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Correlation of local and systemic expression of survivin with histopathological parameters of cutaneous melanoma

Korelacija lokalne i sistemske ekspresije survivina sa patohistološkim parametrima melanoma kože

Milena Jović*, Snežana Cerović*†, Lidija Zolotarevska*†, Milomir Gačević†‡, Ivan Stanojević†§, Karolina Miller‖, Mirjana Djukić¶, Luciano Saso, Ljiljana Jauković† ††, Danilo Vojvodić†§**

***Institute for Pathology and Forensic Medicine, ‡Clinic for Plastic Surgery and Burns, §Institute for Medical Research, ††Institute for Nuclear Medicine, Military Medical Academy, Belgrade, Serbia; †Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia; ‖Dorset County Hospital Foundation Trust, Dorchester, United Kingdom; ¶Department of Toxicology, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia; **Department of Physiology and Pharmacology, Sapienza University, Rome, Italy**

Abstract

Background/Aim. Survivin is a multifunctional protein abundantly expressed in tumors of various types, including melanoma. There are still sparse data regarding relationship of melanoma cell survivin expression with accepted histopathological characteristics as well as serum concentration. The aim of this study was to investigate the association of local tumor survivin expression (primary tumor and metastatic lesions) and serum concentration with clinical and histopathological parameters in melanoma patients. **Methods.** The level of survivin expression was determined immunocytochemically in tumor tissue and with ELISA test in the serum of 84 melanoma patients diagnosed from 2009 to 2013 at the Institute for Pathology and Forensic Medicine and Institute for Medical Research at Military Medical Academy, Belgrade, Serbia. **Results.** The intensity of survivin expression was significantly higher in the patients whose tumor had ulceration, higher mitotic index, higher Clark and Breslow stage, that made vascular invasion or spread through lymphatic vessels in primary tumor, and was significantly higher in the patients with metastatic disease. Survivin expression and the number of survivin positive cells in metastatic lesions were significantly associated with the duration of disease free interval (DFI). The patients with high expression score had almost double shorter DFI comparing to those with weak local survivin expression and a small number of survivin+ cells (9 ± 7 *vs* 19 ± 13 months, respectively). The degree of tumor infiltrating lymphocytes presence in tumor tissue was significantly associated with serum survivin concentration, with lowest average level detected in samples of patients with the highest degree of infiltration. Serum survivin concentrations were highest in samples of melanoma patients with IA American Joint Commission on Cancer (AJCC) clinical stage, pT1a histological stage, patients whose tumors were still in horizontal growth phase, without signs of lympho-hematological disease spreading, with the highest number of mitoses and the smallest Clark index. **Conclusion.** Survivin expression in tumor tissue and its serum concetration significantly correlate with clinical and histopathological parameters. Serum levels could be important in initial follow-up as indicators of those patients that would have aggressive local tumor growth and spreading. Survivin determination in tumor tissue is of great significance in estimation of DFI.

Key words:
neoplasm proteins; biological markers; melanoma; histology; immunohistochemistry; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Survivin je multifunkcionalni protein bogato ispoljen u tumorima različite vrste, uključujući i melanom. Retki su radovi koji opisuju odnos ispoljavanja survivina u melanomskim ćelijama sa njegovom serumskom koncentracijom kao i sa histopatološkim karakteristikama melanoma. Cilj rada bio je da se ispita udruženost lokalne ekspresije survivina u tumoru (primarni tumor i metastatske promene) i serumske koncentracije sa kliničkim i histopatološkim parametrima kod bolesnika sa melanomom. **Metode.** Nivo ekspresije survivina određivan je imunocitohistohemijski u

Correspondence to: Milena Jović, Institute for Pathology and Forensic Medicine, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: alhemastro@gmail.com

tumorskom tkivu i ELISA testom u serumu 84 bolesnika sa melanomom, dijagnostikovanih u periodu od 2009. do 2013. na Institutu za patologiju i sudsku medicinu i Institutu za medicinska istraživanja na Vojnomedicinskoj akademiji, Beograd, Srbija. **Rezultati.** Intezitet ekspresije survivina bio je značajno veći kod bolesnika čiji su tumori bili ulcerisani, sa visokim mitotskim indeksom, visokim Clark i Breslow indeksom, sa prisutnom vaskularnom i limfnom invazijom, kao i kod onih sa metastatskom bolesti. Ispoljavanje survivina i broj survivin pozitivnih ćelija u metastatskim lezijama bio je značajno udružen sa trajanjem intervala bez bolesti (*disease free interval* – DFI). Bolesnici sa visokim skorom ekspresije imali su skoro dvostruko kraći DFI u odnosu na one sa slabom lokalnom ekspresijom survivina i malim brojem survivin pozitivnih ćelija (9 ± 7 *vs* 19 ± 13 meseci). Stepen prisustva tumor infiltrišućih limfocita u tumorskom tkivu bio je značajno udružen sa koncetracijom survivina u serumu, sa najnižim prosečnim vrednostima detektovanim u uzorcima bolesnika sa najvećim stepenom infiltracije. Serumske koncentracije survivina bile su najveće u uzorcima bolesnika sa melanomom IA kliničkog stadijuma *American Joint Commission on Cancer* (AJCC), pT1a histološkog stadijuma, bolesnika čiji su tumori bili u horizontalnoj fazi rasta, bez znakova širenja limfohematogenim putem, sa najvećim brojem mitoza i koji su imali najmanji Clark indeks. **Zaključak.** Ekspresija survivina u tumorskom tkivu i njegova serumska koncentracija značajno korelišu sa kliničkim i histopatološkim parametrima melanoma. Serumski nivo može biti važan kao inicijalni indikator kod onih bolesnika koji bi mogli imati agresivan lokalni tumorski rast i širenje. Određivanje survivina u tumorskom tkivu, kako u primarnom tumoru tako i u metastazama, od velikog je značaja u utvrđivanju trajanja DFI.

Ključne reči:
proteini, onkogeni; biološki pokazatelji; melanom; histologija; imunohistohemija; osetljivost i specifičnost.

Introduction

Melanoma is the deadliest form of skin cancer. It is recognized that in humans, the malignant transformation of normal melanocytes into melanoma cells is due to specific genetic predisposition and the influence of environmental factors [1]. Recent studies of the role of survivin in the pathogenesis of malignant tumors were extensive and primarily directed into its role as a biomarker. The latest publications suggest that survivin might have an important role in melanomas.

Survivin is a multifunctional protein with an important role in the inhibition of apoptosis, regulation of mitotic activity and angiogenesis. External and intrinsic pathways of apoptotic signals are interrelated at the levels of effector enzymes called caspases. Caspases 3 and 7 are targets for suppression by a family of endogenous inhibitors of apoptotic proteins (IAPs) that in humans is composed of 8 proteins such as X-IAP, cIAP1, cIAP2, ML-IAP (Livin; K-IAP), Naip, ILP2 (TS-IAP), Apollon/Bruce and survivin) [2]. Survivin is the inhibitor of apoptosis through its effect on various caspases, through binding and inhibition of mitochondrial protein SMAC/Diablo and stabilization of XIAP proteins by blockade of ubiquitination and degradation of proteasome activity.

Under normal physiological conditions, expression of survivin is regulated by the cell cycle and connected to the G2M phase. Survivin is a part of mitotic spindle in connection with tubulin and is important regulator of mitosis. Malignant tumor cells and human fetal cells have increased expression of survivin while it is absent in the mature and well-differentiated human tissues. The results of the most recent investigations show that survivin correlates well with progression and with outcome of various types of solid tumors and hematological malignancies. It has been shown that high concentrations of survivin in malignant tumors induce resistance of tumor cells on chemotherapy and ionizing radiation.

Immunocytochemical studies show that increased expression of survivin is not just a sign of increased mitotic rate in tumor but that its increase is independent from tumor mitotic rate [3]. Furthermore, in the vast majority of tumors, survivin is increased not only during cell mitosis but in all phases of the cell cycle [4–6]. Out of many acquired genetic alterations in melanoma cells, the best described are mutations of BRAF, HRAS or NRAS, increased telomerase activity, as well as defects in signaling cascade and retinoblastoma gene and p53 [1, 4]. Although the mechanisms responsible for increased expression of survivin in the transformation of normal melanocytes into malignant melanoma cells are unknown, the epigenetic, genetic and post-translational mechanisms for regulation of survivin gene are described in other types of cells. The role of survivin in cancerogenesis is not limited only on the inhibition of apoptosis and subsequently chemoresistance of malignant cells but survivin is also important for neoangiogenesis.

In the animal model of melanoma it has been shown that expression of survivin is increased in melanoma cells in comparison with normal melanocytes and that survivin is necessary for viability of melanoma cells and that in these animals exposure of melanocytes to UV light leads into malignant transformation of melanoma cells and their metastatic potential [3].

Increased expression of survivin has been demonstrated in invasive and metastatic melanoma and it is believed that this is the consequence of dysregulation of apoptosis, mitosis and angiogenesis [3]. DNA microarray analysis has shown that survivin gene is one out of four most important genes with increased expression in melanoma. Immunocytochemical studies performed on melanocytic lesions and melanoma cases show different results in survivin expression in relation to the phase of this malignant disease and variation of survival localization in different cell compartments such as cytoplasm, nucleus or in both, nucleus and cytoplasm simultaneously [5–7].

The aim of this study was to assess the values of localized and systemic expression of survivin in melanoma patients as well as the correlation between expression of survivin and disease progression and histopathological parameters [clinical stage, histological stage, growth phase, mitotic rate of the tumor, tumor infiltrating lymphocytes (TIL), Clark's

level, Breslow's thickness, tumor ulceration, histological subtype and tumor regression].

Methods

The tumor survivin expression was determined immunohistochemically in tissue samples of 84 patients, 48 male, 36 female, aged from 25 to 78 years, diagnosed in the Institute for Pathology and Forensic Medicine, Military Medical Academy (MMA), Belgrade, Serbia in a time interval from 2009 to 2013. Serum survivin concentration was determined by commercial ELISA (R&D Systems, USA) in samples of the same patients, at the Institute for Medical Research, MMA, Belgrade, Serbia.

The level of survivin expression was determined immunocytochemically in tumor tissue and with ELISA test in the serum of 84 melanoma patients of which 48 were male and 36 female, aged from 25 to 78 years, diagnosed at the Institute for Pathology and Forensic Medicine of the MMA, Belgrade, Serbia. All the patients were stratified according to the American Joint Commission on Cancer (AJCC) clinical stage in the following groups: stage I 23 patients, stage II 17 patients, stage III 28 patients and stage IV 12 patients. The control group was composed of 20 patients with dysplastic and 20 patients with classic naevi; for testing of survivin level in the serum, control group was composed of 20 healthy persons without melanoma.

as follows: 0 for no staining, 1+ for weak staining, 2+ for moderate staining and 3+ for strong staining and according to the percentage of positive tumor cells results were evaluated as: 0 – (< od 5%); 1 – (5–25%); 2 – (25–50%); 3 – (50–75%) i 4 – (> 75%) [8].

Blood samples were left to completely coagulate, serum samples were centrifuged (1000 × g) for 15 min and stored at -70°C until tested for human survivin using Human Survivin Immunoassay, R&D ELISA Quantikine USA, cat. no. DSV00.

Statistical analysis of our data was performed with GraphPad Prism software using ANOVA test (with Bonferroni post testing), Mann-Whitney test and Wilcoxon test.

Results

Tumor tissue samples from melanoma patients showed a significantly higher average survivin expression in comparison with the samples of dysplastic naevi and benign melanocytic lesions of the control group ($p < 0.0001$) (Table 1). Analysis of survivin tissue expression in patients samples according to the AJCC clinical staging showed that even the patients with stage Ia had significant local tumor production. Comparison of survivin expression between the patients with different clinical stages showed the lowest values in stage IA and highest in stages IIIA and IIIC (Table 1). Survivin tissue expression in the melanoma patients stage IIA was

Table 1

Survivin tissue expression according to the investigated parameters ($\bar{x}$ ± SD of intensity score)

Clinical stage	Histological grade	Mitoses	Clark	Breslow	Ulceration	Vascular invasion	Spreading	Patients
IA 1.1 ± 1.5	pT1a 1.1 ± 1.4	0 1.6 ± 1.1	I nd	< 1 1.3 ± 1.3	- 1.6 ± 1.1	- 1.7 ± 1.0	none 1.5 ± 1.0	CN 0.3±0.5
IB 1.6 ± 1.1	pT1b 1.8 ± 1.0	1 1.9 ± 0.8	II 1.0 ± 1.0	2 1.6 ± 1.1	+ 2.1 ± 0.8	+ 2.2 ± 0.8	L 2.0 ± 1.0	DN 0.4±0.5
IIA 1.3 ± 0.5	pT2a 1.5 ± 1.2	2 1.3 ± 1.2	III 1.5 ± 1.0	3 1.4 ± 0.6			L+H 1.6 ± 1.0	MP 2.2±1.0
IIB 1.6 ± 0.6	pT2b 1.0 ± 0.8	3 2.5 ± 0.6	IV 1.9 ± 1.0	4 1.7 ± 0.8				
IIC 1.8 ± 0.9	pT3a 1.3 ± 0.5	4 2.5 ± 1.0	V 2.0 ± 0.9	5 2.4 ± 0.5				
IIIA 2.0 ± 0.9	pT3b 1.5 ± 0.6	6 1.0 ± 0.7		> 5 1.9 ± 0.9				
IIIB 2.0 ± 0.8	pT4a 2.4 ± 0.7	> 6 2.3 ± 0.5						
IIIC 2.2 ± 0.7	pT4b 2.2 ± 0.8							
IV 1.8 ± 0.8								
IA /IIB *	pT1a /pT4a *	3 / > 6 *	II /IV *	< 1 5 *	- /+ *	- /+ *	none /L *	CN /MP ***
IIA /IIIA *	pT1a /pT4b *	6 / > 6 *	II /V **	3 5 **				DN /MP ***
IIA /IIIC *	pT2b /pT4a *							
	pT2b /pT4b *							
	pT3a /pT4a *							
	pT3b /pT4b *							

Spreading (L – lymphatic, L+H – lympho-hematological); Patients (CN – control naevus, DN – dysplastic naevus, MP – melanoma patients); (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$, Mann Whitney test).

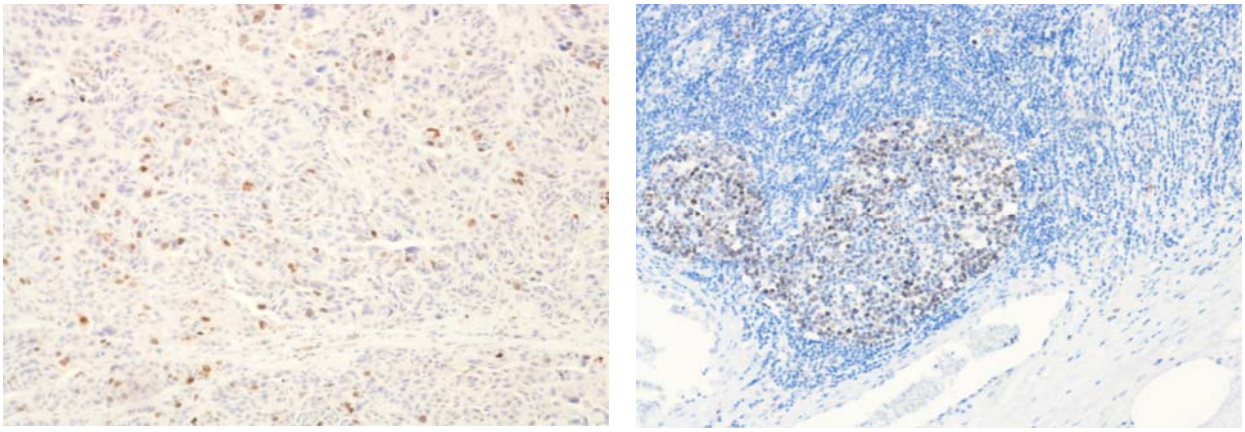
Tissue samples from patients' melanomas were fixed in 4% buffered formalin, dehydrated, cleared in xylen and paraffin impregnated in a Leica ASP300 tissue processor, and paraffin embedded tissue was sliced in 4μ thin tissue sections. The DAKO anti-survivin mouse monoclonal primary antibody 1:100 was used after microwave antigen retrieval in the DAKO retrieval solution pH 6.0. The CSA II DAKO labelling system was also used. DAKO mouse anti IgG2a antibody was used for negative control in the same 1:100 dilution as the primary antibody. The intensity of staining for survivin was determined using the semi-quantitative method

statistically lower than in tumor tissue in the patients in stages IIIA i IIIC ($p = 0.0371$; $p = 0.0428$). We found a graduate and constant increase of survivin tissue expression level following the disease progression reflected in advancing clinical stages. The correlation of values of survivin tissue expression with histological stage of melanoma showed similar results. The lowest average values of survivin expression score were detected in the samples of tumor tissue from the patients with pT1a stage, significantly lower than values found in the patients stage pT4a ($p = 0.0486$) and pT4b ($p = 0.0286$), that had the highest expression of survi-

vin in tumor cells (Table 1). Average survivin expression score in the melanoma tissue of the patients in pT4a and pT4b was significantly higher comparing to the patients in stage pT2a ($p = 0.0276$; $p = 0.0256$) and pT3a ($p = 0.0321$; $p = 0.0418$). Comparing expression of survivin in melanoma tissue with the Clark levels we found that the intensity of survivin expression in Clark level II was significantly lower than in Clark level IV and V ($p = 0.0180$; $p = 0.0102$) (Table 1). Analysis according to the Breslow score showed similar findings with the highest average values of survivin found in the tissue samples of the patients with the Breslow thickness 4–5mm and lowest in the patients with the Breslow thickness < 1 mm. The patients with the highest Breslow score had significantly higher survivin tissue expression in their tumors comparing to the patients that had Breslow less than 1 mm ($p = 0.0353$) and the patients that had Breslow 2–3 mm thick melanomas ($p = 0.0087$) (Table 1). Ulcerated melanomas showed increased survivin expression comparing to non ulcerated ones ($p = 0.0476$) (Table 1). The intensity of expression of survivin was statistically significantly higher in melanomas with the highest mitotic activity (> 6 mitosis *per* mm^2) than in melanomas with 3 mitosis ($p = 0.0334$) and melanomas with 6 mitoses ($p = 0.0371$) (Table 1). We found the highest values of survivin expression in melanomas without intratumoral lymphocytic infiltration (TIL) and the lowest values in melanomas with the brisk tumor infiltrating lymphocytes (TILs). This difference was not significant.

There was no significant difference between the intensity of survivin staining and vertical or horizontal phases of melanoma growth, histological type of tumor and the presence or the absence of tumor regression. Positive immunohistochemical staining to survivin in tumor cells of primary and metastatic melanoma is shown in Figures 1a and b.

Analysis of survivin expression and the number of survivin positive cells in metastatic lesions of stage IV patients showed a significant association with the duration of disease free interval (DFI) (Figure 2). The patients with high expression score had almost double shorter DFI comparing to those with weak local survivin expression and a small number of survivin+ melanoma cells (9 ± 7 *vs* 19 ± 13 months).



a) **b)**

Fig. 1 – Survivin positive tumor cells in primary melanoma (a), and metastatic melanoma (b) (survivin original magnification ×100 catalized system amplification – CSA II).

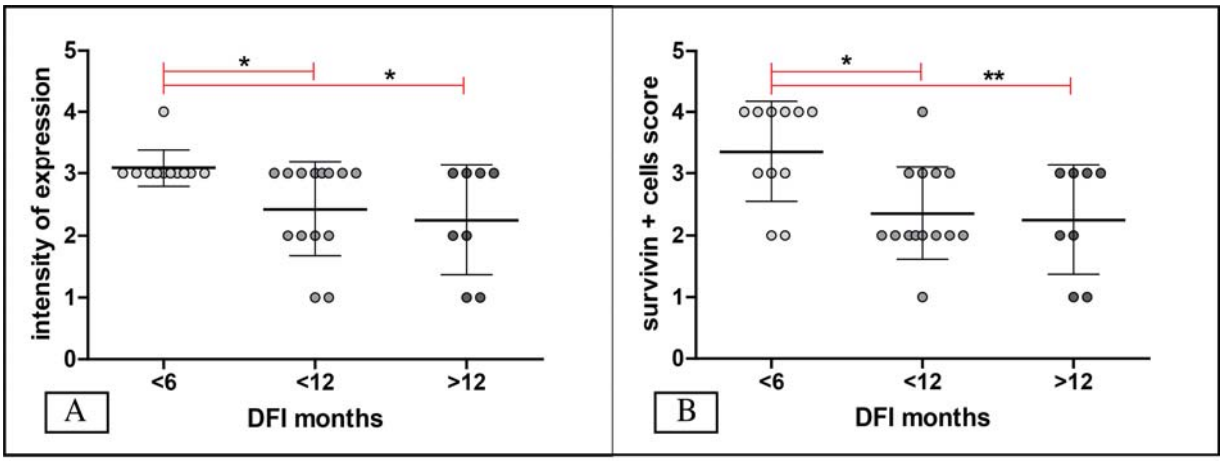


Fig. 2 – Association of disease free interval (DFI) duration with intensity of survivin expression and the number of survivin positive cells in metastatic melanoma lesions. A – Survivin expression in metastatic tumor lesions according to DFI duration; B – Number of survivin positive tumor cells in metastatic lesions according to DFI duration; ($\bar{x} \pm SD$, intensity score, *$p < 0.05$, ** $p < 0.01$, Mann Whitney test).

The average survivin serum concentration was significantly increased in melanoma patient samples comparing to samples of examinants with dysplastic naevi, benign pigmented skin shanges and control healthy persons. Analysis of survivin concentration according to clinical stages showed that the patients in IA stage had the highest average value, significantly higher than the patients in stage IB ($p = 0.0363$) (Figure 3A). The melanoma patients in IIIC clinical stage had the lowest average survivin concentration, significantly lower comparing to the patients of IA ($p = 0.0495$), IIB ($p = 0.0286$) and IIIA stage ($p = 0.0286$). Histological staging of primary tumors showed that the patients with pT1a stage had the highest average serum survivin values, similarly to data found when the patients where classified according to the AJCC staging system. The patients with melanomas classified as pT2b had the lowest average survivin concentration, significantly less than those patients whose tumors were pT1a ($p = 0.0424$), pT1b ($p = 0.0294$), pT3b ($p = 0.0286$), pT4a ($p = 0.0159$) and pT4b ($p = 0.0120$) (Figure 3B). Lymphocyte infiltration of primary tumor was significantly associated with survivin concentration. The patients with the highest degree of tumor infiltration by lymphocytes had a significantly decreased average survivin level comparing to the patients with no detectable infiltrating lymphocytes ($p = 0.0114$), or with mild ($p = 0.0098$) or moderate ($p = 0.0036$) degree of infiltration (Figure 3C). The highest survivin concentration was detected in samples of the patients with tumor with the highest mitotic activity, significantly higher comparing to the patients with 1 mitosis/mm^3 of tissue ($p = 0.0276$) (Figure 3D). The patients in horizontal growth tumor phase had significantly more survivin values than those in vertical growth phase ($p = 0.0211$) (Figure 3E). Aggressive melanoma spreading according to Clark level was associated with lower survivin serum concentration. The patients with melanomas qualified as Clark II had a significantly increased survivin concentration comparing to those with Clark IV ($p = 0.0290$) or Clark V ($p = 0.0285$) tumors (Figure 3F). Anatomical localization of primary tumor was significantly associated with survivin concentration (Figure 3G). The patients with melanoma localized at the foot had a significantly decreased survivin concentration comparing to the patients with melanoma locali-

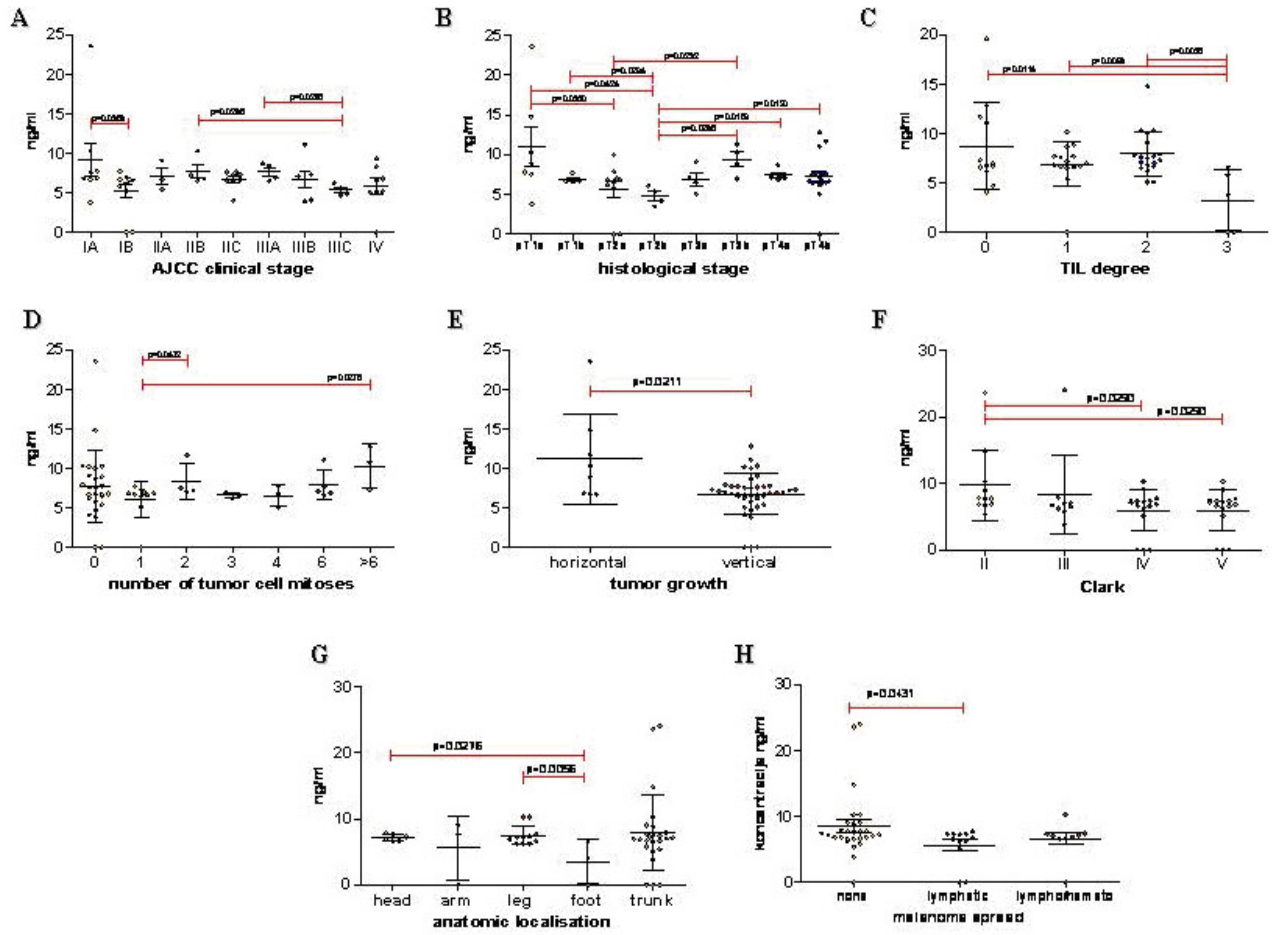


Fig. 3 – Survivin serum concentration ($\bar{x} \pm SD$ ng/mL) in melanoma patients samples. A – clinical stage of melanoma patients (AJCC); B – histological grade of primary tumor; C – tumor infiltration (lymphocytes degree in primary tumor); D – number of mitoses estimated in tumor cells; E – phase of primary tumor growth; F – Clark score; G – anatomic localization of primary tumor; H – direction of melanoma spread.

zed at the head (p = 0.0276). Finally, the type of spreading was significantly related to serum survivin values. The patients without histological evidence of tumor spreading through lymphatic or blood vessels had a significantly increased average survivin concentration comparing to the patients with lympho and/or hematological spread tumor (p = 0.0431) (Figure 3H).

Survivin concentration did not differ significantly in the patients with different histological type of tumor with different Breslow score, the presence or the absence of tumor regression, ulceration or metastases. Also, survivin concentration did not differ significantly in the patients that had stable disease or clinical progression, and the patients who survived or died.

Finally, when we analyzed survivin expression *vs* serum concentration of the same patient, we found that the patients with lowest intensity of tissue expression had a significantly higher serum level than those with intensive local tissue expression (p = 0.0153) and also that the patients with the smallest number of survivin+ cells had the highest value of survivin serum concentration (Figures 4A and B).

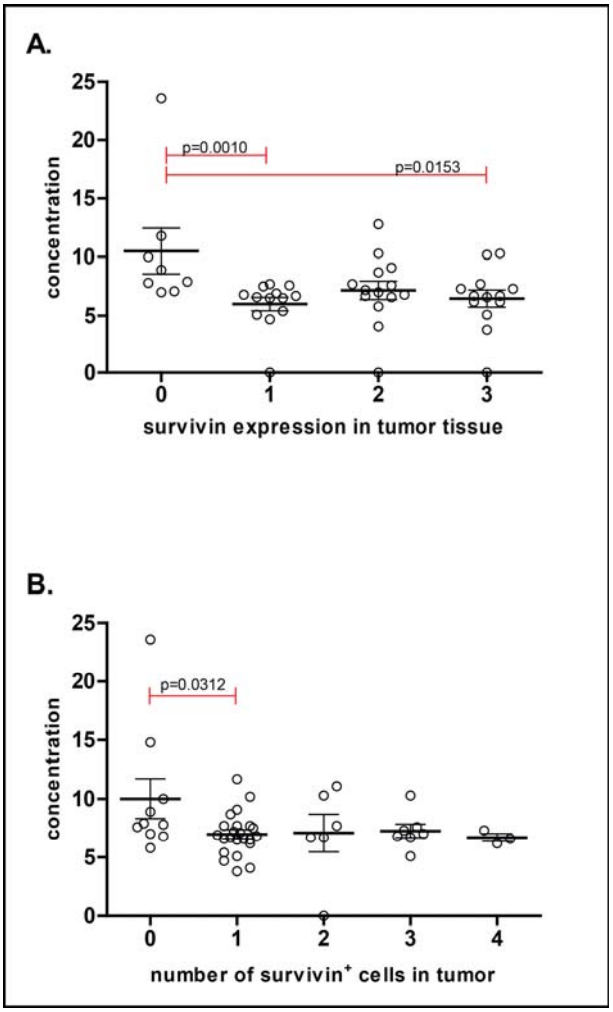


Fig. 4 – Survivin serum concentration ($\bar{x}$ ± SD ng/mL) in relation to survivin expression in tumor tissue. A – average survivin serum concentration according to the degree of survivin expression in primary tumor tissue; B – average survivin serum concentration according to the degree of survivin positive tumor cells. (Mann Whitney test).

Discussion

Survivin is the only member of IAP family that is able to interact with the mitotic apparatus and has several functions, serving as mitotic regulator, cell death inhibitor and a regulator of cell migration/metastasis [9, 10]. It has been proposed that multiple function of survivin are associated with its structure modifications and distinct localization in different cell compartments. Nuclear localization seems to represent survivin potential to control the cell cycle, while cytoplasm/mitochondrial localization are associated with inhibition of the programmed cell death process [11]. Therefore, survivin performs both, cycle-dependent and non-cycle-dependent functions, important in response to hematopoietic and vascular remodeling cytokines. Additionally, there is a mode that cell use to transport survivin inside out, which is stress-induced and executed by exosome extracellular release. This extracellular survivin retains both antiapoptotic and proliferative functions [12–14].

Both clinical and experimental data showed survivin is excessively expressed in human melanoma samples [15], making it the important initial factor for melanoma growth [16], having significant influence on melanoma cell migratory potential [17]. Finally, comprehensive bioinformatic analysis of immunohistochemical and gene array studies of expressed genes and proteins, in order to define melanoma prognosis biomarkers, selected proliferating cell nuclear antigen (PCNA) and survivin among 254 others as priorities for further melanoma biomarkers examination [18].

Our results showed that detectability and average score of survivin expression were lowest in the patients in IA AJCC stage, constantly increasing towards advanced disease stages, with a significantly highest value in the patients that were in stages IIIA and IIIC. These findings are consistent with several other studies. Piras et al. [19] showed that survivin detection increase in melanoma samples with disease progression from 67% in initial stage I to over 90% in patients in stage II. Survivin expression was mainly localized to nuclear compartment and that nuclear expression was significantly associated with melanoma thickness. Samples from primary melanomas of our patients showed predominantly nuclear localization (> 91%), with weak cytoplasmic staining. Analysis of metastatic lesions showed increment of cytoplasmic localization of survivin from 9% to 25%, comparing to primary tumors of the same persons, which implicate different regulation of local survivin production, at least in some melanoma metastases.

Survivin expression in metastatic lesions from stage IV melanoma patients documented both by immunohistochemistry (IHC) and mRNA level was significantly associated with survival [20]. Although stage IV melanoma patients represent a very inhomogeneous population respective to their disease progression and more important, to response to adjuvant therapy, mRNA survivin was detected in almost all (98%) of samples. But there was a significant distinction between numbers of survivin mRNA copies in metastatic lesions. These melanoma patients whose lesions had low survivin mRNA load showed double longer median survival interval than those with high number of

survivin mRNA copy number (24 *vs* 11 months). Immunohistochemical findings correlated well with molecular data, characterized by intense survivin staining in tumors with high mRNA survivin load and *vice versa*. There was no significant correlation between clinicopathology factors and survivin mRNA copy number of tumors from 63 patients.

In the group of the stage IV patients the differences of survivin expression in metastatic lesions were not significantly associated with the differences in survival interval. But, the intensity of survivin expression and the number of survivin positive cells in metastatic lesions were significantly associated with DFI. The patients with a high expression score had almost double shorter DFI comparing to those with weak local survivin expression and a small number of survivin+ melanoma cells (9 ± 7 *vs* 19 ± 13 months).

Analysis of survivin expression according to histological grade showed similar results as seen in different clinical stages. In 50% of our patients with pT1a stage survivin was absent and other half had weak local expression detected in a small number of tumor cells. Contrary, all the patients with pT4a and pT4b tumors had strong local expression in numerous tumor cells, significantly higher than other histological stages. A significant correlation of survivin expression with histologic grade and stage was reported not only in melanoma [21] but also in tumor samples of the patients with endometrial carcinoma [22], ovarian carcinoma [23], hepatocellular carcinoma [24] and breast cancer [25, 26].

The intensity of survivin expression was significantly higher in the patients whose tumor had ulceration, higher mitotic index, higher Clark and Breslow stage, that made vascular invasion or spread through lymphatic vessels in primary tumor, and was significantly higher in the patients with metastatic disease. Local survivin expression in tumor tissue was directly associated with the presence of ulceration, at least in experimental condition [27], which was explained by the mutually exclusive mechanism which regulates expression of caspase 3 and survivin. Takeushi et al. [20] showed that high level of survivin expression in metastatic melanoma lesions were associated with shorter median survival interval.

The presence of TIL is considered as independent prognostic factor for melanoma patients. It is reasonable to assume that some TIL is specific for survivin overexpression in tumor tissue. There are several lines of evidence supporting this viewpoint. Patients suffering from cancers of different origin frequently show spontaneous anti-survivin response mediated by specific T lymphocytes [28, 29]. In a study on TIL of melanoma patients, Hadrup et al. [30] identified CD8+107a+ cytotoxic lymphocytes specific for MAGE 1,3,4 NY-ESO-1 antigens. Although strong in tumor expression of survivin, they failed to demonstrate survivin specific T cells. But, when they monitored specific response in a melanoma patient with long term complete remission after interleukin (IL-2) therapy, they identified population of T lymphocytes specific for survivin (HLA-A11 restricted) [31]. These T lymphocytes were detectable during remission period, 7 years. Ellebaek et al. [32] further confirmed that TIL from melanoma patients contain significant distinct populations of CD8+ CD107a+ cells that were cytotoxic to autologous target cells expressing survivin (SUR53-62) in context of HLA-A3+/A11+, and also showed significant potential to lyse autologous tumor cells [32–34]. We did not find any significant difference in tumor tissue survivin expression whether there was no infiltrating lymphocytes or most intensive lymphocyte infiltration. But, the degree of TIL presence in tumor tissue was significantly associated with serum survivin concentration, with lowest average level detected in samples of patients with the highest degree of infiltration.

Serum survivin concentrations were highest in samples of melanoma patients with IA AJCC clinical stage, pT1a histological stage, patients whose tumors were still in horizontal growth phase, without signs of lymphoid hematopoietic disease spreading, with the highest number of mitoses and that had the smallest Clark index. All these indicate that melanoma in the initial phase have abundant local survivin production, underlying the importance of exosome survivin compartment at the disease beginning. Experimental data showed that extracellular survivin is essential in stimulating melanoma cell motility through upregulation of α5 integrin function [35], implicating that significant survivin production could enable early melanoma cells spread, both local and systemic.

In a study on serum anti-apoptotic markers Tas et al. [36] investigated survivin and BCL2 concentration in serum samples of 45 melanoma patients. They did not find any significant difference in survivin values between control subjects and patients, nor between patients according to standard prognostic parameters. But they did find a significantly higher survivin concentration in patients that had lymph node involvement and in patients that had metastatic disease and underwent dacarbazine (DTIC)-based chemotherapy. Contrary, in patients with early stage breast cancer survivin concentration significantly correlated with Ki67 and p53 concentration, histological and nuclear grade of tumor [37].

Finally, when we analyzed survivin expression *vs* serum concentration of the same patients, we found that the patients with the lowest intensity of tissue expression and the smallest number of survivin+ cells had significantly higher serum level than those with intensive local tissue expression. Those differences could be addressed to methods sensitivity, with s 100 EIA kit being more sensitive than immunohistochemistry. But, again, these findings underline that even smallest melanoma lesion, without signs of local survivin expression had significant capacity to secrete survivin, probably in exosome form, and to mediate all tumor biological functions that are important for further growth and disease spreading.

Conclusion

According to the obtained results we could conclude that local survivin expression in tumor tissue (primary tumor, metastatic tissue) and its serum concentration significantly correlate with clinical and histopathological parameters of melanoma. Serum levels could be important in initial follow up as indicators of those patients that would have aggressive local tumor growth and spreading. Survivin determination in tumor tissue, both in primary tumors and metastases, is of great significance in estimation of disease free interval.

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