STUDY OF EZH2 GENE TYROSINE 641 MUTAION IN EGYPTIAN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA

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Introduction

Diffuse large B-cell lymphomas (DLBCLs) are phenotypically and genetically heterogeneous. Gene-expression profiling has identified two subgroups of DLBCL; activated B-cell–like (ABC) and germinal-center Bcell–like (GCB) according to cell of origin. Diagnosis relies on a combination of morphologic findings (peripheral blood, bone marrow, or lymph node), immunohistochemistry, immunophenotyping, cytogenetics, fluorescence in situ hybridization (FISH) and molecular genetics. Enhancer of zeste homolog 2 (EZH2) is the catalytic subunit of the polycomb repressive complex 2 (PRC2) which implements transcriptional repression by trimethylation at lysine 27 of histone H3 (H3K27me3). It is important for normal immunoglobulin VDJ recombination in pre-B cells. EZH2 (Y641) mutations result in increased EZH2 stability and increased H3K27me3 activity, resulting in the repression of tumor suppressor genes. Its catalytic activity is closely correlated with tumor aggressiveness, drug resistance, and poor prognosis. Here, we studied 50 DLBCL biopsy samples using chain termination sequencing.

Aim of the work

The aim of the study was to study the mutational status of enhancer of zeste homolog 2 (EZH2) gene tyrosine 641and its relation with different clinicopathological parameters in DLBCL patients.

Patients and Methods

The study was conducted on 50 newly diagnosed patients with diffuse large B cell lymphoma diagnosed by routine histopathology using H&E staining and immunohistochemical basis on formalin-fixed paraffin-embedded (FFPE) tissue sections of lymph node and/or bone marrow biopsies employing the current World Health Organization Classification 2018 obtained from the Pathology and/or Clinical Pathology departments of Faculty of Medicine, Alexandria University.

Patients aged more than 18 years and had neither co-exiting malignancies nor previous immunosuppressive therapy, chemotherapy or irradiation. All patients included in this study were subjected to full history taking clinical examination and routine laboratory investigations.

DNA extraction was done from FFBE then DNA quantity and purity was determined. The DNA samples were subjected to conventional PCR then the obtained PCR products were tested by gel electrophoresis. The DNA products were purified, tested by gel electrophoresis and re-measured. The purified DNA were subjected to chain termination analysis.

Results

Of all the 50 studied patients, EZH2 mutation was detected in a total of 18 patients (36%) were mutated and 32 patients (64%) were non-mutated. 5 (10%) patients showed (p.Ser42Thr) missense mutation, 1 (2%) patient exhibited (p.Tyr44Phe) missense mutation while others had non-coding mutations.

Table 1: Distribution of the studied cases according to EZH2 sequencing analysis (n = 50)

	No.	%
Result		
Non mutated	32	64.0
Mutated	18	36.0

Table 2 : EZH2 sequencing analysis supplemental data

Chromosomal position	Mutations	no	Effect	Amino acid change	Mutation nature	Previously known in DLBCL/ de novo
Ch7: 72650	T -> A	3	Missense	(p.Ser42Thr)	Substitution	De novo
Ch7: 72657	A -> T	1	Missense	(p.Tyr44Phe)	Substitution	De novo
Ch7:72590	T -> A	2	Missense	(p.Ser22Thr)	Substitution	De novo

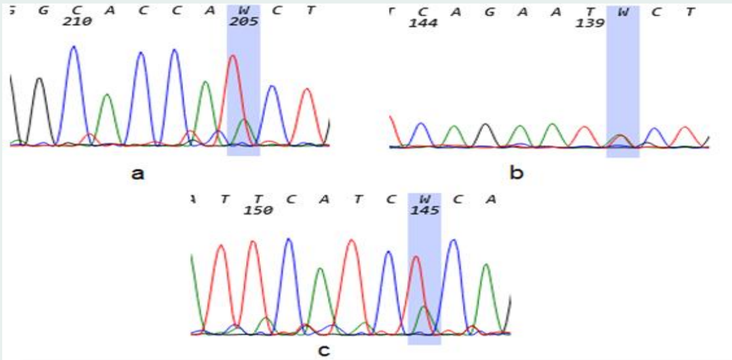

Fig 1 : **Electrograph of DNA sequencing analysis of EZH2 mutations (a) Ser->Thr (b) Tyr->Phe (c) Ser->Thr**

Conclusion

From this study we can conclude the following:

1. EZH2 in exon 16 is recurrently mutated in DLBCL.
2. Multiple novel mutations have been detected in exon 16 including missense and non-coding mutations.
3. EZH2 mutations seem to be a pathogenetic mechanism in DLBCL development.

In conclusion, our data support the implementation in the clinic of the analysis of recurrent somatic mutations of EZH2 to diagnose and guide salvage therapy based on molecular targets.