Abstract

Leukemia is the most common malignant disease in children patients. In our laboratory (CLIP) a novel subtype of B-cell precursor Acute Lymphoblastic Leukemia (BCP-ALL) with lineage switch during early phase of treatment towards myeloid lineage (swALL) was recently documented. SwALL incidence is almost 4 % of all BCP-ALLs (Slámová et al., 2014). DNA methylation (presence of 5-methylcytosine) is together with post-translational histone modifications and non-coding RNAs an epigenetic mechanism which regulates gene expression without changes of genetic code. DNA methylation is easily detected by bisulphite conversion and subsequent sequencing. The aim of this work was to compare genome-wide DNA methylation patterns between patients with swALL and control BCP-ALLs.

The first step in achieving that was revision and improvement of bioinformatic processing protocol for eRRBS data from massive parallel sequencing. To improve the sequence adapter trimming I tested four bioinformatic tools – FAR, cutadapt, Trimmomatic and fastx_clipper. I implemented the fastest and most effective - Trimmomatic into the processing protocol.

As a next step I analysed the data with improved protocol and extended the analysis in R programming environment where the comparison of studied groups was performed.

The comparison of methylation patterns of swALL and control BCP-ALL patients revealed 84338 significantly differentially methylated cytosines, 321 significantly differentially methylated CpG islands and 2295 significantly differentially methylated 100 bp tiles. The majority of significantly differentially methylated cytosines laid within gene regions (promoter, exon or intron). I found 176 genes with promoters differentially methylated between swALL and control BCP-ALL. I also added number of genes of interest to form a list of candidate genes. I visually inspected those genes and selected 20 genes with 1) continuously methylated or non-methylated region, 2) similar methylation status inside each group and 3) different methylation status between groups at the same time. Among them were for instance CEBPA, CEBPE, KLF14 and other genes related to myeloid differentiation. Such genes are candidates for explaining the mechanism of lineage switch in swALL patients.

Key words: childhood leukemia, B-cell precursor Acute Lymphoblastic Leukemia, BCP-ALL, DNA methylation, methylation patterns, eRRBS, massively parallel sequencing, bioinformatics