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Assessing the expression of differentiation antagonizing non-protein coding RNA (DANCR) in newly diagnosed Egyptian acute myeloid leukemia patients

Nour Mohammed Rasheed[1*], Howaida Attia Nounou[1], Soad Mohamed Eltabakh[1], Nahla A. M. Hamed[2] and Ayman Ahmed Darwish[1]

Abstract

Background Role of Long non-coding RNAs in cancer research in the recent years have been highlighted with evidence to their involvement in cancer disease pathogenesis and progression. One of these emerging long non-coding RNAs is differentiation antagonizing non-protein coding RNA (DANCR). DANCR distinct expression in different cancers and implication in tumor signaling pathways made it a promising therapeutic target for cancer.

The purpose of this study was to evaluate DANCR expression in de novo acute myeloid leukemia (AML) patients and to assess DANCR expression in relation to cytogenetics and French American British (FAB) AML classification as well as correlate DANCR expression with patients' response to treatment.

The present study included 60 newly diagnosed AML patients and 30 healthy subjects as controls. Relative DANCR expression was done using real time qPCR method.

Results DANCR was significantly downregulated in AML patients compared to controls ($p=0.038$). In addition, DANCR showed significantly lower expression in M4 and M5 compared to M0, M1, and M2 groups ($p<0.001$). Furthermore, DANCR expression was significantly downregulated in cytogenetically normal AML patients compared to the controls ($p=0.011$).

Conclusion Significant downregulation of DANCR in AML suggests a potential tumor suppressor role and variable expression of DANCR among AML subtypes suggests that DANCR action may be different among AML subtypes. Also, M1 subtype patients with higher DANCR expression were less refractory to treatment and therefore less resistant to cytarabine.

Keywords DANCR, Long noncoding RNA, Acute myeloid leukemia, Gene expression, Adult leukemia

Background

Acute Myeloid leukemia (AML) is the most common form of all leukemias affecting adults, making about 25% of all leukemia types with an incidence rate of 4 per 100,000 and with a high mortality rate being the sixth highest cancer related death in male population in the United States [1]. AML is characterized by uncontrolled proliferation of cells and arrest of differentiation of myeloid progenitors in different stages [2]. AML is commonly

*Correspondence:
Nour Mohammed Rasheed
Nourelhoda.mohammed@alexmed.edu.eg
[1] Department of Medical Biochemistry, Alexandria Faculty of Medicine, Alexandria, Egypt
[2] Department of Internal Medicine (Hematology Unit), Alexandria Faculty of Medicine, Alexandria, Egypt

classified using two staging systems, the French-American-British (FAB) classification which classifies AML into subtypes from M0 to M7 depending on the cell morphology and the type of cell that AML arises from [3] and the more recent WHO classification which included the variable cytogenetic abnormalities that occur in AML [4].

AML is a disease of complex pathogenesis, and is known for its genetic heterogeneity, where there is more than one mutation found in the majority of the patients [5]. Around half of the patients have chromosomal abnormalities, and the other half is cytogenetically normal AML (CN-AML), with or without somatic mutations in nucleophosmin (NPM1), or FMS-like tyrosine kinase 3 internal tandem duplication (FLT3-ITD) which are in-frame duplications of variable size, ranging from three to more than one thousand nucleotides or mutations in CEBPA [6, 7].

Identification of these genetic mutations in AML focused on protein-coding genes to understand complex molecular pathogenesis of the disease. Despite aggressive treatment regimen, response to treatment is still unsatisfactory and long-term survival is still low [8], thus identifying potential molecular therapeutic targets has become essential to improve disease prognosis.

High throughput technologies highlighted the various roles played by non-coding RNAs (ncRNAs), which are transcribed from the human genome, but are not encoded into proteins. ncRNAs that exceed 200 nucleotides in length are Long non-coding RNAs (lncRNAs) [9]. Though they are not encoded into proteins, they are involved in various cellular functions such as regulating the expression of different genes involved in cell cycle, proliferation, differentiation, stem cell differentiation, apoptosis, invasion, migration and autophagy [10]. Subsequent studies suggested that several lncRNAs are deregulated in cancers and may play an oncogenic role through cancer development [11, 12].

Among these lncRNAs is differentiation antagonizing non-protein coding RNA (DANCR), which is 855 bases in length [13]. It was suggested that DANCR is an oncogenic lncRNA and was found to be overexpressed in various cancers such as gastric cancer [14], lung cancer [15], glioma [16], colorectal cancer [17], hepatocellular carcinoma [18], and cervical cancer [19]. DANCR was shown to play a role in cancer proliferation, metastasis and resistance to therapy through acting on various signaling pathways [20]. However, other studies [21, 22] showed that DANCR was downregulated in cancer cells and can act as a tumor suppressor via inhibiting tumor progression and invasion. Despite few studies regarding DANCR in AML, DANCR role remains controversial.

The aim of the current study was to evaluate the expression of lncRNA DANCR in de-novo AML patients and assess DANCR expression as regards to cytogenetics and AML subtypes, as well as correlate DANCR expression with patients' response to treatment.

Methods

Study group and material

The study was conducted in the period between January 2021 to March 2022 and included 60 newly diagnosed Egyptian adult AML patients above 18 years taken from the Hematology Unit of Alexandria Main University Hospital. Patients with the following criteria were excluded; patients who started induction therapy, relapsed AML, acute promyelocytic leukemia (M3), or those with associated other malignancies. Whole blood samples were withdrawn soon after patients' diagnosis prior to start of therapy. Following FAB criteria [3], 21 (35%) AML patients were M5, 20 patients (33.3%) were M1, 13 patients (21.7%) were M4, 4 patients (6.7%) with M2, and 2 (3.3%) patients were M0. Whole blood samples were also collected from 30 demographically matched healthy volunteers who were included as a control group. Upon sample withdrawal, an informed consent was taken from all subjects included in the study. The study was conducted according to the institution protocols that follow Declaration of Helsinki and an approval by the Ethics Review Board of the Alexandria University, Faculty of Medicine was obtained under the number of 0201437.

AML patients were diagnosed based on hypercellular bone marrow with more than 20% blasts, morphologic findings, cytogenetics and immunophenotyping. Genetic testing was done to detect NPM1 or FLT3-ITD mutations for all patients using real time quantitative polymerase chain reaction (RT-qPCR).

Patients risk stratification was done according to 2017 European Leukemia Net (ELN) recommendations [23]. Follow-up of patients was done after standard induction therapy with 7 plus 3 regimen (7 days of cytarabine plus doxorubicin on days 1 to 3) to track response to treatment. Patients who had less than 5% of bone marrow (BM) blasts as well as showed recovery of peripheral blood counts without circulating blasts were considered complete remission. Partial remission was defined as less than 25% of blast cells in the BM and BM is functioning normally. Patients who were unresponsive to treatment were considered refractory.

RNA isolation and cDNA synthesis

Isolation of total RNA from fresh blood samples collected on ethylenediamine tetraacetic acid (EDTA) tubes was performed by Qiagen® miRNeasy Mini Kit. (Cat. No. 217004) according to kit protocol. The purity and quantity of RNA was done by Nanodrop Spectrophotometer. After RNA extraction, RNA was reverse transcribed to

complementary DNA (cDNA) using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) in accordance with the kit's instructions. The reaction was programmed as follows; 10 min at 25 °C, 120 min at 37 °C, 5 min at 85 °C. Resultant cDNA was stored for further usage in RT-qPCR at − 20 °C.

Real-time quantitative PCR

RT-qPCR was performed on Rotor Gene Q PCR system (QIAGEN, Germany). Maxima SYBR Green qPCR Master Mix (2X) kit was used (Thermo Scientific, Cat. No. K0251), and for an endogenous control, Glyceraldehyde 3-phosphate dehydrogenase (GADPH) was employed.

Specific primers used for DANCR and GADPH were;

DANCR forward primer: 5'-AGCTGCCTCAGT TCTTAGCG-3'
DANCR reverse primer: 5'-CATGGTGATGTGCAA AGCGG'-3
GADPH forward primer: 5'-GAAGGTGAAGGT CGGAGTCAAC-3'
GADPH reverse primer: 5'-CAGAGTTAAAAG CAGCCCTGGT -3'.

The following were pipetted into each reaction tube; 10 µl Maxima SYBR Green qPCR Master Mix (2X), 1 µl forward Primer (50 pmol), 1 µl reverse primer (50 pmol), 6.5 µl nuclease free water, 1.5 µl cDNA (~ 75 ng/reaction) and no ROX. Each sample was carried out in duplicates. In each run, a no template control was done. RT-qPCR was programmed for an initial activation cycle of 50 °C for 2 min then 95 °C for 10 min; followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for DANCR and GAPDH for 30 s and extension at 72 °C for 30 s.

For PCR products, a melting curve was done to ensure specificity and identity. The relative quantification approach ($RQ = 2^{-\Delta\Delta CT}$) was used to determine the fold change between a sample and a normal control for DANCR [24]. Results were analyzed using Rotor-Gene Q Software 2.3.4.3.

Statistical analysis of the data

Data was analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Numbers and percentages were used to illustrate categorical data. To compare between AML patients and healthy controls, the chi-square (χ^2) was used. For continuous data, they were tested for normality by the Kolmogorov- Smirnov test. Range (minimum and maximum), mean, standard deviation and median were used to express quantitative data. For comparing normally distributed quantitative data between two groups, Student t-test was used. On the other hand, for comparing not normally distributed quantitative data between two groups, Mann Whitney test was used. For correlation between two markers that are not normally distributed, Spearman coefficient was used. To analyze whether DANCR expression was associated with AML FAB subtypes or cytogenetics or prognosis, AML patients were divided into two groups based on the median value into low and high DANCR expression and the relationship was analyzed by χ^2 test. Receiver operating characteristic (ROC) curve was used to determine the diagnostic performance of DANCR, with an area under the curve (AUC) more than 50% gives acceptable performance and AUC about 100% is the best performance for the test. Significance of the acquired results was assessed at the 5% level, and a p value ≤ 0.05 was considered statistically significant.

Results

Demographic characteristics of the studied groups were shown in Table 1. In the current study, there were no statistically significant differences regarding gender and age distribution ($p = 0.058$, $p = 0.309$ respectively). Complete blood count of the two studied groups is shown in Table 1. BM blasts at diagnosis ranged from 28 to 97% with a median of 78%.

FLT3-ITD mutation was absent in 59 (98.3%) patients, while NPM1 mutation was absent in 56 (93.3%) patients. 28 patients were CN-AML, 13 patients were cytogenetically abnormal of variable chromosomal abnormalities and in 19 patients no metaphase chromosomes were acquired, and thus excluded from risk stratification. Response to treatment was variable, 12 patients showed complete remission, 3 patients showed partial remission. 19 patients were refractory to treatment, while 19 patients died. 7 patients were lost during follow-up (Table 2). By assessing DANCR expression, we found that DANCR expression in AML patients ranged from 0.07 to 6.09 and was significantly lower in AML patients with a median of 0.64 than in the control group which ranged from 0.2 to 5.68 with a median of 1.02 ($p = 0.038$) (Table 1).

Correlation between lncRNA DANCR expression and patients' age, gender, FAB subtypes, cytogenetics and response to treatment

AML patients were divided into two groups based on the median value (0.64) into low DANCR expression group [$n = 30$] and high DANCR expression group [$n = 30$] (Table 3). DANCR expression was significantly associated with FAB subtypes ($p \leq 0.05$). However, there was no significant relationship between DANCR expression with other features as age, gender, cytogenetics or response to treatment.

Table 1 Comparison between the two studied groups according to age, gender, CBC, BM blasts and DANCR expression

	AML patients ($n=60$)	Control ($n=30$)	Test of Sig	p
Gender				
Male	24 (40%)	6 (20%)	$\chi^2=3.600$	0.058
Female	36 (60%)	24 (80%)		
Age (years)			t = 1.022	0.309
Mean ± SD	38.5 ± 10.5	36.07 ± 10.17		
Hb (g/dl)			t = 17.480*	< 0.001*
Mean ± SD	7.88 ± 1.06	12.15 ± 1.15		
WBCs (10^9/L)				
Median (Min. – Max.)	32 (1.62 – 116.7)	4.80 (3.9 – 6.5)	U = 57.500*	< 0.001*
Platelets (10^9/L)				
Mean ± SD	49.67 ± 16.78	254.5 ± 43.12	t = 25.088*	< 0.001*
BM blasts at diagnosis (%)				
Mean ± SD	72.82 ± 17.07			
Median (Min. – Max.)	78.0 (28.0 – 97.0)			
$2^{-\Delta\Delta CT}$ DANCR				
Median (Min.-Max.)	0.64 (0.07–6.09)	1.02 (0.20–5.68)	**U** = 657.50*	0.038*

SD Standard deviation, *t* Student t-test, χ^2 Chi square test, *U* Mann Whitney test

p: *p* value for comparing between the two studied groups

*: Statistically significant at $p \leq 0.05$

Table 2 Characteristics of the studied cases

Patient characteristics	Frequency (%)
FLT3-ITD	
Absent	59 (98.3%)
Present	1 (1.7%)
NPM1	
Wild	56 (93.3%)
Mutated	4 (6.7%)
Cytogenetics [a]	
Normal	28
Abnormal cytogenetics	13
Genetic risk stratification (ELN 2017) [a]	
Favorable	1
Intermediate	33
Poor	7

[a] 19 patients were excluded from the classification

Since the majority of AML M1 subtype patients showed high DANCR expression, further analysis of M1 subtype with high DANCR [$n=16$], was done to compare their response to treatment with the remaining AML patients with low DANCR [$n=30$]. AML patients of M1 subtype with high DANCR (above 0.64) were significantly less refractory to treatment and thus less resistant to cytarabine, whereas AML patients of different subtypes with low DANCR expression (below 0.64) were more refractory to treatment and therefore resistant to cytarabine (Table 4).

Comparison between FAB subtypes M0, M1 and M2, with M4 and M5 AML patients and healthy controls in relation to DANCR expression

To investigate the relation between FAB subtypes and DANCR expression, we further grouped AML patients into immature M0, M1 and M2 subtypes into one group and a second group with the more differentiated M4 and M5 subtypes. Relative expression of DANCR was significantly lower in M4 and M5 subtypes [$n=34$] compared to M0, M1 and M2 subtypes [$n=26$] ($p<0.001$), as well as compared to the control group [$n=30$] ($p<0.001$). M4 and M5 group had a median value of 0.53 and expression was ranging from 0.07 to 3.17, while M0, M1 and M2 group had a median value of 1.19 and levels were ranging from 0.11 to 6.09. However, there was no significant difference between AML patients of M0, M1 and M2 subtypes and controls ($p=0.808$) (Fig. 1).

Comparison between cytogenetically normal AML patients and healthy control group in relation to DANCR expression

DANCR expression in CN-AML patients included in our study [$n=28$] was compared to controls [$n=30$], and DANCR expression was significantly lower in CN-AML patients with a median of 0.58 and expression ranging from 0.11 to 1.98 ($p=0.011$).

Table 3 The relationship between DANCR expression and different parameters in AML

Variable	lncRNA DANCR				χ^2	p
	Low (≤ 0.64) (*n* = 30)		High (> 0.64) (*n* = 30)			
	No	%	No	%		
Age (/years)						
18 – 30	9	30.0	9	30.0	0.889	0.641
> 30 – 45	14	46.7	11	36.7		
> 45 – 65	7	23.3	10	33.3		
Sex						
Male	12	40.0	12	40.0	0.000	1.000
Female	18	60.0	18	60.0		
FAB subtypes						
M0	0	0.0	2	6.7	15.510*	^{MC}p = 0.001*
M1	4	13.3	16	53.3		
M2	2	6.7	2	6.7		
M4	8	26.7	5	16.7		
M5	16	53.3	5	16.7		
Cytogenetics						
Normal	16	53.3	12	40.0	1.071	0.301
Abnormal cytogenetics	6	20.0	7	23.3	0.098	0.754
Undefined [a]	8	26.7	11	36.7	0.693	0.405
Response to treatment						
Partial remission	2	6.7	1	3.3	0.351	^{FE}p = 1.000
Complete remission	5	16.7	7	23.3	0.417	0.519
Refractory	11	36.7	8	26.7	0.693	0.405
Died	9	30.0	10	33.3	0.077	0.781
Lost in Follow-up	3	10.0	4	13.3	0.162	^{FE}p = 1.000

χ^2 Chi square test, *MC* Monte Carlo, *FE* Fisher Exact

p: *p* value for comparing between Low (≤ 0.64) and High (> 0.64)

[a] AML patients with no metaphase on karyotyping

*: Statistically significant at $p \leq 0.05$

Table 4 Comparison between AML with M1 subtype with high (> 0.64) DANCR (*n* = 16), and remaining AML patients with low (< 0.64) DANCR (*n* = 30) in relation to their response to treatment

	$2^{-\Delta\Delta ct}$ DANCR				Test of Sig	*p*
	Low (≤ 0.64) (*n* = 30)		AML M1 High (> 0.64) (*n* = 16)			
	No	%	No	%		
Response to treatment						
Partial remission	2	6.7	1	6.3	$\chi^2 = 0.003$	^{FE}p = 1.000
Complete remission	5	16.7	4	25.0	$\chi^2 = 0.460$	^{FE}p = 0.698
Refractory	11	36.7	1	6.3	$\chi^2 = 5.007$*	^{FE}p = 0.035*
Died	9	30.0	7	43.8	$\chi^2 = 0.870$	0.351
Lost in Follow-up	3	10.0	3	18.8	$\chi^2 = 0.704$	^{FE}p = 0.405

χ^2 Chi square test, *FE* Fisher Exact

p: *p* value for comparing between Low (≤ 0.64) and FAB M1 with High (> 0.64)

*: Statistically significant at $p \leq 0.05$

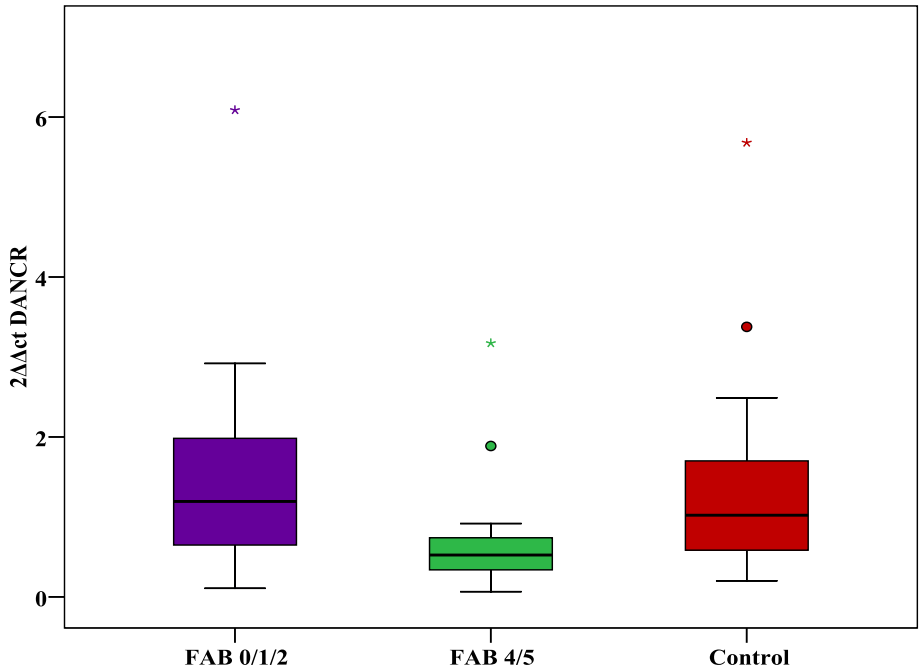

Fig. 1 DANCR expression in different FAB subtypes and healthy controls

Comparison between AML patients with abnormal cytogenetics and healthy control group in relation to DANCR expression

DANCR expression in AML patients with abnormal cytogenetics [$n=13$] was compared to controls [$n=30$], and there was no significant difference between the two groups, where AML patients with abnormal cytogenetics had a median of 0.75 and expression ranging from 0.12 to 6.09 ($p=0.397$).

Comparison between intermediate risk AML patients and high-risk patients in relation to DANCR expression

DANCR expression in intermediate risk patients [$n=33$] was compared to high-risk patients [$n=7$], and there was no significant difference in DANCR expression. ($p=0.337$).

Correlation between DANCR expression in AML patients with BM blasts at diagnosis and WBCs count

There was no significant correlation between DANCR expression and percentage of BM blasts at time of diagnosis ($r_s=-0.084$, $p=0.523$), as well as WBCs count of AML patients ($r_s=-0.091$, $p=0.489$).

Diagnostic value of DANCR in AML patients

ROC curve and AUC were used to investigate DANCR as a potential marker of AML on data from all subjects. The ROC curve showed separation between AML patients [$n=60$] and healthy individuals [$n=30$] with an AUC of 0.635 at the cut off value of ≤ 0.917, sensitivity was 71.67% and specificity was 63.33% (95% CI: 0.512 – 0.757, $p=0.038$) (Fig. 2a). ROC curve also showed strong separation between AML patients of M4 and M5 subtypes [$n=34$] and healthy controls [$n=30$] with AUC of 0.758 at the cut off value of ≤ 0.917; the sensitivity was 94.12% and specificity was 63.33% (95% CI: 0.634 – 0.883, $p<0.001$) (Fig. 2b). In addition, ROC curve was used to discriminate AML patients of M 0, M1 and M2 subtypes [$n=26$] from AML patients of M4 and M5 subtypes [$n=34$] and showed separation between both groups at the cut off value of ≤ 0.886 with an AUC of 0.764; sensitivity was 91.18% and specificity was 61.54% (95% CI: 0.632 – 0.897, $p<0.001$) (Fig. 2c). ROC curve was also applied to discriminate between CN-AML patients [$n=28$] and healthy controls [$n=30$] and showed a separation with AUC of 0.707 at the cut off value of ≤ 0.917; sensitivity was 85.71% and specificity was 63.33% (95% CI: 0.570 – 0.843, $p=0.007$) (Fig. 2d).

Discussion

In the current study, we assessed the expression of lncRNA DANCR in AML patients as well as healthy volunteers, where DANCR expression varied among patients with a significantly lower median than that of the control group. In previous studies regarding DANCR in AML, Bill et al. found that DANCR expression was upregulated in adult CN-AML patients when compared to controls and demonstrated that DANCR may have a

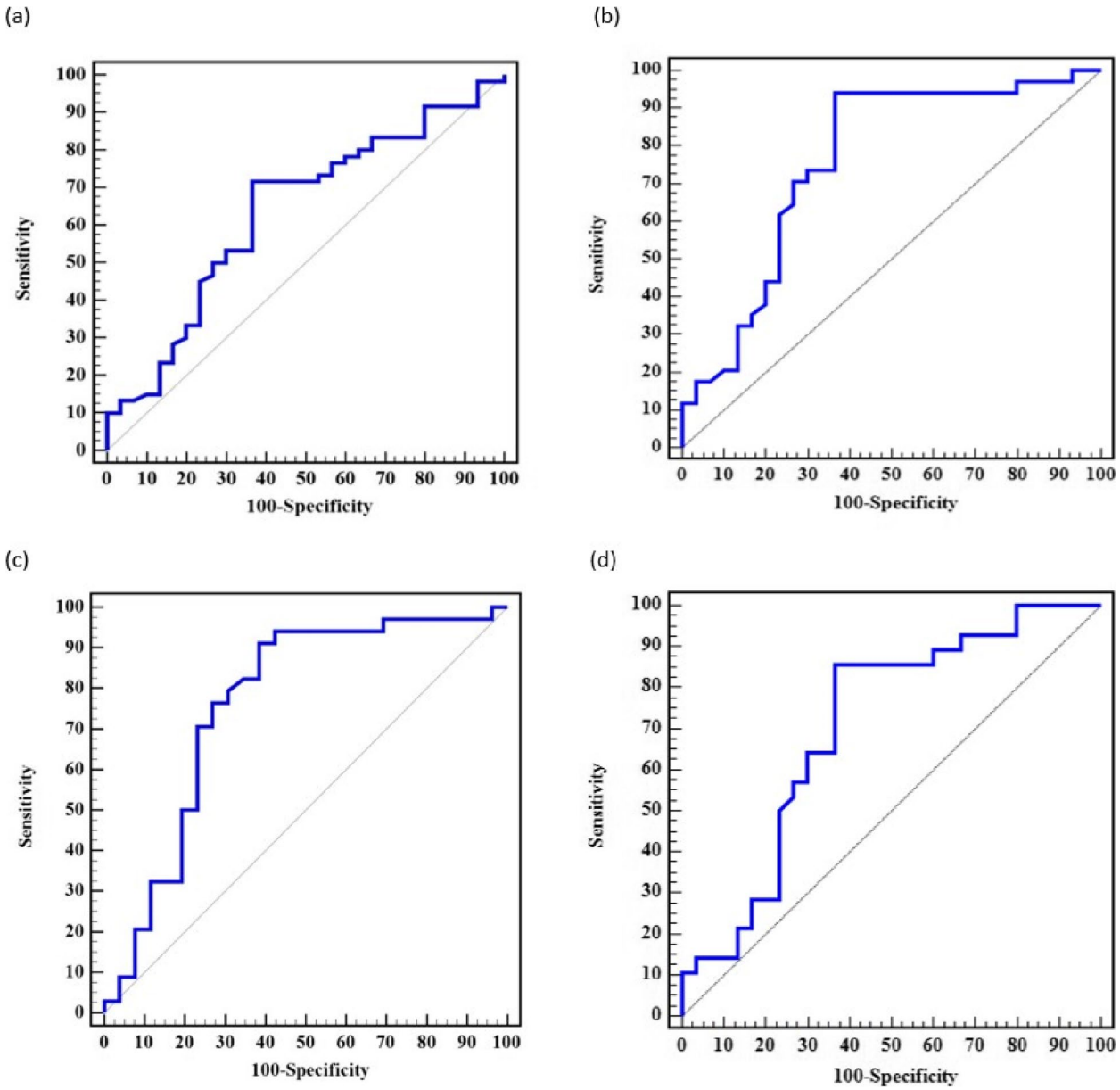

Fig. 2 ROC curve for diagnostic value of DANCR for; **a** differentiating AML patients [*n* = 60] from healthy controls [*n* = 30], **b** for differentiating M4 and M5 subtypes [*n* = 34] from heathy controls [*n* = 30], **c** for differentiating M4 and M5 subtypes [*n* = 34] from M0, M1 and M2 subtypes [*n* = 26] and **d** for differentiating CN-AML patients from healthy controls

role in regulation of leukemic stem cells through the regulation of WNT pathway by using animal models with AML expressing both MII partial tandem duplication/FLT3-ITD [25]. However, in this study DANCR expression was significantly downregulated in CN-AML patients. We might speculate the difference may be due to the absence of FLT3-ITD mutation in the majority of the patients (98.3%) included in the current study. Another reason may be due to differences in AML subtypes included in each study as in this study DANCR showed significant difference in expression among different AML subtypes.

Patients were further classified according to FAB criteria [3] into two groups and DANCR expression in M0, M1 and M2 subtypes was compared to M4 and M5 subtypes (no M6 patients were tested in the present study) and to the control group. DANCR was found to be significantly downregulated in M4 and M5 subtypes compared to M0, M1 and M2 subtypes and compared to the control group. However, there was a non-significant increment

in DANCR expression in M0, M1 and M2 subtypes compared to the controls.

By further analyzing 16 patients in M1 subtype with higher level of DANCR and comparing them to other AML subtypes with lower DANCR expression regarding response to treatment, M1 subtype with higher DANCR expression showed less chemoresistance to cytarabine. However, Zhang et al., showed that treating cultured AML cell lines and primary AML cell lines isolated from newly diagnosed pediatric patients with cytarabine was associated with dose dependent elevation in DANCR expression, where DANCR overexpression was associated with cytarabine resistance, while its knockdown diminishes cytarabine resistance [26]. Thus, DANCR relation to cytarabine resistance may be different in adult AML.

A study by Zhang et al., on papillary thyroid cancer, showed that DANCR was downregulated in cancerous tissue compared with adjacent cancer-free healthy tissue [22]. It was suggested that DANCR may act as a tumor suppressor rather than an oncogenic lncRNA. An explanation might be provided by a study in breast cancer by Li et al., which demonstrated that DANCR acts as a tumor suppressor by controlling the epithelial mesenchymal transition (EMT) pathway and cancer metastasis through attaching to enhancer of zeste homolog 2 (EZH2) and promoting its subsequent phosphorylation and degradation [21]. A study in osteoblasts by Zhu et al., found that DANCR interaction with EZH2, decreased the expression of runt-related transcription factor 2 gene (Runx2) [27], where Runx2 was found to be upregulated in AML [28].

Although many studies showed that DANCR acts as an oncogene that is overly expressed in cancers and promotes tumor invasion [29], DANCR was downregulated in papillary thyroid cancer [22] and multiple myeloma [30] and renal cell carcinoma [31]. Moreover, DANCR induction in-vitro was proved to inhibit tumor invasion and metastasis in cancers as multiple myeloma [30] breast cancer [32] and non-small cell lung cancer [33]. Thus, DANCR may hold a therapeutic potential where increasing DANCR expression in human cancers may prevent tumor progression and invasion.

Furthermore, in our study, the diagnostic value of DANCR was tested and multivariable logistic analysis indicated that DANCR could discriminate AML patients from the control group with a sensitivity of 71.67%. This sensitivity increased to 94.12% when DANCR was used to discriminate between M4 and M5 subtypes of AML from the control group.

To the best of our knowledge, this is the first study to evaluate DANCR expression in different AML FAB subtypes, a classification that is surpassed by WHO classification. However, varying expression of lncRNA DANCR among different FAB subtypes might highlight the role played by the predominate leukemic cells in each subtype in AML pathogenesis.

In conclusion, DANCR expression was downregulated in patients which might suggest that DANCR has a tumor suppressor function in AML. DANCR was also significantly downregulated in M4 and M5 subtypes compared to M0, M1 and M2 subtypes; and AML M1 patients who showed higher DANCR expression were less resistant to cytarabine. However, there was no relation found between DANCR expression and age, sex, WBCs, and BM blasts count. Further research is recommended on AML patients with FLT3-ITD mutations as well as NPM1 mutations to further elucidate the role of DANCR in the presence of these mutations. Research is also recommended to further verify DANCR relation to EZH2 and Runx2, which are highly implicated in AML but were not correlated with DANCR in AML patients.

Abbreviations

AML	Acute myeloid leukemia
AUC	Area under curve
BM	Bone marrow
cDNA	Complementary deoxyribonucleic acid
CI	Confidence interval
CN-AML	Cytogenetically normal acute myeloid leukemia
DANCR	Differentiation antagonizing non-protein coding RNA
EDTA	Ethylenediamine tetraacetic acid
ELN	European Leukemia Net
EMT	Epithelial Mesenchymal transition
EZH2	Enhancer of zeste homolog 2
FAB	French American British
FLT3-ITD	FMS-like tyrosine kinase 3 internal tandem duplication
GADPH	Glyceraldehyde 3-phosphate dehydrogenase
lncRNA	Long non-coding ribonucleic acid
ncRNA	Non-coding ribonucleic acid
NPM1	Nucleophosmin
ROC	Receiver operating characteristic
RT-qPCR	Real time quantitative polymerase chain reaction
Runx2	Runt related transcription factor 2
r_s	Spearman coefficient
WBC	White blood cell

Authors' contributions

All authors contributed equally to the study. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was conducted using human blood samples and approved by the Ethics Review Board of the Alexandria University, Faculty of Medicine in January 2021 under number 0201437.

Informed consent was obtained from each subject included in this study upon sample withdrawal.

Consent for publication
Available consent for publication.

Competing interests
The authors declare that they have no competing interests.

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