# Overexpression of miR-210 is Associated with Poor Prognosis of Acute Myeloid Leukemia

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Source of support: The study was supported by Chongqing Science and Technology Commission (NO.cstc2012jjA10130)

**Background:** MicroRNAs play important roles in regulation of the initiation and progression of AML. MiR-210 is closely related with cancer development; however, whether miR-210 expression level correlates with clinical correlation in AML is unknown. Thus, the aim of this study was to investigate the potential relationship between miR-210 expression and AML prognosis.

**Material/Methods:** Real-time quantitative PCR was carried out to examine the expression level of miR-210 in bone marrow and serum obtained from AML patients and healthy controls. Then the correlation between miR-210 expression and a variety of important clinical parameters (such as overall survival, relapse-free survival, and prognostic value) were further studied.

**Results:** The expression level of miR-210 was significantly higher in the bone marrow and serum of AML patients than that of healthy controls (p<0.001). Moreover, miR-210 expression was associated with various AML clinicopathological parameters, including FAB classification and cytogenetics. The serum miR-210 expression level was reduced significantly when the patients achieved complete remission (p=0.02). The high miR-210 expression group had both poorer relapse-free survival (p=0.015) and worse overall survival (p=0.008). In the multivariate analysis model, miR-210 was identified as an independent prognostic marker.

**Conclusions:** MiR-210 up-regulation was associated with poor prognosis in AML and it might be useful as a marker for predicting the clinical outcome of AML patients.

**MeSH Keywords:** Leukemia, Myeloid, Acute • MicroRNAs • Prognosis

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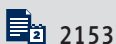
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## Background

Acute myeloid leukemia (AML), a clinically, cytogenetically, and molecularly heterogeneous disease, is characterized by maturation arrest and malignant proliferation of clonal myeloid precursors. The clinicopathological features and prognosis of AML patients are largely influenced by genetic abnormalities such as somatic gene mutations, chromosomal translocations, and deregulated gene expression [1]. Thus, karyotypes and several gene alterations, such as mutations in FLT3, NPM1, CEBPA, KIT, MLL, WT1, NRAS, and KRAS genes, can be employed for diagnoses and prognosis assessment. Although the chemotherapy-based regimen and allogeneic stem cell transplantation have made great progress in the past decades, relapse remains the major cause of treatment failure, and the 5-year survival rates remain low, especially in elderly patients [2]. It is urgent and necessary to screen sensitive and effective biomarkers for predicting the prognosis of AML, which is important for clinicians to stratify the patients, evaluate disease risk, and monitor treatment response.

Except for the genetic mutation mechanism, the epigenetic event has been shown to be closely correlated with the initiation and progression of AML. MicroRNAs (miRNAs) are a class of small, noncoding RNA molecules, which negatively regulate target gene expression. Increasing evidence has revealed that miRNAs play important roles in tumorigenic processes [3]. Various studies showed that aberrant miRNA expression was identified in patients with AML. Zhu et al. found that the expression level of miR-29a was significantly lower in AML patients compared with healthy controls. Moreover, miR-29a down-regulation was associated with advanced clinical features and poor prognosis of pediatric AML patients [4]. Similarly, miR-378 overexpression is a common event in patients with AML and might have an adverse impact on prognosis [5]. Chen et al. showed that miR-124-1 under-expression in AML patients might predict a favorable prognosis [6].

Located within the genomic *loci* of transcript AK123483, miR-210 is an intronic miRNA and can be induced by hypoxia in both normal and transformed cells, indicating an essential role of miR-210 for cell adaptation to hypoxia [7]. In addition, MiR-210 is involved in regulation of various important physiological processes, such as cell survival, proliferation, cell cycle arrest, mitochondrial metabolism, protein modification/transport, DNA damage repair, and angiogenesis [8,9]. MiR-210 overexpression is detected in many solid tumors, including lung carcinoma, head and neck cancer, breast cancer, and pancreatic cancer [10–13]. In most types of cancer, miR-210 up-regulation means poor clinical outcome. However, Mei et al. showed that that lower miR-210 expression in acute lymphoblastic leukemia patients predicts relapse and poorer treatment outcome [14].

MiR-210 has been showed to be involved in the progression of various cancers; however, it remains unknown whether miR-210 expression level correlates with clinical correlation in AML. Thus, the purpose of this present study was to investigate the correlation of miR-210 expression with clinicopathological features and prognosis of AML.

## Material and Methods

### Study population

This study was approved by the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University. All patients and healthy controls who were recruited for participation in this study gave their written informed consent before bone marrow/serum sample collection. All specimens were handled and made anonymous according to the ethical and legal standards.

The samples were collected from 212 newly diagnosed AML patients who underwent therapy at the Department of Hematology, The First Affiliated Hospital of Chongqing Medical University. The age of the 212 patients were ranged from 5 to 78 years and there were 108 males and 104 females. The diagnosis and classification of AML patients were based on the French-American-British (FAB) and World Health Organization classification system. The clinical characteristic of 212 patients with AML is summarized in Table 1.The control group consisted of 40 healthy volunteers with no clinical symptoms of cancer or other diseases.

### Real-time PCR

Up to 5 ml whole blood was collected from each participant, and the serum was isolated from the blood by centrifuging at 3000 rpm for 5 min at room temperature, then centrifuged at 12 000 g at 4°C for 5 min. Mononuclear cells were first isolated by Ficoll-Hypaque density gradient centrifugation of 2 mL bone marrow samples. Total RNA in either serum samples or bone marrow samples was then extracted from cells using a QIAamp RNA Blood kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Reverse transcription (RT) reactions were performed using the Prime-Script RT reagent kit (TaKaRa, Dalian, China). The PCR conditions were 95°C for 5 min, followed by 40 cycles at 95°C for 15 s, 55°C for 30 s and 72°C for 34 s. Real-time PCR was performed on the Applied Biosystems 7500 Fast platform (Applied Biosystems, CA, USA). The relative expression of miR-210 was calculated by the comparative $2^{-\Delta\Delta Ct}$ method using U6 small nuclear RNA levels as internal control. All experiments were performed at least 3 times.

**Table 1.** Association of serum miR-210 level with clinical characteristics of 212 newly diagnosed AML patients.

| Variable | No. of patients (%) | MiR-210 expression (n,%) | | p |
| --- | --- | --- | --- | --- |
| | | Low | High | |
| Gender | | | | |
| Male | 108 | 58 | 50 | 0.658 |
| Female | 104 | 59 | 45 | |
| Age | | | | |
| <60 | 155 | 86 | 69 | 0.887 |
| ≥60 | 57 | 31 | 26 | |
| WBC | | | | |
| <10 | 63 | 36 | 27 | 0.710 |
| ≥10 | 149 | 81 | 68 | |
| Blast in BM | | | | |
| <50% | 96 | 51 | 45 | 0.583 |
| ≥50% | 116 | 66 | 50 | |
| FAB classification | | | | |
| M1-M6 | 194 | 113 | 81 | 0.003 |
| M7 | 18 | 4 | 14 | |
| Extramedullary disease | | | | |
| Absent | 167 | 96 | 71 | 0.195 |
| Present | 45 | 21 | 24 | |
| Cytogenetics | | | | |
| Favorable | 61 | 54 | 7 | 0.000 |
| Intermediate | 102 | 50 | 52 | |
| Unfavorable | 49 | 13 | 36 | |
| Complete Remission | | | | |
| Y | 125 | 65 | 60 | 0.263 |
| N | 87 | 52 | 35 | |

## Statistical analysis

The comparison of miR-210 expression level in bone marrow and serum of AML patients and healthy controls were assessed using the Mann-Whitney U test. With regard to the association of AML clinicopathological parameters with miR-210 expression level, chi-square tests was employed to compare intergroup differences. The Kaplan-Meier method was used to plot overall survival and relapse-free survival curves. Univariate and multivariate analyses were performed using log-rank tests and Cox's proportional hazard model, respectively. Statistics were performed using SPSS 21 software package (SPSS Inc., Chicago, IL, USA) and a p-value of less than 0.05 was considered statistically significant.
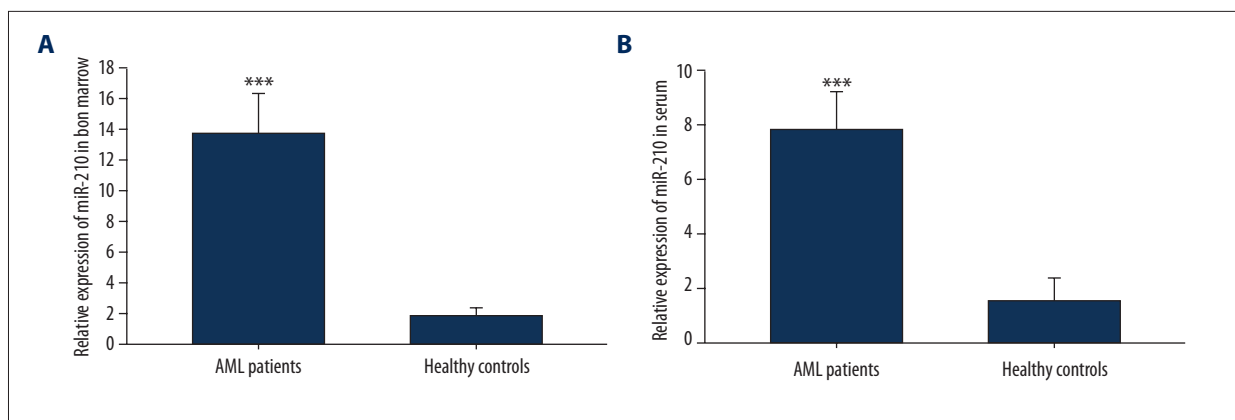
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**Figure 1.** Over-expression of miR-210 in bone marrow and serum of AML patients.

## Results

### Over-expression of miR-210 in bone marrow and serum of AML patients

Our real-time PCR results showed that the expression level of bone marrow miR-210 was significantly higher in AML patients compared with healthy controls (p<0.001, Figure 1A). Similarly, the patients with AML also had a higher serum miR-210 expression level than the controls (p<0.001, Figure 1B)

### The relationship between serum miR-210 expression with AML clinical parameters

We first determined the average expression level of miR-210 in the healthy volunteers, and then the relative expression level of miR-210 in all 212 AML patients was compared with the average expression level of miR-210 in healthy volunteers. Because the data were not normally distributed, the median expression level of serum miR-210 (5.62-fold) was used as the cut-off points to define the high expression level or low expression level. A statistically significant difference was found between serum miR-210 and FAB classification (p=0.003) and cytogenetics (p=0.000). However, there was no correlation between serum miR-210 expression level and age (p=0.658), sex (p=0.887), WBC (p=0.710), blast in BM (p=0.583), extramedullary disease (p=0.195), and complete remission (p=0.263) (Table 1).

### The association between serum miR-210 expression level and treatment response

We compared the expression level of serum miR-210 in AML patients (the high miR-210 expression group) before or after achieving a complete remission. The results showed that the serum miR-210 expression level was reduced significantly when the AML patients achieved a complete remission (p=0.02),
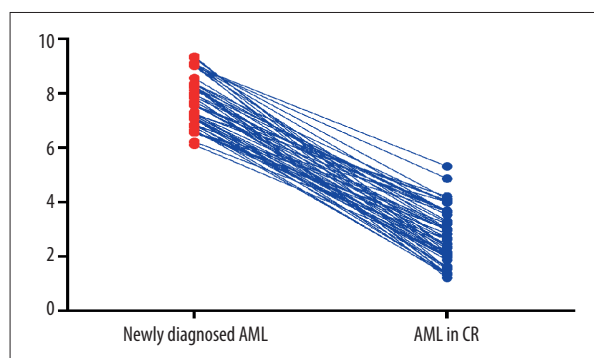


**Figure 2.** The association between serum miR-210 expression level and treatment response.

indicating that serum miR-210 expression is strongly correlated with treatment response (Figure 2).

### Survival analysis

Kaplan-Meier method was used to evaluate the association between serum miR-210 expression level and the overall survival/relapse-free survival of AML patients. Our results revealed that the AML patients in the high miR-210 expression group had poorer overall survival rate (p=0.008) and worse relapse-free survival rate (p=0.015) (Figure 3).

### Univariant analysis and multivariant analysis of prognostic factors in AML

The univariant analysis showed that FAB classification (p=0.031) and cytogenetics (p<0.001) and serum miR-210 expression level (p= 0.002) were significant prognostic indicators for AML (Table 2).

In the multivariant analysis model, cytogenetics (p=0.004) and serum miR-210 expression level (p= 0.012) were independent prognostic factors for AML (Table 2).
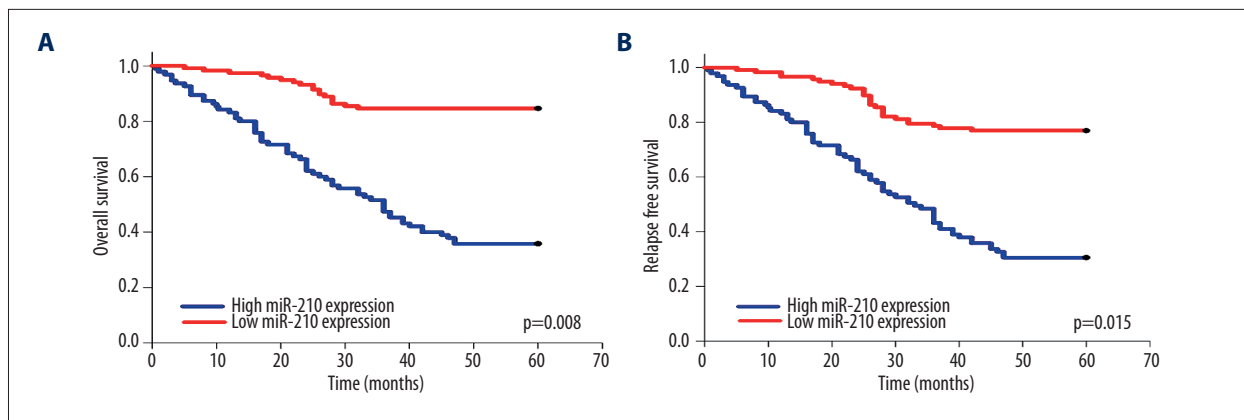
**Figure 3.** High serum miR-210 expression was associated with poorer survival.

**Table 2.** Univariate and multivariate analyses of prognostic factors in AML.

| Independent factors | Univariate p | Multivariate p | HR (95%CI) |
|---|---|---|---|
| Cytogenetics | <0.001 | 0.004 | 6.35 (1.76–8.64) |
| Unfavorable *vs.* favorable/intermediate | | | |
| FAB classification | 0.031 | 0.156 | 1.21 (0.86–1.89) |
| M7 *vs.* M1–M6 | | | |
| MiR-210 expression | 0.002 | 0.012 | 3.65 (1.31–4.28) |
| High *vs.* low | | | |

## Discussion

AML is a malignant disease that attacks the bone marrow and blood, leading to high mortality.

In the past few years, many investigations have been carried out to reveal the relationship between the expression of a specific miRNA (or group of miRNAs) and AML. MiRNA expression is thought to have utility in predicting the prognosis and treatment response of AML, indicating its great potential for clinical application. The expression level of miR-210 was significantly higher in the bone marrow and serum of patients with AML than that of normal controls. Moreover, miR-210 expression was associated with various AML clinicopathological parameters and the serum miR-210 expression level was reduced significantly when the patients achieve a complete remission. Moreover, we found that miR-210 overexpression predicts both worse relapse-free survival and overall survival, and miR-210 was an independent unfavorable prognostic marker for AML. To the best of our knowledge, ours is the first study to reveal the clinical significance of miR-210 in AML patients.

MiRNAs are highly conserved in mammals and are involved in the regulation of various important biological processes, such as cell proliferation, survival, differentiation, and organ development [15]. The length of miRNAs ranges from 18 to 25 nucleotides and miRNAs can inhibit the target genes' expression through mRNA degradation or transcription suppression. MiRNA overexpression or ablation play important roles in cancer development and miRNAs are rapidly being adopted in clinic al use as diagnostic biomarkers and potential therapeutic targets. MiR-210 is up-regulated in all cell types investigated so far and hypoxia has long been recognized as a common feature of tumors, thus it is possible that miR-210 is involved in tumor initiation and progression [7]. Zhang et al. showed that over-expression of miR-210 could promote cancer cell proliferation by down-regulating MNT directly and activating c-MYC indirectly [16]. MiR-210 overexpression has also been found to be correlated with tumor invasion, metastasis, angiogenesis, immunosuppression, apoptosis inhibition, and DNA repair, which are all important steps in cancer progression [7].

Our results show that the expression level of serum miR-210 was significantly higher in patients with AML than in healthy controls. AML is a malignant disease of the bone marrow. Low oxygen tension is found in AML bone marrow and the hypoxic environment might directly increase HIF-1α expression level in AML cells. There is a positive feedback loop between HIF-1α and miR-210. HIF-1α can promote miR-210 expression, and

miR-210 promotes HIF-1α stabilization [17]. Thus, the miR-210 expression is up-regulated in AML patients.

We found that miR-210 expression was associated with many important clinicopathological features and might serve as an unfavorable prognostic factor in AML patients. Consistent with our study, increased levels of serum/tissue miR-210 expression have been found to be directly correlated with poor clinical outcomes in various types of cancers. Lai et al. revealed that glioblastoma patients had 7 times greater serum miR-210 expression than did normal controls. Moreover, higher serum miR-210 was strongly associated with glioma grade and poor clinical outcome [18]. Serum miR-210 expression level was increased in hepatocellular carcinoma (HCC) patients and higher serum miR-210 was an independent prognostic factor predicting poor overall survival. In addition, HCC patients who had higher baseline miR-210 levels had poorer transarterial chemoembolization response [19]. Various studies have reported that circulating miR-210 was increased in patients with pancreatic cancer and might serve as a promising early diagnostic biomarker [20,21]. As regards the relationship between tissue miR-210 and cancer, tissue miR-210 over-expression was correlated with lymph node metastasis, late disease stages, and poor prognosis in patients with lung adenocarcinoma [10]. Samaan et al. showed that the clear cell renal cell carcinoma patients had higher expression of tissue miR-210 than healthy controls. In addition, they found that tissue miR-210-positive patients had a higher chance of relapse and shorter overall survival [22]. Similarly, miR-210 was reported to be up-regulated in colorectal cancer tissues, and its over-expression was correlated with various clinicopathological parameters, such as tumor size, lymph node metastasis, advanced clinical stage, and poor prognosis [23].

Although miR-210 works as an oncogene in most types of cancers, it might play a completely different role in certain types of cancer, such as esophageal squamous cell carcinoma (ESCC), non-small cell lung cancer, and ALL. Tsuchiya et al. reported that miR-210 was down-regulated in ESCC tissues and cell lines. Moreover, miR-210 can inhibit cancer cell survival and proliferation by inducing cell death and cell cycle arrest, indicating the tumor suppressive role of miR-210 [24]. Mei et al. showed that the pediatric ALL patients who suffered from disease recurrence and induction failure had significantly lower miR-210 expression. In addition, lower miR-210 expression level meant poorer treatment response, suggesting that miR-210 may be a good prognostic factor for pediatric ALL patients [14]. Also, contradictory findings on the relationship between non-small cell lung cancer and miR-210 expression have been reported [10,25]. Larger-scale investigations are needed to address this problem. The exact mechanism that responsible for the contradictory roles of miR-210 in different cancers or even in the same type of cancer remains unknown. It is possible that the concrete function of miR-210 depends on the complex microenvironment *in vivo* and the cancer types investigated.

## Conclusions

Our data offer convincing evidence that the over-expression of miR-210 is significantly associated with various clinicopathological parameters of AML. In addition, miR-210 might serve as a novel prognostic marker of this disease. Further investigations should focus on how miR-210 might affect AML development at cellular and molecular levels.

### Conflict of interest

We declare that we have no competing interests.

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