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Original article

The impact of TP53 and PTEN tumor suppressor genes on response to different breast cancer treatment modalities

Nikola Tanić[1], Tatjana Dramićanin[2], Nejla Ademović[1], Tijana Tomić[2], Blagoje Murganić[2], Zorka Milovanović[3], Milica Nedeljković[3], Nasta Tanić[2,4]

[1]University of Belgrade, Institute for Biological Research "Siniša Stanković", National Institute of Republic of Serbia, Department of Neurobiology, Belgrade, Serbia

[2]University of Belgrade, Institute of Nuclear Sciences "Vinča", National Institute of Republic of Serbia, Department of Radiobiology and Molecular Genetics, Vinča, Serbia

[3]Institute of Oncology and Radiology of Serbia, Department of Pathology, Department of Experimental Oncology, Belgrade, Serbia

[4]State University of Novi Pazar, Department of Natural and Mathematical Sciences, Study Program of Biology, Novi Pazar, Serbia

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Corresponding author:
Nikola Tanić, MD, PhD
Bulevar despota Stefana 142,
11060 Beograd
nikolata@ibiss.bg.ac.rs

Summary

Introduction. Breast cancer (BC) is the most frequent type of malignancy and the leading cause of cancer related death among women worldwide. BC is exceptionally heterogeneous disease and therefore distinct treatment modalities are necessary to address these differences. The aim of our study was to investigate the impact of TP53 and PTEN tumor suppressor genes (TSGs) inactivation on BC response to different treatment modalities and their possible cooperation, on post-operative BC samples.

Methods. Patients were classified, based on applied adjuvant therapy, into four distinct groups: those that received hormonal therapy (HT) only, hormonal therapy combined with chemotherapy (HT/CHT), hormonal therapy combined with chemo and biological therapy (HT/CHT/H), and other systemic therapies that exclude HT. Functional inactivation of TP53 and PTEN TSG's were studied by mutation, loss of heterozygosity (LOH) and hypermethylation analysis.

Results. Our results revealed that TP53 gene was altered in 63 out of 90 specimens (70%), while the frequency of PTEN alterations was slightly lower, 54 out of 90 (60%). Simultaneous inactivation was detected in 43 tested samples (48%) with significant association between two analyzed TSGs. Further, we found that TP53 status has significant influence on patients' therapy response. Contrary to this, no significance was found between mutational status of PTEN and various treatment modalities. However, significant association was found between the type of applied therapy and simultaneous alterations of these two TSGs ($p = 0.00001$).

Conclusion. Patients with wtTP53 show significantly better therapy response regardless of the type of therapy, compared to carriers of altered TPp53 gene.

Keywords: breast cancer, p53, PTEN, adjuvant therapy

Introduction

Breast cancer (BC) is the most frequent type of malignancy and the leading cause of cancer related death among women worldwide [1]. More than 70% of all diagnosed invasive BCs express steroid receptors and, as such, are subjected to endocrine therapy [2]. Steroid receptor positive BC is not a single disease, rather, it encompasses several entities with significant differences in clinical course [2]. Distinct treatment modalities are necessary to address these differences especially since BCs often develop endocrine therapy resistance. Therefore, endocrine therapy is often combined with other types of systemic adjuvant therapies - chemotherapy and/or targeted biological therapy (trastuzumab and more recently mTOR and cyclin-dependent kinase 4/6 inhibitors) [3–5]. Whether the management of endocrine responsive BCs will use combined treatment strategies or not, depends on clinical, pathohistological and molecular characteristics of the tumor including lymph node invasion, tumor size, human epidermal growth factor receptor 2 (HER2) status, molecular subtype etc. Numerous factors and their interplay determine BC response to therapy and clinical outcome.

Tumor suppressor genes have a vital role in inhibiting neoplastic transformation. Among so far studied, TP53 (p53) and phosphatase and tensin homologue (PTEN) are the most frequently altered in human cancers [6, 7]. TP53 and PTEN inactivation may occur either through mutation, allelic losses, promoter hypermethylation, non-coding RNA-associated gene silencing, protein sequestration or due to alterations of the genes involved in their regulation [8–11]. p53 is activated in response to cellular stress and has a central role in an immensely complex anti-proliferative network that incorporates numerous biological processes including apoptosis, senescence, cell cycle regulation, differentiation, DNA repair, metabolism, angiogenesis and immune response [7, 12]. Mutations are a frequent mechanism of TP53 inactivation and are identified in about 30% of steroid receptor positive BCs [13]. TP53 mutations not only abrogate p53 protein's tumor suppressor role but may give rise to new (gain-of-function) capabilities that promote tumorigenesis and progression of BC [11]. Mutations in one TP53 allele are commonly accompanied by the loss of the wtTP53 allele (loss of heterozygosity (LOH)) [14]. It seems that during tumor progression, there is a strong selective pressure for TP53 LOH [14]. According to Silwal-Pandit et. al, TP53 LOH was detected in 81% of BCs with one mutated allele, and in up to 52% of steroid receptor positive BCs with wtTP53 [15]. Estrogen receptor alpha (ERα) is highly expressed in steroid receptor positive BC and associated with tumor initiation and growth. ERα and p53 engage in a complex interplay of mutual regulation [16]. There is a positive feedback loop between ERα and p53 – they enhance each other's transcription [16]. Moreover, ERα can regulate p53 on post-transcriptional level and directly interact with p53 to modulate its function [16]. ERα stabilizes p53 by blocking MDM2 inhibition of p53, but, on the other hand, prevents p53 induced apoptosis, blocs the transactivation of the p21 promoter etc. [16]. ERα clearly has a dual role in regards to p53. The fate of BC may depend on the fine balance between ERα and p53.

Phosphoinositide-3 kinase (PI3K) / AKT-/ mammalian target of rapamycin (mTOR) signaling pathway is a crucial mechanism that stimulates cellular survival, growth, proliferation and migration [17]. Activation of PI3K/AKT/mTOR pathway has been associated with initiation and progression of numerous malignancies including BC [18]. PI3K is frequently hyperactivated in steroid receptor positive BC due to PIK3CA mutations [19]. The main 'brake' is the tumor suppressor PTEN which negatively regulates the pathway and attenuates PI3K activation [17]. LOH is the most common mechanism by which

PTEN function is lost in steroid receptor positive BC [20]. Unsurprisingly, PTEN loss was associated with poor outcome and resistance to endocrine and chemotherapy in BC [20,21]. There is ample evidence of PTEN/p53 interaction and complex crosstalk [22]. p53 was shown to stimulate PTEN transcription and PTEN to enhance p53 stability [22]. The loss of PTEN and TP53 may have a synergic effect in tumor promotion [22].

In the present study, we aimed to investigate the impact of TP53 and PTEN inactivation on the BC response to different treatment modalities as well as their possible cooperation.

Methods

This study was performed on 90 invasive breast cancer (BC) and corresponding normal tissue samples collected after surgery, from the Institute of Oncology and Radiology of Serbia, in a period between 1988 and 2013. The age of patients ranged from 29 to 78 year's. Diagnoses of BC's and hormonal status, histological grade and regional lymph node involvement have been determined after hematoxylin-eosin staining. The most of analyzed samples were steroid receptor-positive (94.5%) and classified as invasive ductal, 53 out of 90 (58.9%) or invasive lobular, 37 out of 90 breast carcinomas (41.1%). All relevant clinical and patohistological parameters

Table 1. Clinical and histopathological characteristics of samples

Parameters		Values
Age at onset, years (mean)		29 – 78 (59)
Follow-up, months (mean)		11 – 228 (80)
Number of patients (%)		90 (100%)
Type of Breast Carcinoma	Invasive Ductal (IDC)	53 (58.9%)
	Invasive Lobular (ILC)	37 (41.1%)
Histological grade	Grade 1	5 (5.6%)
	Grade 2	74 (82.2%)
	Grade 3	11 (12.2%)
Steroid receptor status	ER+/PR+	68 (75.6%)
	ER+/PR-	17 (18.9%)
	ER-/PR-	5 (5.5%)
Lymph node status	Negative (N0)	24 (26.7%)
	Positive (N1)	51 (56.7%)
	Positive (N2)	10 (11.1%)
	Positive (N3)	5 (5.5%)
Distant metastases	Present	30 (33.3%)
	Absent	60 (66.7%)
Type of therapy	HT only	56 (62.2%)
	HT/CHT	23 (25.6%)
	HT/CHT/H	4 (4.4%)
	Other Th	7 (7.8%)
Severity of malignancy	Mild	41 (45.6%)
	Severe	49 (54.4%)

ER - Estrogene receptor; ***PR*** - Progesterone receptor; ***HT*** - hormonal therapy; ***HT/CHT*** - hormonal therapy combined with chemotherapy; ***HT/CHT/H*** - hormonal therapy combined with chemo and biological therapy; Other ***Th*** - other systemic therapies that exclude hormonal therapy

(age, tumor type, pTNM stage, steroid receptor status, type of therapy, histological grade) were retrieved from patient's medical records and summarized in table 1.

The patients were classified, based on applied adjuvant therapy, into four distinct groups: those that received hormonal therapy (HT) only, hormonal therapy combined with chemotherapy (HT/CHT), hormonal therapy combined with chemo and biological therapy (HT/CHT/H), and other systemic therapies that exclude HT, for example CHT or H.

Functional inactivation of TP53 and PTEN TSG's by mutations, loss of heterozygosity (LOH) and hypermethylation have been determined on genomic DNA extracted from paired samples of tumor and adjacent normal tissue.

Genomic DNA was extracted using phenol/chloroform/isoamylalcohol precipitation protocol [23]. The quality, concentration and purity of genomic DNA was verified by electrophoresis and spectrophotometry (NanoDrop Technologies, Wilmington, DE, USA). Isolated and purified DNA was stored at +40C until further analyzes.

Loss of heterozygosity (LOH analyses), was performed by fragment analysis with two sets of highly polymorphic microsatellite markers chosen to cover loci where TP53 and PTEN genes mapped at 17p13 and 10q23, respectively. Microsatellite markers used in this study were selected according to the official criteria of heterozygosity, i.e. heterozygosity greater than 0.7 in different human populations. The choice of microsatellite markers and locus-specific PCR conditions were taken from published sources [24–25]. Forward primers for both sets of selected markers were 5'-labeled with fluorescent dyes.

The set for LOH analyzes of TP53 included following markers: Fam labeled TP53 pentanucleotide, PET labeled TP53 dinucleotide, Ned labeled D17S1537 and D17S786 labeled with Vic. Another set of five polymorphic microsatellite markers selected to cover deletions at the whole PTEN locus included: D10S579, D10S1765, D10S215, and D10S541, labeled with Fam and AFM086wg9 which was labeled with PET dye.

Locus specific amplicons, mixed with HiDi formammide and GeneScan-500 LIZ Size Standard, were separated by capillary array electrophoresis on an ABI Prizm 3130 automated sequencer (Applied Biosystems). Subsequently, collected data were analyzed with GenneMapper software (Applied Biosystems). Each analyzed tumor specimen had its own reference, i.e. DNA isolated from normal breast tissue of the same patient was used as a control. The DNA from normal breast tissue adjacent to tumors of the same patient was used as reference, ie each analyzed tumor sample had its own control.

The occurrence of only one peak in the reference, referred that selected marker was uninformative (homozygous). Opposite, a marker was considered informative when two allelic peaks were identified in a control specimen (heterozygous). To determine allelic imbalance we compered (for all informative cases) peak high ratios of microsatellite allels between normal and tumor tissue of the same patient and calculated it automatically by GeneMapper software using the following formula: (peak height of normal allele 2)/(peak height of normal allele 1) divided by (peak height of tumor allele 2)/(peak height of tumor allele 1). This procedure has been done for all informative cases. A sample was defined as an LOH candidate for particular locus if the ratio values were less than 0.66 and higher than 1.5. When the ratio values were less than 0.66 and higher than 1.5, a sample was considered to be an LOH candidate for particular locus.

Frequently mutated exons of the TP53 gene (5–9) were amplified by PCR and screened for mutations using PCR–single-stranded conformational polymorphism (PCR-SSCP) analysis, according to Orita et al [26]. Amplimers and PCR conditions were described in Sakai et al [27]. In order to avoid false positive and/or false negative results, all samples were ex-

amined for the presence of mutations from at least 3 independent PCR amplifications and under at least two different experimental SSCP conditions [28–29]. The DNA isolated from the blood of healthy individuals was used as a negative control.

In order to confirm the results obtained by PCR-SSCP, mutated samples were subjected to sequencing. Sequences were determined with Applied Biosystems Incorporated dye terminator sequencing kit according to the manufacturer's instructions on an ABI Prism 3130 automated sequencer (Applied Biosystems, Foster City, Calif).

The methylation status of PTEN tumor suppressor gene was determined by methylation-specific PCR (MSP). The genomic DNA extracted from breast tumor tissues were modified by sodium bisulfite treatment according to procedure described by Herman et al [30]. In this study, two different sets of primers (set I and set III) were used for MSP reactions [31]. Both set of primers were created to avoid PTEN pseudogene amplification. Commercially available, Unmethylated and CpG Methylated Human Male genomic DNA (Thermo ScientificTM) served as positive control.

The comparison of TP53 and/or PTEN functional inactivation (by mutations, LOH and/or hypermethilation), type of subjected therapy and relevant patohistological parameters, with each other and with the survival (disease free survival, overall survival and breast cancer specific survival) were performed by univariate and multivariate analysis using the Cox proportional hazards model and the Kaplan-Meier test. The level of significance was set at 0.05.

Results

This study included 90 women with breast cancer, classified as invasive ductal (53/90) and invasive lobular (37/90) breast carcinomas. Clinical and histopathological characteristics of examined breast cancer specimens are summarized in table 1. Study included seventy-nine postmenopausal and eleven premenopausal women, most of whom were steroid receptor (ER and/or PR) positive (94.5%). The age at disease onset ranged from 29 to 78 years (mean 59), while the mean overall survival was 80 months (11–228 months). The specimens were further stratified into mild or severe group depending on disease severity. Histological grade, TNM status and tumor type were used as criteria for this distribution. Patients were subjected to different systemic adjuvant therapy depending on steroid receptor status and histopathological and clinical criteria:

- hormonal therapy only (HT)
- hormonal therapy combined with chemotherapy (HT/CHT)
- hormonal therapy combined with chemo and biological therapy (HT/CHT/H)
- other systemic therapies that exclude HT, for example CHT or H only.

Tamoxifen (TAM) was the drug of choice among hormonally treated patients in almost all cases. Namely, only one patient out of fifty-six received anastazol - aromatase inhibitor, while all the others were tamoxifen treated. On the other side, patients from the second therapy group whose treatment was based on chemotherapy (HT/CHT), received CMF (Cyclophosphamide Methotrexate Fluorouracil), FAC (5-Fluorouracil, Doxorubicin, Cyclophosphamide), Taxotere, EC (Epirubicin and Cyclophosphamide) or any combination of listed drugs in addition to TAM.

To evaluate efficiency of different treatment regimens on the overall survival, Kaplan-Meier survival curves were generated. According to obtained results, survival of patients who underwent hormonal therapy only, was significantly longer (Figure 1) then the survival of those treated with other therapy combinations. The greatest statistical significance in overall survival was detected between HT and HT/CHT therapy groups

(Figure 1). Further analyses (Cox regression) confirmed obtained results, suggesting that patients receiving hormone therapy had at least 3 times greater survival rates compared to patients on other therapies (Table 2).

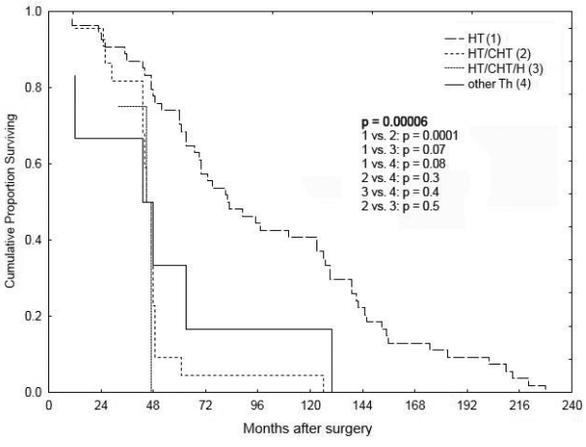

Figure 1. Kaplan-Meier survival curves for different treatment regimens

Women treated with hormonal therapy only (HT), lived significantly longer compared to other three therapy groups: HT/CHT - hormonal therapy combined with chemotherapy; HT/CHT/H - hormonal therapy combined with chemo and herceptin; other TH - therapies that exclude tamoxifen. Survival rate was considered significantly different if $p < 0.05$.

To determine the potential influence of tumor-suppressor genes (TSGs) on the response to therapy we analyzed alterations of TP53 and PTEN genes in 90 breast tumor specimens. Namely, functional inactivation of TP53 by mutations and/or loss of heterozygosity and PTEN by loss of heterozygosity and/or promoter hypermethylation, were tested. As a result, altered TP53 gene was found in 63 out of 90 specimens (70%), while the frequency of PTEN alterations was slightly lower, 54 out of 90 (60%) patients had inactivated PTEN. At the same time, simultaneous inactivation of both TSGs was detected in 43 out of 90 tested (48%). Alterations in either one of tested TSGs were found in 31 out of 90 (34%), while 16 out of 90 (18%) had no alterations at all. Statistical analyses showed significant association of TP53 alterations with malignancy type and disease severity (Table 3). An 11-point severity scale was used to rate the severity of 16 symptoms: alopecia, anxiety, poor appetite, constipation, cough, depression, diarrhea, dry mouth, dyspnea, fatigue, nausea/vomiting, pain, peripheral neuropathy, difficulty remembering, sleep disturbances, and weakness.

Table 2. The influence of therapy type on survival rates of persons with breast malignancy with respect to other forms of treatment

Type of therapy	Cox Hazard Ratio	Significance (p value)	CI (95%)
HT vs. Oth	0.07	*p<0.05*	0.004 – 1.07
HT/CHT vs. Oth	1.33	*p>0.05*	0.12 – 14.70
HT/CHT/H vs. Oth	4.27	*p=0.057*	0.95 – 19.10
HT vs. HT/CHT	0.29	*p<0.001*	0.16 – 0.51
HT/CHT vs. HT/CHT/H	0.60	*p>0.05*	0.20 – 1.78
HT vs. HT/CHT/H	0.17	*p=0.002*	0.06 – 0.52

H - hormone therapy, HT/CHT - Hormone combined with chemo therapy, HT/CHT/H - Hormone combined with chemo and biological therapy, Oth - Other types of therapy

Further, Spearman's correlations revealed significant association between two analyzed TSGs (Table 3). Specifically, inactivation of PTEN was significantly more often detected in tumors with altered TP53. In addition to this, we have shown that alterations of at least one of analyzed TSGs occur more frequently in samples with severe disease status. On the other hand, wild type forms of both genes are significantly more frequent in mild disease form (Figure 2).

Survival analyses showed that TP53 alterations, as well TP53/PTEN co-alterations significantly decrease patients' survival times.

According to generated Kaplan-Meier survival curves, patients with altered TP53 gene, lived significantly shorter (p = 0.00074; Figure 3A) when compared to those with wild type (wt) gene. Survival analyses also suggest that PTEN aberrations have no influence on patients' survival rates (p = 0.7; Figure 3B) while co-alterations with p53 have (p = 0.03; Figure 3C). In other words, the survival of patients with both tumor suppressors altered was significantly shorter than the survival of those with wt genes (p = 0.024; Figure 3C).

To examine whether the outcome of different therapeutic treatments depend on inactivation of studied TSGs (separate or simultaneous), survival analyses have been done. According to our results, TP53 status has significant influence on patients' therapy response. Patients with wild type TP53 show significantly better therapy response regardless of type of therapy, compared to carriers of altered TP53 gene (Figure 3A).

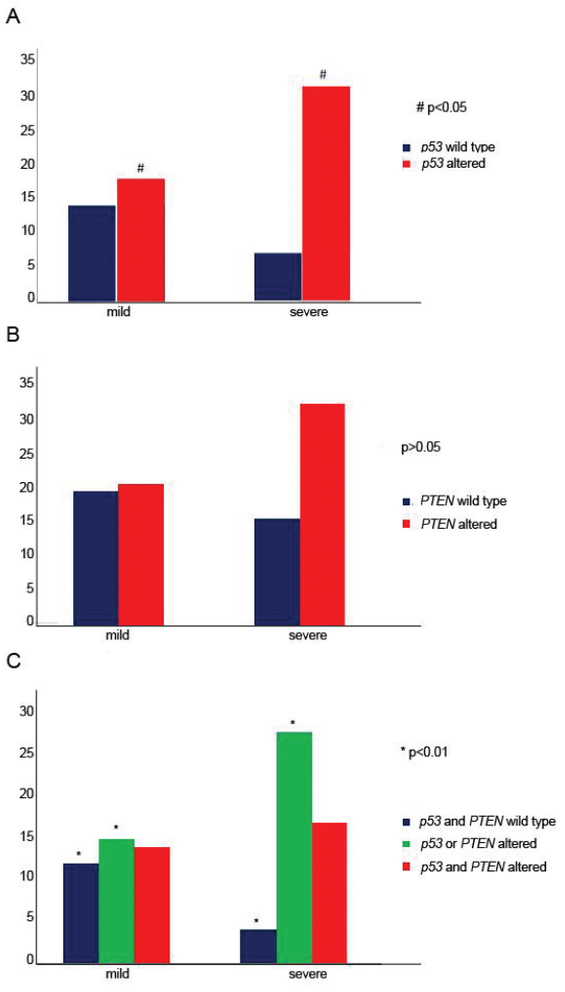

Figure 2. Distribution of genetic alterations upon disease severity

(A) TP53 alterations are significantly more frequent in patients with severe disease. (B) Alterations of PTEN are also more frequent in severe disease form, although without statistical significance. (C) Wild type forms of both genes (TP53 and PTEN) are significantly more frequent in mild disease form ($p < 0.01$).

Table 3. Intercovariate Spearman's correlations

Bivariate Spearman's correlation	Spearman's coefficient	Significance (p value)
p53 alterations/PTEN alterations	0.26	p<0.05
p53 alterations/Malignancy type	0.21	p<0.05
p53 alterations/Malignancy grade	0.11	p>0.05
p53 alterations/Severity of malignancy	0.29	p<0.01
PTEN alterations/Malignancy type	-0.02	p>0.05
PTEN alterations/Malignancy grade	0.03	p>0.05
PTEN alterations/Severity of malignancy	0.16	p>0.05

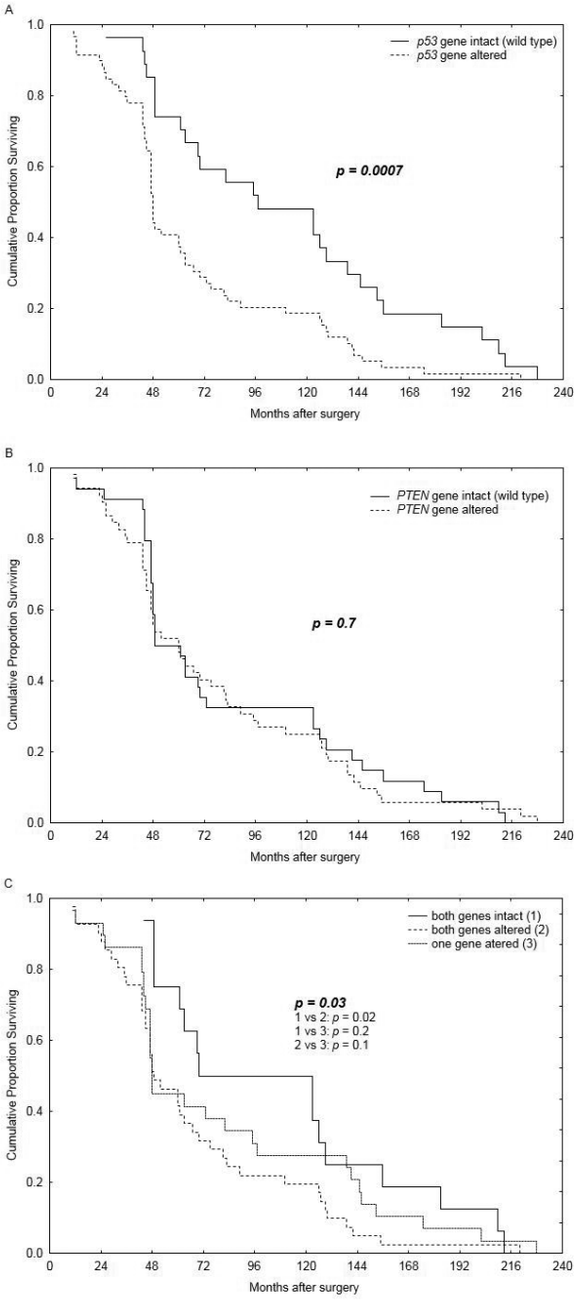

Figure 3. Kaplan-Meier survival curves

The impact of (A) TP53 alterations, (B) PTEN alterations and (C) simultaneous TP53/PTEN gene co-alterations on patients' survival rate is shown. Survival rate was considered significantly different if $p < 0.05$.

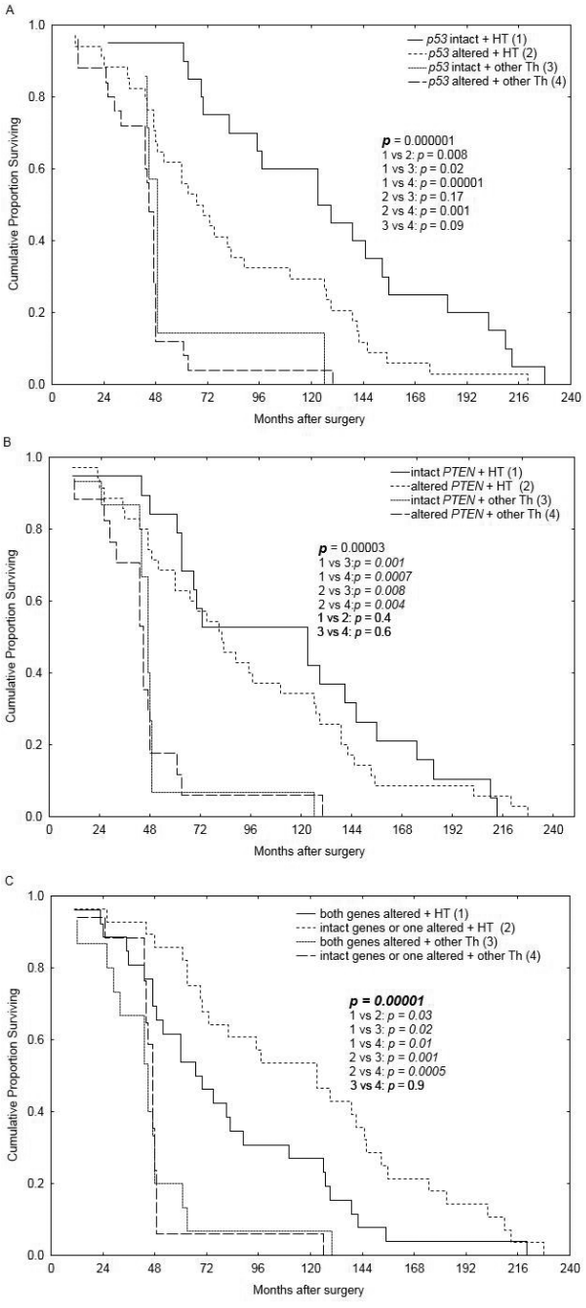

Figure 4. Kaplan-Meier survival curves of patients treated with tamoxifen and other TH combinations in relation to mutational status of (A) TP53, (B) PTEN and (C) both TSGs.

Survival rate was considered significantly different if $p \leq 0.05$.

In support of this we have shown that hormonally treated women with intact (wt) TP53 gene had significantly longer survival rate (p = 0.000001; Figure 4A) when compared to: (i) hormonally treated women with aberrant TP53 gene, (ii) women with intact (wt) p53 subjected to any of remaining three therapy combinations (HT/CHT or HT/CHT/H or therapy that exclude HT), and (iii) women with altered TP53 that belong to second (HT/CHT), third (HT/CHT/H) or forth (systemic Th that exclude HT) therapy group. Moreover, it appeared that even those with altered TP53 gene if treated with tamoxifen only, lived significantly longer than those treated with other therapy combinations (Figure 4A, 2 vs 4, p = 0.001).

Contrary to this, no significance was found between mutational status of PTEN and treatment with tamoxifen as the main HT drug (Figure 4B, 1 vs 2, p = 0.4). Generally speaking, survival rate of HT-treated patients was almost the same regardless of PTEN status. Finally, survival rate does not depend on the mutational status of PTEN gene but it does depend on the type of subjected therapy, herein on HT-only (p = 0.00003), as shown in figure 3B and figure 4B.

In addition, the joint effect of TP53 and PTEN alterations on overall survival of breast cancer patients subjected to four different treatment regimens was also analyzed. According to generated survival curves, significant association between the type of applied therapy and simultaneous alterations of two most commonly altered TSGs in human cancers, does exist (p = 0.00001; Figure 4C). Namely, we found that women who received tamoxifen only and who had both TSGs altered lived significantly shorter than those on HT therapy with both or at least one tumor suppressor intact (Figure 4C, 1 vs 2 p = 0.03).

Finally, HT treated patients with both genes altered lived significantly longer compared to patients on other therapy regiments regardless of their TSGs status. (Figure 4C, 1 vs. 3, p = 0.02; 1 vs 4, p = 0.01).

Discussion

Tumor suppressor genes are, in general, regarded as autonomous anti-cancer genes/proteins. However, at the molecular level, autonomy of genes/proteins appears to be a remote concept since gene expression and protein function are regulated through different cell networks, sinergistic and antagonistic ones. Therefore, linking the action of tumor suppressors may be the key to understanding and predicting their role in tumorigenesis and response to various treatment regiments. TP53 and PTEN are two most highly mutated tumor suppressors in human cancers and it is tempting to speculate that they cooperate in tumor suppression, specifically when having in mind that PTEN has been attributed to the cytoplasm while the site of action of p53 is associated with the nucleus. Consequently, the aim of our study was to investigate the impact of TP53 and PTEN inactivation on the BC response to different treatment modalities as well as their possible cooperation.

Our results reviled that TP53 mutational status has significant influence on patients' response to therapy. Namely, patients with wild type TP53 show significantly better therapy response regardless of type of therapy, compared to carriers of mutated p53 gene. This finding is expected and in concordance with some previous reports [11]. However, some recent studies reviled that patients with mutant TP53 response better to therapy, specifically to chemotherapy, due to lack of arrest in „mutant" tumors, tumors that carry mutated TP53, which results in aberrant mitoses, cell death and a superior clinical response [32]. We cannot agree less with these findings because our study unambiguously shows the opposite.

Contrary to this, we did not establish any significance between mutational status of PTEN gene alone and various treatment modalities, although the trend is unequivocal. Apparently, this contradicts our previous findings on the role of the PTEN gene in the

resistance of hormone-positive breast tumors to tamoxifen [20]. We have few explanations of this contradictory. First, we think that PTEN is primarily responsible for acquired resistance, not inherent one, and will be inactivated during therapy. Secondly, and maybe more important, PTEN and p53 crossreact. Namely, quite neglected study of Freeman et al [33] showed that there is a crosstalk between PTEN and p53 tumor suppressors and that PTEN could regulate the function of WT p53 by both phosphatase-dependent and –independent mechanisms, but not mutationally altered p53. Therefore, altered p53 gets all the credits for bad response to therapy, which our results support.

The question is, what happens if p53 and PTEN are simultaneously inactivated? According to our results that is the worst scenario. We showed that patients with simultaneous inactivation of these two tumor suppressors develop resistance to all therapy regiments and live dramatically shorter compared to patients with only one gene altered or patients with WT tumor suppressor genes. Therefore, we suggest that mutational screening of TP53 and PTEN genes should be done previous to describing therapy regiment and, in case of observed alterations in either of these genes, particularly in both, therapy should be designed to target both PTEN and p53 or their controlled pathways.

Conclusion

Patients with wtTP53/wt*PTEN* showed significantly better therapy response regardless of the type of therapy, compared to carriers of altered *TP53*/*PTEN*. Patients with simultaneous inactivation of these two tumor suppressors develop resistance to all therapy regiments and live dramatically shorter compared to all other patients. Analysis of mutational status of *TP53* and *PTEN* is a prerequisite to the decision of therapy regiment and, in case of observed alterations in either of these genes, particularly in both, therapy should be designed to target both *PTEN* and p53, or their controlled pathways.

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Ethical approval. The Ethics Committee of the Institute for Oncology and Radiology, Belgrade, Republic of Serbia, number 4321-01, approved the study and informed consent was obtained from all individual respondents. The research was conducted according to the Declaration of Helsinki.

Conflicts of interest. The authors declare no conflict of interest.

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Uticaj tumor supresorskih gena TP53 i PTEN na odgovor na različite načine lečenja raka dojke

Nikola Tanić[1], Tatjana Dramićanin[2], Nejla Ademović[1], Tijana Tomić[2], Blagoje Murganić[2], Zorka Milovanović[3], Milica Nedeljković[3], Nasta Tanić[2,4]

[1]Univerzitet u Beogradu, Institut za biološka istraživanja „Siniša Stanković", Nacionalni institut Republike Srbije, Odeljenje za neurobiologiju, Beograd, Srbija

[2]Univerzitet u Beogradu, Institut za nuklearne nauke „Vinča", Nacionalni institut Republike Srbije, Odeljenje za radiobiologiju i molekularnu genetiku, Vinča, Srbija

[3]Institut za onkologiju i radiologiju Srbije, Odeljenje za patologiju, Odeljenje za eksperimentalnu onkologiju, Beograd, Srbija

[4]Državni univerzitet u Novom Pazaru, Odeljenje za prirodno-matematičke nauke, Studijski program Biologija, Novi Pazar, Srbija

Uvod. Rak dojke (RD) je najčešći tip maligniteta i vodeći uzrok smrti od raka kod žena širom sveta. RD je izuzetno heterogena bolest i stoga su neophodni različiti modaliteti lečenja da bi se pokrile ove razlike. Cilj našeg istraživanja je bio da se ispita uticaj inaktivacije TP53 i PTEN tumor supresorskih gena (TSG) na odgovor RD na različite modalitete lečenja, kao i njihova moguća saradnja u tome, na postoperativnim uzorcima RD.

Metode. Pacijentkinje su klasifikovane, na osnovu primenjene adjuvantne terapije, u četiri različite grupe: one koje su primale samo hormonsku terapiju (HT), hormonsku terapiju u kombinaciji sa hemoterapijom (HT/CHT), hormonsku terapiju u kombinaciji sa hemoterapijom i biološkom terapijom (HT/CHT/H) i druge sistemske terapije koje isključuju HT. Funkcionalna inaktivacija TP53 i PTEN TSG je proučavana analizom mutacionog statusa, gubitka heterozigotnosti (LOH) i metilacionog statusa.

Rezultati. Naši rezultati su pokazali da je TP53 gen izmenjen kod 63 od 90 pacijenata (70%), dok je učestalost promena PTEN gena bila nešto niža, 54 od 90 (60%). Simultana inaktivacija je detektovana u 43 testirana uzorka (48%) sa značajnom povezanošću između dva analizirana TSG-a. Dalje, pokazali smo da status TP53 ima značajan uticaj na odgovor pacijenata na terapiju. Suprotno ovome, nismo pokazali značajnu asocijaciju između mutacionog statusa PTEN-a i različitih modaliteta lečenja. Međutim, utvrđena je značajna povezanost između primenjenih terapija i simultanih inaktivacija ova dva TSG-a ($p = 0,00001$).

Zaključak. Pacijenti sa wtTP53 pokazuju značajno bolji terapijski odgovor bez obzira na vrstu terapije u poređenju sa nosiocima mutiranog TP53 gena.

Ključne reči: rak dojke, p53, PTEN, adjuvantna terapija