RESEARCH ARTICLE

Lack of Altered BECN1 Gene Expression in Iranian Patients with Acute Myeloid Leukemia

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Abstract

Acute myeloid leukemia (AML), one of the most prevalent leukemia types in adults, demonstrates great heterogeneity in molecular and clinical terms. Hence, there is a necessity to the mechanisms involved in AML generation in order to determine optimal treatment. This cross sectional study aimed to assess changes in BECN1 gene expression in with blood samples from 30 AML patients, compared with samples from 15 healthy persons. RNA was extracted and cDNA was synthesized and Real Time PCR applied to determine BECN1 gene expression. The results showed no significant differences in BECN1 gene expression between patients with AML and normal controls ($P > 0.05$). It appears that expression of BECN1 does not play a significant role in genesis of AML leukemia.

Keywords: Leukemia- BECN1- changes in gene expression

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Introduction

Autophagyisa processes associated with lysosomes, which can lead to induction of destruction and digestion of Organelles and cytoplasmic proteins. The process can cause induction of digestion of Organelles and cytoplasmic proteins physiologically in reaction to the environment and cellular stimulation. Moreover, for autophagy, destructive cellular function is presented as induction of apoptosis, along with Bcl2 loss and mTOR stimulation and there are many disputes on the two functions of autophagy process (Shi et al, 2013). Clearly, in case of declined performance of the cellular cycle, waste organelles such as mitochondria or by-products of cellular proteins remain in ectopic form and produce reactive oxygen species (ROS) or materials with oxidation property and cause dysfunction in vital performances and more importantly, cause mutations in DNA sequence and this can cause more mutations and as a result, more tumorigenesis (tumor creation). However, as it was mentioned, increased activity of the process can also help higher stability of cell, which is in line with cancerous cells property (Grander et al, 2010). Hence, there are disputes on this dual function for this process. In early researches and in rats, it is demonstrated that haploinsufficiency for these genes can lead to increased susceptibility to a variety of tumor. In later studies and on cancer samples, changes in expression of these genes compared to normal samples are confirmed.

For example, changes in expression of genes in this process like Beclin1, ATG7, p63 and Bif were investigated in some prevalent cancers like breast cancer, intestine cancer and prostate cancer and reduction or increase in genes is demonstrated. Moreover, the prognosis value of the reduction or increase of expression is emphasized in prediction for risky patients (Maiuri et al, 2009). However, it should be mentioned that reported changes in gene expression are presented in form of reduction or increase in expression, which is a matter to think about and it is still ambiguous that is this paradox depended on type of cell or depended on cancer stage and this needs more investigations and further studies (Wang et al, 2008).

Impairment in autophagy pathway can affect creation and formation of hematologic malignancies such as AML. For example, it has been found that prescription of some drugs with therapeutic potential can cause reduction of the division of cancer cells and increase the maturity of these cells and such effect is attributed to autophagy process induction (Wang et al, 2008).

In Acute myeloid leukemia (AML), it has been shown that AML cells are mainly sensitive to autophagy

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stimulations and this could create this idea to mind that autophagy can be associated with cytotoxic medication of AML. Despite to this important and probable point, today, there is no comprehensive research to demonstrate status of autophagy genes in AML. As an important mediation, Beclin1can affect initiation and progression of autophagy and a variety of researches have referred to removal of BECN1 recurrence and its diversity in wide range of tumors.

The main purpose of this research is to find frequency of variances in BECN1 gene expression in patients with de novo AML. On the other hand, this research investigated information of individuals with different expression variances.

Materials and Methods

In this research, peripheral blood samples were taken from 30 patients with de novo AML referred to 501 Army Hospital (Imam Reza) and 15 normal individuals participated in the study as control group after signing the consent letter and their blood samples were also taken. Peripheral blood sample to 4cc was mixed with 200µl of EDTA and was transferred in 15-ml falcon to laboratory of Department of Genetics of Faculty of Medical Sciences of Shahid Beheshti University. After confirmation of diagnosis of Oncology specialist, blood samples were transferred to the lab. Obtained results from real-time PCR reactions in this study have been analyzed using LinReg and REST-2009 software. Statistical results of <0.05 are considered as considerable and significant values.

Data for all patients were collected for analysis. Study and patients were assigned on the basis of national/ international breast cancer protocols and approved according to local law and regulations, by the Institutional Review Boards of each participating referral hospital. The study was performed Department of Genetics of Faculty of Medical Sciences of Shahid Beheshti University, in adherence to the guidelines of the Declaration of Helsinki, and was approved by the ethics committee of this University.

DNA extraction

Extraction of DNA was done on patient and normal samples and as a result, density and purity of samples was obtained using biophotometer. Ten 10µl of each sample was electrophoresed on 2% agarose gel in buffer 0.5 X (TBE). Density of RNA was about 40-50µg/ml and OD260/280 was in range 1.6-1.9, which showed favorable quality of RNA. To test quality of RNA, extracted RNA was electrophoresed on 2% agarose gel.

Determining quality of cDNA

To determine accuracy and quality of the cDNA made, PCR reaction of ABL1 housekeeping gene was performed on all samples and the length of product was obtained to 170pb. Performing the reaction can be a reason for accuracy of cDNA synthesis.

For this evaluation RNA quality was evaluated using Eppendorf spectrophotometry and beta-actin was used as housekeeping gene in PCR analysis. RNA of lymph node and breast tissue samples were showed to be of less value if either of the housekeeping gene gave signals that was at least 3.5 cycles over the optimum of lymph node and other specimen checked. Low quality RNA samples were not found. There were 30 patients with same number lymph nodes included in the final data set from this study. The RNA was undergone to reverse transcription reaction to make a cDNA before performing RT-PCR. The obtained RNA was stored in form of separate 10µl in -20 °C until next processing. RNA was used in 4µg concentration, 26 ng/µl oligo dT (Biolab, New England) was added and incubated at 63 °C for 5 minutes and the product transferred on ice. Then in final 25 µl reaction volume, included 1×protoscript buffer, 10mmol/L dithotheriol, 0.5 mmol/L dNTP, 10 U/µl M-MLV reverse transcriptase enzyme (Biolab, first strand cDNA synthesis kit) 0.25 U/µl RNase inhibitor (Biolab). This mix incubated in 37°C for 1 hour and after that, incubated in 92°C for 6 minutes. The obtained product (cDNA) was diluted 1:5 with distilled water and stored at -20°C. Real-Time assay was conducted for mammoglobin marker and beta-2-macroglobinas housekeeping gene.

ABL 1 melting curve (internal control)

The reaction was performed on normal and patient samples in duplicate form. Obtained results are resulted in form of melting curve.

Beclin1 gene expression

The reaction was performed on normal and patient samples in duplicate form. Obtained results from the reaction are discussed.

To determine specificity of product of Real Time RT-PCR reaction

Products of real time RT-PCR reaction were electrophoresed on 2% agarose gel to confirm specificity of the product through performing the reaction.

Results

Results of analysis of real-time RT-PCR raw data

Raw data of RT-PCR reaction were analyzed using LinReg and REST software after determining efficiency of PCR and determining base level and the results are

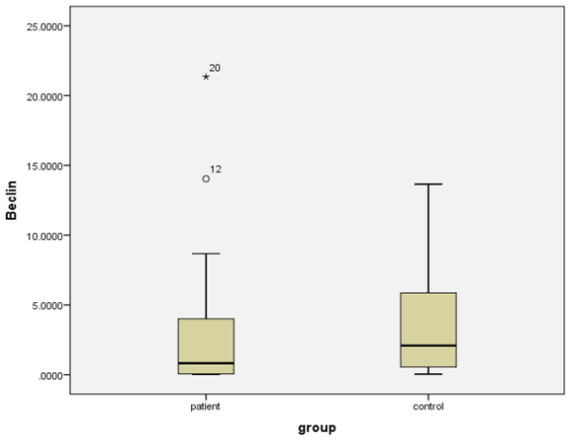

Figure 1. Results of Analysis of Real-Time RT-PCR Data in Patient and Control Groups

Table 1. Group Statistics

	group	N	mean	std. Deviation	std. error mean
Beclin	patient	30	3.2	4.9	0.9
	control	15	3.5	4.1	1.04

Table 2. Primer Sequence

Primers
FLT3-ITD Forward TTGTCTCCTCTTCATTGTCGTTTT
Reverse CTGTTGCGTTCATCACTTTTCC
NPM1 Forward TTTATTGATGTCTATGAAGTGTTGT
Reverse CTGACCACCGCTACTACTATGT
BECN1 Forward AACCAGATGCGTTATGCCCA
Reverse CTGTCCACTGTGCCAGATGT
ABL1 Forward GCTGTTATCTGGAAGAAGCCCT
Reverse GGGTCCAGCGAGAAGGTTTT

presented as follows Figure 1.

The mean value and SD of BECN1 in both groups is presented as follows:

Obtained results from this research are presented here as headlines:

• RNA concentration was about 40-50µg/ml and OD260/280 was in range 1.6-1.9, which shows favorable quality of RNA.

• Extracted RNA on 1% agarose gel (existence of 28 s, 18s, 5s bands shows quality of extracted RNA)

• PCR reaction of ABL1 housekeeping gene was performed on all samples and the length of product was obtained to 170pb. Performing the reaction could be a reason for accuracy of cDNA synthesis.

• Electrophoresis of products for BECN1 gene showed that the reaction and its specificity are confirmed.

• Obtained results from this study showed that BECN1 gene expression in patients showed no change and variance compared to normal samples.

Discussion

Currently, cancer is being considered as a genetic disease, in which changes in genome can result in impairment in expression of oncogenes or tumor suppressor genes and as a result, can cause cancer. Available data show that change in sequence of nucleotides can't be the one and only factor for change in gene expression or impairment in mechanism of gene regulation (Liang et al, 1998). One mechanism that plays vital role in creation and progression of cancer is epigenetic changes. Epigenetic changes can be considered as an additional level of gene expression, which can conduct many natural mechanisms such as tissue differentiation during fetal life and unnatural mechanisms that can cause cancer or other genetic diseases (Baspinar, 2013).

Investigation of changes in expression of genes involved in progression of cancer cells can play key role in advancement of diagnosis methods, medication and prognostic of different human cancers. BECN1 family genes such as BECN1 play key role in Apoptosis process. Cancer cells need escaping from inhibitory processes such as apoptosis to have uncontrolled growth (Khodapasand, 2013). As a result, it is expected that relevant gene expressions of this process such as BECN1can be changed abundantly in cancer cells. BECN1 gene is an autophagy gene that increases formation of autophagy vesicles and plays key role in protecting cells against chromosome instability. Increased level of this gene can predict optimal survival of different types of cancer (Yang et al, 2015). BECN1plays vital role in autophagy regulation, apoptosis and separation and also growth of cancer (Baspinar et al, 2013). Hence, investigating these changes and the relationship between them and different factors of disease can play key role in researches in field of cancer.

Blood cancer can be the most prevalent group of cancer and it seems that it is created as a result of impairment in cell differentiation. Hence, there is a strong correlation between this type of cancer and changes in gene expressions as a result of epigenetic changes (Park et al, 2011). Acute myeloid leukemia has encompassed 15% of leukemia cases of childhood, which can be diagnosed by clinical and biological properties and has lower cure rate than acute lymphocytic leukemia.

In addition to conventional chromosomal translocation, mutation of oncogenes and loss of tumor suppressor genes play key role in AML pathogenesis. For example, abnormality in special genes like FLT3 (Birg et al, 1992), Nucleophismin (NPM) (Falini et al, 2005), RAS (Ahuja et al, 1990), BECN1 (Wang et al, 2008) and mutation destruction of tumor suppressive genes like P53 (Mori et al, 2005) are common in AML cells. BECN1 is a tumor suppressor gene, which is involved in gene transcription and translation (Lankat Buttgereit et al, 2009). However, obtained results from this study show lack of selected

Table 3. Clinical and Genetic Characteristics of the Patients According to BECN1 Status

Clinical and biological Characteristics of the patients:		Clinical and biological Characteristics of the controls	
Age	46 (18 – 73)	Age	41 (18 – 65)
Sex(male/female)	16/14	Sex (male/female)	8/7
Hemoglobin (gr/l)	6 (49 – 111)	Hemoglobin (gr/l)	148 (124 – 163)
WBC (109/L)	5.3 (0.9 – 342)	WBC (109/L)	7.3 (5.2 – 8.9)
Platelet count (109/L)	48.5 (3 – 434)	Platelet count (109/L)	286 (193 – 412)
Bone marrow blast (%)	70 (0 - 98)		

changes in gene expression in De Novo AML and they are important from this perspective. It has been demonstrated that BECN1 plays key role in evolution and metastasis of tumor in both rat and human samples (Wei et al, 2012).

In this research, the main purpose has been to analyze expression changes of this gene in AML and obtained results showed lack of changes in BECN1 gene expression assumed in De novo AML. Also, obtained results from the research showed that BECN1 expression was not significantly different between two patients and normal groups (Wei et al, 2012). BECN1 gene expression has decreasing process in many other human tumors. Ding et al have investigated Beclin1 gene expression in mRNA and protein levels in 63 specimens of gastrointestinal stromal tumor. They found that BECN1 expression was reduced in 68% of specimens compared to adjacent normal gastrointestinal tissues (Ding et al, 2010; Ozpolat et al, 2007).

It seems that reductive regulation o BECN1 in different types of human cancers is along with accelerated growth of tumor, metastasis and weak prognostic (Wang, 2008). At the present study, no correlation was observed between level of BECN1 expression and other clinical parameters like patients' sex, platelet count and hemoglobin concentration.

For further investigations, the authors tend to implement this study in larger sample size and in frame of a big project. In larger sample size, although Beclin1gene expression shows no significant difference between patients and control group, reduction of Beclin1 gene expression was along with BECN1 mutation, more number of white blood cells, monosomies karyotype and older age (Mourgues et al, 2015). In 60-80% of patients with AML in age range below 60 years old, complete remission after induction therapy was observed. Complete remission is defined as the presence of less than 5% blasts in the bone marrow and modification of peripheral blood cell count: Neutrophil count more than 1,000 cells per microliter, the platelet count more than 100,000 cells per microliter, hemoglobin more than 10gr/dl and no presence of blasts in peripheral blood. Moreover, more than 20% bone marrow cellularity should be existed with evidence of hematopoiesis all three cell lines (Smith et al, 2004). No significant correlation was also observed between expression level of Beclin1 of patients with complete remission and those didn't enter to remission phase. Also, Beclin1 gene expression level in patients with complete remission was relatively similar to those who didn't enter to remission phase.

However, BECN1 regulatory mechanisms in AML and its role in natural hematopoiesis and also probable correlation of it with outcome of patients need further studies.

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Conflict of interest

The authors have no financial relationships to disclose and no conflicts of interest to report.

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