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Research Article

Expression of Myeloid Cell Leukemia-1 (Mcl-1) Predicts the Survival of Patients with Metastatic Gastric Cancer

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Abstract

Objectives: The aim of this study is to investigate the effect of myeloid cell leukemia-1 expression on overall survival in metastatic gastric cancer patients.

Methods: The files of 57 metastatic gastric cancer patients who were followed up in our clinic were reviewed retrospectively. The expression of Mcl-1 in the tumor tissue was immunohistochemically examined. The level of Mcl-1 staining was given in percentage (range: 0-100%). The correlation between the clinicopathological characteristics, progression-free survival, overall survival and Mcl-1 was analyzed.

Results: Of the 57 patients in our study, 41 were male and 16 were female. The median age was 66 years. The median Mcl-1 expression value was 70. Median progression-free survival was 7.6 months, median overall survival was 9.3 months. In the multivariate analysis, Mcl-1 expression ≤70 and responsiveness to first line therapy were found to be independent good prognostic factors for overall survival, respectively [17 vs 6 months ($p<0.001$); 16.3 vs 8.7 months ($p=0.04$)]. The expression of Mcl-1 was higher in pure adenocarcinoma, well-differentiated carcinoma and visceral organ metastasis ($p=0.005$, $p=0.01$, $p=0.006$, respectively). No relationship was found between Mcl-1 value and gender, age, smoking, performance score, Her-2/neu expression, tumor localization and omental involment status.

Conclusion: In our study, we found that the myeloid cell leukemia-1 expression and responsiveness to first line therapy are good prognostic factors in terms of overall survival at metastatic gastric cancer patients.

Keywords: Metastatic gastric cancer, myeloid cell leukemia-1, overall survival

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Worldwide, gastric cancer is the fourth most common cancer, accounting for 6 percent of total cancer incidence, and is the third leading cause of death, accounting for 8 percent of cancer-related deaths. Despite a steady decline in the rates of incidence and mortality observed worldwide for several decades, this trend has been lessened.[1] Men and women are not equally affected; incidence rates are approximately twofold higher in men than in women.[2] Surgery is a still the most effective treatment for gastric cancer and good survival can be achieved if tumor is resectable. However, most gastric cancer is either diagnosed at an advanced stage or relapses after apparently curative surgery. The standard of care for patients with metastatic gastric cancer (MGC) is palliative chemotherapy with best supportive care. With the increasing use of cytotoxic chemotherapy agents in various types of cancer, chemotherapy has also been used for MGC, and various studies have demonstrated the superiority of systemic chemotherapy over best supportive care.[3–5] However, despite the established efficacy of chemotherapeutic agents, prognosis is still poor with median survival being less than 1 year.

The Bcl-2 (B-cell lymphoma-2) family of proteins, which consists of anti-apoptotic and pro-apoptotic members, is a

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Table 1. Correlation between Mcl-1 expression and clinicopathological parameters of gastric cancer

Variables	MCL high (>70) (n)	MCL low (≤70)(n)	p
Gender			
Male	31	10	p=0.1
Female	12	14	
Age			
≥65	24	6	p=0.39
<65	19	8	
Grade			
Differantiated	29	4	**p=0.01**
Undifferantiated	14	10	
Localization			
Proximal	30	12	p=0.31
Distal	13	2	
Primary operated			
Yes	8	5	p=0.27
No	35	9	
Her-2/neu status			
Negative	14	7	p=0.5
Positive	8	2	
Unknown	21	5	
Smoking history			
Yes	18	8	p=0.31
No	25	6	
ECOG performance			
0–1	29	12	p=0.3
≥2	14	2	
Response to first line therapy			
Yes	14	6	p=0.1
No	19	6	
5Fu/oxaliplatin treatment			
Yes	14	6	p=0.5
No	29	8	
5Fu/cisplatin treatment			
Yes	9	5	p=0.29
No	34	9	
Visseral metastasis			
Yes	30	4	**p=0.006**
No	13	10	
Histology			
Signet ring cell	5	7	**p=0.005**
Pure adenocarcinoma	38	7	
Omental involved			
Yes	10	7	p=0.06
No	33	7	

Mcl-1: Myeloid cell leukemia-1; ECOG: Eastern Cooperative Oncology Group.

critical regulator for the mitochondrial pathway of apoptosis through controlling the integrity of the outer mitochondrial membrane.[6] The pro-apoptotic members control the release of cytochrome c, and subsequent activation of caspases. In contrast, anti-apoptotic members such as Bcl-2, Bcl-xL, Bcl-w, A1 and myeloid cell leukemia-1 (Mcl-1) promote cell survival by inhibiting pro-apoptotic proteins, including Bim, Bax, and Bak.[7–9]

Mcl-1 is a member of the anti-apoptotic bcl-2 family of proteins, is frequently upregulated or overexpressed in malignant cells and exerts its anti-apoptotic function by heterodimerizing with other Bcl-2 family members and preventing the permeabilization of the mitochondrial outer membrane.[10,11] Increased expression of Mcl-1 occurs in a variety of human cancers and is strongly associated with resistance to therapies, tumor progression, and poor prognosis in most cancers, including gastric cancer.[12–17]

In our study, the expression of Mcl-1 was investigated for clinicopathological and prognostic significance in patients with metastatic gastric carcinoma.

Methods

The study included 57 patients with metastatic gastric cancer. Local ethics committee approval was obtained for study. Clinicopathological characteristics, treatments administered, and treatment responses were retrospectively recorded from the patient files. Progression-free Survival (PFS) was considered as the time from diagnosis to progression and overall survival (OS) was considered as the time from diagnosis to death or last follow-up. The expression of Mcl-1 was immunohistochemically examined in the tissue biopsy of patients at the time of diagnosis (endoscopic material or gastrectomy material). For immunohistochemical analysis, we was used for formalin-fixed and paraffin-embedded specimens. Cut sections to 4 µm and dry at 80°C for 15 min. Dilute anti-Mcl-1 polyclonal antibody (sc-74437; Santa Cruz Biotechnology, Santa Cruz, CA, USA) 1:50–1:100 (antibody diluent from Ventana) and fill into a Ventana antibody dispenser. The Ventana staining procedure includes pretreatment with Cell Conditioner 2 (pH 6) for 60 min (standard), followed by incubation with 1:50–1:100 diluted antibody at 37°C for 32 min. Upon antibody incubation perform Ventana standard signal amplification, ultraWash, counter- staining with one drop of Hematoxylin for 4 min and one drop of bluing reagent for 4 min. For chromogenic detection use ultraView Universal DAB Detection Kit (Ventana). Remove slides from stainer, wash in water with a drop of dishwashing detergent and mount. Stained tissues were viewed and photographed using a light microscope (Olympus, Tokyo, Japan). The staining pattern was evaluated in percentage (0%=no staining; 100%=high staining). Statistical analysis was performed with the Statistical Package for the Social Sciences 17. The expression of Mcl-1 with relation to various clinicopathological

Table 2. Univariate and multivariate analysis for PFS

Variables	Number of patients n (%)	Univariate analysis for PFS (month)	p (univariate)	Multivariate analysis
Gender				
Male	41 (71.9)	7.7	0.05	
Female	16 (28.1)	6.7		
Age				
≥65	30 (47.4)	7.7	0.50	
<65	27 (52.6)	6.7		
Grade				
Differantiated	33 (57.9)	6.4	0.34	
Undifferantiated	24 (42.1)	7.7		
Histology				
Signet ring cell	12 (21.1)	7	0.85	
Pureadenocarcinoma	45 (78.9)	7.7		
Localization				
Proximal	42 (73.7)	7.6	0.77	
Distal	15 (26.3)	5.9		
Primary operated				
Yes	13 (22.8)	7.7	0.35	
No	44 (77.2)	7		
Her-2/neu status				
Negative	21 (36.8)	7.7	0.16	
Positive	10 (17.5)	5.4		
Unknown	26 (45.6)	6.7		
Smoking history				
Yes	26 (45.6)	6.4	0.76	
No	31 (54.4)	7.7		
ECOG performance				
0–1	41 (71.9)	7.8	**0.01**	NS
≥2	16 (28.1)	4.3		
5Fu/cisplatin treatment				
Yes	14 (24.6)	7.7	0.24	
No	43 (75.4)	7		
Oxaliplatintreatment				
Yes	20 (35.1)	7.8	0.62	
No	37 (64.9)	5.4		
Visseral metastasis				
Yes	34 (59.6)	6.7	0.53	
No	23 (40.4)	7.6		
Omental involved				
Yes	17 (29.8)	7	0.73	
No	40 (70.2)	7.6		
Mcl-1 status				
Low	14 (24.6)	8.6	**0.02**	**p=0.02, HR: 2.5, %95 CI: 1.1–5.8**
High	43 (75.4)	7		

PFS: Progression-free survival; Mcl-1: Myeloid cell leukemia-1; ECOG: Eastern Cooperative Oncology Group; NS: nonsignificant; HR: Hazard ratio; CI: Confidence interval.

parameters was assessed with the X^2 test and Fisher's exact test. The survival rates of patients was estimated with the Kaplan-Meier method and analyzed using a log-rank test. Differences were considered significant when $p<0.05$.

Results

The study included 57 patients. All patients were in the metastatic stage at the time of diagnosis. Of the patients, 41 were male and 16 were female. The median age was 66

Table 3. Univariate and multivariate analysis for OS

Variables	Number of patients n (%)	Univariate analysis for OS (month)	p (univariate)	Multivariate analysis
Gender				
Male	41 (71.9)	9.3	0.34	
Female	16 (28.1)	8.9		
Age				
≥65	30 (47.4)	9.1	0.8	
<65	27 (52.6)	9.3		
Grade				
Differantiated	33 (57.9)	9.3	0.48	
Undifferantiated	24 (42.1)	9		
Histology				
Signet ring cell	12 (21.1)	8.2	0.98	
Pureadenocarcinoma	45 (78.9)	9.3		
Localization				
Proximal	42 (73.7)	9.1	0.34	
Distal	15 (26.3)	10.2		
Primary operated				
Yes	13 (22.8)	15.4	0.24	
No	44 (77.2)	8.9		
Her-2/neu status				
Negative	21 (36.8)	13.6	**0.03**	NS
Positive	10 (17.5)	8.2		
Unknown	26 (45.6)	4.4		
Smoking history				
Yes	26 (45.6)	9.1	0.39	
No	31 (54.4)	9.3		
ECOG performance				
0–1	41 (71.9)	9.1	0.3	
≥2	16(28.1)	4.9		
Response to first line therapy				
Yes	22 (38.6)	17	**<0.001**	**p<0.001. HR: 0.1, %95 CI:0.08-0.4**
No	35 (61.4)	6		
5Fu/cisplatin treatment				
Yes	14 (24.6)	15.4	0.009	NS
No	43 (75.4)	8.7		
Oxaliplatin treatment				
Yes	20 (35.1)	9.3	0.43	
No	37 (64.9)	8.6		
Visseral metastasis				
Yes	34 (59.6)	9.3	0.86	
No	23 (40.4)	9		
Omental involved				
Yes	17 (29.8)	8.9	0.4	
No	40 (70.2)	9.6		
Mcl-1 status				
Low	14 (24.6)	16.3	**0.01**	**p=0.04, HR: 2.1, %95 CI: 1.0-4.4**
High	43 (75.4)	8.7		

Mcl-1: Myeloid cell leukemia-1; ECOG: Eastern Cooperative Oncology Group; NS: Nonsignificant; OS: Overall survival; HR: Hazard ratio; CI: Confidence interval.

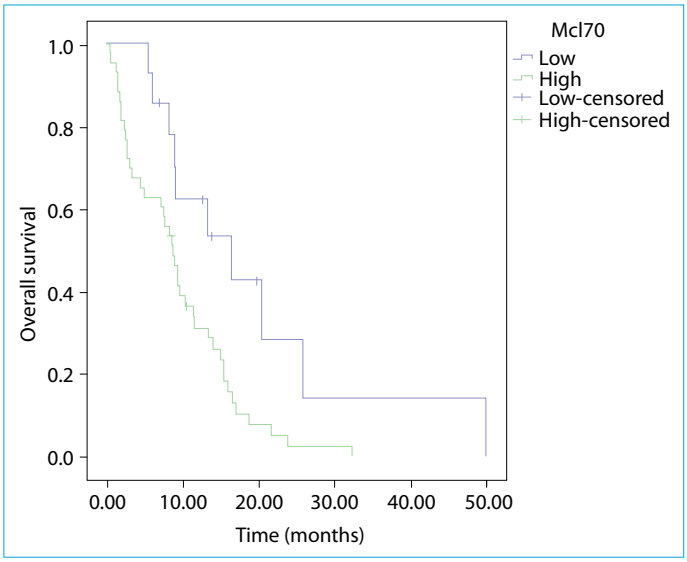

Figure 1. Relationship between Mcl-1 expression and OS.

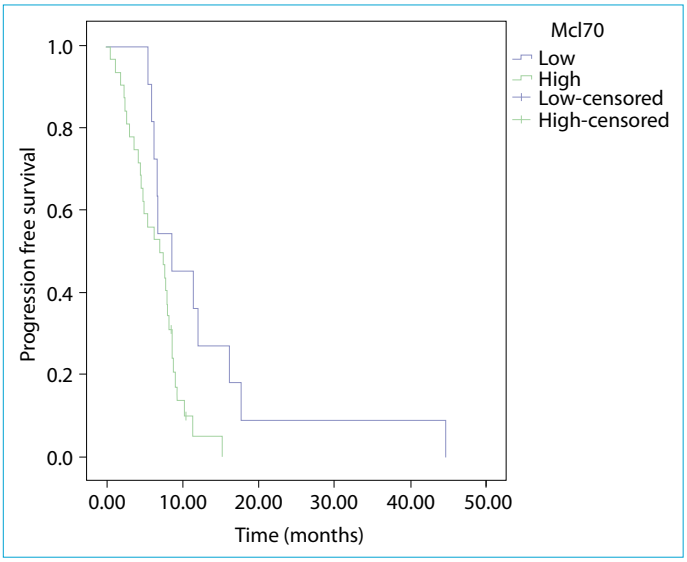

Figure 2. Relationship between Mcl-1 expression and PFS.

years. The median progression-free survival (PFS) and overall survival (OS) were 7.6 months and 9.3 months respectively. The median Mcl-1 value was 70%. The comparison of the Mcl-1 levels and clinicopathological characteristics showed that the expression of Mcl-1 was higher in the patients with pure adenocarcinoma histology, well differentiated carcinoma and visceral organ metastasis (Mcl-1>70) (Table 1).

The PFS was 8.6 months in the patients with a low Mcl-1 level (Mcl-1≤70), while it was 7 months in the patients with a high Mcl-1 level (p=0.02) (Table 2).

According to the univariate and multivariate analysis, the patients, who responded to the first-line treatment and who had low Mcl-1 levels had longer overall survival [17 vs. 6 months (p<0.001); 16.3 vs. 8.7 months respectively (p=0.04)] (Table 3).

Mcl-1 was found to be an independent good prognostic factor for both PFS and OS (Figs. 1, 2).

Discussion

In the last decade, the development of targeted therapies and the optimization of already available chemotherapeutic drugs has expanded the available treatment options for advanced gastric cancer and granted better survival expectations to the patients. Although expression of different biomarkers, such as tumor EBV, MSI, PD-L1 and Her-2/neu status are associated with both prognosis and treatment response of certain agents, there is limited biomarker for predicting prognosis of patients with gastric cancer. This study has shown that; a Mcl-1 level of ≤70 improved PFS and OS. The favorable outcome in patients with low Mcl-1 expression suggests that this marker can be used as an emerging biomarker.

Apoptosis, or programmed cell death, is critical for tissue homeostasis and normal development, and its dysregulation plays a major role in the development of cancer.[18] Moreover, compromised apoptosis is directly associated with resistance to cytotoxic agents as well as to targeted therapies. The Bcl-2 family of proteins are key regulators of apoptosis.[19] Mcl-1 protein is a member of the Bcl-2 protein family. It inhibits apoptosis, and overexpression of Mcl-1 has been associated with tumor progression and resistance to both traditional and targeted therapy, including Bcl-2 inhibitors.[20] Mcl-1 is highly expressed in a variety of human cancers. Furthermore, expression of Mcl-1 is associated with advanced stages and poor clinical outcome of many human cancers including gastric cancer.[12–17] For example, cholangiocarcinoma cells upregulate Mcl-1 expression via the interleukin-6-mediated Stat3 pathway.[21] Melanoma cells upregulate the Mcl-1 level upon endoplasmic reticulum stress.[22] In gastric cancer, the Mcl-1 level was evaluated by an immunohistochemical technique, and the expression level of Mcl-1 was suggested as a prognostic marker.[16] This study showed that an Mcl-1 expression of ≤70 improved PFS and OS. The favorable outcome in patients with low Mcl-1 expression suggests that Mcl-1 may be used as an emerging biomarker.

In this study, the level of Mcl-1 was immunohistochemically examined in tumor tissue of patients with metastatic gastric cancer. The levels of Mcl-1 staining were given in percentages. The median Mcl-1 value was 70%. The patients were divided into two groups based on the median value: high Mcl-1 (>70) and low Mcl-1 (≤70). The comparison of the clinicopathological characteristics showed that the Mcl-1 level was relatively higher in differentiated tumors with no signet-ring cell component (pure adenocarcino-

ma) and visceral organ metastasis. There was no statistically significant difference between Mcl-1 and age, gender, history of smoking, tumor localization, HER-2/neu expression, performance score, and treatment administered. Although previous studies have shown that the Mcl-1 expression is associated with the development of resistance to chemotherapeutic agents such as cisplatin and 5-fluorouracil (5-FU).[23] This study found no difference between the patients treated with 5-FU/cisplatin and 5-FU/oxaliplatin in terms of Mcl-1 expression. The univariate analysis showed better overall survival in the patients treated with 5-FU/cisplatin, while this was insignificant in the multivariate analysis.

PFS and OS were higher in the patients with a low Mcl-1 expression. The patients who responded to the first-line treatment had longer overall survival. Independently of the type of first-line treatment regimen (either cisplatin/5-FU or oxaliplatin/5-FU), response to the first-line treatment was an independent good prognostic factor. Again, independently of the agents used for the first-line treatment, overall survival was better in the low Mcl-1 group. A Mcl-1 level of ≤70 was a good prognostic factor for overall survival. According to the literature, studies to date have evaluated the Mcl-1 level in operated gastric cancer patients and the positive Mcl-1 expression has been evaluated to be associated with poor prognosis.[16, 17] In our study, all patients were in the metastatic stage. Only one patient had an Mcl-1 expression level of 0%. Survival was poorer in the patients with an Mcl-1 expression of >70. This result suggested that the Mcl-1 expression may be a potential therapeutic target and Mcl-1-inhibiting agents may be used for the treatment of these patients.

The small sample size and retrospective design were the limitations of our study. Unknown HER-2 expression status of some patients was another limitation of our study.

In conclusion, our study suggests that mcl-1 plays an important role in metastatic gastric cancer progression by modulating tumor cell proliferation. It may be used as a molecular marker for the prediction of clinical outcomes at metastatic gastric cancer patients.

Disclosures

Ethics Committee Approval: The ethics committee of University of Health Sciences Kocaeli Derince Training and Research Hospital provided the ethics committee approval for this study (11.06.2020-2020/20).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – E.T.B.; Design – E.T.B.; Supervision – E.T.B.; Materials – E.T.B., G.S.O.; Data collection &/or processing – E.T.B., G.S.O.; Analysis and/or interpretation – G.S.O.; Literature search – G.S.O.; Writing – E.T.B; Critical review – E.T.B.

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