TO STUDY THE MECHANISM OF RUNX1 AND ITS MUTANTS IN DNA BINDING AND ALTERED GENE EXPRESSION

ABSTRACT

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AbstracT

Runt related transcription factor 1 or RUNX1, though normally functions to regulate the development of hematopoietic system in concert with various transcriptional co-regulators, its malfunction is known to be the major cause of several variants of acute leukemia. RUNX1 gene is the most common target of chromosomal translocations and mutations in its runt domain are frequently associated with leukemogenesis. In mammalian system, in addition to RUNX1, two more genes comprise the family, namely RUNX2, which is essential for osteoblast differentiation and RUNX3, which is essential for the anti-proliferation and apoptosis of gastric epithelium, for differentiation of certain root ganglion nerve cells, and for the establishment of mature CD8+ cytotoxic T-lymphocytes. Structural studies of the runt domain of RUNX1-DNA complexes provide details of the direct contacts formed between the runt domain of Runx1 and DNA. CBFβ, an interacting partner, hetero-dimerizes with Runx1 and increases its affinity to bind with the DNA. The amino acid residues, Lys83 and Arg174, of runt domain directly interact with the consensus sequence of the promoters and found to be frequently mutated in the leukemic patients. Most of the structure-functional studies, till date, are confined to the binding of runt domain with synthetic DNA fragments of 8-10 bp).

To understand the role of mutant RUNX1 in the progression of leukemia, an attempt was made to study the conformation of mutant protein using biophysical parameters and physiological changes due to mutations in RUNX1 gene by analyzing the expression of direct/indirect target genes of RUNX1 in leukemic patients. Initially, in silico binding of Runx1 and its mutants with DNA was carried out using AMBER and Insight II software, followed by its functional evaluation. The ternary complex of runt domain of Runx1/CBFβ and DNA, which was available in protein databank, was utilized to carry out in-silico studies. With a view to monitor a change in the protein conformation, the ternary complex was modified to runt domain/DNA
(RUNX3/LAT) complex using InsightII and energy minimized using AMBER 8.0 software. The substitution of Lysine 83 to Glutamic acid and Arginine 174 to Glutamine were also carried out using InsightII along with energy minimization. The RMSD values inferred from the superimposed structures of mutant Runx1 on wild type Runx1 complexed with RUNX3/LAT promoter suggested a change in the conformation of the protein due to the point mutations. To get a better understanding of the change, the hydrogen bonds, hydrogen bond lengths and newly synthesized hydrogen bonds were also compared in the mutant complex (with RUNX3/LAT promoter) with respect to the wild type Runx1 complexes with RUNX3/LAT promoter.

The structural changes observed through our *in-silico* studies were further verified by in vitro studies using Electrophoretic Mobility Shift Assays (EMSA) assays and also analyzed through CD spectroscopy and Fluorescence spectroscopy using the purified Runx1 protein. The results obtained suggested that both the point mutations (Lys83Glu/Arg174Gln) lead to a change in the conformation of the full length Runx1 protein (as evident from *in-silico* study), which in turn affects its binding with the DNA. We observed that the DNA binding affinity of the mutated Runx1 with RUNX3 and LAT promoters was about 5-10 folds lower than that of wild type Runx1. Expression analysis of the target genes of Runx1 suggest a decrease in the expression of Runx3 and GM-CSF and FoxP3 and claudin1 in most of the patients while LAT and Pu1 expression was not effected in several patients with mutant Runx1. These results suggest that besides mutations in RUNX1, there may be some other mechanism involved in the alteration of expression of its target genes, which could lead to the progression/severity of acute myeloid leukemia.