Received: 23 December 2018 | Revised: 5 February 2019 | Accepted: 14 February 2019

DOI: 10.1002/jcb.28796

RESEARCH ARTICLE

WILEY Journal of Cellular Biochemistry

Interaction between 12p chromosomal abnormalities and Lnc-HOTAIR mediated pathway in acute myeloid leukemia

Nashwa El-Khazragy[1] | Sherief Ghozy[2] | Safa Matbouly[3] | Walid Zaki[4] | Gehan Safwat[5] | Ghada Hussien[5] | Omar Khalifa[5]

[1]Clinical Pathology and Haematology Department, Faculty of Medicine, Ain Shams University Biomedical Research Department, Cairo, Egypt

[2]Neurosurgery Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt

[3]Department of Paediatric, Faculty of Medicine, Ain Shams University, Cairo, Egypt

[4]Department of Biochemistry, Faculty of Medicine, Ain Shams University, Cairo, Egypt

[5]Department of Molecular Biology, Faculty of Biotechnology, October University for Modern Sciences and Arts (MSA), Cairo, Egypt

Correspondence
Nashwa N. El-Khazragy, Clinical Pathology and Haematology Department, Faculty of Medicine, Ain Shams University Biomedical Research Department, Cairo, Egypt, Global Research Labs, Medical Center, clinic no 403, Nasr city, Cairo, 11787, Egypt.
Email: nashwaelkhazragy@med.asu.edu.eg; nashwa@globalresearchlabs.com

Abstract

Objectives: To investigate the correlation of homeobox (HOX) transcript antisense RNA expression with clinicopathological features and the clinical prognosis of the patients with chromosome 12p abnormalities associated acute myeloid leukemia (AML). We also investigate the association of 12p chromosomal on the expression of HOTAIR, miRNA-193a, and c-kit gene as targeting genes for HOTAIR in AML.

Methods: AML patients with 12p chromosomal abnormalities were recruited and compared to AML with other chromosomal abnormalities rather than 12p. The long noncoding RNA (lncRNA) "HOTAIR," miR-193a, and c-Kit genes expression were measured in bone marrow samples using Syber green based real-time polymerase chain reaction.

Results: We found a significant difference for the expression levels of HOTAIR, c-kit, and miR-193a between 12p abnormalities associated AML and those without. The survival analysis revealed that patient's with low expression levels of HOTAIR and c-kit had significantly better survival and leukemia free survival. In contrast, miR-193a was associated with better overall survival but not leukemia free survival.

Conclusion: 12p abnormalities associated AML were associated with worse prognosis. Our results proved that HOTAIR, miR-193a, and c-kit genes are independent prognostic predictors in 12p chromosomal associated AML; therefore it may represent a novel therapeutic application in AML in the future.

KEYWORDS

12p aberrations, 12p deletion, acute myeloid leukemia, c-KIT, homeobox transcript antisense RNA, miR-193a

1 | INTRODUCTION

Acute myeloid leukemia (AML) is acknowledged as the main type of leukemia of adult and old age accounting for 80% of cases in this age group.[1] Somatic genetic abnormalities were evident in 30% to 50% of cases of AML.[2] Periodic abnormalities include loss or deletion of chromosome 7, Y, 5, 9 and translocation in different chromosomes.[1] Moreover, some somatic mutations had good prognosis including t(8;21), (q22;q22) or *inv* (16) (p13;q22). Poor-risk patients have a deletions including chromosome 5 or 7, translocations in 11q23, or chromosome 3 aberrations.[2,3]

[Correction added on 26 June 2019, after first online publication: affiliation of the co-author Gehan Safwat changed from 4 to 5]

One of the prevalent chromosomal aberrations is aberrations in the short arm of chromosome 12 which is usually associated with poor prognosis.[4,5] The 12p chromosome aberrations include translocations, deletions, additions and duplications the patients with small 12p deletions had better prognosis in general than other 12p aberrations with less incidence of chromosomal rearrangement. The deletions could not have specific cytogenetic subgroups or disease specific changes.[4,6] Another form of 12p aberrations is translocation which is usually associated with loss and deletions in 12p. The most frequent translocation is the one involving the ETV6 gene at 12p13 which is usually translocated including the 5′ end of ETV6 with 3′ end of the partner. It is usually accompanied by the deletion of ETV6 in the corresponding allele. t/dic (12;13) is one of the commonest translocations representing 20% and usually associated with poor prognosis. Additions are usually frequent imbalanced translocations while duplications are described in benzole agent exposure in one case.[4]

Homeobox (HOX) transcript antisense RNA (HOTAIR) is long noncoding RNA that is located on chromosome 12, and it is responsible for inhibition of multiple genes through activation of polycomb repressive complex 2 that cause H3K27 methylation. HOTAIR has a regulatory rule on different biological process includes proliferation, differentiation, cell cycle, and apoptosis. HOTAIR is considered as an oncogenic factor. Overexpression of HOTAIR was associated with high incidence of metastasis in cervical and breast cancer and poor prognosis in many cancers.[7,8] Under expression of HOTAIR in ovarian tumor cells has antitumorigenesis and antimetastatic effect.[9] Downregulation of HOTAIR induces apoptosis; inhibit proliferation, and metastasis.[10] A study which has explored the role of HOTAIR in AML, they demonstrated its upregulation in AML.[11] HOTAIR expression in AML was associated with poor outcome with less survival time. Furthermore, knockdown of HOTAIR in AML was associated with inhibited cell proliferation and induced apoptosis.

Another regulatory microRNA for c-KIT is miR-193a which was found to inhibit cell Proliferation and induce apoptosis by targeting c-KIT in AML cells.[11] A study found that HOTAIR competitively bind to miR-193a which inhibits its action on c-KIT. Furthermore, HOTAIR inhibits expression of miR-193a thus increasing the effect of c-KIT.[11] These interactions were found to explain the relationship of c-KIT, miR-193a, and HOTAIR Figure 1.

Gao et al found that the expression of miR-193a was downregulated in AML. The study suggests the prognostic efficacy of the HOTAIR in AML as regulatory modulator of different genes.[11] Another study found that HOTAIR is highly expressed in AML patients and implied its important role for its clinical and pathological stratifications.[12] Moreover, the HOTAIR had poor overall survival and relapse free survival.[13] However, another study found that there is no prognostic value of HOTAIR in Iranian patients.[14]

With this confusing evidence, we have shown the expression of the lncRNA "HOTAIR" in 12p chromosomal abnormalities associated AML, moreover to correlate it with miR-193a and c-Kit gene expressions. In addition; to find out the association between HOTAIR/miR-193a/c-Kit regulatory pathway on prognosis and disease outcome.

2 | METHODS

2.1 | Ethical approval

The study was approved by the ethical committee of Faculty of Medicine; Ain Shams University. The study was conducted according to the World Medical Association Declaration of Helsinki. A written consent was obtained from all participants because this was an observational study and all diagnosis and management

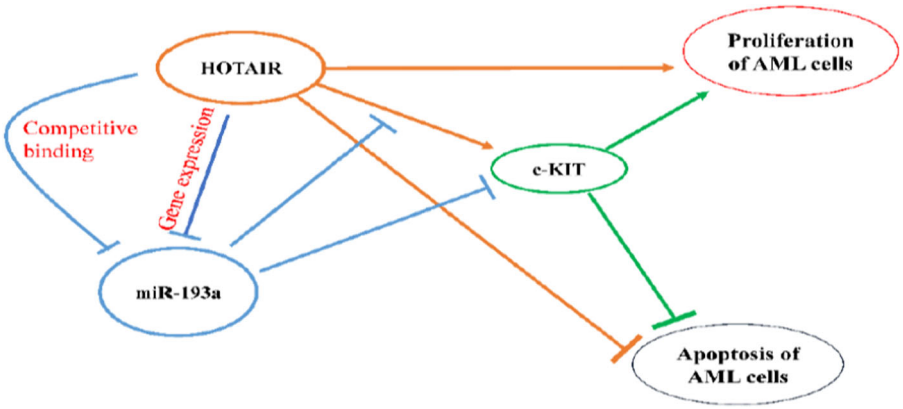

FIGURE 1 Interaction between homeobox (HOX) transcript antisense RNA, miR-193a, and c-KIT in acute myeloid leukemia patients

followed standard of care. Participants were assured that all information generated in this study remained confidential. An exclusive identification number was assigned to each patient at the time of enrolment.

2.2 | Patient enrollment

Bone marrow samples (BM) were collected at diagnosis from 70 patients with AML; the diagnosis was confirmed based on morphological, cytochemical, immunophenotyping, and cytogenetic basics. According to the Cytogenetic results, the participants are categorized into two subgroups. Thirty AML patients were associated with 12p chromosomal abnormalities; while, forty patients were cytogenetic negative or associated with other chromosomal abnormalities rather than short arm of chromosome 12. Minimal residual disease (MRD) was assessed at the day 15th postinduction for the percentage of BM blasts. All patients received a standard regimen of induction chemotherapy consists of vincristine, corticosteroids, L-asparginase and anthracycline. After 4 weeks "the end of induction phase"; the second follow-up sample was collected to assess the response to treatment.

2.3 | RNA isolation and purification and complementary DNA synthesis

The collected BM samples were washed with PBS (pH 7.4) and lysed with TRIzol. MicroRNAs (miRNAs) was extracted using miRNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Concentration and purity of extracted RNA was evaluated spectrophotometrically at 260 and 280 nm; complementary DNA (cDNA) was synthesized using miScript II RT Kit (Qiagen, Hilden, Germany).

2.4 | Bioinformatics analysis

In the present study, the association of lncRNA – HOTAIR with short arm of chromosome 12 abnormalities was investigated using the (http://www.cuilab.cn/lncrnadisease) and (http://www.cuilab.cn/files/images/ldd/rnaseq/HOTAIR.txt) long noncoding databases. The interaction between miR-193a and HOTAIR was predicted from the crosslinking immunoprecipitation RNA sequencing using the starBase platform (http://starbase.sysu.edu.cn/), Finally, the candidate c-Kit target gene for HOTAIR and miR-193a were predicted using ChIPBase (http://deepbase.sysu.edu.cn/chipbase/) and further confirmed using PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), (http://oncodb.hcc.ibms.sinica.edu.tw/index.htm. On the basis of the genomic information that was collected, the regulatory network of the lncRNA-HOTAIR in 12p chromosomal abnormalities was constructed by Cytoscape software version 3.5.0 (http://www.cytoscape.org/index.html).

2.5 | Quantitative real-time polymerase chain reaction

Quantitative real-time polymerase chain reaction (qPCR) was performed using RT2 lncRNA qPCR primer assays and RT2 lncRNA qPCR Kit for amplification of human HOTAIR (*Lnc-RNA HOTAIR*, cat no:330701), the QuantiTect primer assay (Hs_Kit quantiTect primer assay, cat no: 249900, ID: QT01679993), and QuantiTect Syber green PCR Master mix for amplification of c-Kit mRNA. The gene expression was normalized in relation to ACTB as a housekeeper gene. miR-193a was amplified using miScript SYBR Green PCR Kit and miScript primer assay (Hs_miR-193a_1 miScript Primer assay; cat no: Ms00008932); the small nucleolar RNA, C/D box 48 (SNORD48), (NCBI RefSeq: NR_002745.1) was used as a reference gene. Expression assays were purchased from Qiagen, Hilden, Germany and conducted on the 5-plex Rotor Gene PCR System (Qiagen, Hilden, Germany). The reaction mixture and the thermal protocol for each target were performed according to manufacturer's instructions. Expression levels of tested genes were normalized to the housekeeper gene.

2.6 | Statistical analysis

Statistical analysis was done using SPSS v.22 (Chicago, IL).The nonparametric Mann-Whitney *U* test and the Wilcoxon Signed Rank test were performed to evaluate the differences of Lnc-HOTAIR, miR-193a, and c-Kit genes expression between 12p chromosomal abnormalities associated AML and 12p negative chromosomal specimen. Moreover, to assess the correlation of basal level of Lnc-HOTAIR, miR-155, c-Kit levels at with patients' clinic-pathological features. We used Kaplan-Meier Survival analysis for comparison of survival between different groups. Spearman's correlation was used to assess the expression levels of HOTAIR, miR-193a, and c-kit were correlated with patient outcome. Significance was set at level less than or equal to 0.05.

3 | RESULTS

3.1 | Patient characteristics

Total of 70 AML patients were enrolled in this study. We included 30 patients diagnosed as AML with 12p abnormalities (12p AML) and 40 patients with AML-negative for 12p abnormalities (12p negative AML). The

mean age for 12p AML was 50 with 18 patients aged above 40 years old. Meanwhile, the mean age for 12p negative AML was 49.9 with 27 patients aged above 40 years old. The majority of 12p positive AML had a TLC > 50?000 (n = 11) compared to 12p negative abnormality AML (n = 4). Furthermore, The AML who are negative for 12p abnormalities had better survival than positive cases Table 1. There was a difference between both groups regarding clinical response; laboratory tests and status are present in Table 1.

TABLE 1 Descriptive analysis of the studied subjects

Demographic data	AML with 12p abnormalities (N = 30)	AML-negative for 12p abnormalities (N = 40)
Age, y		
Mean ± SD	50.0 ± 14.0	49.4 ± 14.5
Range	28-73	22-77
Sex, n (%)		
Male	19 (37)	32 (63)
Female	11 (58)	8 (42)
Age subgroups, n (%)		
Favorable (=40)	12 (37)	32 (63)
Unfavorable (>40)	18 (51)	27 (49)
TLC ($\times 10^6$/L), n (%)		
=50	19 (35)	38 (66)
>50	11 (73)	4 (27)
Hemoglobin (g/dL), n (%)		
>6	18 (37)	38 (68)
=6	12 (86)	2 (14)
Platelet count ($\times 10^{12}$/L), n (%)		
=30	11 (73)	4 (27)
<30	19 (35)	36 (65)
BM blasts, n (%)		
=25%	15 (29)	37 (71)
>25%	15 (83)	3 (17)
MRD at day 15, n (%)		
=0.01	13 (27)	36 (73)
>0.01	17 (81)	4 (19)
Clinical response, n (%)		
Remission	10 (22)	11 (24)
Relapse	5 (15)	36 (78)
Status, n (%)		
Died	15 (65)	8 (35)
Alive	15 (32)	32 (68)
Overall Survival (OS)		
Median (IQR)	15 (11)	20 (4)
Range	2-24	5-24
Leukemia free survival (LFS)		
Median (IQR)	8 (10)	16 (1.8)
Range	0.8-22	0.4-7.7

Abbreviations: 12p: short arm of chromosome 12; BM, bone marrow; MRD, minimal residual disease .

3.2 | Analysis for HOTAIR, miR-193a, and c-Kit between different studied subgroups and different risk groups

Although, the three studied markers are increase in AML vs the control group, we had established that 12p AML had higher log expression of HOTAIR than 12p negative AML (median = 34.8 vs median = 19.4, $P = 0.006$) Table 2. The expression levels of c-kit were also the same as HOTAIR (median = 50 vs median = 30.4, $P = 0.001$) Figure 2.

In contrast, 12p AML had lower log expression of miR-193a than 12p negative AML (median = 2.6 vs median = 5.2, $P = 0.01$) Table 2.

3.3 | Analysis for HOTAIR, miR-193a, and c-Kit between different risk groups

In addition, we found significant different levels of gene expression of HOTAIR, miR-193a, and c-Kit among studied risk groups of 12p abnormalities positive and negative AML. In case of 12p AML, there was significantly higher level of log HOTAIR in patient with TLC more than 5×10^7 cells ($P = 0.02$). The same was found for hemoglobin levels in which there was significantly higher level of log HOTAIR in patient with hemoglobin levels more than 6 mg/dL ($P = 0.04$). The median log HOTAIR expression level was significantly higher in platelet count less than 30 (median = 84 (IQR = 42)). moreover, the median log HOTAIR expression level was significantly higher in MRD at 15 days more than 0.01 and BM blasts more than 70 (median = 22 and 80, respectively, $P < 0.01$) Table S1. There was no significant difference of the median log HOTAIR expression level among different age, sex, or clinical status.

Meanwhile, the gene expression levels of log miR-193a was higher in patients with less than 5×10^7 cells (median = 3.4, $P = 0.008$). In addition, gene expression levels of log miR-193a was higher in patients with hemoglobin less than 6 mg/dL (median = 3.3, $P = 0.05$) Table S1. Furthermore, the median log miR-193a expression level was significantly higher in platelet count more than 30 (median = 3.8 (IQR = 2.8), $P = 0.01$). The median log miR-193a expression level was significantly higher in MRD at 15 days less than 0.01 and BM blasts less than 70 (median = 4.1 and 4 respectively, $P < 0.01$) Table S1. There was no significant difference of the median log miR-193a expression level among different age, sex, or clinical status.

For c-kit expression levels, we found that it was higher in hemoglobin less than 6 mg/dL (median = 70), platelet counts less than 30 x10^{12}/L (median = 71), BM blasts

TABLE 2 Comparative analysis for *HOTAIR*, *miR-193a*, and *c-KIT* between different studied subgroups

	Median (IQR) range				Statistics: *U* Mann-Whitney test value (*P* value)			
Variables	AML (n = 70)	12p abnormalities positive AML (n = 30)	12p abnormalities negative AML (n = 40)	Control (n = 20)	AML/ control	12p abnormalities positive AML/ control	12p abnormalities negative AML/ control	12p abnormalities positive/ negative AML
Lnc-HOTAIR (log^{10})	22 (54.8) 6.5-133.4	34.8 (66.3) 6.5-133.4	19.4 (15.4) 8.8-113.6	8.8 (6.3) 0.8-18.0	106 (0.001)*	28 (0.001)*	78 (=0.01)*	370 (0.006)*
miR-193a (log^{10})	3.3 (4.4) 0.2-8.5	2.6 (7.7) 0.2-7.9	5.2 (4.2) 0.2-8.5	12.8 (12.5) 4.9-32.0	53.5 (0.001)*	6.0 (0.001)*	48 (=0.01)*	384 (0.01)*
c-Kit (log^{10})	32.4(25) 8.6-88.0	50.0 (38) 22-88	30.4 (13) 8.6-61.0	13.4 (7.5) 6.8-22.0	42 (0.001)*	2.0 (0.001)*	40 (=0.01)*	235 (0.001)*

Abbreviations: 12p, short arm of chromosome 12; AML, acute myeloid leukemia; IQR, interquartile ratio; Lnc, long noncoding; n, number of subjects.
*Mann-Whitney *U* test is significant at $P = 0.01$.

more than 70 (median = 70), MRD at day 15 more than 0.01 (median = 70) and in relapses (median = 64). There was no significant difference of the median log miR-193a expression level among different age or sex.

For patients with 12p negative AML patients, only miR-193a was significantly higher in platelet count more than 30×10^{12}, MRD at day 15 less than 0.01 and in relapse (median = 5.5 (IQR = 4.2), $P = 0.01$), (median = 5.5

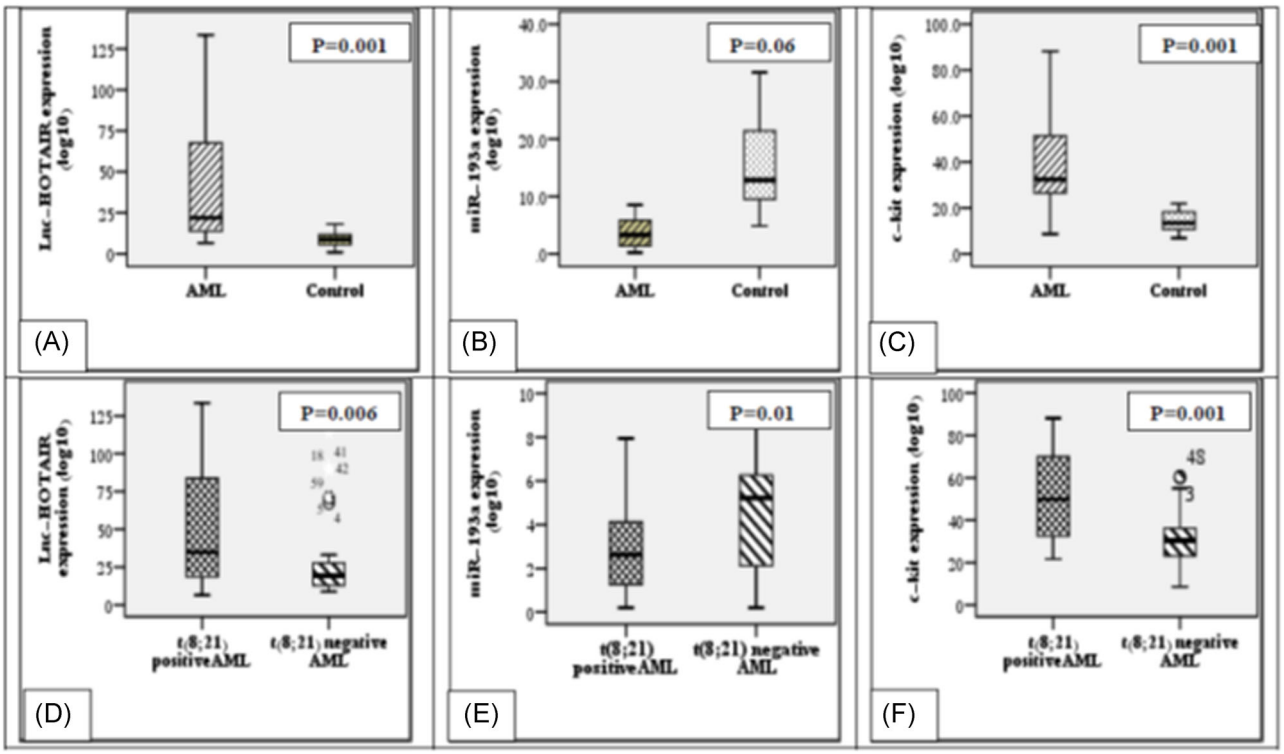

FIGURE 2 Box-plots illustrating the difference in expression levels of Lac-HOTSTAR (A) MaR-193a (B) and C-kit (C) between with chromosome 12p abnormalities associated acute myeloid leukemia (AML) and healthy control a high significant difference was obtained between ML and Control for the three measured parameters ($P = 0.01$). Moreover when the expression levels were compared between 12p abnormalities positive and negative AML patients; a high significant difference was observed for Lnc-HOTSTAR (D) maR-193a (E) and c-kit (F) ($P = 0.01$) 12p chromosomal abnormalities associated AML patients had higher expression levels of Lnc-HOTSTAR and c-kit however; maR-193a was downregulated in this group

(IQR = 4.2), $P = 0.04$), and (median = 53 (IQR = 21), $P = 0.001$), respectively.

3.4 | Association of HOTAIR, miR-193a, and c-Kit gene expression with overall survival and leukemia free survival

Generally, log expression levels of HOTAIR, c-kit and miR-193a was significantly correlated with overall survival and Leukemia free survival ($P = 0.001$) in AML patients whether in 12p abnormalities associated AML or 12p abnormalities negative AML. With exceptions in some patients, It was found that the log expression of miR-193a in 12p AML and log expression of c-kit was not significantly associated with overall survival and Leukemia free survival Table S2**.** The log expression of miR-193a in 12p negative AML was not significantly correspond with the overall survival.

To explore the impact of the biomarkers expression levels on disease outcome in 12p positive abnormities AML; the cut-off value for each is determined and Kaplan-Meier curve was plotted; we demonstrated that gene expression values above the cut-off value of HOTAIR and c-kit (34.8 and 50); respectively were significantly associated with poor overall survival and shorter leukemia free survival (LFS) ($P < 0.01$) Table 3. On opposing; Patients with high miR-193a expression more than the cut-off value (2.6) had significantly better survival time Figure 3B, but no difference was observed for LFS Figure 3B.

4 | DISCUSSION

This study was set out with the aim to understand the genetic and epigenetic interactions underlying the acute myeloid leukemia associated with 12p chromosomal abnormalities and how it affects different laboratory parameters and clinical outcomes in patients of acute myeloid leukemia (AML).

In our study, we classified our patients into AML with 12p abnormalities and those with no 12p abnormalities, and then we tested the effect of epigenetic markers including HOTAIR and miR-193a on c-KIT genes in each type and its impact on clinical outcome.

Per our results, we have revealed a significant difference for the expression levels of HOTAIR, c-kit and miR-193a between AML patients with 12p abnormalities and those with no 12p abnormalities. The level of c-kit expression was higher in AML patients with 12p abnormalities and those with no 12p abnormalities (median = 50.0, IQR = 38) and (median = 30.4, IQR = 13), respectively. Similar results were obtained for HOTAIR expression (median = 34.8, IQR = 66.3) and (median = 19.4, IQR = 15.4). Meanwhile, the expression of miR-193a was higher in patients Who were negative for 12p abnormalities than those with abnormality (median = 2.6, IQR = 7.7) and (median = 5.2, IQR = 4.2), respectively. Furthermore, a significant association was observed for HOTAIR, miR-193a and c-Kit genes expression with high risk subgroups of 12p associated AML including total leukocytic counts, MRD at day 15, platelets count, clinical outcomes, BM blasts and hemoglobin levels. Meanwhile, in 12p abnormalities negative AML, the gene expression of c-kit and miR-193a was significantly different in MRD at day 15, platelets count and clinical outcomes.

The survival analysis showed that Patients with low HOTAIR and c-kit expression had significantly better survival and leukemia free survival. In contrast,

TABLE 3 Impact of high/low expression levels of Lnc-HOTAIR, miR-193a, and c-Kit on overall and progression free survival in 12p abnormalities positive AML

		Overall survival, mo					Leukemia free survival, mo				
			95% confidence interval		Log rank (Mantel-Cox)			95% confidence interval		Log rank (Mantel-Cox)	
Variables	Cut-off	Estimate OS	Lower	Upper	χ^2	*P* value	Estimate LFS	Lower	Upper	χ^2	*P* value
Lnc-HOTAIR (log^{10})	=34.8	22.8	21.3	24.3	29	0.001	15.3	12.0	18.6	26	0.001
	>34.8	7.5	5.3	9.6			3.8	2.7	5.2		
miR-193a (log^{10})	=2.6	12.0	8.6	15.4	3.5	0.06	8.7	5.4	12.0	0.08	0.9
	>2.6	18.7	14.7	22.7			10.7	7.0	14.4		
c-kit (log^{10})	=50	21.3	18.3	24.0	11.9	0.001	16.6	12.6	20.6	17.5	0.001
	>50	9.5	6.7	12.4			5.9	3.0	7.2		

Abbreviations: χ^2, Chi-square value, HOTAIR, homeobox (HOX) transcript antisense intergenic RNA; LFS, leukemia free survival; Lnc, long noncoding; OS, overall survival.

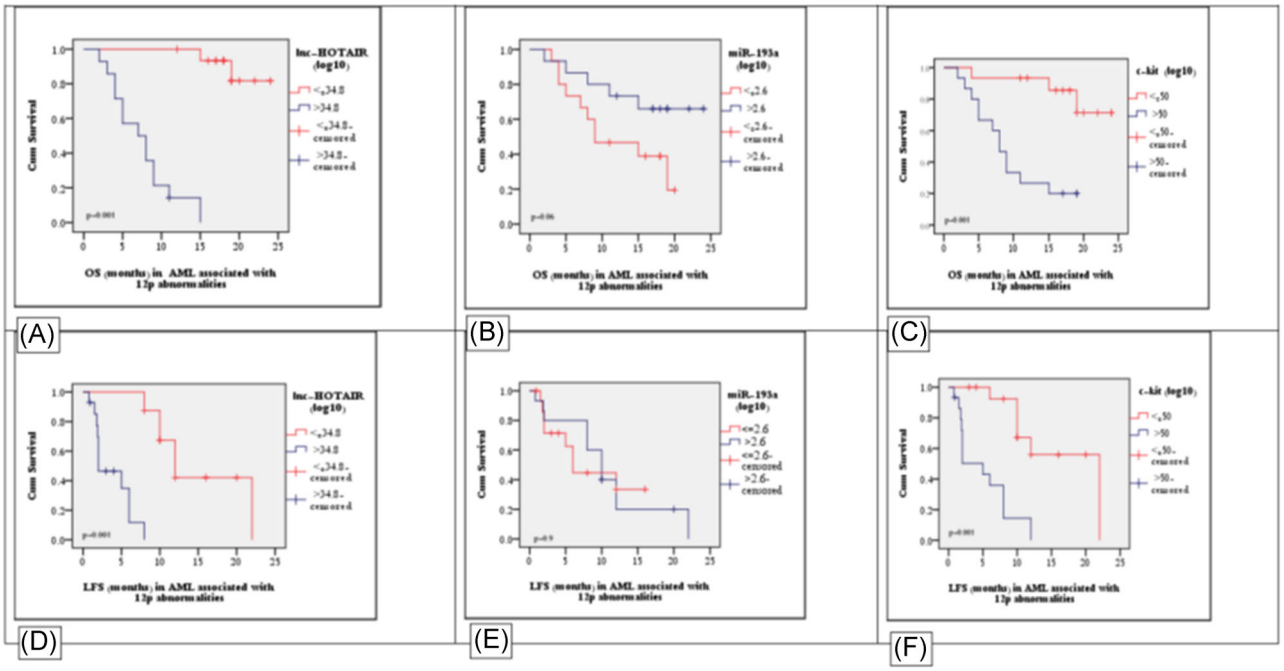

FIGURE 3 Kaplan-Meier survival curves of patients with high and low Linc-HOTSTAR miR-193a and c-Kit expression in 12p abnormalities associated acute myeloid leukemia patients were dichotomized into high and low genes expression groups based on median expression value patients with low Lnc-HOTSTAR and c-Kit expression had significantly better survival (A,C) and leukemia free survival (D,F) respectively than those with higher expression on contrary patients with high maR-193a expression had significantly better survival (B) but no obvious difference was observed for leukemia free survival (E)

miR-193a was associated with better overall survival but not leukemia free survival.

Our study results proved that AML can be classified into two subgroups based on the presence or absence of 12p abnormalities. 12p abnormalities are common in hematological pregnancy. In our study, 12p abnormalities positive patients represents 42% of all cases. There is no study in literature that compared between 12p abnormalities positive patients to 12p negative AML. In chronic lymphocytic leukemia, 12p aberrations were associated with worse outcome and prognosis.[15] Another study found that 12p aberrations is usually present in AML after exposure to mutagen in myelodysplastic syndromes.[16] Braulke et al[17] indicated that there is underestimation of 12p aberrations in AML. Furthermore, they found that 12p aberrations were associated with better prognosis in myelodysplasia and less conversion to AML.[17] The most important genes that is carried on 12p and was identified as one of the genes involved in the pathogenesis of AML is ETV6.[18,19] This gene is a member of transcription regulators and responsible for DNA binding activities.[18] In our study, we found that the overall survival and leukemia free survival was less in 12p AML than 12p negative AML. This consistent with literature that found that 12p aberrations were associated with worse prognosis.[4]

For underlying genetic expression in each subgroup in our study, we tested the interaction between HOTAIR, miR-193a, and c-KIT as evidenced in literature as one of the influencers of the AML prognosis. In our study, we found that there was significant high expression level of HOTAIR and c-KIT in 12p AML than 12p negative AML. Meanwhile, there was a higher expression of miR-193a in 12p negative AML. Our results explain the worse survival associated with 12p aberrations.[4] On the basis of the study by Boissel et al[20] the c-KIT is usually associated with higher rate of relapse and worse survival. Ayatollahi et al[21] confirmed that c-KIT has worse prognosis in CBF-AML. However, another study found no relation between the outcome and c-KIT expression which is inconsistent with our results.[22] Furthermore, the c-kit was found to be upregulated by HOTAIR which by itself had poorer prognosis in AML.[11] The protective miR-193a was found to cause worse outcome if it is knocked down.[11] Moreover, high expression of HOTAIR was associated with inhibition of miR-193a activity[11] Figure 1. These studies explained why the 12p AML had worse prognosis in our study. Even the difference of expression of HOTAIR, c-KIT and miR-193a were among the different risk groups and resulted in difference of clinical parameters. This can be explained by the association between the expression values of these genes and poor prognosis of t12p abnormality.

Surprisingly, we found that miR-193a expression was not correlated with leukemia free survival in 12p AML and c-KIT was not correlated with leukemia free survival. This is consistent with Boissel et al[20] who found no significant effect of c-KIT on disease free survival. Furthermore, another study implied that there was no significant effect of c-KIT on the overall survival and prognosis.[22]

HOTAIR expression was associated with worse outcome in AML. Furthermore, knockdown of HOTAIR inhibited cell proliferation in AML.[11,13,23] Furthermore, another study recommended determination of the level of HOTAIR in AML patients for risk factors stratification and prognosis determination.[25]

On the basis of our results, 12p abnormalities should be considered when dealing with AML cases. Then, it would be stratified based on expression of c-KIT, HOTAIR, and miR-193a for better dealing with patients and for prediction of the prognosis in AML patients.

5 | CONCLUSION

12p abnormalities is considered a lifestyle threating hassle which do represent the highest mortality price rate in AML patients; therefore; it is a must be treat as a single separate entity of AML. Primarily based on our effects, it had a worse analysis, significantly higher c-Kit, HOTAIR expression, and coffee miR-193a expression and related to poor outcome.

ACKNOWLEDGMENTS

We thank our colleagues from (Clinical Hematology Department, Ain Shams University Hospitals) who provided insight and expertise that greatly assisted the sample collection and patients data for this study, although they may not involve with all of the interpretations/conclusions of this paper. We would also like to show our gratitude to the patients who are the backbone of this study for their cooperation and sharing their pearls of wisdom with us during the course of this study, and we thank three "anonymous" reviewers for their so-called insights. We are also immensely grateful for their comments on an earlier version of the manuscript, although any errors are our own and should not tarnish the reputations of these esteemed persons.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

NE-K conceived of the presented idea, developed the theory, performed the computations and verified the analytical methods. Encouraged Walid Zaki, SM to investigate supervised the findings of this study. All authors discussed the results and contributed to the final manuscript. OK and GH carried out the experiment. SG wrote the manuscript with support from NE-K, who helped supervise the project. Walid Zaki and SM conceived the original idea. NE-K supervised the project. Walid Zaki developed the theoretical formalism, performed the analytic calculations and performed the numerical simulations. Both NE-K and SG authors contributed to the final version of the manuscript. NE-K supervised the project.

ORCID

Nashwa El-Khazragy http://orcid.org/0000-0001-6646-4674

REFERENCES

1. Deschler B. Acute myeloid leukemia: epidemiology and etiology. *Cancer*. 2006;107(9):2099-2107.
2. Heim S, Mitelman F. Cytogenetic analysis in the diagnosis of acute leukemia. *Cancer*. 1992;70:1701-1709.
3. Grimwade D, Walker H, Harrison G, et al. The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML); analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. *Blood*. 2001;98:1312-1320.
4. Bilhou-Nabera C. 12P abnormalities in myeloid malignancies. *Atlas Genet Cytogenet Oncol Haematol*. 2011;2:125-126.
5. de Rooij J, Beuling E, Fornerod M, et al. Aberrations are a recurrent event in pediatric acute myeloid leukemia with poor clinical outcome. *Blood*. 2014;124:1012.
6. Crane MM, Strom SS, Halabi S, et al. Correlation between selected environmental exposures and karyotype in acute myelocytic leukemia. *Cancer Epidemiol, Biomarkers Prev*. 1996;5:639-644.
7. Chase A, Reiter A, Burci L, et al. Fusion of ETV6 to the caudal-related homeobox gene CDX2 in acute myeloid leukemia with the t(12;13)(p13;q12). *Blood*. 1999;1999(93):1025-1031.
8. Hajjari M, Salavaty A. Review HOTAIR: an oncogenic long non-coding RNA in different cancers. *Cancer Biolo Med*. 2015;2015:1-9.
9. Wang J, Chen D, He X, et al. Downregulated lincRNA HOTAIR expression in ovarian cancer stem cells decreases its tumorgeniesis and metastasis by inhibiting epithelial-mesenchymal transition. *Cancer Cell Int*. 2015;15:24.
10. Han L, Zhang H-C, Li L, Li C-X, Di X, Qu X. Downregulation of long noncoding RNA HOTAIR and EZH2 induces apoptosis and inhibits proliferation, invasion, and migration of human breast cancer cells. *Cancer Biother Radiopharm*. 2018;2018(33):241-251.

11. Xing C-Y, Hu X-Q, Xie F-Y, et al. Long non-coding RNA HOTAIR modulates c-KIT expression through sponging miR-193a in acute myeloid leukemia. *FEBS Lett*. 2015;2015(589):1981-1987.
12. Hao S, Shao Z. Original article HOTAIR is upregulated in acute myeloid leukemia and that indicates a poor prognosis. *Int J Clin Exp Pathol*. 2015;8:7223-7228.
13. Wu S, Zheng C, Chen S, et al. Overexpression of long non-coding RNA HOTAIR predicts a poor prognosis in patients with acute myeloid leukemia. *Oncol Lett*. 2015;10:2410-2414.
14. Sayad A, Hajifathali A, Hamidieh AA, Roshandel E, Taheri M. HOTAIR long noncoding RNA is not a biomarker for acute myeloid leukemia (AML) in Iranian patients. *Asian Pacific J Cancer Prev*. 2017;2017(18):1581-1584.
15. Marín IG-G, Hernández-Sánchez M, Rodríguez-Vicente AE. A high proportion of cells carrying trisomy 12 is associated with a worse outcome in patients with chronic lymphocytic leukemia. *Hematol Oncol*. 2016;34(2):84-92.
16. United Kingdom Cancer Cytogenetics Group. Translocations involving 12p in acute myeloid leukemia: association with prior myelodysplasia and exposure to mutagenic agents. *Genes, Chromosomes and Cancer*. 1992;5:252-254.
17. Braulke F, Müller-Thomas C, Götze K, et al. Frequency of del (12p) is commonly underestimated in myelodysplastic syndromes: results from a German diagnostic study in comparison with an International Control Group. *Genes Chromosomes Cancer*. 2015;2015(817):809-817.
18. Eguchi M, Eguchi-Ishimae M, Tojo a, et al. Fusion of ETV6 to neurotrophin-3 receptor TRKC in acute myeloid leukemia with t(12;15)(p13;q25). *Blood*. 1999;93:1355-1363.
19. Tosi S, Harbott J, Teigler-schlegel A, et al. t (7; 12)(q36; p13), a new recurrent translocation involving ETV6 in infant leukemia. *Genes, Chromosomes Cancer*. 2000;332:325-332.
20. Boissel N, Leroy H, Brethon B, et al. Incidence and prognostic impact of c-Kit, FLT3, and Ras gene mutations in core binding factor acute myeloid leukemia (CBF-AML). *Leukemia*. 2006;20:965-970.
21. Ayatollahi H, Shajiei A, Sadeghian MH, et al. Prognostic importance of C-KIT mutations in core binding factor acute myeloid leukemia. *Hematol Oncol Stem Cell Ther*. 2017;10:1-7.
22. Riera L, Marmont F, Toppino D, et al. Core binding factor acute myeloid leukaemia and c-KIT mutations. *Oncol Rep*. 2013;2013(29):1859-1866.
23. Garzon R, Volinia S, Papaioannou D, et al. Expression and prognostic impact of lncRNAs in acute myeloid leukemia. *Proc Natl Acad Sci USA*. 2014;111:18679-18684.
24. Zhang Y-Y, Huang S-H, Zhou H-R, Chen CJIE. Role of HOTAIR in the diagnosis and prognosis of acute leukemia. *Oncol Rep*. 2016;36:3113-3122.
25. Díaz-Beyá M, Brunet S, Nomdedéu J, et al. The lincRNA HOTAIRM1 , located in the HOXA genomic region, is expressed in acute myeloid leukemia, impacts prognosis in patients in the intermediate-risk cytogenetic category, and is associated with a distinctive microRNA signature. *Oncotarget*. 2015;6:31613-31627.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: El-Khazragy N, Ghozy S, Matbouly S, et al. Interaction between 12p chromosomal abnormalities and Lnc-HOTAIR mediated pathway in acute myeloid leukemia. *J Cell Biochem*. 2019;120:15288-15296. https://doi.org/10.1002/jcb.28796