631. CHRONIC MYELOID LEUKEMIA: BIOLOGY AND PATHOPHYSIOLOGY, EXCLUDING THERAPY | DECEMBER 02, 2016

Novel Molecular Findings in Protein Tyrosine Phosphatase Receptor Gamma (PTPRG) Among Chronic Myelocytic Leukemia (CML) Patients Studied By Next Generation Sequencing (NGS): A Pilot Study in Patients from the State of Qatar and Italy

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Abstract

Background: Chronic Myelocytic Leukemia (CML) is a clonal myeloproliferative disorder characterized by constitutive phosphorylation of Protein Tyrosine kinases (PTKS) that continuously activates multiple proliferative and antiapoptotic signaling pathways.

Protein Tyrosine Phosphatases (PTPs) on the other hand is potential natural inhibitory mechanism for regulating the tyrosine kinase activities in which phosphorylation is reciprocally controlled and maintained in equilibrium state by PTKs and PTPs.

As a member of PTPs family, Protein Tyrosine Phosphatase Receptor Gamma (PTPRG) was found to act as a tumor suppressor gene. This negative regulatory mechanism of PTPRG was observed to be down-

regulated and disabled in CML and one of the possible mechanisms that alter the negative regulatory effect of PTPs is mutations.

Several mutations have been identified in PTPs in many different leukemias such as Acute Myeloid Leukemia (AML), Juvenile MyeloMonocytic Leukemia (JMML), Myelodysplasic Syndrome (MDS), B-cell Acute Lymphoblastic Leukemia (B-ALL) and these mutations are associated with hyper-cellular proliferation, disease progression and poor outcome.

However, relatively little is known about PTPRG mutations and no studies on CML are available in the literature while mutations inBCR-ABL1tyrosine kinase have been extensively characterized.

Thus, understanding the role of PTPRG in antagonizing the PTK phosphorylation of BCR-ABL1 will be important to determine its role in CML development and progression.

Aim:

1) To identify potential genetic alterations causing inactivation of PTPRG and 2) correlate the PTPRG findings with patients' response to the Tyrosine kinase Inhibitors.

Methods:

16 CML patients, 9 from Qatar and 7 from Italy respectively, were studied for PTPRG mutations by exome sequencing. Custom primers were designed for Human PTPRG gene (5 Kb of exonic region of interest) using Ion AmpliSeq Designer. Target regions were enriched and amplified for the 16 DNA samples using Ion AmpliSeq Library kit 2.0.

The amplicons were partially digested with FuPa reagent and phosphorylated prior to ligation of Ion Xpress Barcode Adapters followed by cleanup using HighPrep reagent. The adapter ligated molecules were enriched with adapter specific primers using a limited cycle PCR followed by a cleanup using HighPrep reagent. The final libraries were quantified on Qubit Flurometer using Qubit dsDNA HS Assay Kit and Agilent Bioanalyzer using Agilent High Sensitivity DNA Kit. All samples were pooled according to the concentrations on the Bioanalyzer and loaded on Ion 318TM Chip kit V2 to be sequenced on Ion Personal Genome Machine (PGM) system.

European Leukemia Net (ELN) 2013 criteria were employed to assess the response/resistance of patients to treatment. Responses are defined at the hematological, cytogenetic and molecular levels. Patients response was classified into optimal and failure

Results:

Four mutations/variants were identified in PTPRG genes, three were missense Y92H, G574S, S561Y and 1 was frameshift Y285fs in the 16 CML patients.

PTPRG Y92H was identified in 5 (1 Homozygous and 4 heterozygous alleles) patients and the 5 patient failed the Imatinib Mesylate (IM) treatment.

On the other hand, The PTPRG G574S was identified in 6 (2 homozygous and 4 heterozygous alleles) patients. Out of the 6 patients, 4 were classified as failure to the treatment and 2 responded optimally.

In addition, the PTPRG S561Y and Y285fs were identified on 1 and 3 patients respectively and these patients responded optimally to IM treatment.

Discussion and Conclusions:

This is the first prospective pilot study to investigate PTPRG gene mutations as underlying mechanism to explain treatment failure.

Our preliminary data showed that the identified variant PTPRG Y92H might be associated with IM failure although it has been reported as Single Nucleotide Polymorphisms (SNPs) (rs62620047) and this could be attributed that some polymorphisms might behave like a mutation.

On the other hand, PTPRG G574S variant (rs2292245) showed various clinical outcomes regardless to its allele zygosity as 67% (4/6) of patients failed the TKIs treatment.

From the results of our pilot study we recommend carrying out PTPRG sequencing in a significantly larger cohort of patients to further explore and pinpoint the crucial mutations that can be correlated with CML resistance/response to treatment.

Disclosures

No relevant conflicts of interest to declare.

Author notes

*Asterisk with author names denotes non-ASH members.