Expression analysis of PTEN gene in acute myeloid leukemia

IV. ULUSLARARASI KATILIMLI DENEYSEL HEMATOLOJİ KONGRE BİLDİRİSİ

Rozhgar A. KHAILANY[1,2], Amer A. HASAN[3], **Naser GILANI[1,4]**, Khandakar A. S. M. SAADAT[1], Belan KANABE[5], Ahmet ARSLAN[6]

[1]Department of Medical Biology and Genetics, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey; [2]Department of Biology, College of Science, Salahaddin University, Erbil, Iraq; [3]Department of Medical Laboratory, College of Technical Health, Sulaymani Polytechnique University, Sulaymani, Iraq; [4]Farabi Medical Laboratory, Erbil, Iraq; [5]Department of Biology, Gaziantep University, Gaziantep, Turkey; [6]Department of Medical Biology, Faculty of Medicine, Namık Kemal University, Tekirdag, Turkey

Abstract

Background: Acute myeloid leukemia (AML) is a molecularly and clinically heterogeneous disease and biologically complex, its incidence is increasing as the population ages. Large chromosomal translocations as well as defects in the genes involved in hematopoietic proliferation and differentiation result in the accumulation of poorly differentiated myeloid cells. Phosphatase and tensin homolog (*PTEN*) gene, act as a tumor suppressor, located on 10q23.31, that is involved in a large number of cancers at high frequency. The goal of this study was to measure the mRNA expression level of *PTEN* in AML patients.

Methods: The study included 18 cytogenetically normal AML cases and 15 controls, samples were grouped according to the clinical characteristics of patients which include gender and the mean age. The mRNA expression level was measured by quantitative real time polymerase chain reaction (qRT-PCR).

Results: The *PTEN* gene expression level was significantly reduced (down-regulated) in AML in compared with normal samples.

Conclusion: As a result, we conclude that the aberrant expression in *PTEN* gene might be a risk factor for AML. However for confirming of this relation further studies should be done.

Key word : Acute myeloid leukemia, PTEN, Expression analysis, qRT-PCR.

INTRODUCTION

Acute myeloid leukemia (AML) is a cancer of the blood and bone marrow, in which the bone marrow makes abnormal myeloblasts, red blood cells, or platelets. It is the most common type of AML in adults. There are some known risk factors for AML includes: smoking, exposure to certain chemicals, being treated with certain chemotherapy drugs, high-dose radiation exposure, certain blood disorders, some genetic mutations , positive family history, older age and male gender[2].PTEN gene is an essential tumor suppressor that acts mostly as a phosphatase that de-phosphorylates the Phosphatidylinositol (3,4,5)-trisphosphates (PIP3), therefore counteracting the activation of PI3-kinase (Figure 1). Beside this major mechanism of action, PTEN tumor suppressive functions can be independent of its phosphatase activity. In particular, PTEN was shown to act in the nucleus, where it mediates cellular proliferation through the interaction with the APC/CDH1 complex and promoting the stabilization of the genome(Figure 2). Disruption of PTEN causes centromeric instability and spontaneous DNA double-strand breaks.[3] Similarly, nuclear PTEN was also shown to regulate DNA repair and sensitivity to genotoxic stress.[4] PTEN is regulated in an highly complex network.[1] The function of the protein is indeed affected by proper levels of expression, which in turn are mediated by genetic and non-genetic mechanisms.[1,5] In particular, PTEN level of expression are regulated by several miRNAs, with important implications in cancer pathogenesis.[6] PTEN phosphatase activity is also controlled by post-trasductional modifications.[1] Especially, PTEN phosphorylation by Casein Kinase II promotes PTEN inactivation through the stabilization of PTEN in a closed conformation.[7] Notably, even proper PTEN cellular compartmentalization is essential for the regulation of its tumor suppressive functions.[8] Essentially, loss of the nuclear pool of a genetically wild-type PTEN was associated with development of cancer.[9] PTEN shuttling from the nucleus to the cytoplasm is regulated by mono-ubiquitination[9] and sumoylation.[4] Both PTEN phosphorylation, changes in protein levels and cellular compartmentalization are associated with tumorgenesis leading to the definition of PTEN as the paradigm for the non genomic loss of function of tumor suppressors.

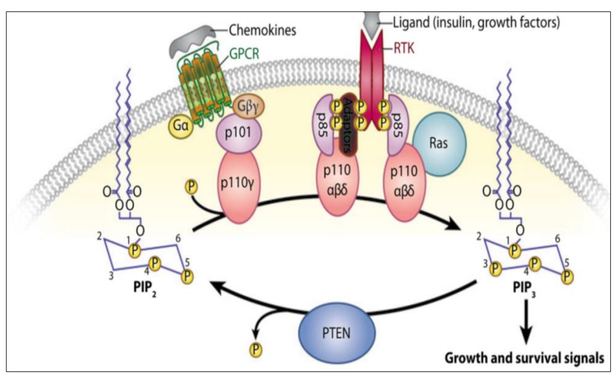

Figure 1.PTEN regulation of PI3K-AKT signaling

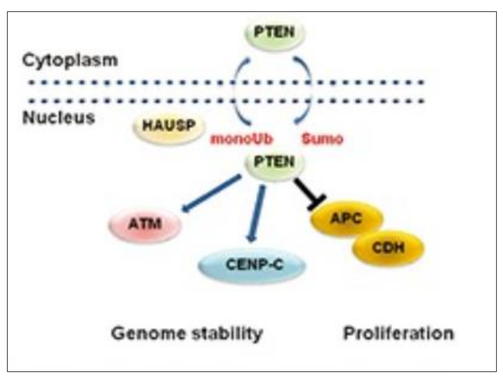

Figure 2. PTEN function in nucleus

MATERIALS AND METHODS

Patients

The study included 18 cytogenetically normal AML cases and 15 controls, samples were grouped according to the clinical characteristics of patients which include gender and the mean age.

RNA Extraction

Total RNA extraction was carried out upon confirmation by hematological and histopathological analysis. RNA samples were obtained by using extraction kits (Qiagen RNA Isolation Kit, Catalog No. 74104, Hilden, Germany) according to the instructions of the manufacturer. Quantification of RNA concentrations were performed by using spectrophotometer (NanoDrop, ND-1000, USA).

Complementary DNA synthesis and qRT-PCR

Complementary DNA was synthesized using the protoScript First Strand cDNA Synthesis Kit (Catalog no:E6300S NEB, England). The cDNA was amplified by qRT-PCR and used the expression primers. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene was used as a housekeeping gene for the normalization of *PTEN* gene expression data.

RESULTS

The expression level of *PTEN* gene was obtained from 18 cytogenetically normal AML cases and 15 controls,; mRNA expression of *PTEN* in AML samples were decreased (down-regulated) according to expression level of normal control samples, p=0,0001 and statistically it is significant counted on ($p < 0,05$). The mRNA expression levels for both normal controls and AML cases are shown in Figure 3.

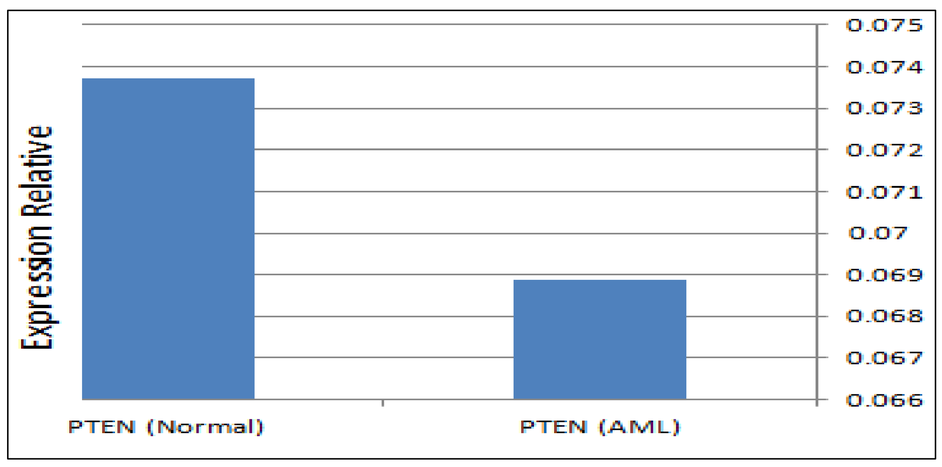

Figure 2. The mRNA expression levels of normal controls and AML cases

CONCLUSION

As a result, we conclude that the aberrant expression in *PTEN* gene might be a risk factor for AML. However for confirming of this relation further studies should be done.

REFERENCES

1. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3–kinases as regulators of growth and metabolism. Nat. Rev. Genet. 2006;7:606–619.
2. https://www.cancer.org/cancer/acute-myeloid-leukemia.html.
3. Nobukuni T, Joaquin M, Roccio M, Dann SG, Kim SY, et al. Amino acids mediate mTOR/raptor signaling through activation of class 3 phosphatidylinositol 3OH-kinase. Proc. Natl. Acad. Sci. USA. 2005;102:14238–14243.
4. Wurmser AE, Emr SD. Novel PtdIns(3)P-binding protein Etf1 functions as an effector of the Vps34 PtdIns 3-kinase in autophagy. J. Cell Biol. 2002;158:761–772.
5. Mora A, Komander D, van Aalten DM, Alessi DR. PDK1, the master regulator of AGC kinase signal transduction. Semin. Cell Dev. Biol. 2004;15:161–170.