EXPRESSION OF ANTIAPOPTOTIC PROTEIN SURVIVIN IN DYSPLASTIC NEVI

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INTRODUCTION

Cells of malignant tumors show a certain degree of resistance to apoptosis, while some types of tumor cells are remarkably resistant. Malignant melanoma, one of the most aggresive tumors, is characterized by high resistance to therapeutic drugs and by elevated capacity to metastasize [1, 2]. Inherited or common acquired nevi can give rise to dysplastic nevi, which may progress into early neoplastic lesions. Dysplastic nevi form, both clinically and histologically, a continuum extending from a common nevus to a superficial spreading melanoma [3].

The inhibitor of apoptosis protein (IAP) survivin is the only known apoptosis regulator expressed both in nevi and malignant melanoma, its expression was very rarely studied in dysplastic nevi [1, 2, 3, 5, 6].

In present study, we determine the expression pattern of survivin in dysplastic nevi stressing its overall cytoplasmic distribution. We claim that nuclear locations of surviving antigen may be a marker for malignant progression.

MATERIAL, METHODS AND RESULTS

Samples of 27 dysplastic nevi were enrolled onto this study. Each representative paraffin block was cut into four micrometer sections subjected to immunohistochemical staining. 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). 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).

In each case, the following features were assessed: 1. The intensity of staining, 2. The number of positively stained cells, 3. The subcellular localization of staining, 4. The degree of cell atypia.

The above mentioned parameters were evaluated by two pathologists separately (ĽL, MA) and scored using unified and clear cut-off criteria, to achieve good reproducibility. The results of expression profiling are sumarized in Table 1. Nevertheless, some more significant findings are worth comment: survivin expression was observed as a cytoplasmic staining in 18 cases (66,7%), combined nuclear and cytoplasmic positivity was found in 5 cases (18,5%). No one case showed only nuclear staining. Interestingly, in 4 of 5 cases with NC staining, severe dysplasia was detected (Table 2).

Table 2 NC localization of survivin and the degree of dysplasia

	Subcellular survivin localization	Dysplasia	Size of skin lesion	Sex	Age
1.	NC	+ + +	10 mm	F	18
2.	NC	+ + +	7 mm	F	32
3.	NC	+	5 mm	F	18
4.	NC	+ + +	6 mm	F	29
5.	NC	+ + +	8 mm	F	31

NC: nuclear and cytoplasmic localization; **Dysplasia:** + mild, + + moderate, + + + severe

DISCUSSION

Survivin is a multifunctional protein that inhibits apoptosis, regulates cell division and enhances angiogenesis. It is rarely expressed in terminally differentiated normal adult tissues. Upregulation of survivin is found in most premalignant lesions and malignant tumors [4, 7].

There is large difference in expression between malignancy and corresponding normal tissue, therefore survivin is an attractive target as tumor marker.

Survivin can be found in different subcellular localizations. Immunohistochemistry or subcellular fractionation revealed two main pools of survivin: the nucleus and cytoplasm. Recently, a further pool of survivin was identified in mitochondria from tumor cells [8]. The mitochondrial fraction of survivin appears to play critical role in tumor progression. Moreover, the subcellular localization of survivin may also change during malignant transformation from premalignant lesion into developed malignancy. Ding et al. [9] recently reported, that survivin is variably expressed in the cytoplasm in the entire spectrum of melanocytic lesions, with nuclear expression detectable only in malignant melanomas. These findings may underscore the importance of nuclear survivin in progression to melanoma.

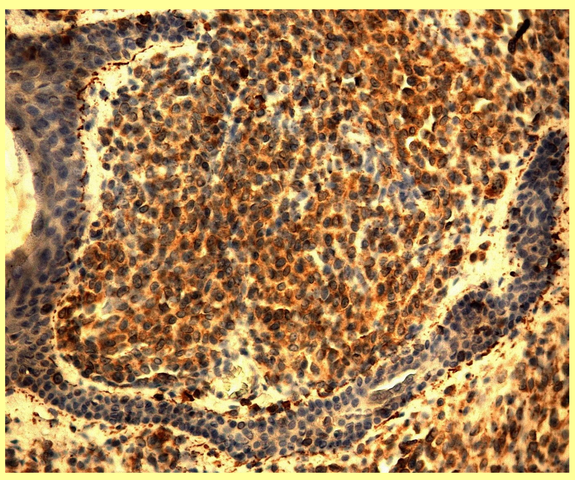

Fig. 1. Cytoplasmic survivin positivity in nevi cells.

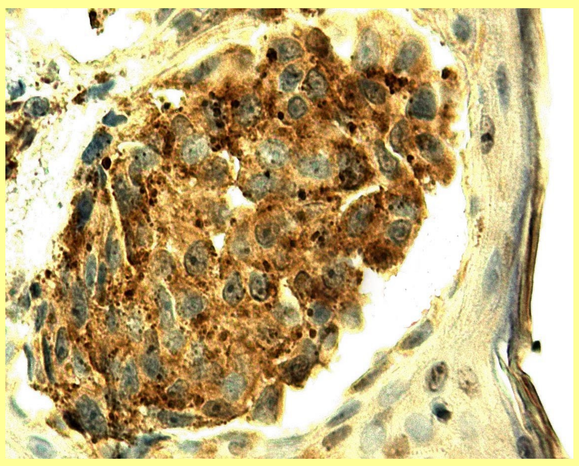

Fig. 2. Cytoplasmic survivin positivity in nest of nevi cells.

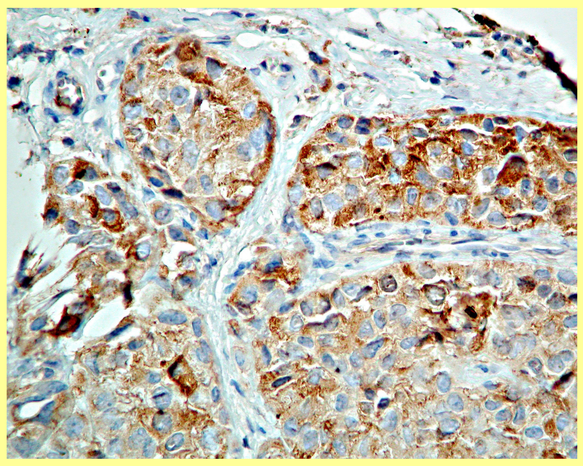

Fig. 3. Cytoplasmic survivin positivity in nests of nevi cells.

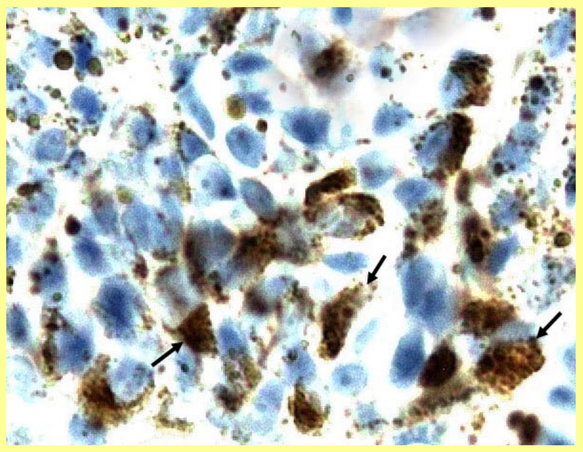

Fig. 4. Nuclear survivin positivity in nevi cells (arrows).

Alonso et al. [10] demonstrated nuclear survivin expression in 61% of malignant melanomas, showing notably increased expression with melanoma progression, and nuclear expression was negative in all nevi.

Table 1 Panel of 27 dysplastic nevi submitted to survivin staining

	S. l.	I	%	Nevus diagnosis	Size of skin lesion	Dysplasia
1.	C	+	>10%	compound	6 mm	+
2.	C	+	>10%	congenital	8 mm	+
3.	NC	+ +	>10%	compound	10 mm	+ + +
4.	A	–	–	compound	6 mm	+
5.	C	+ + +	>10%	compound	6 mm	+ +
6.	C	+	<10%	compound	5 mm	+ +
7.	C	+	>10%	compound	10 mm	+ +
8.	C	+ +	>10%	compound	5 mm	+ + +
9.	C	+ + +	>10%	compound	6 mm	+
10.	NC	+ +	>10%	compound	7 mm	+ + +
11.	C	+	>10%	compound	5 mm	+ +
12.	A	–	–	congenital	6 mm	+ +
13.	A	–	–	intradermal	9 mm	+ +
14.	C	+ +	>10%	compound	7 mm	+
15.	C	+ + +	>10%	compound	5 mm	+
16.	C	+	>10%	compound	5 mm	+
17.	A	–	–	compound	4 mm	+ +
18.	NC	+ +	>10%	compound	5 mm	+
19.	NC	+ +	>10%	compound	6 mm	+ + +
20.	C	+	>10%	compound	9 mm	+ + +
21.	C	+	<10%	compound	5 mm	+
22.	C	+ +	>10%	compound	8 mm	+ +
23.	C	+ +	>10%	compound	10 mm	+
24.	C	+ +	>10%	compound	7 mm	+ +
25.	C	+ +	>10%	compound	5 mm	+
26.	C	+ +	>10%	compound	10 mm	+
27.	NC	+ + +	>10%	compound	8 mm	+ + +

S. l. – subcellular localization of survivin positivity: **A** – absent, **N** – nuclear, **C** – cytoplasm,**NC** – nuclear and cytoplasm; **I** – intensity of immunoreactivity; **%** – % of labeled cell; **Dysplasia:** + mild, + + moderate, + + + severe

We found in this study, that survivin was variably expressed in panel of 27 dysplastic nevi. Survivin immunoreactivity was absent in 4/27 cases (14,8%). Cytoplasmic localization of immunoreactivity for survivin was detected in 18/27 cases (66,7%). Nuclear localization only was not expressed. These results are in agreement with the literature, many studies have revealed similar findings [8, 9]. In both localizations, nuclear and cytoplasmic, survivin expression was detected in 5/27 cases (18,5%), with predominance in cytoplasmic compartment. Nuclear staining pattern was diffuse finely granular. There are only very rare data which describe nuclear and cytoplasmic survivin expression in melanocytic nevi. Vetter et al. [11] demonstrated survivin in both localizations in dermal and congenital nevi. Most studies presented cytoplasmic subcellular positivity [9, 12]. Interestingly, 4/5 NC cases (80%) are associated with severe dysplastic changes, while 2/18 C cases (11,1%) only present severe dysplasia. These findings suggest that nuclear survivin expression may be an important in early step in the trasformation from nevus to its malignant counterpart. For development of malignant melanoma, the strongest single risk factor is the presence and number of dysplastic nevi [13].

For the first time, this study calls attention to the association of nuclear and cytoplasmic survivin immunoreactivity with severe dysplasia in melanocytic nevi**.** Nevertheless, this data represent a further confirmation of the role of survivin in the progression of dysplastic nevi to melanoma.

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