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DNMT3A gene is down-regulated in an Italian cohort of AML patients

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INTRODUCTION: The scientific community has recently focused our attention on both structural and functional analysis of methyltransferase genes. Gene alterations of these enzymes have been related to Acute Myeloid Leukemia (AML)1,2. DNMT3A plays an important role in de novo methylation, paternal and maternal imprinting and as transcriptional co-repressor too2. The presence of SNPs (single nucleotide polymorphisms) in DNMT3A gene could affect its role3,4. This study aims to look inside DNMT3A structure in order to assess the gene mutational state and to identify novel SNPs with possible pathogenic significance. The present investigation is also direct to the quantification of gene expression in order to better define the DNMT3A role as AML molecular marker.

PATIENTS AND METHODS: A cohort of 8 AML patients was recruited from the Clinic of Hematology, Hospital-University Company "Ospedali Riuniti" in Ancona (Italy). The sex ratio group was 4M:4F. We stratified our patients based on the previous structural analysis and we selected four patients for the downstream analysis of gene expression. Total RNA was extracted at disease onset using TempusTM Spin RNA Isolation kit (Applied Biosystems); cDNA was obtained by means of RetrotrascriptTM (Ambion) using according to the manufacturer's instructions. Quantitative Real-time PCR (qPCR) was performed using Power SYBR Green PCR Master Mix system (Applied Biosystems) according to the manufacturer's instructions. Beta-actin was employed as housekeeping gene and three healthy subjects were used as calibrators. The Fold change method was used for relative quantification of gene expression.

RESULTS: Quantitative analysis showed that DNMT3A gene is down regulated when the gene is mutated. The Fold change target-control values were statistically different (Student's t test; $p<0.05$). DNMT3A mRNA was average expressed about 500 times less than calibrator gene, that is the average control sample (Fold Change values were compared). We obtained single peak dissociation curves and single gel bands of predicted size; both confirmed the PCR specificity.

CONCLUSIONS: The identification of causative mutations in AML patients is responsible for down-regulation of DNMT3A gene. For this reason, the protein induces no transcriptional repression. The over-expression of downstream genes involved in AML pathway could have functional consequences. Further investigations are required to clarify the role of this gene as cancer modulator, also in relation to the potential involvement of this gene in other neoplastic diseases

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