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ORIGINAL ARTICLE

The prognostic significance of Caspase-3 and survivin expression in colorectal cancer patients

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Summary

Purpose: *To investigate the expressions of caspase-3 and survivin in colorectal cancer patients and their possible associations with clinicopathological parameters and the oncological outcome.*

Methods: *Between January 2008 and December 2011, 85 patients with sporadic colorectal cancer were submitted to colectomy with curative intent. Postoperatively, all patients were followed every three months up to the 36th month. Immunohistochemical detection of the apoptosis-related proteins was carried out on 4-μm-thick deparaffinized sections from all primary tumors. Univariate and multivariate analyses were performed by using the R software for Windows, version 3.3.2.*

Results: *Setting the cut-off point for caspase-3 positivity at 5%, 48% of the patients were characterized as caspase-3(+). Caspase-3 positivity was not found related either to any clinicopathological parameter or to the oncological outcome. Choosing simple survivin positivity as the cut-off point for its expression, 78% of the patients were considered as survivin(+). Survivin inexpression predisposed to poorly differentiated tumors of advanced T stage. However, neither a dismal nor a favorable prognostic role for survivin expression or inexpression was disclosed. By dividing all enrolled patients in four different groups, a trend for worse 3-year overall survival rate in the caspase-3(-)/survivin(-) subgroup of patients was noticed (p=0.067).*

Conclusion: *Caspase-3 expression was unrelated to the oncological outcome in colorectal cancer patients. The proposed favorable prognostic role for survivin inexpression was not confirmed. On the contrary, survivin(-) tumors were mainly of poor differentiation and advanced T stage. An inverse relationship between caspase-3 and survivin expressions was also not confirmed. Future studies focusing on specific survivin isoforms expression or inexpression may give answers on apoptotic-antiapoptotic interactions on cancer cell death*

Key words: *caspase-3, survivin, colorectal cancer, apoptosis, prognosis, cell death*

Introduction

Cell death is a fundamental process for tissue homeostasis. By eliminating harmful, unwanted or damaged cells and by removing redundant cellular structures, cell death controls the total number of cells and promotes their growth and differentiation further [1,2] .

Apoptosis or programmed cell death, plays a crucial role in biological homeostasis and defense against external or internal injury in mammals [3]. Normally, there is a fine balance between apoptosis and regeneration. Defective regulation of apoptosis may play a role in the etiology of cancer, autoimmune and degenerative diseases [4].

Cystein-aspartic proteases (Caspases) are proteolytic enzymes holding a key-role in cell death and inflammation. Based on their function, caspases are defined as apoptotic or inflammatory, while the apoptotic ones are subdivided further to initia-

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tors and executioners [2]. Apoptosis is defined as a caspase-dependent variant of cell death and it can be initiated by intracellular (intrinsic) or extracellular (extrinsic) stimuli [1-3,5].

Intrinsic (or mitochondrial) apoptosis is activated in response to cellular stress (e.g., cytotoxic drugs) and obligatorily requires the participation of the Bcl-2 family of proteins [6]. The apoptotic stimuli release cytochrome *c* from the intermembrane space into the cytosol and upon its release, cytochrome *c* binds to apoptotic peptidase-activating factor 1 (Apaf-1). Multiple copies of procaspase-9 bind to the Apaf-1 ring to form the apoptosome, the central signal initiator for caspase-9 activation [7,8].

On the other hand, FasR and FasL are cell surface molecules of the TNF superfamily [9]. Upregulation of FasR expression on cancer cells promotes their ligand binding on the surface of T lymphocytes [10]. Death induced signaling complex (DISC) makes the first link of the extrinsic apoptosis signal [11], recruiting and activating the initiators caspases-8 and -10 [12].

Upon their activation, initiator caspases mediate the activation of the executioners caspases-3, -6 and -7 [4] which cleave essential cellular substrates and dismantle cells [12]. Lysosomal rupture may be determinant for inducing apoptosis [13]. Whenever irreversible plasma membrane permeabilization and/or complete fragmentation of the cell occur, these cells are considered as dead [14].

The survivin gene is located in chromosome 17q25 encoding a wild-type 16.5kDa multifunctional protein (survivin), the smallest member of the antiapoptotic proteins (IAP) [15]. Survivin is almost absent in well differentiated tissues, but it is expressed in embryonal and fetal tissues [16] and in nearly all cancer types [17]. Survivin expression in tumor cells is negatively regulated by the tumor suppressor gene *p53* [18] and is upregulated by the Wnt/β-catenin pathway [19]. Survivin is present in different cellular compartments such as nucleus, cytosol and mitochondria, exerting distinct functions. The nuclear survivin contributes as a chromosomal passenger complex protein to the proper alignment of chromosomes, and via binding to tubulin to the spindle formation [20]. Cytoplasmic survivin stabilizes the phosphorylated X-linked inhibitor of apoptosis protein (XIAP) and by degradating caspase-3 and caspase-9 inhibits their apoptotic activity [21].

The purpose of this study was to investigate the expressions of caspase-3 and survivin in colorectal cancer patients and their possible associations with clinicopathological parameters and the oncological patient outcome.

Methods

Patients

All colorectal cancer patients who were referred for further investigation and treatment to the 1st Department of Surgery in "Agia Olga" Hospital in Athens, were presented and discussed in the Cancer Meeting. The most suitable therapeutic strategy was planned and adopted by all surgeons.

All patients suffered from sporading colorectal cancer having undergone colonoscopy and biopsies for histological confirmation of the disease, all had undergone at least computed tomography (CT) of the thorax and abdomen for staging of disease, while patients suffering from rectal tumors had been further submitted to magnetic resonance imaging (MRI) of the pelvis for loco-regional staging [22].

Excluded patients were as follows: (i) who were diagnosed with histological types others than adenocarcinoma, (ii) who were classified as having locally advanced disease and referred for neo-adjuvant therapies, (iii) who had multiple distant metastases and referred for system-

Table 1. Clinicopathological characteristics of the enrolled patients

Characteristics	*No of patients (n=85))*
Gender	
Male	55
Female	30
Age (Median + IR)	70 (61-76)
Differentiation	
Well	4
Moderate	60
Poor	21
Localization of the primary tumor	
Right colon	31
Left colon	34
Rectum	20
Size of the primary tumor (Median + IR)	45 (35-60)
T (mm)	
T1	3
T2	14
T3	66
T4	2
N	
N0	52
N1	22
N2	11
Stage	
I	14
II	38
III	33
Caspase-3 (Median + IR)	4 (2-5)
Survivin (Median + IR)	10 (2-20)

atic chemotherapy, (iv) who were diagnosed as stage IV, even though a curative resection was performed and (iv) who were operated for palliation. All the others were considered as eligible for oncological colectomy as first therapeutic option with curative intent.

Between January 2008 and December 2011 85 colorectal cancer patients were submitted to colectomy and constituted the material of the present study. Demographics, clinical data, type of operation, postoperative complications, histological findings, adjuvant therapies, follow-up and time elapsed to either local or distant recurrence were recorded and retrospectively analyzed. For the needs of the present study, all patients were followed every three months, up to the 36th month.

Oncological outcome

The pathological stage of the disease was based on the 7th TNM Classification [23], while the tumor grade was based on the WHO classification [24]. During the follow-up, the period elapsed from the initial operation to recurrence development, the site and the organ of recurrence, the therapeutic strategies and the final outcome were documented, in order to estimate the disease free survival (DFS) and the overall survival (OS).

Immunohistocemistry (IHC)

IHC detection of apoptosis-related proteins was carried out on 4-μm-thick deparaffinized sections. Before IHC, the sections were subjected to heat-induced epitope retrieval by incubation in a 0.01 M sodium citrate solution (pH 6) at 120°C for 10 min, followed by a 2-h cool-down. Primary antibodies were diluted in the following buffer: 0.1 M phosphate buffered saline (PBS), 0.3% (m/v) bovine serum albumin (BSA), 0.1% (m/v) sodium azide, 0.06% (m/v) n-ethyl-maleimide, and 20% (v/v) glycerol (PAB). Active caspase-3 was detected with a species-unspecific rabbit polyclonal antibody (1:1000 diluted; BD Biosciences, Le Pont-de-Claix, France) that specifically recognizes the large fragment (17 kDa) of the active protein but not full-length caspase-3. The AF886 antibody (RD Systems; Bad Nauheim, Germany, 1:400) was applied to detect survivin protein. Stained speci-

Table 2. Univariate analysis between expression of caspase-3 ≥5% and evaluated parameters and multivariate analysis among several factors possibly affected by caspase-3 expression

Factors	*Univariate analysis*				*Multivariate analysis*		
	No of pts	*Caspase-3 <5% (n=44)*	*Caspase-3 ≥5% (n=41)*	*p value*	*RR*	*p value*	*95% Confidence interval*
Survivin, % (Median + IR)		10 (1.25-20)	10 (4.5-20)				
Survivin						0.4941	-0.20064-0.411959
Negative	19	11	8				
Positive	66	33	33				
Gender	55	27	28			0.5669	0.30244-0.166921
Male	30	17	13				
Female		70 (60-75.5)					
Age (Median + IR)			70 (63-76.5)			0.8804	0.01016-0.011822
Differentiation	64					0.3074	-0.14181-0.4441
Well/Moderate	21	34	30				
Poor		10	11				
Localization of the primary tumor	65					0.4444	0.38185-0.169172
Colon	20	32	33				
Rectum		12	8				
Size of the primary tumor (mm)						0.9854	-0.26056-0.265398
<40	27	14	13				
≥40	58	30	28				
T						0.5472	-0.42024-0.224526
T1/T2	17	7	10				
T3/T4	68	37	31				
N							
N0	52	24	28				
N1/N2	33	20	13			0.3472	-0.35991-0.128144

mens were viewed at an objective magnification of ×100 and ×200 by two investigators. Good immunostaining data (i.e., low background staining and good precision) were obtained with both antibodies.

Statistics

All statistical calculations were performed with the use of the R software for Windows, version 3.3.2. The data were entered into Microsoft excel sheet and imported to R. Chi-square was used for categorical data analysis. Mann-Whitney U test was used for statistical analyses of quantitative data. A p value<0.05 was considered as statistically significant. DFS and OS were calculated for all patients and Kaplan-Meier curves were generated. Survival differences were evaluated with log-rank test. Multivariate analysis of factors that might influence the recurrence, DFS and OS was carried out using the Cox proportional hazard method.

Results

There were 56 male and 29 female patients with a median age of 70 years (IR: 61-76 years). The clinicopathological characteristics of the enrolled patients are presented in Table 1. In 31 among the enrolled patients the cancer had been developed on adenoma, while in the remaining 54 was it developed *de novo*.

Caspase-3 expression

Choosing as a cut-off point for caspase-3 positivity the 5% (Table 2), 48% of the patients (41 out of 85) were characterized as positive. Neither the univariate analysis between the caspase-3(+) and the caspase-3(-) tumors nor the multivariate analysis among several factors possibly affected by the caspase-3 expression disclosed any statistically significant difference.

Survivin expression

For survivin expression we set different cut-off points and we compared them to several variables in various combinations. We found that simple expression of survivin was giving the most utilizable

Table 3. Univariate analysis between survivin(-) and survivin(+) tumors and multivariate analysis among several factors possibly affected by surviving expression

Factors	*Univariate analysis*				*Multivariate analysis*		
	No of pts	*Survivin(-) (n=19)*	*Survivin(+) (n=66)*	*p value*	*RR*	*p value*	*95% Confidence interval*
Caspase-3, % (Median+IR)		4 (2-5)	4.5 (2-5)				
Caspase-3						0.4941	-0.11093-0.227768
Negative	44	11	33				
Positive	41	8	33				
Gender						0.7545	-0.14724-0.202298
Male	55	12	43				
Female	30	7	23				
Age (Median+IR)		71 (55-74)	70 (62.5-76.25)			0.3380	-0.00419-0.012056
Differentiation				<0.001	-3.975	0.0001	-0.59789- -0.19785
Well/Moderate	64	8	56				
Poor	21	11	10				
Localization of the primary tumor						0.3567	-0.1093-0.299705
Colon	65	15	50				
Rectum	20	4	16				
Size of the primary tumor (mm)						0.3188	-0.09639-0.292128
<40	27	6	21				
≥40	58	13	45				
T				0.013	-2.021	0.0467	-0.4717- -0.00355
T1/T2	17	0	17				
T3/T4	68	19	49				
N						0.7653	-0.15498-0.209835
N0	52	10	42				
N1/N2	33	9	24				

Table 4. Characteristics of the recurrence

Gender	*Age (years)*	*Caspase-3 value*	*Survivin value*	*Localization of the tumor*	*Grade*	*Stage*	*Site of recurrence*	*Time of recurrence (months)*
F	78	2	1	Right colon	Moderate	T3N2	Peritoneal seedlings	3
F	74	2	0	Left colon	Moderate	T3N2	Liver	3
M	60	2	20	Right colon	Poor	T3N2	Liver	5
F	76	5	60	Right colon	High	T4N1	Peritoneal seedlings	6
M	54	10	10	Right colon	Moderate	T3N0	Liver	6
M	72	10	2	Rectum	Moderate	T3N1	Liver	6
M	70	5	5	Left colon	Moderate	T3N2	Liver	6
F	76	2	40	Rectum	Poor	T3N0	Liver + Lung	6
M	52	3	2	Rectum	Moderate	T3N1	Liver + Lung	7
M	59	1	20	Rectum	Moderate	T3N2	Liver	10
F	72	1	2	Rectum	Moderate	T3N0	Liver	12
M	66	3	0	Rectum	Poor	T3N1	Liver + Lung	16
M	65	5	25	Left colon	Moderate	T3N2	Local recurrence	19
M	38	2	15	Left colon	Moderate	T3N2	Liver	33

M: male, F: female

Table 5. Univariate analysis between recurrence and the evaluated parameters and Multivariate analysis among factors possibly affecting the recurrence

Factors	*Univariate analysis*		*Multivariate analysis*			
	Recurrence (n=14)	*No recurrence (n=71)*		*RR*	*p value*	*95% Confidence Interval*
Caspase-3					0.7061	-0.1876-0.127691
Positive	5	36				
Negative	9	35				
Survivin					0.4895	-0.13811-0.285911
Positive	11	55				
Negative	3	16				
Gender					0.7159	0.19205-0.132533
Male	9	46				
Female	5	25				
Age (Median + IR)	68 (57.75-74.5)	70 (61-76.75)			0.5802	-0.0097-0.00547
Differentiation					0.9187	-0.21401-0.193084
Well/Moderate	10	54				
Poor	4	17				
Localization of the primary tumor					0.1642	-0.05627-0.325426
Colon	8	57				
Rectum	6	16				
Size of the primary tumor (mm)					0.8713	-0.16666-0.196264
<40	4	23				
≥40	10	48				
T			0.04		0.1469	-0.05894-0.38703
T1/T2	0	17				
T3/T4	14	54				
N			<0.001	2.65	0.0098	0.055943-0.394688
N0	3	49				
N1/N2	11	22				

results. So, we chose simple survivin positivity as the cut-off point. Based on that, 66 out of the 85 patients (78%) were considered as survivin(+) (Table 3).

Univariate analysis between the survivin(+) and survivin(-) groups of patients disclosed that survivin inexpression predisposed to poorly differentiated tumors of advanced T stage. Multivariate

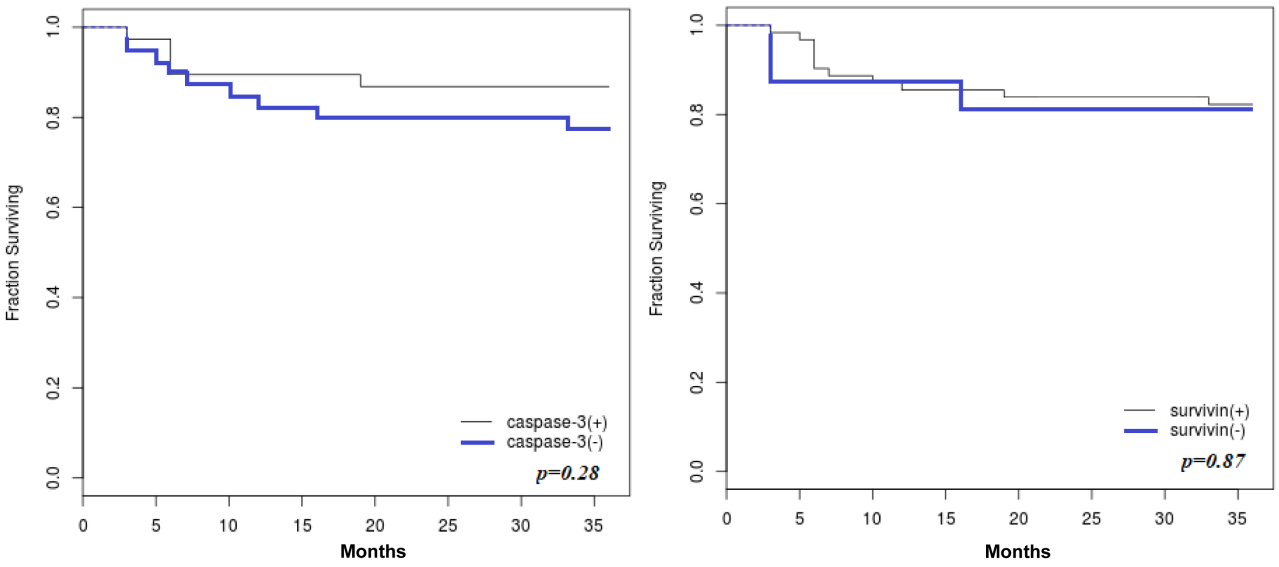

Figure 1. Kaplan-Meier survival curves for disease free survival.

Table 6. Multivariate analysis among factors possibly affecting the overall survival

Parameter	*RR*	*p value*	*95% Confidence Interval*
Caspase-3		0.7875	-1.94331-1.479367
Positive			
Negative			
Survivin		0.2861	-0.18113-1.793866
Positive			
Negative			
Gender		0.2327	-2.89462-0.716513
Male			
Female			
Age (Median + IR)		0.4685	-0.11927-0.055462
Differentiation	2.415	0.0185	0.466578-4.91722
Well/Moderate			
Poor			
Localization of the primary tumor		0.1691	-3.50372-0.627212
Colon			
Rectum			
Size of the primary tumor (mm)		0.5430	-2.59077-1.376139
<40			
≥40			
T		0.8228	-2.14198-2.685285
T1/T2			
T3/T4			
N		0.9499	-1.85322-1.973939
N0			
N1/N2			
Recurrence		0.3217	-3.77135-11.31673
Disease free survival	5.830	<0.0001	0.511146-1.043561

analysis among several factors possibly affected by the survivin expression disclosed that the lack of its expression was independently related to poor differentiation and to advanced T stage of the tumors.

Oncological outcome

None of the patients died postoperatively and none was lost during the follow up, thus all the patients were enrolled in the long term follow up. During the follow up, 7 patients died of diseases unrelated to their colorectal cancer, namely cerebrovascular accident (n=2), heart disease (n=2), road traffic accident (n=1), lung cancer (n=1) and lymphoma (n=1), being however, disease-free from their colorectal cancer. Hence, we included all of them for the recurrence calculation, but we excluded them for the disease-free survival (DFS) and the overall survival (OS) calculation.

Recurrence

Fourteen out of the 85 (16.5%) enrolled patients developed disease recurrence (Table 4). Univariate and multivariate analyses (Table 5) disclosed the well-known factor of metastatic infiltration of the regional lymph nodes as independently related to the recurrence. Neither caspase-3 nor survivin expressions were found as related to that.

Disease free survival (DFS)

Eleven patients recurred within 12 months, 2 patients recurred between the 12th and the 24th month and one more patient recurred during the 3rd year of follow up. Apart from the well-known dismal prognostic role of the recurrence, no other parameter among the examined ones was found as related to the DFS in the multivariate analysis. Neither caspase-3 nor survivin affected DFS in statistically significant levels (Figure 1).

Overall survival (OS)

Six patients died within the 1st year since the initial operation, 3 patients died within the 2nd year and 2 more patients died during the 3rd year of follow up. Three patients who had recurred on 5th, 19th and 33rd month respectively, were still alive on the

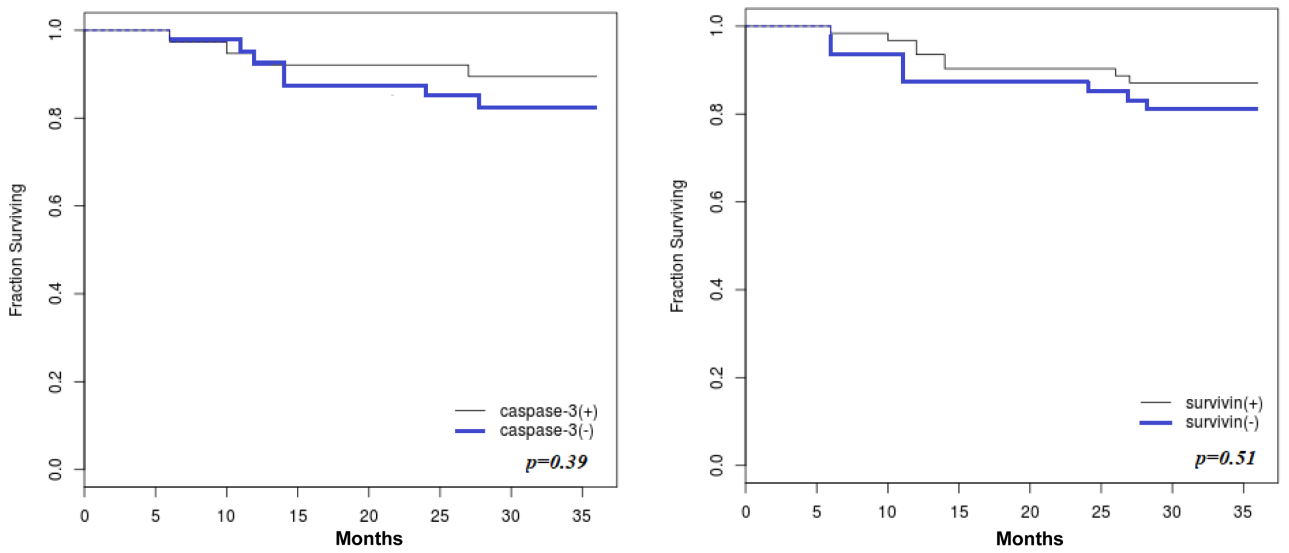

Figure 2. Kaplan-Meier survival curves for overall survival.

Table 7. Comparison between the four different groups of patients

Parameter of outcome	*No. of patients*	*Group A Caspase-3(+) Survivin(-)*	*Group B Caspase-3(-) Survivin(+)*	*Group C Caspase-3(+) Survivin(+)*	*Group D Caspase-3(-) Survivin(-)*
Recurrence	85	(n=8)	(n=33)	(n=33)	(n=11)
		0	6	5	3
Overall survival (%)	78	(n=7)	(n=31)	(n=31)	(n=9)
One-year survival		7 (100)	30 (97)	28 (90)	7 (77)
Two-year survival		7 (100)	28 (90)	28 (90)	6 (67)
Three- year survival		7 (100)	27 (87)	27 (87)	6 (67)

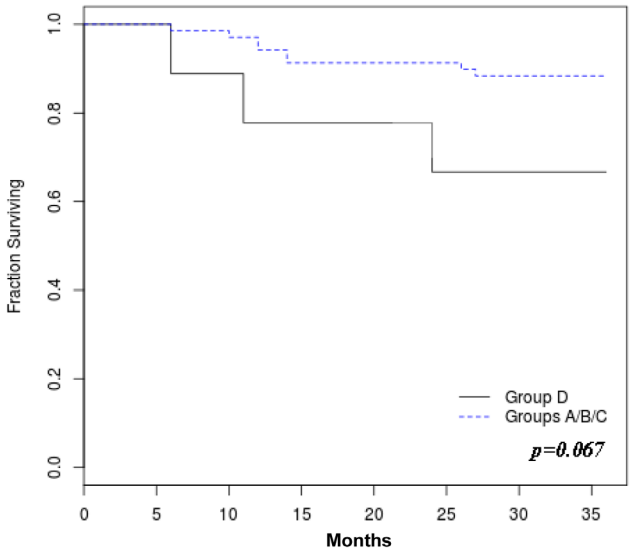

Figure 3. Three-year OS between the groups.

36th month. Finally, among the 78 patients enrolled in the long term follow up, 72 were alive on the 12th month (1-year OS 92%), 69 were alive on the 24th month (2-year OS 88.5%) and 67 were alive after three years of follow up (3-year OS 86%). Multivariate analysis among factors probably affecting the OS (Table 6) disclosed poor differentiation of the tumor and DFS as adversely related to the OS. Neither caspase-3 nor survivin expression affected OS (Figure 2).

Comparison of different groups of patients

Using the proposed cut-off points, the enrolled patients could be divided in four different groups (Table 7). Although the sizes of the groups were not suitable for credible results, we found that the group A [caspase-3(+)/survivin(-)] had the best and the group D [caspase-3(-)/survivin(-)] the worst outcome in terms of recurrence, DFS and OS. Comparing group A to the groups B/C/D (7 versus 71 patients), no statistically significant differences were detected. On the other hand, comparing group D to all other groups (9 versus 69 patients) a trend for worse 3-year OS rate in the former group was noticed (66% versus 89%, p=0.067) (Figure 3).

Discussion

The main purpose of the host immune system [25] as well as the applied antineoplastic therapies [26,27] is to induce tumor cell death. Among the several types of cell death, apoptosis has been extensively studied.

Activation of the executioner caspase-3 represents the final step in the apoptotic process initiated by both the extracellular and the intracellular pathways. Caspase-3 exists in the form of zymogen in the cytoplasm, is activated at an early stage of the apoptotic process eventually leading to cell death, but at a late stage its activity significantly decreases [28]. It could be hypothesized that the higher the caspase-3 levels, the higher the apoptotic rate, thus more malignant cells are led to death, hence a favorable prognostic role is reserving for its expression.

However, the prognostic significance of its expression in colorectal cancer patients remains controversial. Other authors [29-32] addressed its expression as related to a favorable outcome, while others [28,33-36] stated that its expression was related to a shorter OS, while Reimers et al [37] disclosed that its expression can be used for the calculation of a proposed apoptosis/proliferation index which might have prognostic significance only for left-sided microsatellite stable tumors. Moreover, a recent meta-analysis [38] did not find any relation between caspase-3 expression and OS.

Huang et al [36] disclosed that caspase-3 is activated by dying tumor cells causing increased secretion of PGE2, finally resulting in tumor cell repopulation and resistance to radiotherapy. Moreover, Liu et al [39] reported that caspase-3 promoted genomic instability facilitating carcinogenesis in response to DNA damage. Both findings suggest that paracrine signals released by dying cells promote carcinogenesis [2,40]. Thus, a clear distinction between dying and dead cells is mandatory [2,41].

In the present study we set different cut-off points for caspase-3 expression (from simple positivity up to 40%) and we compared them to several variables in various combinations. We were not able to identify any statistically significant difference between the caspase-3(+) and the caspase-3(-) tumors either in the clinicopahological characteristics or in the oncological patient outcome within three years.

Li et al [42] and Parenti et al [43] reported that survivin expression was inversely related to caspase-3 activation, speculating that survivin (by inhibiting caspase-3 activation) decreases apoptosis. Survivin expression may also obliterate the apoptotic checkpoint allowing the aberrant progression of cancer cells through mitosis [44], enhancing the angiogenesis [16] and increasing the invasion favoring the metastases [45] in colorectal cancer patients. Although these reports may be partly explanatory, they raise the notion that survivin expression inhibits apoptosis, playing a dismal prognostic role, while its inexpression could be related to a favorable outcome. This notion was confirmed in recent meta-analyses [46,47] which unanimously disclosed survivin expression as a dismal prognostic factor for colorectal cancer patients.

The unexpected finding of the present study was that survivin expression was independently related to well/moderate differentiated tumors of T1/T2 stage, imposing an indirect favorable prognostic role for its expression. Although survivin did not independently affect the recurrence, the DFS or the OS, poorly differentiated tumors (predominant in survivin(-) patients) predisposed to shorter OS.

Among the 14 studies enrolled in Huang et al [46] and the 15 studies enrolled in Krieg et al [47] meta-analyses, 8 were coming from countries outside Asia and Far East. Studies from UK [48], Sweden [49], Egypt [50] and Germany [51] revealed a dismal prognostic role for survivin positivity. On the other hand, studies from Australia [52], Turkey [53] and Greece [54] did not find statistically significant correlation between survivin positivity and survival, while in a study from France [55], survivin positivity was indicative, although not statistically significant, for improved survival.

Population studies have shown that among the five known isoforms of survivin, the -31G/C polymorphism was associated with increased colorectal cancer risk in Asians [56], correlating however to an improved survival [57], while in a Caucasian population the same risk was found as related to the -31C/C polymorphism [58].

Based on the above, we feel that the prognostic role of survivin in colorectal cancer patients remains controversial. Data from different countries, probably enrolling patients with different isoforms of survivin expression, with significant differences in the sample sizes, determining the positivity by the use of different antibodies, encountering it in different parts of the tumor or even in different cellular compartments [59] may be possible explanations for the confusing and conflicting published results.

Hypothesizing that caspase-3 and survivin are inversely correlated to the cell death process, caspase-3(+)/survivin(-) tumors (namely tumors with high apoptotic and low anti-apoptotic characteristics) should have the best oncological outcome. In fact, the present study concluded in 100% 3-year survival rate for this subgroup of patients. On the other hand, caspase-3(-)/survivin(+) tumors (namely tumors with low apoptotic and high anti-apoptotic characteristics) should have the worst oncological outcome. The present study disclosed lower 3-year survival rate for this subgroup of patients (87% versus 100%). However, the worst oncological outcome (67% 3-year OS) was noticed in the caspase-3(-)/survivin(-) tumors (namely tumors with low apoptotic and low anti-apoptotic characteristics). Comparing this subgroup of patients to the rest of the patients of the present study, we found that combination as indicative (p=0.067) for unfavorable prognosis. Not surprisingly, the lack of apoptosis in poorly differentiated and advanced T stage tumors reserved the worst prognosis.

We presented the results of a small, retrospective, single-institute study (low level of evidence), which was conducted however on a homogeneous Caucasian population.

Caspase-3 positivity was unrelated either to the clinicopathological variables or to the oncological outcome, indicating that apoptosis by itself may not be sufficient enough for effective cancer cell death. Caspase-3 inexpression may reserve an unfavorable outcome for the caspase-3(-)/survivin(-) subgroup of patients, but the small sizes of the patient groups did not allow a statistical significance to be reached.

The unfavorable prognostic role of survivin expression was not confirmed. On the contrary, survivin(+) tumors were mainly well differentiated of small T stage. However, these indirect favorable correlations do not finally reflect a favorable oncological outcome. An inverse relationship between caspase-3 and survivin expressions was also not confirmed.

Future studies focusing on specific survivin isoforms expression or inexpression may give answers on the apoptotic-antiapoptocic interactions on cancer cell death.

Conflict of interests

The authors declare no conflict of interests.

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