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ORIGINAL ARTICLE

Tissue Expression of the Proteins Fas and Fas Ligand in Colorectal Cancer and Liver Metastases

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Abstract
Background and Aim Our purpose is to study the clinical significance of Fas/FasL expression in colon cancer and liver metastases (LM).
Material and Methods The expression of Fas/FasL in 68 patients with colon cancer was examined immunohistochemically and correlated to the clinicopathological features of the tumors.
Results High expression of FasL, was observed in stage D and in LM ($p = 0.024$). Fas expression was reduced in stage D tumors and in LM, when compared to earlier stages of disease ($p = 0.024$). LM had also shown a decreased expression of Fas ($p = 0.016$). Tumors with low FasL expression upregulate more often their Fas expression ($p = 0.028$). No correlation could be established regarding the patients survival.
Conclusions Low expression of Fas and high expression of FasL are more often in colon tumor stage D and in liver metastasis; these imply tumor aggression, resistance against apoptosis, and could be held as negative prognostic factors.

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Purpose

The aim of the present study is to evaluate the clinical significance of the expression of the apoptosis-related proteins Fas, Fas ligand (FasL) in colorectal cancer and its liver metastasis. Fas receptor and its ligand consist a protein complex that activates the extrinsic pathway of apoptosis [1]. Tissue expression of the above proteins will be related to tumor stage and overall survival.

Materials and Methods

The study evaluated 68 patients (35 men, 33 women) who were operated for colorectal cancer during the last 3 years. None of these had received chemo-, radio- or immunotherapy before resection. There were 14 patients with stage A, nine with stage B, and 16 with stage C disease. Finally, there were 27 with stage D disease. In more details, in six cases, there was only colon specimen, in 13, only the liver metastasis, and in the rest eight cases, colon as well as liver metastasis (synhron or metachron) were available. Patient cohort was separated into two groups with regard to the presence of liver metastasis or not.

Paraffin wax-embedded surgically resected thin tumor sections (3–4 μ) were deparaffinized in xylene and rehydrated before analysis.

A Benchmark XT and Ventana automatic immunohistochemistry machine was used.

Rabbit monoclonal Fas and Fas-ligand antibodies of Cell Signaling Technology with an incubation period of 40 and 32 min, respectively, were applied to all sections. The

Table 1 Tissue sample features

		Number	Percentage (%)
Dukes	A	14	18.9
	B	9	12.2
	C	16	21.6
	D	35	47.3
Tissue origin of stage D	Bowel	14	40
	Liver metastases	21	60
Tumor—T	T2	20	37.7
	T3	32	60.4
	T4	1	1.9
Nodes—N	No	27	50.9
	Yes	26	49.1
Metastasis	No	53	71.6
	Yes	21	28.4
Differ	Well=0	13	17.6
	Moderate=1	45	60.8
	Poor=2	16	21.6
Astler–Coller	B1	14	18.9
	B2	9	12.2
	C1	3	4.1
	C2	13	17.6
	D	35	47.3

evaluation of the results was made using an optic microscope Nikon eclipse 50i with adapted camera Nikon Digital sight DS-S1 (Nikon Corporation,Japan) with capacity of 100-fold magnification.

The expression of the proteins Fas and FasL was evaluated with null to three crosses. No expression or one cross was regarded as low expression, while two or three crosses as high expression. Comparison between the two groups was performed using χ^2 test and where necessary with Fisher's exact test. The significant levels are bilateral and the statistical significance was set at 0.05. The analysis of all data was made with the program SPSS 17.0.

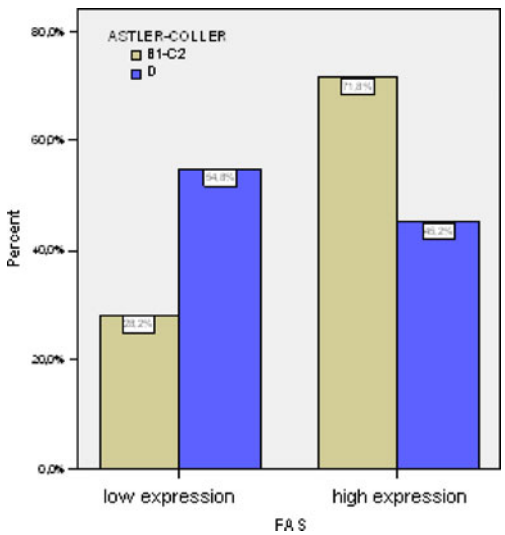

Fig. 1 Low and high expression of Fas protein

Results

The features of all 74 examined tissue samples are exposed in Table 1.

Lymph node involvement and liver metastasis were present in 49.1% and 28.4% of the cases, respectively. A 47.3% of the cases were of stage D disease.

The incidence of low and high expression of Fas protein has been related to the following parameters: tumor stage, differentiation, metastatic tissue, lymph node involvement and survival (Fig. 1). Cancer cells of stage A to C showed higher expression of Fas as compared to that of stage D tumors (Table 2; Fig. 2). Metastatic tissues did also underexpress the Fas protein. Both results were statistically significant.

The respective results regarding the FasL protein are shown in Table 3 and Fig. 3.

FASL expression was upregulated in stage D tumors as compared to earlier stages of disease (Fig. 4). There was no statistically significant difference in the expression rate of FasL from metastatic tissue or tumors with moderate or poor differentiation.

Table 2 Fas expression according to stage

		FAS				
		Low expression		High expression		
		Number	Percentage (%)	Number	Percentage (%)	P χ^2 test
Astler–Coller	B1–C2	11	28.2	28	71.8	0.024
	D	17	54.8	14	45.2	
Nodes	No	8	29.6	19	70.4	0.552
	Yes	9	37.5	15	62.5	
Metastasis	No	16	31.4	35	68.6	0.016
	Yes	12	63.2	7	36.8	
Differ	0	6	54.5	5	45.5	0.328
	1–2	22	37.3	37	62.7	
Death	No	1	33.3	2	66.7	>0.999
	Yes	11	35.5	20	64.5	

The relationship between the two proteins is shown in Table 4. It comes out that the Fas protein is more overexpressed in tumors with downregulation of FasL expression.

Unfortunately, the analysis of survival data gave up no clear conclusions, as most of the patients were treated in different centers, were enlisted in different follow-up protocols and the follow-up period was estimated for the first three postoperative years. Most of them were still alive, while some died from another cause. In that mean, there was no significant difference in survival regarding the FasL expression mentioned. On the other side, the results were opposite than expected for the protein Fas.

Discussion

The FAS and FASL system plays a key role in regulating apoptotic cell death and in human carcinogenesis. Corruption of this signal pathway has been shown to participate in immune escape and tumorgenesis. Its significance has been also evaluated in many other cancers such as HCC [2], gastric [3], esophageal [4], pancreatic [5], lung [6], breast [7], and ovarian [8] cancer.

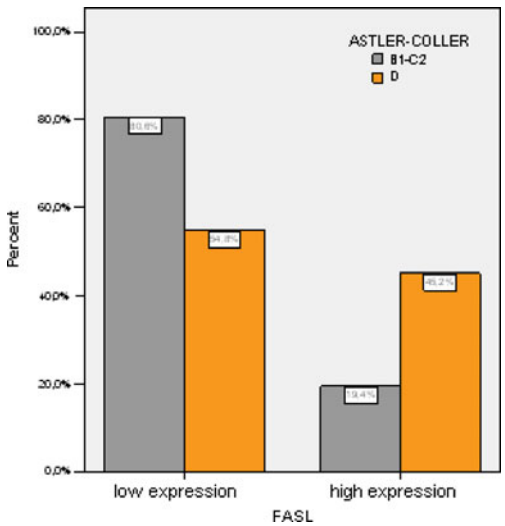

Fig. 2 Low and high expression of FasL—protein

In our study group, 60% and 31% of examined tissues showed high expression for Fas and FasL, respectively. The expression rates were associated with clinical parameters such as tumor stage, differentiation, nodal involvement and survival. The liver metastases were additionally evaluated alone, as a subgroup of stage D samples.

The Fas expression was significantly downregulated in Stage D disease (primaries and liver metastasis; p=0.024). Metastatic tissues did also show a decreased expression of Fas when compared to the total sample of primary colonic tissues (p=0.016). This fact represents a strong antiapoptotic privilege of metastatic tumor cells.

The opposite results were found regarding the FasL protein.

FasL was overexpressed in stage D tumors, as compared to earlier stages (p=0.024). The fact that the tumor cells upregulate their FasL expression gives them immunological privilege contributing to their malignant capacity. Liver metastasis did also show an increased FasL expression, although this upregulation was not statistically significant in our study group. Finally, the Fas expression is higher in tumors with low FasL expression as compared to those that overexpress it (p=0.028).

We could not extract safe conclusions regarding survival, as the follow-up period is relatively short for colon cancer.

Our conclusions are in line with the majority of published literature. According to the existing data the Fas expression is downregulated as the tumor progresses. In that mean, a high Fas-expression index constitutes a favorable prognostic factor and should be associated with longer survival.

Table 3 FasL expression according to stage

		FASL				
		Low expression		High expression		
		Number	Percentage (%)	Number	Percentage (%)	P χ^2 test
Astler–Coller	B1–C2	29	80.6	7	19.4	0.024
	D	17	54.8	14	45.2	
Nodes	No	19	82.6	4	17.4	0.103
	Yes	16	61.5	10	38.5	
Metastasis	No	35	71.4	14	28.6	0.420
	Yes	11	61.1	7	38.9	
Differ	0	9	75.0	3	25.0	0.740
	1–2	37	67.3	18	32.7	
Death	No	3	75.0	1	25.0	>0.999
	Yes	24	82.8	5	17.2	

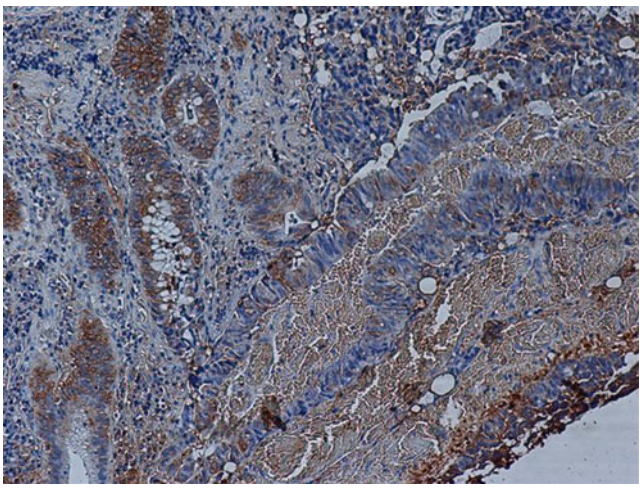

Fig. 3 High cytoplasmic expression of Fas—colon

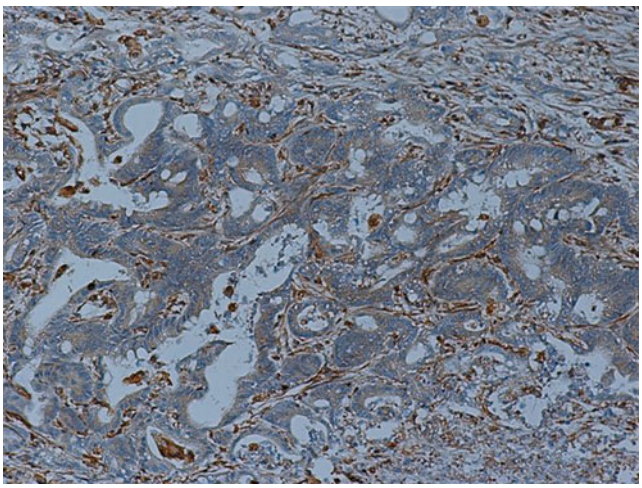

Fig. 4 High cytoplasmic expression of FasL—liver metastasis

In the study of Backus et al., the Fas expression was pretty high in normal colonic mucosa and upsent in colonic tumors and their liver metastasis [9]. On the other side, the expression of FasL should be upregulated in advanced tumor stages, tumors of poor differentiation, and more aggressive tumors, implying a negative prognostic factor [10, 11]. The percentage of FasL expression is negatively associated with overall survival according to a study of 90 points [12].

The study of M. W. Bennett et al. [13] concludes that colonic cancer cells upregulate their FasL-expression during their progression. Lambert C et.al propose an unknown mechanism of colonic tumor growth, partial favored by FasL tumor production [14].

The main theory supports that via FasL expression, the cancer cells can lead the immune tumor-infiltrating lymphocytes (TILs) to apoptosis, preventing their aggregation. In the same way FasL expression contributes to metastatic phenomenon, leading the goal—cells to apoptosis.

Colon cancer patients with microsatellite instability downregulated the FasL expression, as proven by immunohistochemical study on tissues from 91 points. This could explain the higher rate of TILs infiltration and

Table 4 Correlation of Fas/FasL expression

		FAS				
		Low expression		High expression		
		Number	Percentage (%)	Number	Percentage (%)	P χ^2 test
FasL	Low expression	14	31.1	31	68.9	0.028
	High expression	11	61.1	7	38.9	

the better prognosis that characterize these tumors (Houston et.al.) [15].

The apoptosis of cancer cells should follow the Fas-expression index, as long as these cells remain sensitive to that pathway. It is suggested that tumor cells become resistant to Fas-mediated apoptosis during tumor progression. Sugita et.al revealed a clear correlation between the FasL-producing macrophage and the number of tumor cells being in apoptotic procedure. The Fas/FasL pathway is with no doubt crucial to cancer biology [16]. As studied in endometrial cancer, necrosis in contrast to apoptosis is the main way of cell death, as the tumor progresses, implying the apoptotic resistance that malignant cells develop [17, 18]. Mann B et al. [19] proposed that the subpopulation of cancer cells that metastasize, does highly express FasL, as compared to the rest primary cancer cells, fact that enhances their survival- and capacity to spread.

Consideration should be given, if the Fas-mediated apoptosis could be used as treatment pathway, especially for early stages of colon cancer, where Fas protein is overexpressed from tumor cells, cells that still remain sensitive to that way of death. The FasL has already been used to enhance the effect of applied chemotherapy agents, giving promising results (in vitro application of FasL protein in combination with andiamycin in hepatocellular carcinoma) [20].

The cancer biology is very complex and the exact role of Fas/FasL-mediated apoptosis needs to be further investigated.

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