

## SURVIVIN AND Ki67 PROLIFERATIVE INDEX IN BREAST CARCINOMA

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### Summary

Survivin is a member of the inhibitor of apoptosis (IAP) family. It is also involved in the regulation of cell division. Survivin is widely expressed in foetal tissues and in human cancers, but generally not in normal adult tissue. This study examined the expression of survivin protein in a series of 50 cases of invasive primary breast carcinoma. Our study comprised 50 cases of breast cancer, 10 of each immunophenotype. All tumours were diagnosed as invasive ductal carcinoma (Not Otherwise Specified, NOS). Formalin-fixed, paraffin-embedded tissue sections were immunostained for survivin. Survivin immunoreactivity was evaluated as follows: 0(0-5% positive cells); 1(5-20%); 2(21-50%); 3(51-75%); 4(>76%) with cutoff value of 20% that was established as a positive result. Immunohistochemical analysis showed positive expression for survivin in 18 of 50 cases (36%) of breast carcinomas of TNM stages I to III. In previous studies, there was significant relationship between survivin expression and negative prognostic factors like larger tumour size, higher histologic grade and negative hormonal status. What we should emphasize in our study is the correlation between HER2 positive tumours and survivin expression ( $P=0.007$ ) and strong association of survivin expression in cytoplasm in HER2 positive tumours as a predictor of unfavourable outcome. Further larger studies are needed in future to explore and explain the facts about survivin and its connection with breast cancer.

KEY WORDS: *apoptosis, survivin, breast cancer*

### SURVIVIN I PROLIFERATIVNI INDEKS Ki67 U RAKU DOJKE

#### Sažetak

Survivin je protein koji je član obitelji inhibitora apoptoze, a uključen je i u regulaciju stanične diobe. Široko je rasprostranjen u fetalnim tkivima i ljudskim tumorima, ali se u pravilu ne javlja u normalnim odraslim tkivima. Studijom smo ispitali izražajnost survivina u seriji od 50 slučajeva invazivnog primarnog karcinoma dojke. Obrađeno je 50 slučajeva, po 10 od svakog imunofenotipa. Svi tumori su dijagnosticirani kao invazivni duktalni karcinom (NOS). Dijelovi tumorskog tkiva su fiksirani u formalinu i uklopljeni u parafinske kocke te je napravljena imunohistokemijska obrada na survivin. Imunoreaktivnost survivina je procijenjena: 0 (0-5% pozitivnih tumorskih stanica); 1 (5-20%); 2 (21-50%); 3 (51-75%) i 4 (> 76%) uz graničnu vrijednost od 20%, koja je ustanovljena kao pozitivan rezultat. Imunohistokemijska analiza pokazala je pozitivnu izraženost survivina u 18 slučajeva (36%) TNM stadija I do III. U prethodnim studijama vidljiva je značajna povezanost izraženosti survivina i negativnih prognostičkih čimbenika kao što su veličina tumora, visoki histološki gradus i negativni hormonalni status. Treba naglasiti da je prediktor nepovoljnog ishoda pozitivna korelacija HER2 pozitivnih tumora i izražajnosti survivina u njihovoj citoplazmi ( $P=0.007$ ). Potrebne su buduće studije koje će istražiti i objasniti činjenice vezane uz survivin i njegov odnos s karcinomom dojke.

KLJUČNE RIJEČI: *apoptoza, survivin, karcinom dojke*

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## INTRODUCTION

Apoptosis is the process of programmed cell death where senescent or damaged cells that are beyond repair are eliminated. It is a cascade of molecular events regulated by proteins that promote or prevent cell death. It is believed to be an important mechanism by which therapeutic chemotherapy and radiation therapy destroy cancer cells (1). Aberrant inhibition of apoptosis interferes with normal cell regulation and promotes tumour development (2).

Survivin is an anti-apoptotic protein that is overexpressed in most human cancers. Survivin regulates the G<sub>2</sub>/M phase of the cell cycle by associating with mitotic spindle microtubules, and it directly inhibits caspase-3 and caspase-7 activity. During tumorigenesis, survivin expression is inversely correlated with apoptosis inhibition and positively correlated with proliferation and angiogenesis (3).

Survivin mRNA was found to be diffusely expressed during foetal development, but it was generally not found in normal adult tissues. Survivin is also overexpressed in most human cancers including bladder (4), blood (5,6), colon (7,8), liver (9), brain (10,11,12), lung (13), pancreas (14), prostate (15), and kidney (16). In the majority of cancers studied to date, survivin is associated with poor prognosis. Clinicopathological investigations on the role of survivin in breast cancer focusing on its importance as a prognostic factor have been limited (1). In this article, we investigated the prevalence and cellular localization of survivin in a series of 50 primary breast cancers, according to immunophenotype and proliferative index Ki67.

## MATERIALS AND METHODS

### Patient characteristics

In our research, we observed immunohistochemical results of survivin and the relation between survivin and proliferative index Ki67 in 50 cases of breast cancer in accordance with immunophenotype by St. Galen (2015 god.). Our study comprised 50 cases of breast cancer, 10 of each immunophenotype. Patients age was between 29 and 84 years at the time of diagnosis. The size of the tumours varied between 0.5 and 6 cm. The TNM

stage of tumors was as follows: Twenty-four tumours were T1 (less than 2 cm in diameter), 22 tumours were T2 (2-5 cm), 2 tumours were T3 (larger than 5 cm in diameter) and 2 tumours were T4 (involvement of epidermis). All tumours were diagnosed as invasive ductal carcinoma (NOS). Five tumours were grade 1, 17 tumours were grade 2 and 28 tumours were grade 3.

### Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue sections were immunostained for survivin. Sections 3 mm thick were cut, dewaxed in xylene and rehydrated in alcohol. Prior to immunohistochemical staining sections were blocked for endogenous activity with 3% H<sub>2</sub>O<sub>2</sub>. Antigen retrieval was carried in citrate buffer, pH 6.0 in water bath for 40 minutes. The sections were then incubated overnight at 4 °C with mouse monoclonal antibody against survivin protein (clone 12C4, Dako, Carpinteria, USA) at dilution of 1:100 for all cases. Sections were washed in PBS to remove unbound antisera. Bound antibody was detected using En Vision detection kit for 60 minutes (En Vision Flex, kat.no. K8010, Dako, Glostrup, Denmark) and visualised with DAB as a chromogen (10 minutes). Slides were then lightly counterstained with haematoxylin.

### Evaluation of immunohistochemistry results

Survivin immunoreactivity was evaluated semiquantitatively according to the previous studies (1). Nuclear and cytoplasmic tumour cell immunoreactivities were separately assessed at 40 magnification, and were given an arbitrary score: 0(0-5% positive cells); 1(5-20%); 2(21-50%); 3(51-75%); 4(>76%). A cutoff value of 20% was established as a positive result. Invasive tumours with a score of 0 or 1 were considered negative. The results were separately analysed and statistically calculated (1).

We found that of those specimens that were survivin positive, 3 of 18 expressed survivin in the nuclear region of the tumour cell. In 12 specimens, the reactivity was confined to the cytoplasm, and in 6 it was present in both nucleus and cytoplasm as seen in Figure (1). Considering small number of positive cases and relatively small sample, nuclear and cytoplasmic positivity was evaluated in collaboration.

**Statistical analysis**

Statistical analysis was done using chi-squared test. P < 0.05 was considered to be statistically significant.

**RESULTS**

Immunohistochemical analysis showed positive expression for survivin in 18 of 50 cases (36%) of breast carcinomas of TNM stages I to III. In contrast, no expression of survivin in adjacent normal tissue was detected. (figure 1.)

When we consider luminal A type of tumours, our results are negative for survivin in 70% of the cases (7 of 10) and those that are positive show nuclear positivity only (Table 1).

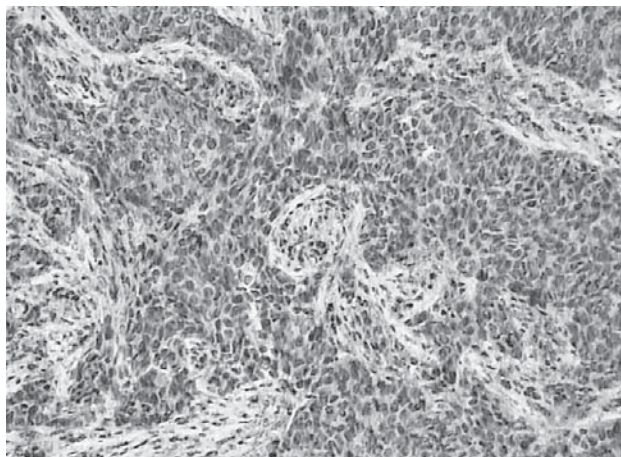


Figure 1. Immunohistochemical reaction of survivin in nucleus and cytoplasm in breast cancer tumour cells.(IHCx40)

Table 1.

TUMOUR IMMUNOPHENOTYPE: LUMINAL A

LUMINAL A					
AGE	SIZE (cm)	GRADE	T	SURVIVIN	Ki67
40	2.2x1,5x1.2	1	2	neg	12,3
52	1.3x0.7x0.4	1	1c	neg	7
73	1.2x0.9x0.8	2	1c	50% n	12
71	0.9x0.6x0.5	1	1b	neg	7,2
49	1x1x1.2	2	1c	80% nc	5
67	1.1x1x1	2	1c	60% n	17,9
46	1.7x1.1x1.5	2	1c	neg	16
73	3x2.2x2.3	2	2	neg	17
50	2.1x1.7x1.2	1	2	neg	12
65	1.2x0.7x1.2	1	1c	neg	8,6

T – tumor stage; Ki67 – proliferative index; c- cytoplasm; n - nucleus

In luminal B type of tumours, 70% of the cases (7 of 10) were negative for survivin, and those that were positive were grade II-III and were larger (T2-T4b). (Table 2).

In luminal B, HER2positive tumours result for survivin was negative in 6 of 10 tumours (60%) and positive in 4 of 10 tumours (40%). Those cases that were positive for survivin were grade II-III and T1b-T2, and showed no relation with proliferative index Ki67 (Table 3).

Triple negative tumours were survivin negative in 80% of the cases (8 of 10), and positivity in nucleus and cytoplasm was found in cases of small-sized tumours (T1a and T1b). (Table 4).

According to our results survivin is diffusely expressed in Her-2 positive tumours in 9 of 10

Table 2.

TUMOUR IMMUNOPHENOTYPE: LUMINAL B, HER-2 NEGATIVE

LUMINAL B HER2 NEG.					
AGE	SIZE (cm)	GRADE	T	SURVIVIN	Ki67
78	3x3x1.7	3	2	25% c	24,5
49	3.5x2.4x3	3	2	neg	90
38	1.2x0.9x0.8	3	1c	neg	25,6
56	1.5x1.3x1.3	2	1c	neg	35
66	3.2x3x3	2	2	90% c	28,5
74	6x4x2.7	3	4b	40% c	24,5
58	1.1x0.6x0.6	2	1c	neg	34
74	2.2x2.2x1.5	2	2	neg	43
77	4x2x3.5	3	2	neg	33
63	0.9x0.4x0.5	2	1b	neg	23

T – tumor stage; Ki67 – proliferative index; c- cytoplasm; n - nucleus

Table 3.

TUMOUR IMMUNOPHENOTYPE: LUMINAL B, HER-2 POSITIVE

LUMINAL B HER2 POSITIVE					
AGE	SIZE (cm)	GRADE	T	SURVIVIN	Ki67
36	1.5x1,2x1	2	1c	neg.	31
71	0.6	2	1b	20% n	31
67	2.5x1.7x1.3	2	2	neg.	45
64	3.5x2.8x1.8	3	2	30% n 50% c	14,3
60	1.8x1.3x1,,5	2	1c	70% nc	17
65	2x2x1	3	1c	neg.	47
62	1.3	3	1c	neg.	18
40	1.8x1	3	1c	90% c	24,2
72	2.5x2.5x2	2	2	neg.	9,4
79	2.2x1.8x1.5	3	2	neg.	31,7

T – tumor stage; Ki67 – proliferative index; c- cytoplasm; n - nucleus

Table 4.  
TUMOUR IMMUNOPHENOTYPE: TRIPLE NEGATIVE

TRIPLE NEGATIVE					
AGE	SIZE (cm)	GRADE	T	SURVIVIN	Ki67
65	4.2X3.8X3.5	3	2	neg.	70
68	1.3X1.1X0.9	3	1c	neg.	70
58	1.9X1.5X1.5	3	1c	neg.	64
39	4X2.3X3.9	3	3	neg.	90
68	2.5X2X2.5	3	2	neg.	47
43	3.5X2.5X3	3	2	neg.	90
84	0.9X0.8X0.5	2	1b	30% nc	14,8
41	0.5X0.5X0.4	3	1a	30% nc	55
75	4X3X3.5	3	4	neg.	33
76	4X3X2	3	2	neg.	80

T – tumor stage; Ki67 – proliferative index; c- cytoplasm; n - nucleus

Table 5.  
TUMOUR IMMUNOPHENOTYPE: HER-2 POSITIVE

HER2 POSITIVE					
AGE	SIZE (cm)	GRADE	T	SURVIVIN	Ki67
77	5.5X3X3.5	3	3	50% c	37
53	1.5X1.2X1.4	3	1c	80% nc	17,2
69	1.9X1.3X1.5	3	1c	30% c	32
49	1.9X1.8X1.5	3	1c	90% c	45
51	4X2.6X1.6	3	2	30% c	35,2
83	2.2X1.2X1.5	3	2	70% c	40
37	2.5X2X1	3	2	80% c	50,6
73	2.5X2X1	3	2	80% c	43
74	2.1X1.8X0.9	2	2	60% c	13,3
29	2.5X1.9X1.5	3	2	neg.	38,2

T – tumor stage; Ki67 – proliferative index; c- cytoplasm; n - nucleus

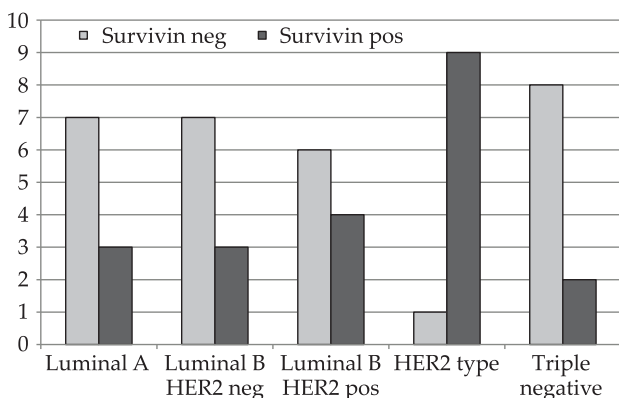


Figure 2. Chart representing number of survivin positive and survivin negative tumours regarding immunophenotype. HER-2 positive tumours show positivity in 90% of all cases.

cases (90%) and shows 30-90% cytoplasmic positivity.

There is no correlation between survivin and Ki67 proliferative index, because according to table 5., there is a large extent between 13.3 and 50.6% which are all survivin positive.

## DISCUSSION

The role of survivin has been studied in many cancers but little has been reported about the role of survivin in breast cancer (18). High expression of survivin is associated with poor prognosis in most human cancers as well as in breast carcinoma (19). There are some studies that show that association of survivin with prognosis is ambiguous (20), some studies that report survivin prognostically irrelevant (21) and some studies that connect survivin with good prognosis (1,22).

Survivin is a member of the inhibitor of apoptosis (IAP) family that plays important role in cell proliferation and carcinogenesis in many organs in human. In previous studies, there was significant relationship between survivin expression and negative prognostic factors like larger tumour size, higher histologic grade and negative hormonal status (1,17,18). The positivity of survivin can be nuclear or cytoplasmic. It is important to emphasize that nuclear positivity goes along with better outcome, and that cytoplasmic positivity is an indicator of adverse prognosis (1,22,23).

Statistical analysis in our study showed that there was complete consistency with expression of survivin and tumour size over 5 cm in diameter, although there was no consistency with survivin expression and tumour size under 5 cm (P=0,597). When we consider tumour grade, there are some significant results if we separate grade 1 in one group and put grade 2 and grade 3 in another group. The latter group is accordant with survivin expression (P=0.0449). We did not find significant relationship between survivin expression and age of the patients (P=0.492) or survivin expression and proliferative index Ki67 (P=0.603). There was no strong correlation between survivin expression and positive oestrogen receptors (P=0.128). What we should emphasize in our study is the correlation between HER2 positive tumours and survivin expression (P=0.007).



What we can interpretate in our research is that survivin is not expressed in luminal A or luminal B types of tumours, or its expression in nucleus is an indicator of better prognosis which is consistent with previous studies (18). Strong association of survivin expression in cytoplasm in HER2 positive tumours is a predictor of unfavourable outcome (18). What stays unclear in our study is lack of survivin expression in triple negative tumour types, or positivity in small sized tumours which does not comply with results in previous studies (18).

## CONCLUSION

In conclusion, survivin expression is related to adverse prognostic parameters in breast cancer as well as in other human cancers. This is a preliminary study and further larger studies are needed in future to explore and explain the facts about survivin and its connection with breast cancer.

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