RESEARCH ARTICLE

ERCC1 as a Biological Marker Guiding Management in Malignant Pleural Mesothelioma

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Abstract

Background: To determine prognostic value of excision repair cross-complementation 1 (ERCC1) in patients with malignant pleural mesothelioma (MPM). Materials and Methods: The study included 60 patients with MPM who were diagnosed and treated in the Radiation Oncology Department of Kayseri Teaching Hospital and Medical Oncology Department of Erciyes University, Medicine School between 2005 and 2013. By using immunohistochemical methods, ERCC1 expression in biopsy specimens was evaluated. We retrospectively assessed whether there is a correlation between ERCC1 and response to anti-neoplastic therapy or survival. Results: There were 50 men and 10 women with median age of 62 years (range: 39-83). Histological type was epithelial mesothelioma in the majority of the cases (85%), most commonly presenting in stage four. Of the cases, 20 (33%) received radiotherapy, 60 (%100) received first-line chemotherapy and 15 (%25) received second-line chemotherapy. In the assessment after therapy, it was found that there was partial response in 12 cases (20%), stable disease in 19 cases (31.4%) and progression in 25 cases (41.7%). ERCC1 was positive in 43% of the cases. Mean OS was 11.7 months and mean DFS was 9.5 months in ERCC1-positive cases regardless of therapy, while they were 19.2 months and 17.1 months in ERCC1-negative cases, respectively. The difference was found to be significant (p<0.05). In univariate analysis, stage, comorbidity, response to treatment and ERCC1 expression were found to be significantly associated with OS (p=0.083; p=0.043; p=0.041; p=0.050). In multivariate analysis, response to treatment remained to be significant for OS (p=0.005). In univariate and multivariate analyses, response to treatment and ERCC1 were found to be significantly associated with DFS (p=0.049; p=0.041). Conclusions: ERCC1 was identified as poor prognostic factor in patients with MPM.

Keywords: Malignant pleural mesothelioma - ERCC1 - prognosis

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Introduction

Malignant pleural mesothelioma is the primary tumor of pleura arising from mesothelial cells which has poor prognosis and no standard treatment (Pagan et al., 2006). It is estimated that there are annually 500-600 new MPM cases in Turkey and overall 30,000 MPM cases worldwide (Metintas et al., 2002; Fennell et al., 2008). It is rarely seen with tendency to increasing incidence. It is seen between 50 and 60 years of age and is more common among men. Asbestos and erionite are two known factors in the etiology (Pagan et al., 2006).

Therapeutic approach is palliation in MPM, as it is refractory to all therapeutic modalities. The commonly used modalities are surgery, radiotherapy and chemotherapy in the management of patients with MPM (Metintas et al., 2002). Five-year survival rate is below 5% and median survival varies from 12 to 17 months. High mortality remains to be an important problem in MPM despite all advances in the diagnosis and management (Santoro et al., 2008). It is still unknown that what are clinical and molecular parameters predicting response to treatment. Thus, identification of biomarkers that can determine or predict response to treatment is of importance. ERCC1 is one of these biomarkers.

ERCC1 is an essential enzyme for life and one of the major proteins involved in DNA repair. The primary function of ERCC1 is nucleotide excision repair of damaged DNA. There are strong evidence coming from preclinical and clinical trials, indicating that ERCC1 has an independent predictive value regarding prognosis, response to treatment, recurrence and overall survival (Olaussen et al., 2006; Ozkan et al., 2010; Betti et al., 2011; Zhang et al., 2012; Zhang et al., 2013; Li et al., 2013; Li XD et al., 2013; Mechanicsville 2013). Although there are studies on ERCC1 expression level and effects of chemotherapy on survival in MPM from several countries, this is a relatively new topic with conclusions covering small groups (Martin et al., 2008; Righi et al., 2010; Zimling et al., 2012).

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In the present study, we aimed to detect ERCC1 expression, a novel protein, that can be used as guide in the selection of platinum-based chemotherapy by using immunohistochemical method and to investigate whether data obtained have an impact on prognosis in MPM.

Materials and Methods

Demographic characteristics

We retrospectively reviewed data of 60 patients with MPM who were managed at Radiation Oncology Department of Kayseri Teaching Hospital and Medical Oncology Department of Erciyes University, Medicine School between 2005 and 2013. The study was approved by Ethics Committee of Erciyes University, Medicine School. The study conducted in accordance to local ethics regulations and Helsinki Declaration. In all patients included, following characteristics were reviewed: age, gender, smoking, asbestos exposure, histopathological subtypes, treatments employed, and overall and disease-free survivals. Patients with missing data and those not attending to controls were excluded from analysis.

Treatments

All patients underwent thoracoabdominal CT scan, MR imaging and/or PET-CT scan before surgery. Pleurectomy/decortication, extrapleural pneumonectomy (EPP) or thoracoscopic biopsy was performed in surgery. American Joint Commission on Cancer (AJCC) 2002 staging system was used for staging.

Chemotherapy: chemotherapy was given to patients with ECOG (Eastern Cooperative Oncology Group) performance status 0-2, those having no severe cardiac problem (coronary artery disease, congestive heart failure, arrhythmia etc.), and those with normal renal (serum creatinine≤1.5 mg/dL; creatinine clearence≥60 mg/kg), hepatic (serum bilirubin≤1.6 mg/dL) and bone marrow functions (leukocyte≥4,000/μL; platelet: 100,000/μL). One of the following regimens was given by 3-weeks interval as first-line chemotherapy: cisplatin plus pemetrexed, cisplatin or pemetrexed. Second-line therapy was given to patients with progression or good performance status, including cisplatin plus pemetrexed, pemetrexed, cisplatin plus gemcitabine and/or gemcitabine.

Radiotherapy: radiotherapy was delivered to surgical scar and drain sites after decortication or prophylactic radiotherapy was delivered to biopsy site to decrease recurrence and pain. In addition, palliative radiotherapy was delivered for symptom palliation. Radiotherapy involving macroscopic mass or painful drain sites was delivered with total dose of 3000-5600 cGy in fractions of 200-300 cGy per day by using Co 60/Linac (6 MC photon) device.

Treatment response and follow-up

Treatment response was assessed according to World Health Organization criteria. Follow-up visits were scheduled by 3-months intervals during first 2 years after treatment; and by 6-months intervals thereafter. In the follow-up visits, all patients were assessed by physical examination, blood tests including complete blood count, and hepatic and renal function tests, and imaging modalities (thorax and abdomen CT scans or PET-CT scan).

Immunohistochemical staining and histopathological evaluation

ERCC1 enzyme activity was studies from paraffin blocks by using immunohistochemical methods. Tonsillar biopsy specimens were used as positive control. Sections of 3μm thickness were obtained from paraffin blocks containing MPM and tonsillar biopsy specimens. Sections obtained were transferred to poly-L-lysine-coated slides. Preparations were incubated for 1 hour at 60°C in oven and preparations were placed into xylene for 30 minutes to induce deparaffinization. Then, they were placed into absolute alcohol for 15 minutes and into 96% alcohol (for 3x5 minutes); followed by rehydration in distilled water. For antigen retrieval, retrieval solution was prepared by adding 90 mL distilled water to 10 mL EDTA solution. Preparations were placed in this solution and heated in microwave oven with maximum power for 5 minutes (repeated 4 times). Then, they were left cooling for 20 minutes at room temperature. After cooling, preparations were placed in 10 nM citrate buffer (pH, 6) and exposed to 200°C heat for 20 minutes in microwave. Then, they were left cooling for 20 minutes at room temperature. This process was repeated after cooling. Preparations were treated with 0.3% hydrogen peroxide for 10 minutes and washed by using phosphate-buffered saline (PBS) solution.

Preparations were incubated with primary anti-ERCC1 antibody for 30 minutes at room temperature. Then, they were treated with biotinylated anti-mouse and anti-rabbit immunoglobulin for 10 minutes in order to stain with streptavidin-biotin immunoperoxidase. Preparations were washed by using PBS. After washing, they were treated with streptavidin conjugate for 10 minutes and re-washed with PBS. Then, they were treated with diaminobenzidine chromogen and washed with deionized water. Contrast staining was achieved by Mayer hematoxylin. After applying balsam, preparations were closed by cover glass and cell count (500 cells per preparation) was performed under light microscope. Staining density in tonsil tissue, vessel and epithelium as positive control was considered as reference, as being staining density +2.

In immunohistochemical evaluation, cell nuclei were assessed according to staining density and staining percent by ERCC1. Staining density was rated (0-3). Final score was obtained by multiply rating score by staining percent. Data were stratified as ERCC1-negative and ERCC1-positive according to median value.

Statistical analysis

SPSS (Statistical Package for the Social Sciences) for Windows version 15.0 was used in data analyses. Continuous variables were expressed as mean ± standard deviation, while categorical variables were expressed as frequency and percentage. Student t test was used to compare age between groups. Chi-square test was used for categorical variables. Kaplan-Meier analysis was used

to determine correlation between ERCC1 expression and survival. Log-rank test was used to assess differences in survival at low and high expression levels. p<0.05 was considered as significant.

Results

Table 1 presents demographic characteristics. There were 50 men and 10 women with median age of 62 years (range: 39-83). There was history of smoking in 27 cases, history of asbestos exposure in 8 cases. Histological type was epithelial mesothelioma in 85% of the cases. There was stage 4 disease in 45% and stage 3 disease in 40% of the cases. The most common regimen used was cisplatin plus pemetrexed in the first-line therapy. After first-line treatment, there was complete response in 4 cases, stable disease in 19 cases, partial response in 12 cases and progression in 25 cases. Second-line chemotherapy was given to 15 cases with progression and good performance status. Palliative radiotherapy was delivered to 20 cases (33%). There was distant metastasis in 8 cases.

Table 1. Demographics, Clinical and Tumor-characteristics

Characteristics		No. of Patients (%)
Gender	Male	50 (83.3)
	Female	10 (16.7)
Age (years)	<65	35 (58.3)
	≥65	25 (21.2
Smoking status	Smoker	22 (36.7)
	Nonsmoker	23 (38.3)
	Unknown	15 (25.0)
Asbestos exposure	No	44 (73.3)
	Yes	8 (13.3)
	Unknown	8 (13.3)
Location	Right	31 (51.7)
	Left	29 (48.3)
Comorbide	Yes	26 (43.3)
	No	30 (50.0)
	Unknown	4 (6.7)
Stage	II	7 (11.7)
	III	22 (36.7)
	IV	31 (51.7)
Performance status	0	15 (25)
	1	45 (75)
Histology	Epithelioid	51 (85.0)
	Biphasic	7 (11.7)
	The others	2 (3.3)
Surgery	EPP	5 (8.3)
	Pleurodesis	8 (13.3)
	Biopsy	47 (78.3)
Firstline chemotherapy		
	Cisplatin+pemetrexed	22 (36.7)
	Cisplatin	19 (31.7)
	Pemetrexed	13 (21.7)
	The others	6 (10)
Secondline chemotherapy		
	Cisplatin+pemetrexed	3 (5)
	Pemetrexed	4 (6.7)
	Cisplatin+gemsitabine	6 (10)
	Gemsitabine	2 (3.3)
Radiotherapy	Yes	20 (33.3)
	No	40 (66.7)
Response	Complete response	4 (6.7)
	Partial response	12 (20.0)
	Stable disease	19 (31.7)
	Progressive disease	25 (41.7)
Distant metastasis	Yes	8 (13.6)
	No	52 (86.4)
Distant metastasis	Bone	3 (5.1)
	Abdomen	2 (3.4)
	Brain	3 (5.1)
ERCC1	Negative	34 (56.7)
	Positive	26 (43.3)

Median follow-up was 10 months (range: 10 days-30 months). Mean overall and disease-free survival were 16.4 and 14.4 months. One-year and 2-years overall survival rates were 54% and 32%, respectively. One-year and 2-years disease-free survival rates were 47% and 34%, respectively (Figure 1).

Table 2 presents ERCC1 distribution according to clinic-pathological characteristics and results of analyses. ERCC1 was positive in 26 of 60 patients (Figure 2). There was a significant difference between ERCC1 groups regarding surgery (p=0.020). No significant difference was observed in other parameters between ERCC1 groups.

Table 3 presents disease-free and overall survivals and p values according to risk groups. Mean overall survival was 15.3 months in patients with comorbidity, while 17.7 months in those without comorbidity (p=0.044). Mean overall survivals in patients with complete response (n=4), partial response (n=12), stable disease (n=19) and progression (n=25) were 22.0, 25.0, 16.2 and 8.0 months, respectively (p=0.001). Disease-free survivals were 20.1, 16.2, 15.1 and 6.9 months, respectively (p=0.042). Regardless of treatment, mean overall survival was 11.7 months (range: 8.4-15.1) in ERCC1-positive cases, while 19.2 months (range: 14.9-23.6) in ERCC1-negative cases.

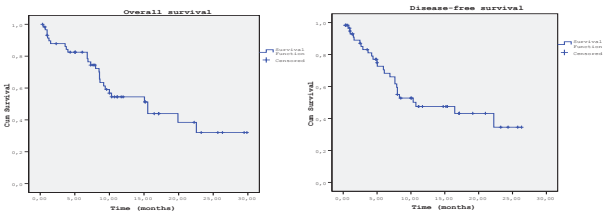

Figure 1. Kaplan-meier Overall and Disease-free Survival Curve for Malignant Pleural Mesothelioma

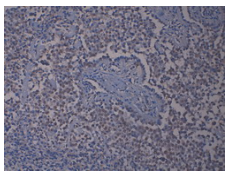

Figure 2. ERCC1-positive Malignant Pleural Mesothelioma

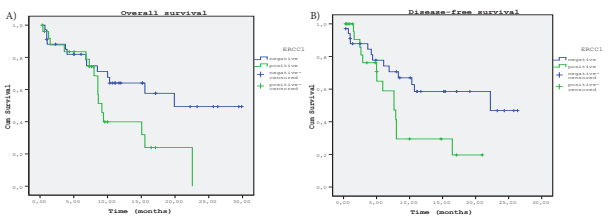

Figure 3. The Overall and Disease-free Survival Curve According to ERCC1 Expression Level

In the Kaplan-Meier survival analysis, 12-months and 24-months survival rates were 40% and 0% in ERCC1-positive cases, while 64% and 49% in ERCC1-negative cases, respectively (p=0.044) (Figure 3a). DFS was found to be 9.5 months in ERCC1-positive cases, while 17.1 months in ERCC1-negative cases. In Kaplan-Meier survival analysis, 12-months survival rate was 30% in ERCC1-positive cases, while 58% in ERCC1-negative cases. The difference was found to be significant (p=0.035; Figure 3b). Although overall and disease-free survivals were higher in patients younger than 65 years of age, women, non-smokers, those without comorbidity, biphasic type, those received radiotherapy and chemotherapy, the difference didn't reach significance.

Table 2. ERCC1 Distribution According to Clinic-pathological Characteristics and p value

Characteristics		ERCC1 ve- (%)	ERCC1 ve+ (%)	p value	Characteristics		ERCC1 ve- (%)	ERCC1 ve+ (%)	p value
Gender	Male	26 (42.1)	24 (40)	0.103	Histology	Epithelioid	30 (50)	21 (35)	0.131
	Female	8 (13.3)	2 (3.3)			Biphasic	2 (3.3)	5 (8.3)	
Age (years)	<65	17 (28.3)	18 (30)	0.134	Surgery	Inop	25 (41.6)	25 (41.6)	0.02
	≥65	17 (28.3)	8 (13.3)			Surgery	9 (15)	1 (1.6)	
Smoking status	Smoker	11 (18.3)	12 (20)	0.492	Firstline chemotherapy				
	Nonsmoker	15 (25)	8 (13.3)			Cisplatin+pemetrexed	13 (21.6)	9 (15)	0.713
	Unknown	8 (13.3)	6 (10)			Cisplatin	12 (20)	7 (11.6)	
Asbestos exposure	No	26 (42.1)	18 (30)	0.493		Pemetrexed	6 (10)	7 (11.7)	
	Yes	3 (5)	5 (8.3)			The others	3 (5)	3 (5)	
	Unknown	5 (8.3)	3 (5)		Secondline chemotherapy				
Location	Right	16 (26.6)	15 (25)	0.414		Cisplatin+pemetrexed	0 (0)	3 (5)	0.146
	Left	18 (30)	11 (18.3)			Pemetrexed	1 (1.6)	3 (5)	
Comorbide	Yes	17 (28.3)	9 (15)	0.492		Gemsitabine	3 (5)	3 (5)	
	No	15 (25)	15 (25)			Cisplatin+gemsitabine	1 (1.6)	1 (1.6)	
	Unknown	2 (3.3)	2 (3.3)		Radiotherapy	Yes	22 (36.6)	18 (30)	0.713
Stage	II	6 (10)	1 (1.6)	0.251		No	12 (20)	8 (13.3)	
	III	12 (20)	10 (16.6)		Response	Complete response	3 (5)	1 (1.6)	0.635
	IV	16 (26.6)	15 (25)			Partial response	8 (13.3)	4 (6.6)	
Performance status	0	11 (18.3)	4 (6.6)	0.133		Stable disease	9 (15)	10 (20)	
	1	23 (38.3)	22 (36.6)			Progressive disease	14 (23.3)	11 (18.3)	

Table 3. Risk Factors for the Overall and Disease-free Survival

Variables		Patients no.	Overall survival		Disease-free survival	
			Survival month mean (95% CI)	p value	Survival month mean (95% CI)	p value
Age	<65	50 (83.3)	17.4 (13.1-21.8)	0.551	15.4 (11.5-19.3)	0.562
	≥65	10 (16.7)	13.2 (9.8-16.6)		12.1 (8.2-15.9)	
Gender	Female	10 (16.7)	20.5 (12.2-28.8)	0.325	15.4 (7.6-23.39	0.897
	Male	50 (83.3)	15.4 (12.0-18.8)		14.1 (10.9-17.2)	
Smoking	Yes	22	13.6 (7.8-19.4)	0.165	10.6 (6.5-14.7)	0.247
	No	23	19.8 (15.0-24.5)		17.2 (12.7-21.6)	
	Unknown	14	12.3 (8.2-16.4)		11.2 (6.8-15.6)	
Asbestos exposure	No	44 (73.3)	14.2 (10.9-17.4)	0.581	12.7 (9.4-15.9)	0.235
	Yes	8 (13.3)	12.6 (7.8-17.4)		12.1 (6.5-17.7)	
	Unknown	8 (13.3)	19.6 (11.7-27.5)		19.8 (12.7-26.9)	
Hemithorax involvement	Right	31 (51.7)	18.6 (13.8-23.5)	0.301	15.1 (11.1-19.2)	0.577
	Left	29 (48.3)	13.9 (10.3-17.4)		13.6 (9.4-17.8)	
Tumor stage	II	7 (11.7)	22.6 (16.0-29.3)	0.057	20.9 (14.3-27.4)	0.357
	III	22 (36.7)	18.9 (13.2-24.5)		12.3 (8.0-16.7)	
	IV	31 (51.7)	12.1 (9.1-15.0)		12.8 (9.2-16.7)	
Performance status	0	15 (25)	20.3 (15.1-25.4)	0.107	17.1 (10.8-23.3)	0.372
	1	45 (75)	14.9 (11.2-18.5)		13.2 (10.0-16.4)	
Pathology	Epitheloid	51 (85)	15.1 (11.7-18.6)	0.499	12.4 (9.5-15.2)	0.123
	Biphasic	7 (13)	17.9 (8.3-27.5)		21.2 (13.5-28.9)	
Response	Complete response	4 (6.7)	22.0 (13.4-30.6)	0.001	20.1 (10.1-30.0)	0.042
	Partial response	12 (20)	25.0 (19.5-30.6)		16.2 (10.5-21.9)	
	Stable disease	19 (31.7)	16.2 (12.7-19.7)		15.1 (11.2-19.0)	
	Progressive disease	25 (41.7)	8.0 (5.4-10.6)		6.9 (4.3-9.4)	
Radiotherapy	No	40 (66.7)	15.4 (11.5-19.3)	0.493	14.5 (10.7-18.2)	0.824
	Yes	20 (33.3)	16.7 (12.0-21.4)		14.0 (9.2-18.8)	
Firstline chemotherapy	Cisplatin	22 (36.7)	19.3 (14.4-24.2)	0.266	16.4 (11.8-21.0)	
	Cisplatin+pemetrexed	19 (31.7)	15.6 (10.3-20.9)		14.9 (9.8-20.0)	
	Pemetrexed	13 (21.7)	10.1 (6.3-13.8)		8.0 (4.4-11.5)	
	The others	6 (10)	10.2 (3.5-16.9)		9.4 (3.5-15.3)	
Secondline chemotherapy	Cisplatin+pemetrexed	3 (5)	9.1 (8.6-9.5)	0.082	5.3 (2.3-8.3)	<0.000
	Pemetrexed	4 (6.7)	10.6 (3.7-17.7)		9.5 (0.9-18.0)	
	Gemsitabine	6 (10)	7.0 (2.2-11.9)		4.7 (0.1-9.5)	
	Cisplatin+gemsitabine	2 (3)	5.9 (1.1-14.5)		2.8 (0.2-5.4)	
ERCC1	Negative	34 (56.7)	19.2 (14.9-23.6)	0.044	17.1 (13.2-20.9)	0.035
	Positive	26 (43.3)	11.7 (8.4-15.1)		9.5 (6.3-12.6)	

*CI: confidence interval, ERCC1: Excision Repair Cross-Complementation 1

Table 4. Univariate Analysis of Risk Factors for the Overall and Disease-free Survival

Risk factors	Overall survive univariate analysis		Disease-free survive univariate analyis	
	OR (95% CI)	p value	OR (95% CI)	p value
Age (<65 or ≥65)	0.8 (0.4-1.7)	0.554	0.8 (0.3-1.7)	0.564
Gender (female or male)	1.8 (0.5-6.0)	0.335	1.0 (0.3-3.1)	0.898
Smoking (yes or no)	1.1 (0.4-2.8)	0.827	1.3 (0.5-3.6)	0.543
Asbestos exposure (yes or no)	1.7 (0.6-5.2)	0.333	2.9 (0.6-12.7)	0.143
Hemithorax involvement (right or left)	0.7 (0.3-1.4)	0.307	0.8 (0.3-1.7)	0.579
Tumor stage				
II	Ref		Ref	
III	0.7 (0.0-1.2)	0.083	0.3 (0.0-1.6)	0.196
IV	0.5 (0.2-1.7)	0.111	1.0 (0.4-2.3)	0.885
Performance status (0 or 1)	0.4 (0.1-1.2)	0.118	0.6 (0.2-1.7)	0.377
Comorbidity (yes or no)	0.2 (0.0-0.9)	0.043		
Pathology (epitheloid or biphasic)	1.5 (0.4-5.0)	0.505	4.3 (0.5-32.7)	0.156
Response				
complete response	Ref		Ref	
partial response	0.1 (0.0-0.9)	0.041	0.0 (0.0-1.3)	0.093
stable disease	0.1 (0.0-0.5)	0.004	0.3 (01.-0.9)	0.049
progressive disease	0.3 (0.1-0.7)	0.011	0.3 (0.1-0.8)	0.025
Radiotherapy (yes or no)	1.3 (0.5-3.0)	0.497	0.9 (0.4-1.9)	0.824
ERCC1 (negative or positive)	0.5 (0.2-1.0)	0.05	0.4 (0.2-0.9)	0.041

Table 5. Multivariate Analysis of Risk Factors for the Overall and Disease-free Survival

Risk factors	Overall survive multivariate analysis		Disease-free survive multivariate analysis	
	OR (95% CI)	p value	OR (95% CI)	p value
Response				
Complete response	Ref	\	Ref	
Partial response	0.2 (0.0-1.1)	0.072	0.1 (0.0-1.5)	0.11
Stable disease	0.1 (0.0-0.6)	0.008	0.4 (0.1-1.1)	0.078
Progressive disease	0.3 (0.1-0.7)	0.007	0.3 (0.1-0.7)	0.009
ERCC1 (negative or positive)	-	-	0.4 (0.1-0.9)	0.026

Table 4 and 5 present the results of univariate and multivariate analyses for overall and disease-free survivals according to risk factors. In univariate analysis, stage, comorbidity, response to treatment and ERCC1 expression were found to be significantly associated with overall survival (p=0.083; p=0.043; p=0.041; p=0.050). In multivariate analysis, response to treatment remained to be significant for OS (p=0.005). In univariate analysis, response to treatment and ERCC1 were found to be significantly associated with DFS (p=0.049; p=0.041). In multivariate analysis, response to treatment and ERCC1 were found to be significantly associated with DFS (p=0.040; p=0.026).

Discussion

Therapeutic approach is palliation in MPM, as it is refractory to all therapeutic modalities (Metintas et al., 2002). Effectiveness of chemotherapy with a single agent is about 10-15% with low response rates and median survival. In the literature, it is suggested that cisplatin is the most effective agent in monotherapy (Pagan et al., 2006). Median survival rate is approximately 12 months and one-year survival is about 60% with currently used chemotherapy regimen of pemetrexed plus cisplatin. In combination therapy, response rate and survival are better; however, response rate is below 50% due to intrinsic drug resistance (Fennell et al., 2008; Santoro et al., 2008).

In MPM, associations between many prognostic parameters and response to treatment and overall survival are being investigated. It is of importance to identify biomarkers which are thought to be able to determine or predict response to treatment. In the present study, it was investigated that whether ERCC1 is a biomarker that demonstrate sensitivity or resistance to therapy in patients with MPM.

Although there are inconsistent results in previous studies, it has been suggested that ERCC1 is a marker that could be introduced into clinical practice and could predict response to treatment. There are strong evidence coming from preclinical and clinical trials, indicating that ERCC1 has an independent predictive value regarding prognosis, response to treatment, recurrence and overall survival (Olaussen et al., 2006; Martin et al., 2008; Ozkan et al., 2010; Li et al., 2011; 2013a; 2013b; Zhang et al., 2013). ERCC1 enzyme is found in all tumor cells. There are many studies showing highly variable expression levels in tumor cells (George et al., 2005; Ceppi et al., 2006; Martin et al., 2008; Joerger et al., 2011; Mechanicsville 2013). It was reported that high tissue level of ERCC1 enzyme is a marker for good prognosis. However, it was found that high ERCC1 level is associated with poor drug response in platinum-based therapies directing DNA. It has been proposed that ERCC1 decrease drug effectiveness by repairing DNA damage caused by platinum-based chemotherapeutics and is poor prognostic factor. It has been reported that ERCC1 is associated with poor prognosis in ovary, bladder, prostate, lung, stomach, colon, head-neck and esophagus cancers (George et al., 2005; Ceppi et al., 2006; Martin et al., 2008; Li et al., 2011; Li et al., 2013). In a study on 137 patients by Joerger et al., it was shown that low ERCC1 mRNA level was associated with marked advantage in response to platinum-based chemotherapy (Joerger et al., 2011). In a review on 90 patients by Horgan et al. (2011) it was found that high ERCC1 mRNA level was associated with resistance to platinum-based chemotherapy (Horgan et al., 2011). It was shown that ERCC1 release had higher sensitivity in prediction of response to treatment in squamous cell carcinomas when compared to that in adenocarcinomas (Ceppi et al., 2006; Martin et al., 2008).

In our study, we assessed effect of ERCC1 on clinical outcome in MPM patients received cisplatin-based chemotherapy. Positive immune reaction by ERCC1 was shown in 43% of the patients in our study. In the literature, it was reported that rate of ERCC1-positive tumor varied from 41% and 61.5% (Olaussen et al., 2006; Mechanicsville 2013). In our study, it was found that ERCC1-positivity was higher in smokers and in those with asbestos exposure, although the difference didn't reach statistical significance. In a study by Lee et al., ERCC1-positivity rate was found as 67.8% in smoker, while 47.5% in non-smokers (p=0.028) (Lee et al., 2008). Higher expression of ERCC1 in smoker could be due to

increase in ERCC1 enzyme as a result of mutations caused by smoking or over-expression of ERCC1 as a result of mutations in ERCC1 gene caused by smoking itself. In another perspective, it could be due to higher rates of ERCC1 release in order to correct metaplasia caused by smoking (Pfeifer et al., 2002). In a study on MPM patients by Betti et al., it was reported that ERCC1 enzyme is increased due to mutations caused by asbestos exposure (Betti et al., 2011).

In our study, it was found that 61.7% of ERCC1-negative and 42.1% of ERCC1-positive patients were survived among the patients received cisplatin-based chemotherapy. Progression was detected in 36% of ERCC1-negative and 54% of ERCC1-positive patients. Regardless of treatment, mean OS and DSF were found to be 11.7 and 9.5 months in ERCC1-positive patients while 19.2 and 17.1 months in ERCC1-negative patients, respectively. When OS and DFS were compared between ERCC1-positive and ERCC1-negative patients, a significant difference was detected, suggesting a correlation between ERCC1 expression and poor prognosis. Zimling et al. (2012) reported that there was higher OS and DFS in MPM patients with low ERCC1 expression who received cisplatin-vinorelbin chemotherapy (Zimling et al., 2012). Righi et al. investigated relationship between survival and expressions of timidilat synthase and ERCC1 genes in 60 MPM patients received cisplatin plus pemetrexed or cisplatin alone and 81 MPM patients who didn't receive pemetrexed. Authors found that OS and DFS were higher in patients with low timidilat synthase expression. However, they failed to find a correlation between survival and ERCC1 median-H score in patients received platinum-based therapy (n=45), but patients in the lower tertile had significantly shorter survival (HR: 3.06; 95% CI: 1.08-8.69; p=0.035). Authors reported that level of timidilat synthase expression is an independent predictor for survival (Righi et al., 2010). In International Adjuvant Lung Cancer Trial (IALT) involving 1024 patients with non-small cell lung cancer, ERCC1 expressions were studied by immunohistochemical techniques. Sufficient tissue sample was obtained in 783 patients. It was found that ERCC1 expression was higher in patients with squamous cell carcinoma, those older than 55 years and those with pleural effusion. In that study, it was found that overall and disease-free survivals were longer in ERCC1-negative patients receiving chemotherapy when control group not receiving chemotherapy and study group receiving chemotherapy were compared regarding ERCC1. When ERCC1-positive patients were assessed, it was found that there was no significant difference between study and control group regarding survival. Significant correlations were detected between ERCC1 and age, histological type and pleural invasion. Authors concluded that ERCC-1 positivity has significant effect on survival.

In conclusion, high ERCC1 expression was identified as poor prognostic factor in cases with MPM. We think that this can have some clinical implications. According to our results, it can be suggested that patients with negative ERCC1 expression have greater benefit from cisplatin therapy. These findings should have to be tested in comprehensive studies.

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