation (17). Deregulation of its expression is critical for the pathogenesis of various cancer types, including lung cancer (5). C-Myc protein is overexpressed in 40–75% of NSCLC patients (6–9). However, data obtained by RT-PCR are not consistent. Wu et al. have reported only 18% NSCLC cases with MYC overexpression (18), while Hsu and colleagues have found overexpressed MYC in 91% of NSCLC patients' samples (19). In our study, we evaluated MYC gene expression by qRT-PCR analysis in 30 NSCLC patients' samples and observed its overexpression in 37% of cases. This result corresponds to most studies on c-Myc protein expression (7, 20). Moreover, we



**Figure 2** Kaplan-Meier overall survival analyses. No difference in survival was observed between patients with overexpressed (MYC+) and non-overexpressed MYC gene (MYC-) (A). Patients with overexpressed MYC and mutated *E2F4* (MYC+/ *E2F4*+) (B) or *PHACTR3* (MYC+/ *PHACTR3*+) (C) showed trend of decreased survival compared to those with overexpressed MYC only (MYC+/ *E2F4*-, MYC+/ *PHACTR3*-).

have observed for the first time that *MYC* overexpression is a significant characteristic of squamous cell carcinoma subtype rather than adenocarcinoma. *c-Myc* overexpression was previously shown to be more frequently present in squamous cell carcinoma but without statistical significance (9), while Xu et al. (8) reported it to be significant characteristic of adenocarcinoma.

Major focus of this study was to analyze *MYC* gene overexpression in relation to alterations in genes associated with NSCLC pathogenesis (11). Tested gene alterations, *PHACTR3* and *E2F4* were shown to be associated with *MYC* overexpression. Moreover, these alterations significantly coincide with *MYC* overexpression in squamous cell carcinoma. *PHACTR3* (Phosphatase and actin regulator 3/ scaffold-associated PP1 inhibiting protein), also known as scapinin, is a protein phosphatase 1-binding protein associated with nuclear non-chromatin structures (the nuclear matrix or nucleoskeleton) (21). It directly interacts with actin and regulates actin cytoskeleton structures influencing cell spreading and motility (22). At the same time, nuclear F-actin activates transcription of *c-Myc* through Wnt/ $\beta$ -catenin signaling (23). We speculate that actin could be one of potential interconnections between *PHACTR3* alterations and *MYC* overexpression. In our previous study *PHACTR3* was shown to be altered in 6 out of 30 patients, mostly with squamous cell carcinoma (11). Recently, significance of *PHACTR3* as parameter for non-invasive lung cancer diagnosis was evaluated along with other diagnostic and risk biomarkers (24).

Considering interrelationship between *MYC* overexpression and mutational status of *E2F4* the observed positive association is not surprising. *E2F4* is a part of complex involved in repression of *MYC* transcription in response to different signals (25). This transcriptional regulator was shown to be necessary for normal development of lung tissue (26). However, its importance and role in pathogenesis of NSCLC was examined only in our earlier publication (11). In that study, altered *E2F4* was detected in 20% of

patients, predominantly with squamous cell carcinoma.

The influence of *c-Myc* overexpression on NSCLC patient survival is inconclusive. Some authors showed *c-Myc* association with shorter survival (9), while others reported no effects on NSCLC prognosis (7). We observed no significant difference in life span between NSCLC patients with overexpressed and non-overexpressed *MYC* gene. However, the presence of altered *PHACTR3* or *E2F4* along with overexpressed *MYC* contributed to shorter survival but without prognostic significance. Considering our previous results this joint effect of *MYC* and altered genes could be mainly attributed to the significant negative effects of altered *PHACTR3* and *E2F4* genes on NSCLC patients' survival (11).

Overall this study implicates frequent presence of overexpressed *MYC* gene in NSCLC patients and its applicative potential as molecular marker of squamous cell carcinoma. Additionally, the results point out the relevance of overexpressed *MYC* in association with altered *PHACTR3* and *E2F4* genes as joint molecular markers of NSCLC and its specific subtypes. Most importantly, we identified positive associations between *MYC* overexpression and aforementioned gene alterations not previously considered in NSCLC nor other cancer types, therefore opening a novel research perspective in studying *c-Myc* role in carcinogenesis. In this context, functional interconnection of *MYC* and altered genes will be of particular interest in our further studies.

**Acknowledgements:** This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No III41031).

### Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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Received: April 22, 2018

Accepted: June 2, 2018