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Survivin: a promising biomarker in breast carcinoma

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The antiapoptotic protein survivin can be detected in most types of malignant tumors, but it is rarely expressed in corresponding normal adult tissues. Therefore, survivin appears to represent a promising diagnostic biomarker. We examined survivin expression in 13 cases of normal breast tissue, 38 cases of fibroadenomas and 80 cases of breast carcinomas by immunohistochemical staining using anti-survivin antibody (DAKO, Clone 12C4). In each section, the intensity of staining, percentage of labeled cells, and the subcellular location of survivin antigen were assessed. Survivin was detected in 4/13 cases of normal breast tissue (30.7%), in 28/38 cases of fibroadenomas (73.7%), and in 67/80 cases of carcinomas (83.8%). Normal breast tissue showed cytoplasmic positivity only. In fibroadenomas, 19 cases (50.0%) revealed cytoplasmic reaction, and in 9 cases (23.7%), small foci of cells with combined nuclear and cytoplasmic location were identified. In carcinomas, cytoplasmic staining was found in 12/80 cases (15.0%), nuclear staining in 10/80 cases (12.5%), and combined cytoplasmic and nuclear staining in 45/80 cases (56.3%). Subcellular location of survivin between benign and malignant lesions revealed significant differences ($p<0,001$). Our findings point at practical use of survivin detection. We confirm the importance of nuclear staining of survivin antigen in breast carcinoma, which seems to be a notable diagnostic marker for estimation of the degree of neoplasia.

Key words: breast, fibroadenoma, carcinoma, survivin, biomarker

Antiapoptotic protein survivin is a structurally and functionally unique member of the inhibitor of apoptosis protein (IAP) family. Survivin gene located on chromosome 17q25 encodes a 16.5-kDa cellular protein. In contrast to related family proteins, survivin contains only a single baculovirus IAP repeat (BIR) and reveals no zink-binding carboxyl terminated fold [1, 2]. Survivin is a multifunctional protein that suppresses apoptosis, regulates cell division and enhances angiogenesis [3]. Under normal circumstances, it is detected in embryonal and fetal tissues, but is almost completely absent in most terminally differentiated adult tissues [4, 5]. Furthermore, survivin is widely expressed in a variety of human malignancies. It was found to be the fourth most highly expressed transcript in common cancers [6]. Therefore, survivin is currently undergoing intense research as a potential tumor biomarker [6, 7, 8, 9]. In addition, several studies dealt with the prognostic significance of survivin [10, 11, 12]. This antiapoptotic protein is also known to be localized both in the cytoplasm as well as in nucleus. Conflicting data were reported on the significance of nuclear and cytoplasmic survivin expression in different types of tumors, including breast carcinomas [13, 14, 15]. In group of breast carcinomas, it is not clearly defined whether overexpression of survivin and its subcellular compartmentalization can be used as reliable tumor marker. In research studies, there is no conformity with the interpretation of immunohistochemical survivin expression in breast tumor cells [14, 16, 17]. Furthermore, the review of literature revealed diametrically opposed results of survivin expression in normal mammary tissue [16, 18, 19]. In the present study, we report the expression pattern of protein in question with respect to relative number of positively stained cells, intensity of staining and subcellular location as detected by immunohistochemical methods in 13 cases of normal breast tissue, in a panel of 38 benign breast tumors and 80 breast carcinomas, respectively. Our aim was to determine the possible role of survivin as diagnostic biomarker for benign and malignant breast lesions.

Materials and methods

Archival blocks of formalin-fixed paraffin-embedded tissue from 38 benign breast tumors and 80 primary breast carcinomas were enrolled into this study. Furthermore, we evaluated 13 cases of normal breast tissue.

Hematoxylin and eosin stained slides for all cases were reviewed by two pathologists (KK, MA) to confirm the diagnosis. Each representative paraffin block was cut into four micrometer sections subjected to immunohistochemical staining, three sections from each have been stained for survivin antigen. For greater adherence of tissue sections to glass slides, we used silanized slides (DAKO, Denmark), which were baked for 2 hours in an oven at 56°C. The sections were deparaffinized in xylene for 20 minutes, rehydrated at decreasing ethanol concentrations and washed with phosphate-buffered saline (PBS). The endogenous peroxidase activity was quenched with 3% hydrogen peroxide for 30 minutes. Antigen unmasking was achieved by heating the sections which had been immersed in target solution (DAKO) within a hot water bath (96 °C) for 45 minutes. Immunohistochemical staining was performed using monoclonal mouse anti-survivin antibody (DAKO, Clone12C4, dilution 1:50). After overnight incubation, immunodetection was performed with the LSAB Vizualization System (DAKO) using 3, 3´– diaminobenzidine chromogen as substrate, according to manufacturer´s instructions. All sections were counter-stained with hematoxylin (DAKO). Negative controls were obtained by omitting the primary antibody.

In each case, the following parameters were assessed:

1) the intensity of staining, 2) the relative number of positively stained cells, and 3) the subcellular localization of survivin antigen.

To achieve good reproducibility, the above mentioned parameters were evaluated semiquantitatively by two observers separately (MA, EH), who scored them using unified and clear cut-off criteria.

Microsoft Excel software package was used for the statistical analysis. $\chi 2$ test was used to demonstrate the differences in survivin expression in normal breast tissue, breast fibroadenoma and breast cancer. P value less than 0.05 was considered to indicate statistical significance.

Results

1. Survivin expression. Survivin was found either in the cytoplasm (C) or nucleus (N) of benign and carcinoma cells or in both locations (NC).

Table 1 Summary of assessed parameters and statistical analysis

survivin expression	normal breast tissue (n=13)	breast fibroadenoma (n=38)	breast cancer (n=80)
survivin expression absent	9 (69.3%)	10 (26.3%)	13 (16.2%)
subcellular localization			
cytoplasmic	4 (30.7%)	19 (50.0%)	12 (15.0%)
nuclear	0 (0%)	0 (0%)	10 (12.5%)
both (nuclear and cytoplasmic)	0 (0%)	9 (23.7%)	45 (56.3%)
p-value			
in total	8.1676E-08		
normal vs fibroadenoma	0.01315		
fibroadenoma vs cancer	2.4125E-05		
normal vs cancer	2.7197E-05		
intensity of immunoreactivity			
+ mild	4 (30.7%)	22 (57.9%)	35 (43.8%)
++,+++ moderate, strong	0 (0%)	6 (15.8%)	32 (40.0%)
p-value	7.2804E-05		
in total	0.0163		
normal vs fibroadenoma	0.0288		
fibroadenoma vs cancer	7.7961E-05		
normal vs cancer			
percentage of labelled cells			
<10%	0 (0%)	0 (0%)	7 (8.8%)
11-50%	4 (30.7%)	3 (7.9%)	12 (15.0%)
>50%	0 (0%)	25 (65.8%)	48 (60.0%)
p-value			
in total	5.78012E-05		
normal vs fibroadenoma	0.0002		
fibroadenoma vs cancer	0.1203		
normal vs cancer	2.7196E-05		

In normal breast tissue, survivin immunoreaction was detected in 4 out of 13 cases (30.7%). In the fibroadenoma group, survivin was expressed in 28 out of 38 cases (73.7%). Immunohistochemical analysis of 80 breast carcinomas showed survivin expression in 67 cases (83.8%).

The results of all expression profiles are summarized in details in Table 1.

Nevertheless, some more significant findings are worth of attention. As mentioned above, in our panel of 131 cases, survivin was expressed in 99 samples (75.6%). The positive cases showed variable subcellular compartment immunoreactivity. Solely nuclear staining was observed in 10 out of 80 carcinoma cases (12.5%), while cytoplasmic positivity was found in all positive cases of normal breast tissue (4/13; 30.7%), in 19 out of 38 fibroadenoma cases (50.0%), and in 12 out of 80 carcinoma cases (15.0%). The combined nuclear as well as cytoplasmic expression of survivin was demonstrated in 9 out of 38 fibroadenoma cases (23.7%) and in 45 out of 80 malignant tumors (56.3%). At higher magnification, nuclear staining exhibited a punctuate pattern, also the nucleoli often showed an intense survivin immunopositivity. Percentage of labeled cells in carcinoma cases with nuclear positivity only was in 5/10 cases (50%) less than 10%, and in remaining 5/10 cases (50%) in the range of 11 – 50%. Cytoplasmic staining pattern was either granular or diffuse or both. Because the survivin-positive cells frequently showed heterogenous survivin immunoreactivity, the dominant pattern was used for scoring.

2.Statistical analysis.

*Subcellular localization.*The $\chi 2$ test confirmed statistically significant differences in the subcellular localization of survivin expression in normal breast tissue, breast fibroadenomas and breast cancers in total and in mutual comparisons between them, too ($p < 0.001$). (Table 1)

Intensity of immunoreactivity. The intensity of immunoreactivity was expressed as mild, moderate and strong. Statistical analysis revealed significant differences in the estimated intensity of survivin immunoreactivity in breast cancer samples, breast fibroadenoma and normal breast tissue in total as well as in mutual comparisons ($p < 0.05$). (Table 1)

Percentage of labeled cells. The percentage of survivin labeled cells was significantly different in total ($p < 0.001$). Mutual comparison of the percentage of survivin labelled cells in normal breast tissue vs breast fibroadenoma and normal breast tissue vs breast cancer confirmed the statistically significant differences ($p < 0.001$). There was however, no significant difference between the percentage of survivin expressing cells in breast fibroadenomas as compared to cancers ($p > 0.05$). (Table 1)

Discussion

Present paper describes the cellular expression pattern of the antiapoptotic protein survivin in 13 cases of normal breast tissue, in 38 benign tumors and in 80 carcinomas, respectively. Survivin can be detected immunohistochemically in different subcellular compartments. It can be found both in the cytoplasm and nucleus, a combined nuclear as well as cytoplasmic positivity is also known [3,10]. As observed by others, survivin is highly expressed in many malignant tumors but rarely found in normal differentiated adult tissues [3]. In the present investigation, normal mammary tissue showed survivin immunoreaction in 4 out of 13 cases (30.8%). The positivity of reaction was mainly seen in the cytoplasm of luminal cells only. However, the peripheral branching part of the lobule, the ducts and acini were lined by two layers of cells, a luminal layer of epithelial cells and a basal layer of flattened myoepithelial cells.

In general, survivin is undetectable in most normal differentiated adult tissues. Some exceptions were found in placenta and thymus [20], in normal basal keratinocytes [21] and in endothelial cells of granulation tissue [22]. Moreover, only few papers described the positivity for survivin in normal breast tissue. Ryan et al. [23] reported positivity in 5 out of 22 cases (22.7%). Zhang et al. [18] noticed positive immunoreaction in 4 out of 96 cases (4.2%). Nassar et al. [14] pointed at survivin expression in breast carcinomas, but also in normal breast tissue, either in nuclei or in cytoplasm. Our observations along with those of others indicate that further studies with large number of cases would be desirable to elucidate the survivin expression in normal adult tissues and to achieve its objective assessment in benign versus malignant counterparts.

In a panel of 38 fibroadenomas, we have seen survivin immunopositivity in 28 cases (73.7%), whereas it was absent or barely detectable in 10 cases (26.3%). Cytoplasmic localization was shown in all of 28 cases. Interestingly, in 9 out of 28 positive cases (32.1%), we detected combined nuclear and cytoplasmic immunoreaction which was found in small foci of proliferating luminal epithelial cells both in the ducts and in acini. In all of these cases, nuclear positivity showed a punctate pattern. In contrast to carcinomas, rare studies were performed on survivin expression in benign breast tumors. Its expression was reported by Ranade et al. [19] in 17 out of 32 fibroadenoma cases (53%), positivity was observed mainly in cytoplasm. In 4 out of 32 of these benign lesions (13%), a distinct nuclear immunoreactions along with cytoplasmic location was shown. In one fibroadenoma, these authors found only nuclear expression. Our results are almost consistent with the observation of Ryan et al. [23], who detected 67.7% survivin positivity in fibroadenomas (21 out of 31 cases). Survivin expression in benign cases is likely to result from the proliferation and /or dysplastic transformation of luminal epithelial cells [19]. It is known that survivin inhibits apoptosis and promotes cell proliferation and angiogenesis. In proliferating cells, survivin contributes to accurate sister chromatid segregation and stabilization of mitotic spindle components during late mitosis [24]. Survivin associates with the microtubules of mitotic spindle and the disruption of this interaction causes loss of its function and activation of caspases 3 and 7 [25].

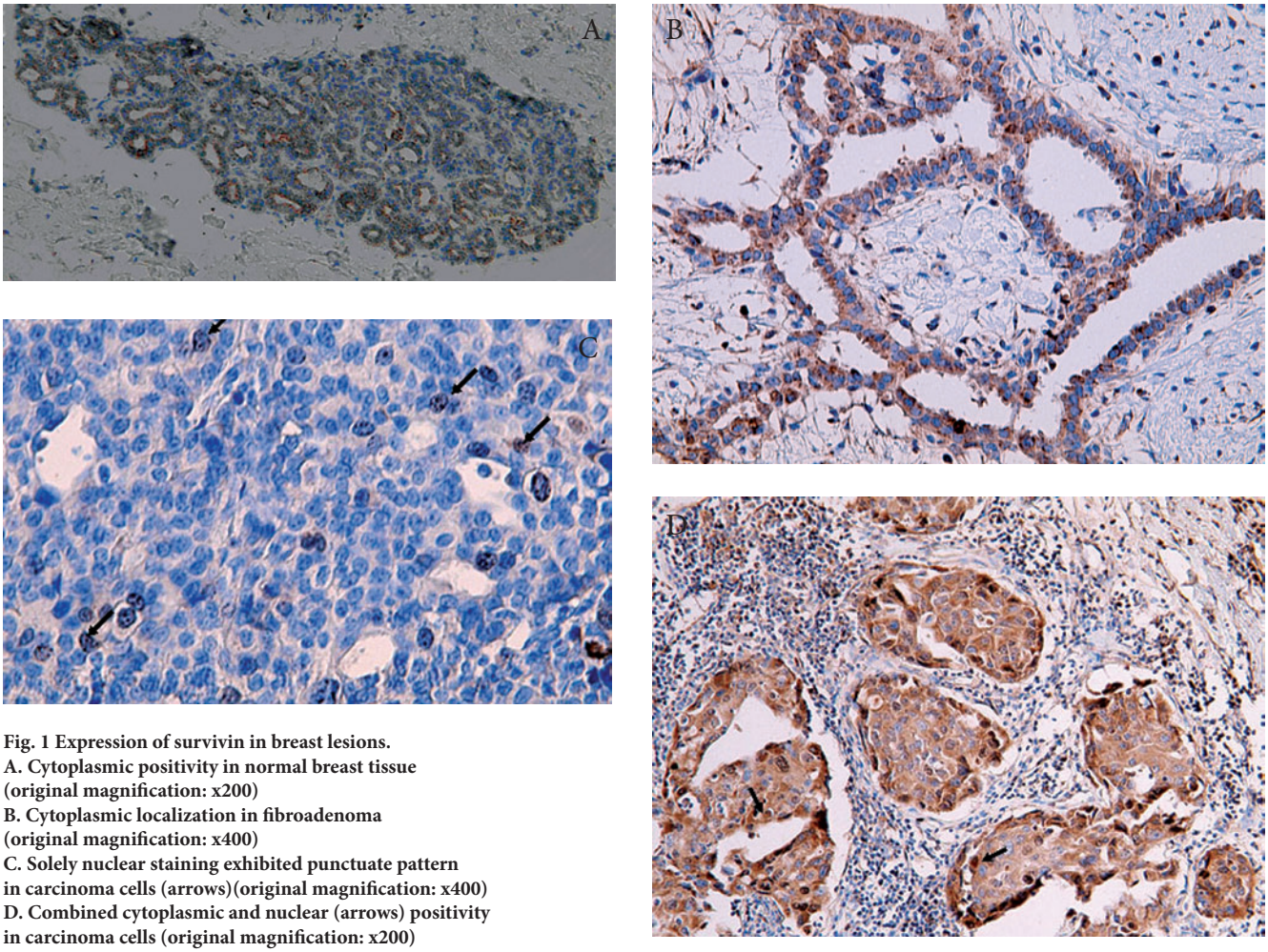

Fig. 1 Expression of survivin in breast lesions.
A. Cytoplasmic positivity in normal breast tissue (original magnification: x200)
B. Cytoplasmic localization in fibroadenoma (original magnification: x400)
C. Solely nuclear staining exhibited punctuate pattern in carcinoma cells (arrows)(original magnification: x400)
D. Combined cytoplasmic and nuclear (arrows) positivity in carcinoma cells (original magnification: x200)

In our group of 80 breast carcinomas, survivin was expressed in 67 cases (83.8%). These results are in agreement with the literature, since many other studies describe similar findings [14, 16, 18, 19, 26]. Taken together, survivin overexpression was noted mainly in human malignant tumors. Furthermore, its presence is associated with poor prognosis in most types of these malignancies. According to our observations, the intensity of staining, the percentage of labeled cells, and the cellular localization of survivin revealed statistically significant differences between specimens from normal breast tissue, benign breast tumors and breast carcinomas, respectively (Table 1).

We found three patterns of immunohistochemical positivity in the nuclei and cytoplasm of carcinoma cells. The subcellular compartmentalization of survivin is still of interest. Immunohistochemical studies or subcellular fractionation revealed two main survivin pools: the nucleus and cytoplasm. A further pool of survivin was identified in the mitochondria of tumor cells [27]. The latter plays an important role in the inhibition of mitochondrial apoptotic pathway. In response to a wide range of apoptotic stimuli, mitochondrial survivin is discharged within a few minutes into the cytosol, where via mediator proteins it prevents caspase activation and /or otherwise inhibits apoptosis. Interestingly, survivin was not found in the mitochondria of normal cells suggesting that mitochondrial survivin is exclusively associated with tumor growth [27]. Moreover, the subcellular localization of survivin may also change during malignant transformation from normal tissue or a premalignant lesion into developed malignant tumor. This is well known in melanocytic lesions. Ding et al. [28] reported, that survivin is variably expressed in the cytoplasm in an entire spectrum of melanocytic nevi, with nuclear expression detectable only in malignant melanomas. These findings may underline the importance of nuclear survivin for progression to malignancy. Therefore, the significance of nuclear – cytoplasmic shuttling of survivin is still under discussion. Recent studies showed that survivin movement is controlled by an active nuclear export signal, which is necessary for its antiapoptotic function [29, 30]. Inhibition of this nuclear export signal may cause an increased susceptibility of tumor cells to apoptosis induced by chemotherapy or radiotherapy [29].

As mentioned above, conflicting data were presented on the localization and significance of survivin expression in

cells of breast clinicomorphological entities. Kayaselcuk et al. [13] described cytoplasmic survivin positivity in breast carcinomas claiming that in normal breast tissue, there was no survivin expression. According to Nassar et al. [16], 84% of breast carcinoma cases showed nuclear reaction, while the normal tissue was negative. Other groups demonstrated three patterns of survivin staining in breast carcinoma cells: nuclear staining only, cytoplasmic only, and combined nuclear as well as cytoplasmic staining. Kennedy et al. [12] found nuclear immunoreaction only in 31% of primary breast carcinomas, cytoplasmic positivity in 13%, and combined nuclear and cytoplasmic staining in 16% of these cases. In similar research, Al-Joudi et al. [31] detected nuclear survivin expression in 16.5% of carcinomas, cytoplasmic expression in 24.1% of cases, and 27.5% of study cases showed both nuclear and cytoplasmic locations simultaneously. In these studies, nuclear survivin immunoreaction was described in 47% and in 44% of carcinoma cases, respectively. In contrast, we identified nuclear survivin in 68.8% of cases (nuclear staining in 12.5% of cases and combined nuclear and cytoplasmic staining in 56.3% of cases).

The prognostic role of subcellular localization of survivin is a further matter for discussion. Some authors concluded that increased nuclear survivin positivity was associated with an unfavorable prognosis, while small number of papers described nuclear survivin as favorable prognostic parameter [15]. In contrast to earlier reports, recent studies found nuclear expression of survivin as a poor prognostic marker and increased nuclear levels of this protein are associated with a proliferative fenotype [17]. On the other hand, cytoplasmic pool of survivin functions as antiapoptotic mechanism [15, 32].

An ideal diagnostic marker should be absent in normal tissues or benign tumors, but should be expressed in malignant counterparts, including even early or small lesions [33]. Summarizing our observations, we confirmed a gradually increasing survivin expression, starting from normal breast tissue through benign tumors to its overexpression in a great majority of primary breast carcinomas. Furthermore, nuclear immunoreaction dominated in carcinoma cases. Subcellular compartmentalization of survivin between benign and malignant lesions revealed significant differences ($p<0.001$). Based on our results, nuclear and combined nuclear and cytoplasmic pattern of immunoreactivity may be considered as important diagnostic marker in breast carcinoma. Current studies are focused on the multifunctional role of survivin not only in cancer formation and progression. Survivin is involved in the regulation of mitosis at the mitotic spindle checkpoint. Survivin promotes angiogenesis and increases chemoresistence [3, 17, 34]. Taken together, the antiapoptotic protein survivin is not only involved in breast carcinogenesis, but may be useful as a diagnostic marker. Furthermore, scoring of survivin can bring significant informations to determine the prognosis of breast carcinomas and to predict their response to anticancer therapy.

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