Relation between expression pattern of p53 and survivin in cutaneous basal cell carcinomas

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Summary

Background: The tumor suppressor gene p53 is a key regulator of cell division and/or apoptosis. Survivin is a multifunctional member of the inhibitor of apoptosis family. Survivin and p53 represent diametrically opposed signals that influence the apoptotic pathway.

Material/Methods: To determine the role of p53 and survivin in basal cell carcinoma (BCC), we evaluated the expression pattern of both proteins with regard to the percentage of positively immunostained tumor cells, the intensity of staining, and subcellular localization among 31 subjects with BCC.

Results: Overexpression of p53 protein was found in 28 of 31 cases (90.3%), whereas survivin accumulation was seen in 27 (87.1%). For p53, moderate and/or strong immunoreactivity was seen in 20 of 28 cases (71.4%), and 26 of 28 cases (92.9%) showed more than 25% reactive tumor cells. Nuclear p53 staining was detected in 23 of 28 cases (82.1%), whereas combined nuclear and cytoplasmic localization was found in only 5 of 28 cases (17.9%). Survivin revealed mild intensity of immunoreaction in 22 of 27 cases (71%), and 25 of 27 cases (92.6%) showed less than 25% labeled tumor cells. Combined nuclear and cytoplasmic survivin localization was present in 26 of 27 cases (96.3%). Statistically significant differences were detected in the assessed expression parameters between those proteins.

Conclusions: Our results suggest that overexpression of wild type p53 protein may suppress the expression of survivin and its antiapoptotic activity in BCC cells.

key words: p53 • survivin • immunohistochemistry • basal cell carcinoma

Abbreviations: **NAIP** – Neuronal Apoptosis Inhibitory Protein; **ILP-2** – IAP-like protein-2; **XIAP** – X-linked IAP; **ML-IAP** – Melanoma-associated IAP; **c-IAP** – cellular IAP; **Apaf-1** – Apoptosis protease activating factor-1; **LSAB** – labeled streptavidin biotin method; **MDM2** – murine double minute 2 oncogene; **Smac/DIABLO** – Second mitochondrial activator of caspases, a.k.a Diablo

BACKGROUND

Basal cell carcinoma (BCC) is the most common form of human skin cancer. Its etiopathogenesis is still not fully understood. Generally, most common genetic alterations in human cutaneous cancers are found at the level of the p53 tumor suppressor gene [1,2]. The p53 tumor suppressor protein plays a critical role in both the induction of apoptosis and carcinogenesis [3].

Under normal conditions, the inactive form of p53 protein is usually present at low levels in the cytoplasm of cells. Cytoplasmic localization in normal cells is controlled by murine double minute (MDM2) oncogene [4]. Murine double minute is able to shuttle continually between the cytoplasm and nucleus because it contains both a nuclear export signal and a nuclear localization signal. In response to DNA damage, for example, by ultraviolet or ionizing radiation, p53 starts accumulating in the nucleus [1,5]. Deoxyribonucleic acid damage can cause activation of p53 by phosphorylation and, consequently, the interaction of p53 and MDM2 is disrupted [6,7]. Tumor protein 53 becomes temporarily free of MDM2 and then accumulates in the nucleus. The intracellular content of p53 depends on the DNA damage response.

Higher content of p53 directs the cells toward apoptosis, whereas low and/or moderate p53 levels result in cell cycle arrest, providing time for DNA repair [8,9]. On one hand, the activated p53 can induce apoptosis or cell cycle arrest through up-regulation of several associated genes. On the other hand, there are genes with antiapoptotic activity that are repressed by p53 at the transcription or translation levels, and this negative regulation is important for the induction of apoptosis [2,3]. Survivin, a unique member of the inhibitor of apoptosis protein (IAP) family, is known to be repressed by the wild-type p53. This repression is one of the mechanisms inducing apoptosis by the activation of the mitochondrial pathway [2,10].

Proteins that belong to the IAP family play a key role in negative regulation of apoptosis. Eight human IAP family members have been identified: c-IAP1, c-IAP2, NAIP, ILP-2, XIAP, apollon, ML-IAP/livin, and survivin [11,12]. Multifunctional survivin possesses a number of distinct features not shared with other IAP members: (1) it is the shortest polypeptide consisting of 142 amino acid residues; (2) the expression of this protein is cell cycle-regulated and occurs in the G2/M phase; (3) it is undetectable in most normal differentiated adult tissues, but is frequently expressed in embryonic and fetal organs, as well as in developed human malignant tumors; (4) it can be found in different subcellular localizations; and (5) it functions in both inhibition of apoptosis and regulation of cell division in contrast to other IAP members regularly restricted to 1 of these 2 functions [13–15]. Survivin is currently undergoing intensive research as a potential tumor marker and prognostic factor [14,16–19,39].

The down-regulation of survivin by wild type p53 in cell cycle is generally well known [3,19], but data that describe the expression pattern and the interaction between survivin and p53 in BCC cells are scarce [20]. Recently, more research groups have been dealing with this topic, mainly in the framework of apoptosis regulation. A variety of non-malignant and malignant cells are being studied, including human melanocytic and keratolytic lesions. The majority of skin lesion studies have focused primarily on squamous cell carcinoma and malignant melanoma [19,21–23]. However, this is not the case with BCC. Review articles on the molecular etiology and pathogenesis of BCC explain the genetic aberrations in human skin cancers at the level of the p53 gene; however, data on survivin expression are limited [20]. Therefore, we evaluated the expression pattern of both proteins with respect to intensity, relative number of positively stained cells, and cellular localization as detected by immunohistochemical methods and the relationship between their expression modes in cutaneous basal cell carcinoma.

MATERIAL AND METHODS

The sample included 31 subjects with cutaneous BCC. The hematoxylin and eosin-stained slides from each subject were independently reviewed by 2 pathologists to ascertain the diagnosis based on morphologic and immunohistochemical parameters and were correlated with clinical data. The standard diagnostic histomorphologic and immunohistochemical criteria were applied as previously described [24–26]. Immunohistochemical staining was performed using monoclonal mouse anti-p53 antibody (Dako, Carpinteria, CA) and monoclonal mouse antisurvivin antibody (Dako, Carpinteria, CA). Negative controls were obtained by simply omitting the primary antibodies.

In each case, the following features were assessed: (1) the intensity of staining, (2) the relative number of positively stained cells and (3), the subcellular localization of p53 and survivin antigens. Microsoft Excel software package was used to perform statistical analyses. Survivin and p53 expression and the correlation between them were analyzed by χ^2 test. A P value less than.05 was considered to indicate statistical significance.

RESULTS

Both p53 and survivin were found in BCC cells either in the nucleus (N), the cytoplasm (C), or both the nucleus and cytoplasm (NC). The expression of antigens among the 31 subjects was scored semiquantitatively as follows:

- *Intensity of p53 staining*: (a) absent or barely detectable; 3/31 cases, 9.7%; (b) weak; 8/31 cases, 25.8%; (c) moderate; 18/31 cases, 58.1%; and (d) strong; 2/31 cases, 6.4%.
- *Intensity of survivin staining*: (a) absent or barely detectable; 4/31 cases, 12.9%; (b) week; 22/31 cases, 71%; (c) moderate: 5/31 cases, 16.1%; and (d) strong: 0/31 cases, 0%.
- *Number of p53 positively stained cells*: (a) more than 25% per field of view: 26/28 cases, 92.9%; (b) less than 25% per field of view: 2/28 cases, 7.1%.
- *Number of survivin positively stained cells*: (a) more than 25% per field of view: 2/27 cases, 7.4%; (b) less than 25% per field of view: 25/27 cases (92.6%).
- *Subcellular localization of p53 staining*: (a) N localization only: 23/28 cases, 82.1%; (b) C localization only: 0/28 cases, 0%; and (c) NC localization: 5/28 cases, 17.9%.
- *Subcellular localization of survivin staining*: (a) N localization only: 1/27 cases, 3.7%; (b) C localization only: 0/27 cases, 0%; (c) NC localization: 26/27 cases, 96.3%.
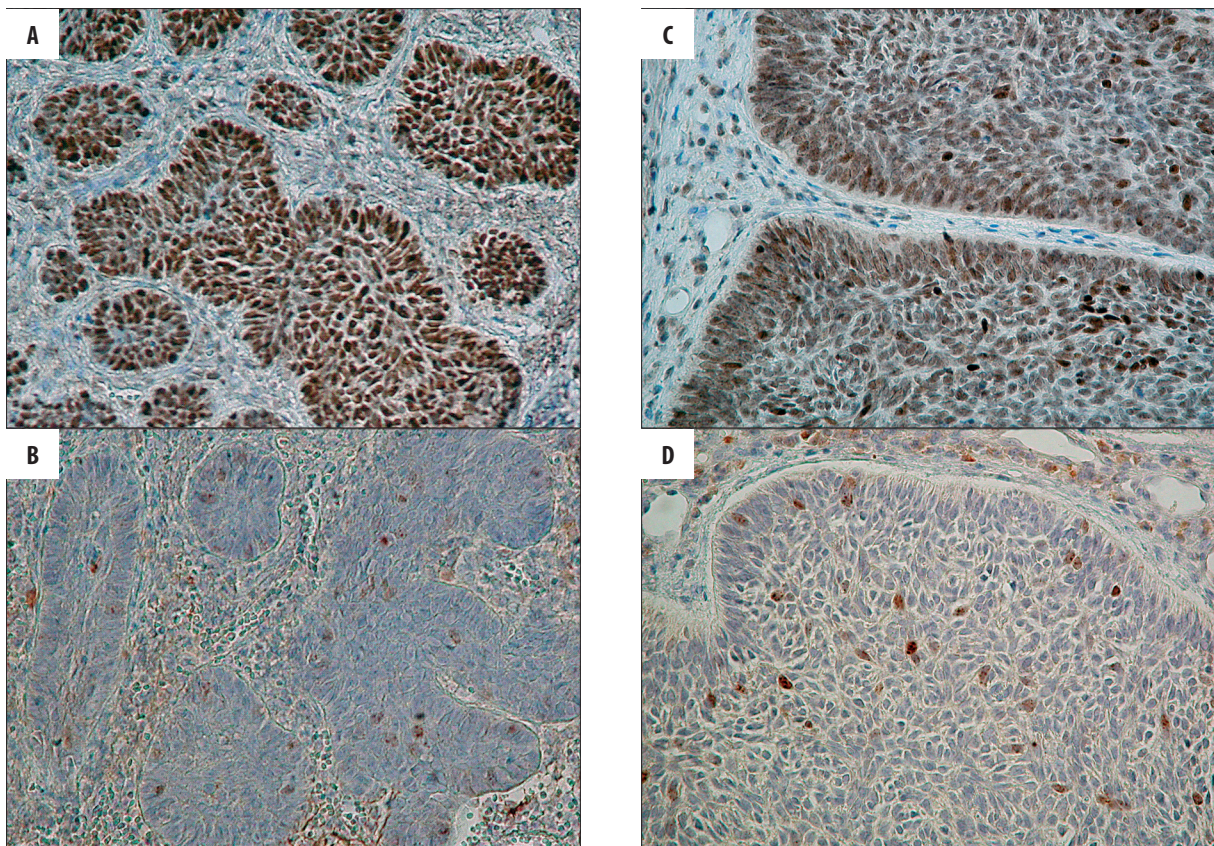
Figure 1. Different number of positively stained cells, subcellular localization, and intensity of immunoreactions of p53 and survivin antigens in BCC cells. (**A**) More than 25% of p53 positively stained tumor cells (case 1, original magnification: ×240). (**B**) Less than 25% of survivin positively stained cells, combined cytoplasmic and nuclear immunoreactivity (case 1, original magnification: ×240). (**C**) Heterogenous intensity of nuclear p53 immunoreactivity (case 8, original magnification: ×480). (**D**) Combined cytoplasmic and nuclear immunopositivity of survivin, nuclear staining exhibited punctuate pattern, and heterogenous intensity of immunoreaction (case 8, original magnification: ×480).

The results of all expression profiles are listed in Table 1. Tables 2 and 3 summarize the number of p53 and survivin positively stained cells and their cellular localization, respectively. The relation between the expression intensity of p53 and survivin is shown in Table 4. Increased p53 expression was present also in adjacent cells of normal epidermis. The χ^2 analysis confirmed a significant correlation between the intensity of survivin and p53 immunoreactivity ($P<.05$), a significant difference in the percentage of survivin- and p53-labeled cells ($P<.001$), and a significant difference in N and NC localization of survivin and p53 ($P<.001$).

DISCUSSION

The results of the current study show significant differences in the intensity of immunoreactions and in the percentage of labeled cells between p53 and survival expression. There were also significant differences in subcellular compartmentalization of both proteins. Positive p53 immunoreactivity was observed in 28 of 31 cases (90.3%). In accord with previous papers [20,26–28], we noted a high frequency of p53 overexpression in BCC. Interestingly, basal layers of the epidermis, adjacent to the tumor lesion, expressed mild and/or moderate immunopositivity. All of our subjects showed cytoplasmic p53-positive reactions only. These findings may be related to early accumulation of p53 immunopositive

clones as a consequence of sun exposure but without any pathological proliferation [29]. Calli-Demirkan and associates [30] found statistically significant differences between the immunoreactivity of p53 protein in normal epithelia adjacent to BCC and age of patients. Bäckvall and associates [31] pointed out that the foci of normal p53-positive keratinocytes are under regulation of more genetic events.

Generally, cells labeled by p53 display nuclear staining, but in some cases, cytoplasmic positivity has also been described [32]. The antiapoptotic protein survivin is also known to be localized both in cytoplasm and nucleus [33,34]. Similar to p53, nuclear export signal has been identified in survivin, as well [35]. It has been reported that survivin is highly expressed in many malignant tissues and is rarely detected in normal differentiated adult tissue [33]. In our study, survivin immunoreactivity was observed in 27 of 31 subjects (86.1%).

For p53, the number of positively stained cells in the majority of cases was more than 25% per field of view, whereas a greater number of subjects with positively stained survivin cells had less than 25% immunopositive cells (Table 2). Consistent with previous data, cellular localization of both proteins showed measurable differences. Results of our study showed nuclear p53 expression in 23 of 28 cases (82.1%)

Table 1. Panel of 31 basal cell carcinoma cases.

	p53			Survivin			Diagnosis -subtype	Age	Sex
	I	P (%)	s. l.	I	P (%)	s. l.			
1.	++	>25	N	++	<25	NC	Micronodular	54	M
2.	+	>25	N	+	<25	NC	Cystic pigmented	79	M
3.	++	>25	N	+	<25	NC	Solid	50	F
4.	+++	>25	NC	+	<25	NC	Multicentric	53	M
5.	++	>25	N	+	>25	NC	Multicentric	61	M
6.	++	>25	N	++	<25	NC	Multicentric	82	F
7.	+++	>25	N	+	<25	NC	Cystic	84	M
8.	++	>25	NC	++	<25	NC	Mixed form	79	F
9.	++	>25	N	+	<25	NC	Cystic	77	F
10.	++	>25	N	+	<25	NC	Multicentric	56	F
11.	++	>25	N	+	<25	NC	Solid	50	M
12.	++	>25	N	+	<25	NC	Cystic	88	M
13.	++	>25	N	+	>25	NC	Solid	57	M
14.	++	>25	N	++	<25	NC	Cystic	72	M
15.	+	>25	N	+	<25	NC	Mixed	64	M
16.	++	>25	N	+	<25	NC	Multicentric	31	F
17.	++	>25	N	+	<25	NC	Cystic	78	M
18.	+	>25	N	+	<25	NC	Multicentric	84	M
19.	+	>25	N	0	–	–	Multicentric	52	M
20.	++	>25	N	0	–	–	Cystic	53	M
21.	+	>25	NC	+	<25	NC	Solid	78	M
22.	+	>25	N	+	<25	NC	Solid	87	F
23.	++	>25	N	+	<25	NC	Multicentric	39	M
24.	++	>25	N	+	<25	NC	Multicentric	68	M
25.	++	>25	NC	+	<25	NC	Adenoid-cystic	75	F
26.	++	>25	N	++	<25	NC	Solid	79	M
27.	+	<25	NC	+	<25	NC	Multicentric	71	M
28.	+	<25	N	+	<25	N	Mixed form	78	M
29.	0	–	–	0	–	–	Cystic	72	F
30.	0	–	–	0	–	–	Cystic	70	F
31.	0	–	–	+	<25	NC	Cystic	59	M

I – intensity of immunoreactivity: 0 – no positivity, s. l. – subcellular localization of survivin and p53; + mild, ++ moderate, +++ strong; C – cytoplasm positivity; P – percentage of labeled cells; N – nuclear positivity; NC – nuclear as well as cytoplasmic positivity.

and combined nuclear and cytoplasmic staining in 5 of 28 cases (17.9%; Table 3).

A more-complicated situation seems to occur with compartmentalization of survivin. Conflicting data were reported with regard to survivin expression in various benign tissues and different types of tumors. Survivin exists in 5 different isoforms as a result of alternatively spliced transcripts: survivin, survivin-2B, survivin-ΔEx3, survivin-3B, and survivin-2a [36–38]. Survivin and survivin-2B are localized in the cytoplasm, particularly in mitochondria; survivin-ΔEx3 is primarily nuclear, survivin-2a is located in both the cytoplasm and nucleus, and the subcellular localization of survivin-3B remains to be determined [7,38,39].

Table 2. Number of p53 and survivin reactive cells.

	Percentage of stained cells		
	<25%	>25%	Total
P53	2 (7.1%)	26 (92.9%)	28/31
Survivin	25 (92.6%)	2 (7.4%)	27/31

Table 3. Cellular localization of p53 and survivin.

	Cellular localization			
	n	C	NC	Total
P53	23 (82.1%)	0 (0%)	5 (17.9%)	28/31
Survivin	1 (3.7%)	0 (0%)	26 (96.3%)	27/31

N – nuclear positivity' C – cytoplasmic positivity; NC – nuclear as well as cytoplasmic positivity.

Table 4. Intensity of survivin versus p53 expression.

Survivin / p53	0	+	++/+++	Total
0	2 (6.5%)	1 (3.2%)	0 (0.0%)	3 (9.7%)
+	1 (3.2%)	7 (22.6%)	0 (0.0%)	8 (25.8%)
++/+++	1 (3.2%)	14 (45.2%)	5 (16.1%)	20 (64.5%)
Total	4 (12.9%)	22 (71.0%)	5 (16.1%)	31 (100.0%)

0 – no positivity; + mild; ++ moderate; +++ strong.

These 5 survivin splicing variants and their unique subcellular compartmentalization create a delicate balance between induction and inhibition of the apoptotic process [36,38]. Discrepancies in the results regarding cytoplasmic versus nuclear survivin localization proceed from the fact that survivin basically exists in 2 subcellular compartments as a result of the properties of different polypeptides specified by alternatively spliced transcripts [40]. In general, malignant tumors are characterized mainly by nuclear localization [33,34,39]. In our study, nuclear or combined nuclear and cytoplasmic survivin immunoreactivity was observed in all of the 27 positive cases (Table 3). Thus, for the first time, we demonstrated a combined nuclear and cytoplasmic pattern of survivin expression in the majority of cutaneous BCC cases (26/27, 96.3%). As shown in our subjects, there were significant differences in the number of positively stained cells and cellular localization between p53 and survivin (P<.001).

There are several genes that play a role in the control of the G2/M phase of the cell cycle that are repressed following induction of wild type p53. Survivin is identified as a gene that is potently repressed at both the RNA and protein levels [3,30]. In the present study, we investigated this interplay at the protein level. Immunoreactivity of p53 was characterized by a predominance of moderate and strong intensity (20/31 cases, 64.5%) over mild intensity (8/31 cases.

25.8%). However, mild intensity of survivin staining dominated (22/31 cases; 71%). By comparison, moderate and strong p53 immunopositivity was accompanied by mild survivin immunopositivity in 16 cases (51.6%). Equal intensity of p53 and survivin was detected in 14 cases (45.2%). Only one case (3.2%) showed no p53 expression accompanied by mild intensity of survivin reaction (Table 4). Our immunohistochemical findings indicated that there are statistically significant differences between the expression intensity of both examined proteins (P<.05).

The basic function of p53 is associated with gene regulations that control apoptosis. Cell cycle arrest induced by p53 permits the repair of DNA damage or p53 can promote apoptosis by activation of the mitochondrial pathway. Wild type p53 leads to the release of mitochondrial cytochrome c, which is considered critical for the intrinsic apoptotic pathway [41–43]. Cytochrome c induces the formation of a large multimeric complex "apoptosome" within cytoplasm, which consists of cytochrome c, the adapter protein Apaf-1, adenosine triphosphate, and procaspase-9. The special 3-dimensional structure of this complex recruits and mediates the autoactivation of initiator caspase-9 with a subsequent caspase cascade [43]. Death receptor-mediated caspase activation may also induce p53-dependent apoptosis [44]. The precise mechanism for how survivin inhibits

apoptosis is not fully understood. Some research groups have reported direct suppression of caspase-3 and caspase-7 [45]. Other investigators have suggested that survivin lacks structural linker sequence, which allows its binding to caspase-3 [46]. Marusawa and associates [47] found that survivin needs to use the cofactor, interacting with the hepatitis B virus coded X protein and binding to and inhibiting procaspase-9. It is known that survivin also may indirectly suppress apoptosis via intermediate proteins, for example, through binding and inactivating Smac/DIABLO [48,49].

Previous studies revealed that many genes involved in the G2/M phase of cell cycle, including a number of genes encoding microtubule components, are subject to negative regulation by wild type p53 [50,51]. Survivin is only expressed in the G2/M phase [52]. It is associated with mitotic spindle by interaction with tubulin during mitosis [50,53]. p53 interacts with the survivin promoter, which is demonstrated as the first promoter to confer p53-dependent repression. Survivin promoter is repressed by both direct (p53 binding) and indirect (induction of p21 protein) mechanisms. Each mechanism may depend on particular stresses, phases of the cell cycle, and cell types, as well [3]. Furthermore, p53 can interfere with bcl-2 proteins in mitochondria, resulting in cytochrome c release [54].

CONCLUSIONS

The results of our study suggest that overexpression of wild type p53 protein may suppress the expression of survivin and its antiapoptotic activity in BCC cells. In addition, *de novo* production or stabilization of p53 is fundamental to triggering apoptosis in BCC [55].

Acknowledgments

Ethical consideration

The study was approved by local Ethics Committee of Jessenius Faculty of Medicine in Martin, registered in Office for Human Research Protection, US Department of Health and Human Services under No: IORG0004721.

Authorship

All authors have read and approved the final version of the manuscript, and all author listed as coworkers met the criteria for authorship.

Conflict of Interest

The authors declared no conflict of interest in relation to the article.

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