HOXA9 Gene Expression and Cytogenetic Analysis in Egyptian Acute Myeloid Leukemia Patients

Hashem Neanaa¹, Nahla A. M. Hamed¹, Ahmad Raafat², Iman Diab³, Ahmed Abdel Rahman¹
¹Department of Hematology, Faculty of Medicine, Faculty of Science, Alexandria University, Egypt, ²Department of Biochemistry, Faculty of Medicine, Faculty of Science, Alexandria University, Egypt, ³Department of Medical Biochemistry, Faculty of Medicine, Faculty of Science, Alexandria University, Egypt

ABSTRACT
Aim: we investigated the frequency of expression of HOXA9 gene in adult Egyptian acute myeloid leukemia (AML) patients and its relation to different cytogenetic abnormalities. Methods: 30 newly diagnosed AML patients (group 1) were the subject of our study. Ten healthy persons of matched age and sex were considered group II (controls). Estimation of HOXA9 expression in AML blasts by RT-PCR was done to both groups. Results: Normal cytogenetic analysis was present in 20 cases (66.6%), t(15,17) in 3 cases, t(8,21) in 3 cases, 45 xy -7 in 1 case, t(16,16) in 1 case, 45 xy-20 in 1 case and 46 XX 1P+ in 1 case. NUP 98 -HOXA 9 gene was not detected in any of the studied case or in the control group. Conclusion: absence of HOXA9 gene in our randomly selected patients may be related to its rarity in Egyptian population. However, further studies including larger population is still needed to confirm this finding with special stress on poor cytogenetic group.

Keywords: HOXA9, Cytogenetics, Acute leukemia

INTRODUCTION
Acute myeloid leukemia (AML) is a genetically heterogeneous disorder characterized by the somatic acquisition of genetic and epigenetic alterations in hematopoietic progenitor cells that disturb normal mechanisms of self-renewal, proliferation and differentiation. In recent years, there has been increasing translation of genetic diagnostics into the clinical setting. In the current World Health Organization classification, more than half of AML cases are categorized on the basis of their underlying genetic defect. Furthermore, cytogenetic and molecular genetic changes have been shown to be among the most powerful prognostic markers. Finally, novel therapies are being developed that target some of these genetic and epigenetic changes.

In humans, there are 39 Class I homeobox (HOX) genes found in four genomic clusters (HOXA, HOXB, HOXC, and HOXD). Their expression is critical for body patterning during development, and for post-developmental regulatory functions. Aberrant HOX gene expression initiates leukemias. HOXA9 particularly is important in leukemia by suppressing differentiation and maintaining self-renewal during myelopoiesis. HOXA9 expression is predominant in myeloid neoplasias. In addition, HOXA9 has been identified as the most reliable predictor of disease outcome.

Aim of the Work
We aimed at investigating the frequency of expression of HOXA9 gene, and its relation to different cytogenetic abnormalities in adult Egyptian AML patients.

METHODS
Patients
This study was performed on 30 newly diagnosed AML patients randomly selected from the Hematology Unit at Alexandria Main University hospital in Egypt. Their age ranged from 18-56 years. 17 patients were males and 13 were females. Based on cytogenetic analysis, patients were categorized into: favorable group (7 patients), intermediate group (22 patients) and unfavorable group (1 patient). The study also included 10 healthy persons of matched age and sex and they were considered group II (controls).
All patients in this study were subjected to: thorough history taking and detailed clinical examination, routine investigations including complete blood picture, bone marrow aspiration, immune-phenotyping, cytochemical staining (myeloperoxidase and Sudan black B), cytogenetic analysis by G-banding and estimation of HOXA9 expression in AML blasts by RT-PCR.

Estimation of HOXA9 expression in AML blasts by RT-PCR
Total RNA was extracted from whole blood EDTA samples using the E.Z.N.A RNA extraction Kit (Omega, bio-tek) (according to manufacturer’s instructions).

For RT-PCR: The master mix RT-PCR beads kit which combines cDNA synthesis from RNA with PCR amplification was used. The following primer set was used: Forward primer: TGTGGTTCTCCTCCAGTTGATAGA
Reverse primer: TCGGTGAGGTTGAGCAGTCGAG, which amplifies a fragment of 267 bp for human HOXA9.

The total reaction volume was 50 μL, containing 10 μL of RNA, 0.4 mM of each primer (forward and reverse primer) were added for each tube containing the PCR master mix beads. The thermal cycler conditions included a step for reverse transcription (42 °C for 15-30 minutes). Then an initial PCR activation step (15 min, 95 °C); 35 cycles consisting of denaturation (1 min, 95 °C), annealing (1 min, 55 °C), and extension (2 min, 72 °C); and a final extension step (10 min, 72 °C).

RESULTS

Cytogenetic analysis: Normal cytogenetic was present in 20/30 cases (66.6%). t(15,17) was present in 3 cases, t(8,21) in 3 cases, 45 xy -7 in 1 case, t(16,16) in 1 case, 45 xy-20 in 1 case and 46 XX 1P+ in 1 case.

NUP 98 -HOXA 9 gene in AML blasts by RT-PCR: was not detected in all AML patients or in the control group, (Figure 1).

Relation between response to chemotherapy and cytogenetic pattern: 22/30 patients were categorized as intermediate risk category. 20/22 patients (90.9%) of them had normal cytogenetic. Among those with normal cytogenetic, 11/20 patients (55%) had achieved complete remission, 4 patients attained partial remission while 3 patients were refractory to chemotherapy (Table 1). 2 patients died before receiving chemotherapy.

DISCUSSION

There is difference in the apparent oncogenicity of HOXA genes in myeloid and lymphoid compartments. It had been suggested that the transcriptional effects of HOX genes are strongly modulated by the spectrum of DNA-binding partners present in a particular lineage. Perhaps, lineage specific patterns of chromatin structure, could also affect the accessibility of HOX proteins to some genomic regions.

HOXA9 gene was expressed significantly more frequently in myeloid cell lines than in lymphoid cell lines. That is why we selected to study acute myeloid leukaemia rather than acute lymphoblastic leukaemia. NUP 98-HOXA 9 gene was not detected in any of our studied patients or control group. A previous study examined the expression of the HOXA cluster genes (HOXA9, HOXA10, and HOXA11) in leukemic cell lines by RT-PCR. The HOXA9, HOXA10, and HOXA11 genes were expressed in 12 of 16 (75.0%), 14 of 16 (87.5%) and 14 of 16 (87.5%) myeloid lineage cell lines.

The absence of NUP 98-HOXA 9 gene expression in all our patients may be attributed to the absence of translocation t(7;11)(p15;p15) in our studied cases. There are some morphological features associated with t(7;11)(p15;p15), including predominantly the AML-M2 subtype with trilineage myelodysplastic features.
AML-M2 was present in seven cases (23.33%) in our study. No dysplastic feature was detected in any case. In the translocation t(7;11), the NUP98 gene (11p15.4), is fused to the class-1 homeobox gene HOXA9 (7p15). The NUP98 gene (11p15.4) codes for a component of the nuclear pore complex that regulates nucleo-cytoplasmic transport of RNA and proteins.14

At least two mechanisms for Nup98-HOXA9 leukemogenesis can be proposed; 1) interference with nuclear transport, e.g., preventing nuclear import of transcription factors critical for myeloid differentiation, or interference with the export of RNAs needed for myeloid differentiation, 2) aberrant transcription. Nup98-HOXA9 may cause leukemia by interfering with the transcriptional programs of hematopoietic differentiation and proliferation13. Splicing in Hox gene is also assigned to play a central task in Hox gene mediated leukemogenesis.15

As regards cytogenetics, 20 cases in our study had normal cytogenetics (66.6%), and 10 cases had cytogenetic abnormalities (33.3%). The numerical abnormalities were present in 10% of cases (monosomy 7, one case, monosomy 20, one case, and 1p+, one case). The most common structural chromosomal abnormalities were t(8;21) in 3 cases (10%), t(15;17) in 3 cases (10%), followed by t(16;16) in 1 case (3.3%). This means that 29/30 cases were either good or intermediate risk cytogenetic while only one case lies in poor risk cytogenetic (-7).

Lower HOXA9 expression was the best predictor of overall/ disease-free survival and response to therapy.16 However this appears to reflect certain category of patients in certain population.

CONCLUSION

From the previous study, we can conclude that HOXA9 was absent in our randomly selected patients. This may be related to geographical distribution. Further study was recommended on wider scale of patients with special stress on bad prognostic category and in those harboring t(7;11)-associated leukemias.

REFERENCES

1. Hartmut D, Verena IG. Impact of genetic features on treatment decisions in AML. Hematology 2011;1:36-42.  
2. Borrow J, Shearman AM, Stanton VP, Jr., et al. The t(7;11)(p15;p15) translocation in acute myeloid leukemia fuses the genes for nucleoporin NUP98 and class I homeoprotein HOXA9. Nat Genet 1996;12: 159-67.  
3. Golub TR, Slonim DK, Tamayo P, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science 1999;286:531-7.  
4. Bruno MC, Justin SS, Ying JC, Heidi SP, Kenneth DA, Giuseppe Z, Janice NC, David J, Jane F, Rui MR, Joseph FC. Reversing HOXA9 oncogene activation by PI3K inhibition: epigenetic mechanism and prognostic significance in human glioblastoma. Cancer Res 2010; 70(2); 453–62.  
5. Christian B, Sebastian B, Dorothee M, Maria-Paz G, Emanuel M, Robert KS. Leukemogenic transformation by HOXAculters genes. Blood 2010; 115; 2910-8.  
6. Frohling S, Scholl C, Gilliland DG. Genetics of myeloid malignancies—pathogenetic and clinical implications. J Clin Oncol 2005; 23:6285-95.  
7. Stasi R, Del Poeta G, Masi M, et al. Incidence of chromosome abnormalities in de-novo acute myeloid leukemia. Cancer Gen Cytogenet 1993; 67: 28-34.  
8. Mallet F, Oriol G, Mary C, Mandraud B. Continuous RT-PCR using AMV-RT, Tag DNA polymerase: characterization and comparison to uncoupled procedures. Bio Techniques 1995; 18: 678-87.  
9. Lawrence H, Fischbach, NA, Largman C. HOX genes: not just myeloid oncogenes any more. Leukemia 2005; 19; 1328–30.  
10. Li Z, Zhang Z, Li Y, Arnovitz S, Chen P, Huang H, Jiang X, Hong GM, Kunjamma RB, Ren H, He C, Wang CZ, Elkahiloum AG, Valk PJ, Dohner K, Neily MB, Bullinger L, Delwel R, Lowenberg B, Liu PP, Morgan R, Rowley JD, Yuan CS, Chen J. PBX3 is an oncogene with a high incidence of fusion with the class-1 homeobox gene, HOXA9. Nat Genet 2003; 33:437–43.  
11. Takeshi T, Tomohiko T, Ryoichi O, Yukio K, Kohmei I, Yasuhide H. The chromosome translocation t(7;11)(p15;p15) in acute myeloid leukemia results in fusion of the NUP98 gene with a HOXA cluster gene, HOXA13, but not HOXA9. Genes, chromosomes & cancer 2002; 34:437–43.  
12. Kwong YL, Chan TK. Translocation (7;11)(p15;p15) in acute myeloid leukemia M2: association with trilineage dysplasia and giant dysplastic myeloid cells. Am J Hematol 1994;47:62–4.  
13. Nakamura T, Largaespada DA, Lee MP, et al. Fusion of the nucleoporin gene NUP98 to HOXA9 by the chromosome translocation t(7;11)(p15;p15) in human myeloid leukemia. Nat Genet 1996;12:154–8.  
14. Maconochie M, Nonchev S, Morrison A, et al. Paralogous Hox genes: function and regulation. Annu Rev Genet 1996;30:529–56.  
15. Stadier CR, VegiN, Mulaw MMA, Edmaier KE, Rawat VP, Dolnik A, Hiddemann W, Dohner K, Dohner H, Feuring-Buske M, Bullinger L, Heilmeier B, Quintanilla-Fend L, Speikermann K, Hiddemann W, Dohner K, Dohner H, Feuring-Buske M, Buske C. The leukemogenicity of HOXA9 depends on alternative splicing. Leukemia 2014; 28(9): 1838-43.  
16. Andreff M, Ruvolo V, Gadgil S, et al. HOX expression patterns identify a common signature for favorable AML. Leukemia 2008; 22: 2041–2047.