A Targeted-enzymatic-methyl-seq approach for cell-free DNA methylation analysis to improve cancer detection, disease following and minimal residual disease monitoring

Mehdi Ben Sassi[1]
Charles Marcaillou[1], Sylvain Guibert[1], Emmanuel Martin[1], Valérie Taly[2] and Pierre Laurent-Puig[2]
[1] IntegraGen SA, Genopole CAMPUS 1 bât. G8, EVRY, Paris, France.
[2] Centre de Recherche des Cordeliers, INSERM, CNRS SNC 5096, Sorbonne Université, Université de Paris, 75006 Paris, France.

Background & objectives

DNA methylation is an epigenetic mark that consists of the addition of a methyl group to cytosine in the CpG specific contexte. Promoter methylation level of a gene is inversely proportional to its level of expression. In cancer cells, the methylation of specific genomic regions is affected, depending on tumor type and origin. In addition, methylation modifications are an early phenomenon in disease progression. Thus, identification of specific methylation loci in cancer cells can lead to early disease detection, treatment response follow-up and Minimal Residual Disease (MRD) monitoring based on cell-free DNA (cfDNA) analysis. Nowadays, several IVD test kits based on cfDNA methylation analysis are available for multiple cancer types. These kits, based on the analysis of differentially methylated regions (DMR) specific for each tumor type, only include a limited number of targets, often resulting in a lack of specificity and/or sensitivity.

The common methods for methylation studies are based on bisulfite conversion or methylation-sensitive restriction enzymes (MSRE). These approaches are not efficient for the discovery of new methylated biomarkers. Bisulfite conversion, used in Next Generation Sequencing (NGS) methods like Reduced Representation Bisulfite Sequencing (RRBS) and Whole Genome Bisulfite Sequencing, or digital PCR, highlights bias issues as well as low yield, difficult to reconcile with the low avaibility of cfDNA. MSRE are not suitable with hight-throughput.

Methods

We have developed a NGS based method with enzymatic conversion of DNA and targeted enrichment of almost 6 millions CpGs. We have worked on improvement of specificity and sensitivity to allow detection of DMR between tumors and healthy tissues from liquid biopsy.

Results

Using this technology on ovarian cancer samples, healthy control tissues including public data and buffy coat matched samples, we have identified tens of thousands of CpGs as potential

biomarkers that could be examined in cfDNA. Furthermore, data collected from our method are compatible with public available bioinformatic tools for cfDNA deconvolution, in order to decipher cell-type origin of fragments.

Conclusion

We have implemented a strategy to discover new methylated biomarkers to meet the medical demand for new diagnostic tests, and evaluate our approach on ovarian cancer samples.