Original Article

Myeloid Cell Leukemia-1 Gene Expression and Clinicopathological Features in Myelodysplastic Syndrome

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A B S T R A C T

Background and Aims: Myeloid cell leukemia-1 (Mcl-1) plays a pivotal role in the survival of hematologic and solid tumors, and is known as a substantial oncogene. Studies have demonstrated the altered expression of Mcl-1 has been linked to malignancy development and poor prognosis. In this research, we have studied the expression of Mcl-1 mRNA in myelodysplastic syndrome (MDS) patients and determined association with clinicopathological factors, MDS subgroups as well as international prognostic scoring system.

Materials and Methods: The relative level of Mcl-1 was determined by real time quantitative real-time polymerase chain reaction and gene expression normalized to Glyceraldehyde-3-phosphate dehydrogenase.

Results: Results indicated amplification of mRNA encoding Mcl-1 in 100% of the cases. The higher level of Mcl-1 existed in MDS patients compared with the healthy controls but there was no statistically difference of Mcl-1 expression between these groups. Fold change in gene expression was higher in advanced stage MDS, high risk MDS, cases with >5% blast and LDH >400 to their corresponding groups. In addition, the correlation between gene expression and cytogenetic prognostic subgroups was statistically significant (p=0.043).

Conclusions: In the present study, we showed that Mcl-1 is expressed in MDS independent of the World Health Organization subgroup and international prognostic scoring system. Therefore, Mcl-1 may be up-regulated already in early stages of leukemogenesis.

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Introduction

Myeloid cell leukemia-1 (Mcl-1) is encoded by the Mcl-1 gene. Alternative splicing of the gene leads to isoform 1 (longer gene product) and isoform 2 (shorter gene product) [1]. Isoform 1, an important pro-survival protein of the *Bcl-2* gene family, is of fundamental importance to the development, differentiation, proliferation and tumorgenesis [2]. The protein promotes cells survival by binding and sequestering their pro-apoptotic counterparts (Bak, Bim, and Bid) and inhibiting the release of cytochrome-c from mitochondria [3-6]. Following genotoxic stress, Mcl-1 is ubiquitinylated and rapidly degraded, therefore cells commit to apoptosis [7]. This protein plays a pivotal role in the survival of hematologic and solid tumors and is known as a substantial oncogene [8]. Overexpression of Mcl-1 has been observed in a broad range of human tumors including breast, prostate, ovarian and colorectal cancers, chronic myelogenous leukemia, multiple myeloma and others [9-13]. Studies have demonstrated that altered expression of Mcl-1 has been linked to malignancy development and poor prognosis [12, 14-18]. Myelodyslastic syndromes (MDS) are a type of hematological malignancy showing variable clinical courses from indolent to aggressive forms. There are few prognostic and predictive biological factors of outcomes in MDS and many investigations are ongoing to find such parameters. Regarding the correlations of Mcl-1 and clinical outcomes in other malignancies, we have studied the expression of Mcl-1 mRNA in MDS patients to determine its association with clinico-pathological factors, MDS subgroups as well as international prognostic scoring system (IPSS).

Materials and Methods

Patient selection and sample collection

Sixty MDS patients who had referred to Shariati and Firouzgar Hospitals (Tehran, Iran) were included in the study. The study was approved by the Ethics Committee of Iran University of Medical Sciences, Tehran, Iran. Informed consent was obtained from all the patients and normal controls. Clinical features of the patients are listed in table 1. According to World Health Organization criteria [19], at the time of sampling, 20, 6, 10, 4, 11 and 3 patients had RA, RT, RCMD, RAEB-1, RAEB-2 and 5q- syndrome respectively. According to IPSS [20], 34 patients were low risk, 7 patients intermediate-1, 6 patients intermediate-2, but 7 patients were at high risk.

Conventional cytogenetic analysis

Conventional cytogenetic investigation was carried out for 54 patients. Karyotypes were investigated according to the method previously described [21].

Gene expression analysis

The relative level of Mcl-1 was determined by real-time polymerase chain reaction (RT-PCR) and gene expression normalized to Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Total RNA was derived by TriPure Isolation Reagent (Roche). 1µg RNA was used for cDNA synthesis using the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany). qPCR was conducted with the

QuantiFast SYBR Green RT-PCR Kit (Qiagen, Hilden, Germany) on rotor gene 6000. Reaction mixes were prepared according to the manufacture's protocol. Forward and reverse primers for cDNA amplification used were Mcl-1, F: 5'CGGCGTAACAAACTGGGGCA-3', R: 5'-TCCACAAACCCATCCCAGCCT-3'(183 bp), GAPDH, F: 5'-CACCAGGGCTGCTTTTAACTCTGGA-3', R:5'-CCTTGACGGTGCCATGGAATTTGC-3'(130bp). Thermal cycling conditions were compromised:

DNA polymerase activation at 95°C for 5 min, 40 cycles of denaturation at 95°C for 10 s and combined annealing/extension at 60°C for 30 s. Each measurement was down in triplicate. ΔΔCt method was used to calculate the relative mRNA expression level of the samples compared to the amount of mRNA in the control samples.

Table 1. Patient characteristics

Characteristics	Median (Range)	N (%)
Age(Years)	61.56 (23-90)	54 (100)
Sex		
Male		29 (53.7)
Female		25 (46.3)
ANC($\times 10^9$/L)	2.69 (0.29-8.47)	
Hemoglobin (g/dL)	9.5 (5.6-14.8)	
Platelets($\times 10^9$/L)	135 (7-752)	
BM Blast (%)		
<5%		39 (72.2)
5-9%		4 (7.4)
10-19%		11 (20.4)
Cytogenetic prognostic subgroups (IPSS)		
Good		40 (74.1)
Intermediate		7 (13)
Poor		7 (13
Cytogenetic risk category (IPSS-R)		
Very good		4 (7.4)
Good		36 (66.7)
Intermediate		8 (14.8
Poor		3 (5.6)
Very poor		3 (5.6)
Ferritin (ng/ml)	386.35 (3-1600)	
LDH (U/L)	410.17 (99-1886)	
IPSS		
Low		34 (63)
Int-1		7 (13)
Int-2		6 (11.1)
High		7 (13)
IPSS-R		
Very Low		20 (37)
Low		13 (24.1)
Intermediate		8 (14.8)
High		4 (7.4)
Very High		9 (16.7)
WHO		
RA		20 (37)
RT		6 (11.1)
RCMD		10 (18.5)
RAEB-1		4 (7.4)
RAEB-2		11 (20.4)
5q-		3 (5.6)

Statistical analysis

Statistical analysis was performed using the SPSS 23.0 software package. Mann–Whitney's U-test, kruskal- wallis test, student's t-test and chi square were utilized as required. For all analyses, a value of $p \leq 0.05$ was considered statistically significant.

Results

Patient characteristics

Demographic and hematologic findings are presented in table 1.

Relationship between expression of Mcl-1 mRNA and clinicopathological data

Levels of Mcl-1 mRNAs were investigated by RT-PCR in 54 MDS patients. Results indicated amplification of mRNA encoding Mcl-1 in 100% of the cases. A higher level of Mcl-1 existed in MDS patients compared with the healthy controls but there was no statistically difference between MDS subgroups (Fig.1).

Fold change in gene expression in cases with >60 years was higher compared with those of <60 years and the difference was statistically significant (31.23 ± 26.59 vs. 17.4 ± 14.01, p=0.046). In addition, the correlation between gene expression and cytogenetic prognostic subgroups (IPSS) was statistically significant (p=0.043) (Fig.2).

Fold change in gene expression was higher in the advanced stage MDS, high risk MDS, cases with >5% blast and lactic dehydrogenase (LDH) >400 to their corresponding groups. Difference in the LDH level (306.9 ± 115.307 vs. 678.67 ± 378.837 U/L, p=0.00) and ferritin (355.98 ± 346.088 vs. 465.33 ± 220.176 ng/mL, p=0.034) was statistically significant between advanced stage MDS and early stage MDS patients in this study. The LDH level (587.54 ± 199.176 vs. 353.93 ± 273.727, p=0.00), ferritin (495.69 ± 223.618 vs. 351.69 ± 337.454, p=0.019) and age (68.077 ± 8.84 vs. 59.48 ± 15.46, p=0.007) of the higher-risk group were significantly higher than those in the lower-risk group (Table.2).
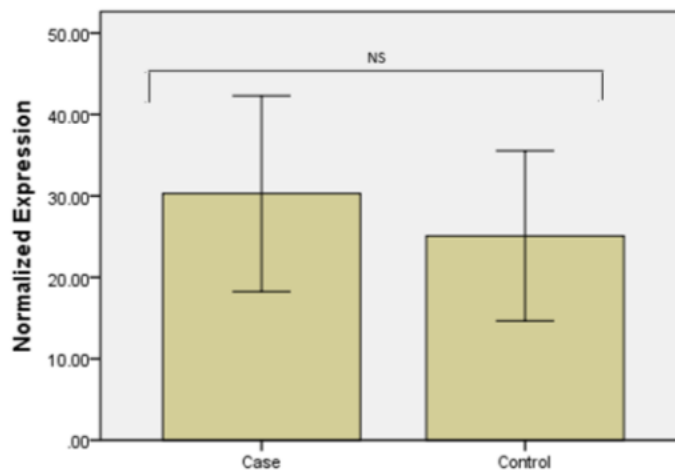
Fig. 1. Real-time quantitative PCR analysis of Mcl-1 expression in samples from MDS patients and controls. The relative mRNA expression of Mcl-1 showed no significant difference between the two groups (p=0.97).
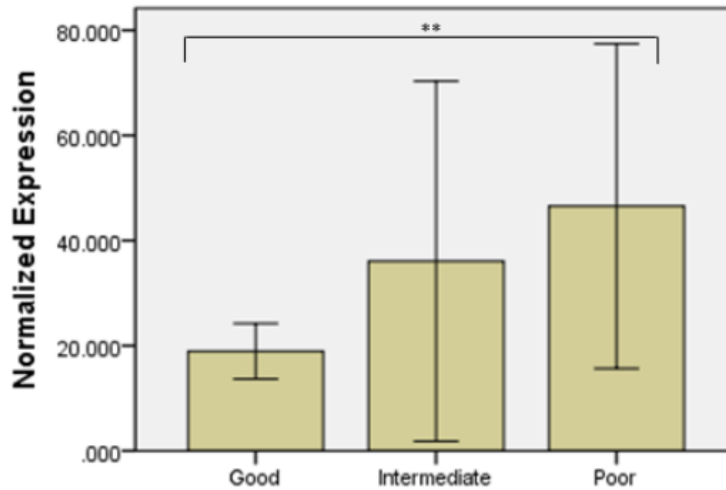
Fig. 2. Real-time quantitative PCR analysis of Mcl-1 expression in samples from cytogenetic prognostic subgroups. The relative mRNA expression of Mcl-1 shows statistically significant differences between cytogenetic prognostic subgroups (p=0.043).

Table 2. Correlation between variables and cytogenetic risk category, WHO subgroups, IPSS and IPSS-R

Groups	Age	ANC	Platelets	Hemoglobin	Lactic dehydrogenase	Ferritin	Mcl-1 mRNA
Cytogenetic risk category	0.282*	0.017	0.233	0.781	0.027	0.258	**0.043**
WHO subgroups	0.56	0.00	0.00	0.001	0.00	0.063	**0.136**
IPSS	0.245	0.02	0.006	0.383	0.001	0.083	**0.27**
IPSS-R	0.004	0.1	0.116	0.017	0.00	0.004	**0.177**

*P-value

Discussion

MDS includes a heterogeneous group of clonal bone marrow disorders that are characterized by aberrant differentiation, cytopenias and leukemic transformation [22, 23]. In the present study, we aimed to clarify the correlation of Mcl-1 expression and clinicopathological findings in MDS. The results revealed significant differences in expression of Mcl-1 gene among cytogenetic prognostic subgroups of MDS. The higher risk-cytogenetic subgroup shows more expression of Mcl-1. Mcl-1 was initially characterized as an early response gene induced during the differentiation of human myeloid leukemia cells the increased expression of which is observed in various hematologic and solid tumors [24].

Mcl-1 has been associated with poor prognosis in advanced ovarian tumors, liver metastases from colorectal cancer and prostate cancer progression [9-11]. Mice expressing Mcl-1 as a transgene were found to develop lymphoma particularly diffuse large-cell lymphoma [25]. Mcl-1 plays a key role in the pathogenesis of myeloid neoplasms. Aichberger et al. demonstrated Mcl-1 detection in isolated primary chronic myeloid leukemia (CML) cells at the mRNA and protein level in all

patients independent of phase of the disease and the levels of Mcl-1 being higher in patients compared to the normal samples. In addition, Mcl-1 expression was also detected in myeloid cell lines including K562 and the basophil cell line KU812. Moreover, they found that targeting of Mcl-1 is related to enhanced sensitivity of leukemic cells against the BCR/ABL tyrosine kinase inhibitor STI571. The researchers concluded that Mcl-1 is a BCR/ABL-dependent survival factor that can be used as a therapeutic target in CML [26]. In the present study, we not only showed that Mcl-1 is widely expressed in MDS samples, but also examined the relationship between Mcl-1 mRNA expression and MDS subgroups, IPSS, cytogenetic risk categories and other variables. Evaluation of Mcl-1 expression in MDS specimens revealed several remarkable conclusions. First, the anti-apoptoticBcl-2 family member, Mcl-1, was detectable in all patient specimens. Not only were levels of Mcl-1 mRNA lower in normal controls but also, not detectable in some control specimens. Second, levels of this mRNA varied widely between different specimens. Third, the important aspect of our investigation was that Mcl-1 is expressed in MDS independent of the WHO and IPSS classification, but average of expression was higher in the advanced stage MDS and high risk MDS compared to the early stage MDS and low risk MDS. Thus, Mcl-1 may be up-regulated already in early stages of leukemogenesis. Fourth, there were a positive correlation between levels of Mcl-1 and increasing age, raising the possibility that increased levels of this anti-apoptotic polypeptide might be a factor that can contribute to the diminished response rate and poor outcomes generally observed in older patients. In addition, there was significant difference between Mcl-1 mRNA expression in cytogenetic risk categories. The highest expression rate was observed in the poor group. Booy et al. indicated that Mcl-1 is a downstream target of epidermal growth factor receptor signaling. In addition, they suggested that overexpression of Mcl-1 in breast cancer cells results in resistance to drug-induced cell death [27].

Kaufmann et al. examined the expression of Bcl-2, Mcl-1, Bcl-xL, and Bax by immunoblotting in human acute leukemia cell lines and acute myeloid leukemia (including antecedent myelodysplastic syndrome and secondary leukemia) and acute lymphoblastic leukemia samples before therapy and at recurrence. They revealed that levels of Bcl-2, Mcl-1, Bcl-xL, and Bax varied over more than 10-fold range between pretreatment specimens. No association was found between pretreatment levels of these proteins and response to initial therapy. However, examination of 19 paired samples revealed about 2-fold increase in Mcl-1 levels in 10 out of 19 pairs at recurrence [28]. Although our observations are in line with Kaufmann's study, our results differ from a previous study reported in MDS patients. Bar et al. examined the gene expression patterns using microarray in MDS patients. They identified several genes related to promotion of apoptosis including Mcl-1, EPOR, and tumor necrosis factor anti-apoptotic modulator (TNFAIP3).

Their data indicated that expression of both variants of Mcl-1 decreases significantly in low and high grade MDS compared to normal bone marrow and down regulation of Mcl-1 is consistent with the promotion of a pro-apoptotic state observed in MDS. They suggested that MDS and progression of MDS are related with not only a decrease in Mcl-1 level, but a shift in transcript in favor of a pro-apoptotic state [29]. With respect to the limited number of patients in our study, correlations which were found here should be examined in a larger study population.

Conclusion

In conclusion, our study revealed a correlation of Mcl-1 expression and high risk cytogenetic categories in MDS. The expression of Mcl-1 generally increased in all MDS subgroups which indicates a possible pathological role of this protein in MDS pathogenesis and progression.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

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