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B7H4, HSP27 and DJ-1 molecular markers as prognostic factors in pancreatic cancer

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ABSTRACT

Objectives: Pancreatic cancer (PC) is one of the most lethal tumors of the gastrointestinal tract. The ability to predict which patients would benefit most from surgical intervention and chemotherapy would be a great clinical tool. A large number of potential markers have been identified lately in pancreatic cancer and their clinical utilities as prognostic tools are under investigation.
Methods: We recruited 41 patients who had undergone radical surgical resection for PC between 2003 and 2010. To investigate the prognostic factors, we evaluated 3 possible markers: B7H4, HSP27 and DJ-1 protein expressions in the tissue specimens of these 41 patients by immunohistochemistry and analyzed the clinical and pathological features of these specimens.
Results: The expression of the three antigens was independently associated with a negative impact of chemotherapy with gemcitabine on patient's survival. Moreover, patients who overexpressed B7H4 had worse prognosis than the ones who did not.
Conclusions: B7H4, DJ-1 and HSP27 may be used in the future as prognostic markers that express resistance of pancreatic cancer patients to chemotherapy with gemcitabine.

1. Introduction

Pancreatic cancer is one of the most aggressive human tumors. It is the fourth most common cause of cancer death in Western society and is a leading cause of cancer death worldwide. The 5-year survival rate is approximately 1%–2% [1], and the median survival time after diagnosis is 4–6 months since most patients present with disease so advanced that surgical resection, which is the only curative option so far, is not possible [2]. Only about 15% of patients are able to undergo curative resection at the time of diagnosis. Gemcitabine appears to be the only clinically effective drug for pancreatic cancer, but it has little impact on the outcome [3].

The resistance of pancreatic cancer to chemotherapy and radiotherapy, in combination with the fact that surgical resection is the only curative option, create the need of an early diagnosis and valid prognosis of pancreatic cancer. Considerable research has focused on identifying molecular pathways in pancreatic carcinogenesis, and their correlation with clinicopathological variables, in order to define better prognostic indicators that would offer a more precise strategy of treatment based upon the subgrouping of patients [4]. At present, serum CA-19-9 (carbohydrate antigen 19-9) is the only Food and Drug Administration-approved biomarker for PC and it has utility as a prognostic marker and as a marker of disease recurrence [5]. Recent studies have shown that there are a few promising new molecules that may be used as prognostic markers in PC, including SMAD4 (Mothers against decapentaplegic homolog 4). SMAD4 expression in PC was associated with local progression, whereas the lack thereof was associated with distant metastases [5]. Prospective validation of SMAD4 expression in PC cytology specimens as a predictive biomarker is warranted and may lead to personalized treatment strategies for patients with localized pancreatic cancer [6]. Mucins also seem to play an important role in carcinogenesis and tumor invasion of pancreatic cancer. The combined status of MUC1, MUC2, MUC4, and MUC5AC expression may

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be useful for the early detection of pancreatobiliary neoplasms and evaluation of their malignancy [7]. MUC1 and also MSLN (mesothelin) are highly significant predictors of early cancer-specific mortality since they are associated with aggressive pancreatic cancer biology [8].

In the present study, we examine the prognostic value of the independent and co- expressing pattern of three antigens that have been previously shown to be prognostic indicators of cancer outcome and survival in pancreatic and/or other types of cancer. Though the diagnostic accuracy of any single marker is inadequate, the combination of several biomarkers could provide the necessary sensitivity and specificity to accurately diagnose pancreatic cancer [9].

B7H4 is a member of the B7 protein family that is expressed in activated T cells and may participate in the negative regulation of cell-mediated immunity in peripheral tissues [10]. Several recent studies have shown that B7H4 protein is frequently overexpressed in certain malignant tumors, including most cases of serous, endometrioid, and clear cell carcinomas of the ovary, both lobular and ductal breast cancer, and in a subset of cases of pulmonary carcinoma [11–13]. B7H4 may play a role as a prognostic factor in endometrioid adenocarcinomas since its overexpression is correlated with a more aggressive biologic potential [14]. B7H4 is also associated with poor prognosis in renal cell carcinoma since patients with tumors expressing B7H4 have been found three times more likely to die from renal cell carcinoma compared to patients lacking B7H4 [15]. In pancreatic cancer, B7H4 has demonstrated a potential diagnostic use for detection of pancreatic adenocarcinoma in resected and EUS-FNA specimens [16].

DJ-1 was originally cloned as a putative oncogene capable of transforming NIH-3T3 cells in cooperation with H-ras [17]. It has also been implicated in fertilization, the regulation of androgen receptor signaling and oxidative stress [18]. Further, mutations of the DJ-1 gene are associated with autosomal early-onset Parkinson's disease [19]. Several lines of evidence suggest that DJ-1 plays a role in human tumorigenesis, including breast cancer, non-small cell lung carcinoma and prostate cancer. Very recently, DJ-1 was identified as a negative regulator of the tumor suppressor PTEN, promoting cell survival in primary breast and lung cancer patients, and it was associated with poor prognosis in non-small cell lung cancer patients [20–22]. DJ-1 has also been correlated with poor prognosis in patients with hepatocellular carcinoma, carcinoma of urinary bladder and glottic squamous cell carcinoma [23–25]. In pancreatic cancer tissue specimens, DJ-1 was overexpressed and was correlated with stage and low survival ranges. It has also been shown to promote invasion and metastatic potential of the pancreatic cancer cells [26]. DJ-1 may play an important role in the chemoresistance of pancreatic tumors to gemcitabine [27].

HSP27 is a chaperone protein. Its main functions are thermoresistance, inhibition of apoptosis, regulation of cell growth and differentiation and signal transducing [28]. HSP27 is found to be expressed in different types of cancer, and is responsible for the resistance of cancer cells to chemotherapeutic agents [29]. In pancreatic cancer, HSP27 overexpression in tumor specimens is associated with higher resistance of pancreatic cancer cells to gemcitabine which suggests that it can be used as a prognostic marker of chemotherapy outcome and also as a chemotherapy target antigen [30,31].

2. Methods

Forty one patients-cases of surgically resected pancreatic adenocarcinomas were studied. Twenty six of them received adjuvant chemotherapy with gemcitabine. Paraffin-embedded tissue samples of pancreatic human adenocarcinomas from pancreatoduodenectomy or distal pancreatectomy were available for all patients. Tissue sections were subjected to conventional hematoxylin and eosin staining (H&E). Unstained slides were used to investigate expression of B7H4, DJ-1, and HSP27 by immunohistochemistry. The clinical data were obtained from the patients' files, including follow-up information. The clinicopathological parameters evaluated were age, sex, stage, differentiation, survival, response to chemotherapy.

The study had received approval by the local Human Investigations Committee and it conforms to the provisions of the Helsinki Declaration.

2.1. Immunohistochemistry

Tissue specimens were fixed in formalin (10% phosphate buffer) and embedded in paraffin according to standard procedures. Four-micron serial sections (4 μm) of representative blocks from each case were deparaffinized, rehydrated, and immunostained by the peroxidase method (Envision System, DAKO, Carpinteria, Calif., USA). Slides were then incubated with the primary antibodies: 60 min with the B7H4 mouse monoclonal antibody at a 1:50 dilution (GeneTex Inc.USA), 30 min with the DJ-1/Park7 rabbit polyclonal antibody (Acris Antibodies GmbH USA) at a 1:50 dilution and 60 min with HSP27 mouse monoclonal anti-human antibody (Acris Antibodies GmbH USA) at a 1:200 dilution.

Control slides were incubated for the same period with non-immunized rabbit serum (negative control). Finally, bound antibody complexes were stained for 10 min with 0.05% diaminobenzidine (DAB). Sections then were briefly counterstained with Mayer's hematoxylin, mounted, and examined under a Nikon Eclipse 50i microscope. A homogenous, light brown staining of the cytoplasm revealed positive cells. Sections with greater than 10% stained tumor cells were considered as being positive. Samples with complete absence of cytoplasmic staining or with weak/incomplete staining (<10%) would be classified as a tumor negative for antigen expression. Staining intensity is described by using a 0–3 score: 0 = negative expression, 1 = mild expression, 2 = medium expression, and 3 = intense expression [32].

2.2. Statistical analysis

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS), version 19.0 (IBM). The normality of quantitative variables was ascertained with Kolmogorov–Smirnov test. Normally distributed quantitative variables were expressed as mean ± standard deviation (SD), while non-normally distributed quantitative variables were expressed as median (interquartile range, IQR). The expression of HSP27, DJ-1 and B7H4 and all other qualitative variables were expressed as frequencies and percentages (%). The chi-square test, Student's *t* test and Mann–Whitney *U*-test were used to evaluate any potential association between HSP27, DJ-1 and B7H4 expression and the demographic and clinicopathological characteristics. Odds ratios and their 95% confidence interval (CI) were calculated by means of simple logistic regression analysis to assess the relation between qualitative variables. As indicator of survival, the disease-specific survival (including only death related to the disease as an event) was investigated. Survival rates were calculated with the Kaplan–Meier method and the statistical difference between survival curves was determined with the log-rank test. Multivariate Cox proportional hazards regression analysis, using a backward selection approach, were performed to explore the independent effect of HSP27, DJ-1 and B7H4 expression on survival. Patients' gender, age, tumor site, histological grade, clinical stage, chemotherapy were

Table 1
Demographic and clinicopathological characteristics of the patients.

	Patients
Gender [no (%)]	
Females	20 (48.8)
Males	21 (51.2)
Age [years; mean (SD)]	67.53 (11.12)
Tumor site [no (%)]	
Head	36 (97.8)
Body	3 (7.3)
Tail	2 (4.9)
Differentiation [no (%)]	
Poor	4 (9.8)
Poor – median	5 (12.2)
Median	19 (46.3)
Median – well	10 (24.4)
Well	3 (7.3)
Stage	
I	4 (9.8)
II	35 (85.4)
III	2 (4.9)
Chemotherapy [yes (%)]	26 (63.4)
CA19.9 [median (IQR)]	75.90 (36.0–319)
CEA levels [median (IQR)]	2.29 (1.38–2.75)
Positive HSP27 [no (%)]	13 (31.7)
Positive DJ-1 [no (%)]	20 (48.8)
Positive B7H4 [no (%)]	16 (39.0)

also included in the multivariate model. All tests were two tailed and statistical significance was considered for p values <0.05.

3. Results

3.1. Patient and tumor characteristics

The study population consisted of 41 pancreatic adenocarcinoma patients that underwent radical resection surgery at the Department of Surgery, General Hospital "Agios Dimitrios," Thessaloniki. Patients' median age was 71 years (range: 44–83 years; mean age ± SD: 67.53 ± 11.12 years). Patients'demographic and clinicopathological characteristic are shown in Table 1. Regarding to clinical stage, the majority of the carcinomas (35 carcinomas, 85.4%) were of stage II, while 4 (9.8%) and 2 (4.9%) carcinomas were of stage I and III, respectively. Thirteen (31.7%) carcinomas were well differentiated, 19 (46.3%) medium, and 9 (22.0%) poorly differentiated. Twenty six (63.4%) underwent adjuvant chemotherapy with gemcitabine.

3.2. Immunohistochemical detection of HSP27, DJ-1 and B7H4 expression

Antigen expression was analyzed by immunohistochemistry in all the above human pancreatic tumors. Cytoplasmic (c) immunoreactivity for HSP27, DJ-1 and B7H4 was detected in the malignant cells and was assessed separately. Representative tissues are shown in Fig. 1. Positive expression of HSP27, DJ-1 and B7H4 was found in 13 (31.7%), 20 (48.8%) and 16 (39.0%) of the patients. In almost 75% of the patients, the same expression of HSP27 and DJ-1 was detected (both negative in 46.3% and both positive in 26.8%); positive expression of DJ-1 was more frequent among positive HSP27 compared to patients with negative HSP27 (84.6% vs. 32.1%, $p = 0.002$; OR = 11.61, 95% CI = 2.12–3.73). No statistically significant association of B7H4 expression was found with HSP27 ($p = 0.460$) and DJ-1 ($p = 0.901$) expression. The association of demographic and clinicopathological features with the expression of HSP27, DJ-1 and B7H4 is given in Tables 2–4. The positive expression of (i) HSP27 was associated with younger age (61.08 ± 11.41 vs. 70.29 ± 9.96, $p = 0.014$) and well differentiated tumors (61.5% vs. 17.9%, $p = 0.005$; OR = 7.36; 95% CI = 1.68–32.26), (ii) DJ-1 was associated with medium or well differentiated tumors (56.3% vs. 22.2%, $p = 0.071$; OR = 4.50; 95% CI = 0.81–25.12) and high CA19.9 levels (median, 160 vs. 70, $p = 0.034$) and (iii) B7H4 was associated with medium or poorly differentiated tumors (50.0% vs. 15.4%, $p = 0.034$; OR = 5.50; 95% CI = 1.03–29.48) and high CA19.9 levels (median, 160 vs. 61.95, $p = 0.031$).

After a median follow up period of 15 months (range, 2–31 months), 36 (87.8%) patients have died as a consequence of disease progression. Among the entire cohort, the mean survival time ±SD was 16.0 ± 1.0 months (95% CI = 13–18 months; median 15.0 months 95% CI = 12–18 months). Results of survival analysis in

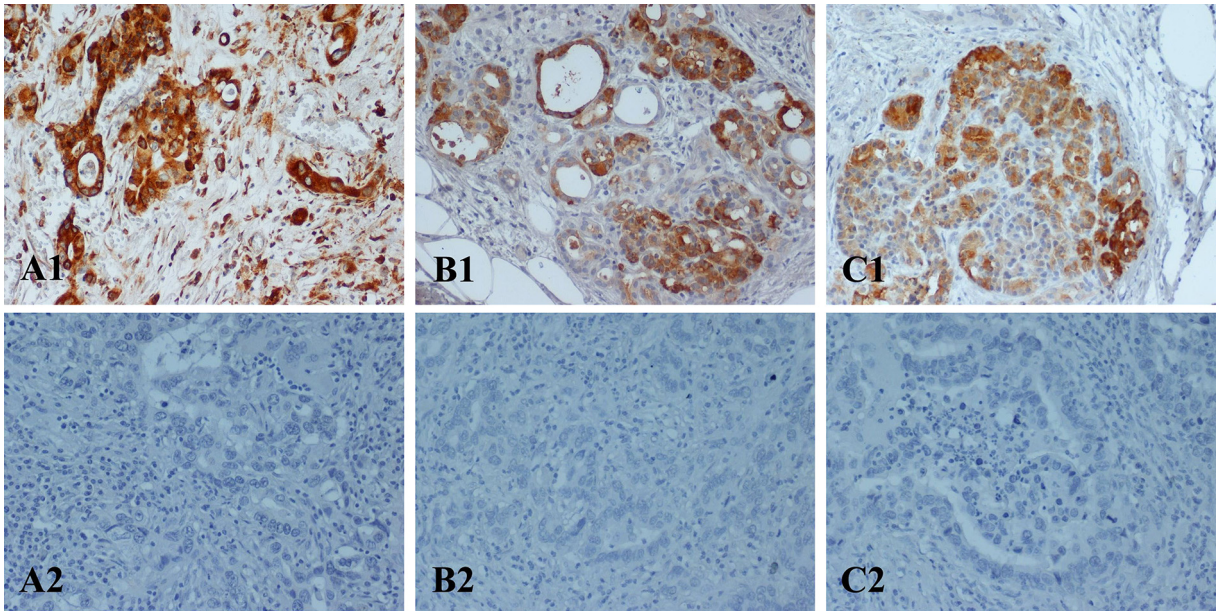

Fig. 1. A1.B7H4 positive expression, immunostaining ×200. A2. B7H4 negative expression, immunostaining ×200. B1. DJ-1 positive expression, immunostaining ×200. B2. DJ-1 negative expression, immunostaining ×200. C1. HSP27 positive expression, immunostaining ×200. C2. HSP27 negative expression, immunostaining ×200.

Table 2
Association of HSP27 status with demographic and clinicopathological characteristics of the patients.

HSP27 status			
Patient's characteristics	Negative	Positive	*p* Value
Gender [no (%)]			0.265
Females	12 (60.0)	8 (40.0)	
Males	16 (76.2)	5 (23.8)	
Age [years; mean (SD)]	70.29 (9.96)	61.08 (11.41)	0.014
Tumor site [no (%)]			0.418
Head	24 (66.7)	12 (33.3)	
Body	3 (100.0)	–	
Tail	1 (50.0)	1 (50.0)	
Differentiation [no (%)]			0.019
Poor/Poor – Median	7 (77.8)	2 (22.2)	
Median	16 (84.2)	3 (15.8)	
Median – Well/Well	5 (38.5)	8 (61.5)	
Stage			0.762
I	3 (75.0)	1 (25.0)	
II/III	25 (67.6)	12 (32.4)	
Chemotherapy [no (%)]			0.221
No	12 (80.0)	3(20.0)	
Yes	16(61.5)	10(38.5)	
CA19.9 [median (IQR)]	80.38 (32–325.75)	75.90 (42.50–386.75)	0.689
CEA levels [median (IQR)]	2.29 (1.71–2.71)	2.01 (1.23–13.20)	0.903

Table 4
Association of B7H4 status with demographic and clinicopathological characteristics of the patients.

B7H4 status			
Patient's characteristics	Negative	Positive	*p* Value
Gender [no (%)]			0.645
Females	13 (65.0)	7 (35.0)	
Males	12 (57.1)	9 (42.9)	
Age [years; mean (SD)]	68.88 (10.85)	65.50 (11.56)	0.354
Tumor site [no (%)]			0.326
Head	22 (61.1)	14 (38.9)	
Body	1 (33.3)	2 (66.7)	
Tail	2 (100.0)	–	
Differentiation [no (%)]			0.098
Poor/poor – median	5 (55.6)	4 (44.4)	
Median	9 (47.4)	10 (52.6)	
Median – well/well	11 (84.6)	2 (15.4)	
Stage			0.120
I	1 (25.0)	3 (75.0)	
II/III	24 (64.9)	13 (35.1)	
Chemotherapy [no (%)]			0.570
No	10(66.7)	5(33.3)	
Yes	15(57.7)	11(42.3)	
CA19.9 [median (IQR)]	61.95 (32.00–259.25)	160.00 (58–1000)	0.109
CEA levels [median (IQR)]	2.13 (1.26–2.63)	2.35 (2.11–4.20)	0.148

relation to HSP27, DJ-1 and B7-H4 are given in Table 5. Regarding the expression of B7-H4, the 1 and 2-year survival rates of patients with negative B7-H4 were 72.00 ± 8.98% and 20.00 ± 8.00%, whereas the respective percentages for patients with positive B7-H4 were 50.00 ± 12.50% and 9.38 ± 8.23%. Furthermore, the median survival time was 19 months (95% CI = 15–23 months) in patients with negative B7-H4 and 9 months (95% CI = 3–18 months) in patients with positive B7-H4. The log-rank test revealed a statistically significant difference between survival rates over time ($p = 0.046$), where patients with positive B7-H4 had worse prognosis (Fig. 2). Moreover, patients with positive B7-H4 were almost twice as likely to die of cancer than those with negative B7-H4 (HR = 1.81, 95% CI = 0.95–3.64, $p = 0.063$). On the contrary, patients' survival was independent of HSP27 (median survival, 15 months in negative HSP27 vs. 18 months in positive HSP27; $p = 0.667$, log-rank test; HR = 0.86, 95% CI = 0.43–1.73, $p = 0.676$) and DJ-1 (median survival, 15 months in negative DJ-1 vs. 17 months in positive DJ-1; $p = 0.292$, log-rank test; HR = 0.71, 95% CI = 0.37–1.37, $p = 0.307$) expression (Table 5).

In multivariate Cox proportional hazards regression analysis, the positive expression of B7-H4 remained a statistically significant independent predictor of poor overall survival (adjusted HR = 2.78, 95% CI = 1.09–7.16, $p = 0.033$). Among the entire cohort, chemotherapy was a statistically significant independent favorable determinant of survival, associated with increased survival (median survival, 18 months, 95% CI = 14–22 months vs. 9 months, 95% CI = 2–16 months, $p = 0.008$) and a 55% reduction in the risk of

Table 3
Association of DJ-1 status with demographic and clinicopathological characteristics of the patients.

DJ-1 status			
Patient's characteristics	Negative	Positive	*p* Value
Gender [no (%)]			0.879
Females	10 (50.0)	10 (50.0)	
Males	11 (52.4)	10 (47.6)	
Age [years; mean (SD)]	69.24 (9.36)	65.63 (12.78)	0.312
Tumor site [no (%)]			0.298
Head	17 (47.2)	19 (52.8)	
Body	3 (66.7)	1 (33.3)	
Tail	1 (100.0)	–	
Differentiation [no (%)]			0.174
Poor/Poor – Median	7 (77.8)	2 (22.2)	
Median	9 (47.4)	10 (52.6)	
Median – Well/Well	5 (38.5)	8 (61.5)	
Stage			0.317
I	3 (75.0)	1 (25.0)	
II/III	18 (48.6)	19 (51.4)	
Chemotherapy [no (%)]			0.393
No	9(60.0)	6(40.0)	
Yes	12(46.2)	14(53.8)	
CA19.9 [median (IQR)]	70.00 (27.75–157.00)	160.00 (46–1000)	0.111
CEA levels [median (IQR)]	2.13 (1.20–2.76)	2.35 (1.45–3.45)	0.560

Table 5
Survival in relation to HSP27, DJ-1 and B7H4 expression.

	Negative	Positive
HSP27 expression		
Patient number	28	13
1-year survival (%)	60.71 ± 9.23	69.23 ± 12.80
2-year survival (%)	12.86 ± 6.61	23.08 ± 11.69
Survival time (years)		
Mean ± SE	15 ± 2	16 ± 2
95% CI	12–18	12–21
Median (95% CI)	15 (12–18)	18 (10–26)
Fatality (%)	24 (85.7%)	12 (92.3%)
p Value (log-rank test)	0.667	
DJ-1 expression		
Patient number	21	20
1-year survival (%)	57.14 ± 10.80	70.00 ± 10.25
2-year survival (%)	9.52 ± 6.41	23.33 ± 9.79
Survival time (years)		
Mean ± SE	14 ± 2	17 ± 2
95% CI	10–18	13–20
Median (95% CI)	15 (6–24)	17 (10–24)
Fatality (%)	19 (90.5%)	17 (85.0%)
p Value (log-rank test)	0.292	
B7H4 expression		
Patient number	25	16
1-year survival (%)	72.00 ± 8.98	50.00 ± 12.50
2-year survival (%)	20.00 ± 8.00	9.38 ± 8.23
Survival time (years)		
Mean ± SE	17 ± 2	12 ± 2
95% CI	14–21	8–15
Median (95% CI)	19 (15–23)	9 (3–18)
Fatality (%)	22 (88.0%)	14 (87.5%)
p Value (log-rank test)	0.046	

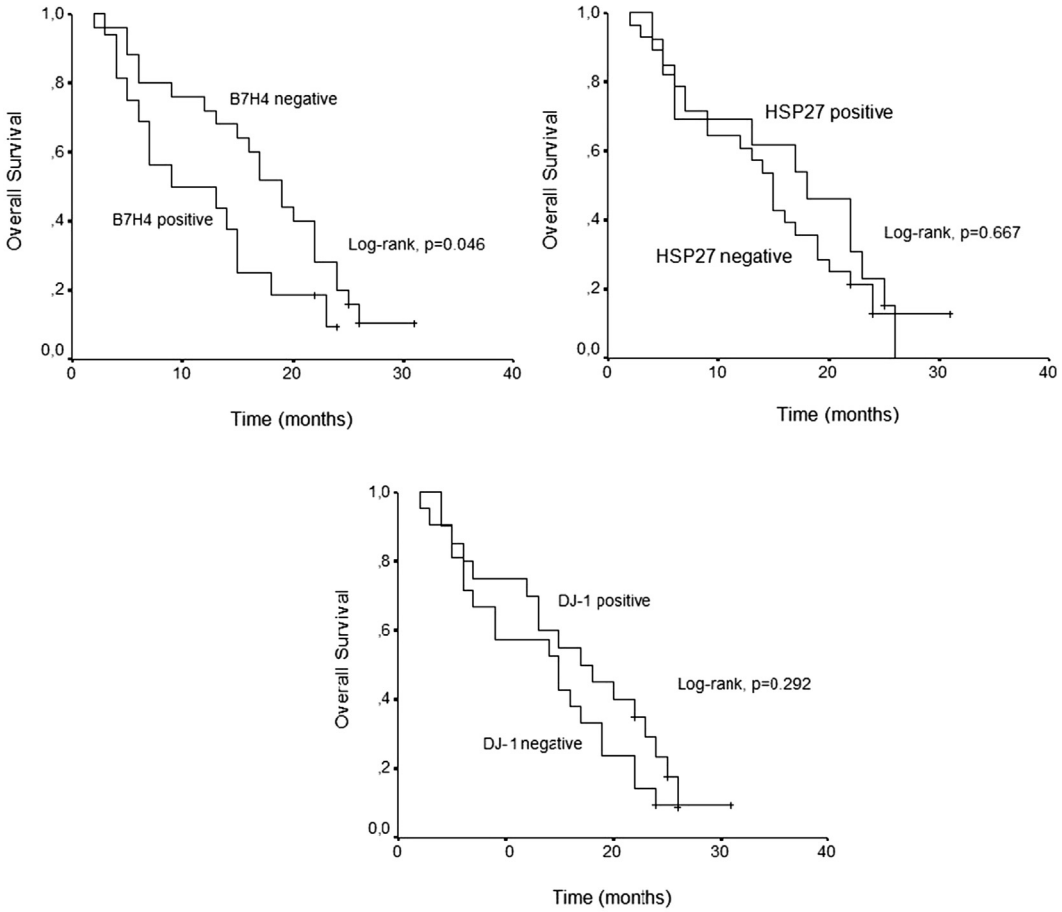

Fig. 2. Overall survival in relation to the expression score of B7H4, DJ-1 and HSP27.

death (aHR = 0.45, 95% CI = 0.21–0.94, $p = 0.035$). When we stratified the analysis according to the expression of HSP27, DJ-1 and B7H4, we observed that the positive impact of chemotherapy on patients' survival was more pronounced among patients with a negative expression of the herein studied markers; in particular, patients who received chemotherapy had statistically significant longer survival and lower risk of death among negative HSP27 (median survival (95% CI), 16 (12–20) months vs. 9 (6–12) months, $p = 0.051$, log-rank test; aHR = 0.19, 95% CI = 0.03–1.61, $p = 0.067$), negative DJ-1 (17 (14–20) months vs. 9 (5–13) months, $p = 0.022$; aHR = 0.20, 95% CI = 0.06–0.66, $p = 0.008$) and negative B7H4 (22 (17–27) months vs. 9 (2–18) months, $p = 0.011$; aHR = 0.19, 95% CI = 0.05–0.70, $p = 0.012$) compared to patients who did not received chemotherapy. On the contrary, the increase in survival and the reduction in the risk of death, which was associated with chemotherapy, did not reach the statistical significance among patients with positive HSP27 (19 (12–26) months vs. 14 (4–24) months, $p = 0.105$; aHR = 0.51, 95% CI = 0.24–1.37, $p = 0.134$), positive DJ-1 (18 (5–31) months vs. 13 (3–23) months, $p = 0.174$; aHR = 0.48, 95% CI = 0.14–1.69, $p = 0.252$) and positive B7H4 (13 (3–23) months vs. 9 (5–13) months, $p = 0.341$; aHR = 0.63, 95% CI = 0.22–2.01, $p = 0.360$).

The association of survival with the co-expression of HSP27, DJ-1 and B7H4 was not statistically significant (co-expression of HSP27 and DJ-1: median survival, 15 months in both negative, 15 months in one positive, 18 months in both positive, $p = 0.648$; co-expression of HSP27 and B7H4: median survival, 17 months in both negative, 14 months in one positive, 5 months in both positive, $p = 0.606$; co-expression of DJ-1 and B7-H4: median survival, 17 months in both negative, 15 months in one positive, 7 months in both positive, $p = 0.484$; co-expression of HSP27, DJ-1 and B7H4: median survival, 17 months in all negative, 15 months in one positive, 17 months in two positive, 5 months in all positive, $p = 0.730$). [5 months in all three positive vs. 15 months in all other, $p = 0.364$; HR = 1.60, 95% CI = 0.56–4.57].

4. Discussion

In the present study, the prognostic value of the independent expression and co-expression pattern of HSP27, DJ-1 and B7H4 in resected pancreatic cancer tissues was examined by correlation to the survival rate of pancreatic cancer patients and the resistance to chemotherapy with gemcitabine.

The positive expression of B7H4 remained a statistically significant independent predictor of poor overall survival since patients with positive B7H4 were almost twice as likely to die of cancer than those with negative B7H4.

Chemotherapy was a statistically significant independent favorable determinant of survival, associated with increased survival. The positive impact of chemotherapy on patients' survival was more pronounced among patients with a negative expression of the herein studied markers; in particular, patients who received chemotherapy had statistically significant longer survival and lower risk of death among negative HSP27, DJ-1 and B7-H4 patients compared to patients who did not received chemotherapy. On the contrary, the increase in survival and the reduction in the risk of death, which was associated with chemotherapy, did not reach the statistical significance among patients with positive HSP27, DJ-1 and B7H4.

However, there was no significant association of the co-expression of HSP27, DJ-1 and B7H4 with the patient's survival, although the positive expression of DJ-1 was more frequent among positive HSP27 compared to patients with negative HSP27.

The mechanisms of gemcitabine resistance are still controversial. The apoptosis-regulating proteins of the bcl-2 family and P-glycoprotein, as well as various other proteins, have been reported to have a role in resistance to chemotherapy [30,33,34].

HSP27 belongs to the family of small heat shock proteins, which are molecular chaperones that modulate the ability of cells to respond to several types of injury and are expressed in virtually all organisms from prokaryotes to mammals. Evidence has been obtained that HSP27 regulates apoptosis by interacting with key components of the apoptotic signaling pathway. HSP27 inhibits etoposide-induced apoptosis by preventing cytochrome c and dATP-triggered activation of caspase-9, which occurs downstream of cytochrome c release. Increased expression of antiapoptotic factor enhances the resistance of tumor cells to chemotherapy. By knocking down HSP27 using siRNA, the gemcitabine sensitivity of pancreatic cancer cells was increased, confirming that HSP27 has a role in gemcitabine resistance [30].

Recently, increasing evidence has suggested that the function of DJ-1 was associated with AKT activation. Down-regulation of DJ-1 inhibits endogenous AKT phos-phorylation in cancer cell lines. DJ-1 as a negative regulator of PTEN (phosphatase and tension homolog deleted on chromosome 10), which directly antagonizes PI3 K to eventually down-regulate AKT [35]. Other studies indicated that AKT activation plays an important role in broad-spectrum chemoresistance and cancer development. The most recent study also demonstrated, by proteomic analysis, that DJ-1 might be a chemoresistance-related gene [36]. Chen et al. have already shown that DJ-1 plays also an important role in chemoresistant of pancreatic tumors to gemcitabine [27].

B7-H4 protein has been found to play an important role in the regulation of antigen-specific immune responses. Administration of B7H4 immunoglobulin fusion protein to mice inhibited the activation of T cells and cytokine secretion and led to cell cycle arrest. There is proof that B7H4 has a potential diagnostic use in pancreatic cancer in combination with p53 [16].

In this study, we show that B7H4 has also a potential use as a negative prognostic biomarker for pancreatic cancer and for chemotherapy outcome.

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