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LncRNA-miRNA-mRNA Networks Associated with BCR-ABL T315I Mutation in Chronic Myeloid Leukemia

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Abstract : Background: The most challenging mutation of the oncokinase BCR-ABL protein T315I, which is commonly known as the “gatekeeper” mutation and is notorious for its strong resistance to almost all tyrosine kinase inhibitors (TKIs), especially imatinib. Therefore, this study aims to identify T315I-dependent downstream microRNA (miRNA) pathways associated with drug resistance in chronic myeloid leukemia (CML) for prognostic and therapeutic purposes. Methods: T315I-carrying K562 cell clones (K562-T315I) were generated by the CRISPR-Cas9 system. Imatinib-treated K562-T315I cells were subjected to small RNA library preparation and next-generation sequencing. Putative lncRNA-miRNA-mRNA networks were analyzed with (i) DESeq2 to extract differentially expressed miRNAs, using Padj value of 0.05 as cut-off, (ii) STarMir to obtain potential miRNA response element (MRE) binding sites of selected miRNAs on lncRNA H19, (iii) miRDB, miRTarbase, and TargetScan to predict mRNA targets of selected miRNAs, (iv) IntaRNA to obtain putative interactions between H19 and the predicted mRNAs, (v) Cytoscape to visualize putative networks, and (vi) several pathway analysis platforms – Enrichr, PANTHER and ShinyGO for pathway enrichment analysis. Moreover, mitochondria isolation and transcript quantification were adopted to determine the new mechanism involved in T315I-mediated resistance of CML treatment. Results: Verification of the CRISPR-mediated mutagenesis with digital droplet PCR detected the mutation abundance of ≥80%. Further validation showed the viability of ≥90% by cell viability assay, and intense phosphorylated CRKL protein band being detected with no observable change for BCR-ABL and c-ABL protein expressions by Western blot. As reported by several investigations into hematological malignancies, we determined a 7-fold increase of H19 expression in K562-T315I cells. After imatinib treatment, a 9-fold increment was observed. DESeq2 revealed 171 miRNAs were differentially expressed K562-T315I, 112 out of these miRNAs were identified to have MRE binding regions on H19, and 26 out of the 112 miRNAs were significantly downregulated. Adopting the seed-sequence analysis of these identified miRNAs, we obtained 167 mRNAs. 6 hub miRNAs (hsa-let-7b-5p, hsa-let-7e-5p, hsa-miR-125a-5p, hsa-miR-129-5p, and hsa-miR-372-3p) and 25 predicted genes were identified after constructing hub miRNA-target gene network. These targets demonstrated putative interactions with H19 lncRNA and were mostly enriched in pathways related to cell proliferation, senescence, gene silencing, and pluripotency of stem cells. Further experimental findings have also shown the up-regulation of mitochondrial transcript and lncRNA MALAT1 contributing to the lncRNA-miRNA-mRNA networks induced by BCR-ABL T315I mutation. Conclusions: Our results have indicated that lncRNA-miRNA regulators play a crucial role not only in leukemogenesis but also in drug resistance, considering the significant dysregulation and interactions in the K562-T315I cell model generated by CRISPR-Cas9. In silico analysis has further shown that lncRNAs H19 and MALAT1 bear several complementary miRNA sites. This implies that they could serve as a sponge, hence sequestering the activity of the target miRNAs.

Keywords : chronic myeloid leukemia, imatinib resistance, lncRNA-miRNA-mRNA, T315I mutation
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