Longitudinal monitoring of cell-free DNA methylation in ALK-positive non-small cell lung cancer patients

Florian Janke[1]
Arlou Kristina Angeles[1], Anja Lisa Riediger[2], Martin Reck[3], Albrecht Stenzinger[4], Marc A. Schneider[5], Thomas Muley[5], Michael Thomas[5], Petros Christopoulos[5] and Holger Sültmann[1]
[1] German Cancer Research Center
[2] Universitätsklinikum Heidelberg and German Cancer Rsearch Center
[3] LungenClinic Grosshansdorf GmbH
[4] Universitätsklinikum Heidelberg
[5] Thoraxklinik Heidelberg GmbH

Background & objectives

DNA methylation (5-mC) signals in cell-free DNA (cfDNA) of cancer patients represent promising biomarkers for minimal-invasive tumor detection. The high abundance of cancer-associated 5-mC alterations permits parallel and highly sensitive assessment of multiple 5-mC biomarkers. Here, we performed genome-wide 5-mC profiling in plasma of metastatic *ALK*-rearranged non-small cell lung cancer (NSCLC) patients receiving tyrosine kinase inhibitor therapy. We established a strategy to identify *ALK*-specific 5-mC changes from cfDNA and demonstrated the suitability of the identified markers for cancer detection, prognosis, and therapy monitoring.

Methods

Longitudinal plasma samples (n = 79) of 21 *ALK*-positive NSCLC patients and 13 healthy donors were collected alongside 15 *ALK*-positive tumor tissue and 10 healthy lung tissue specimens. All plasma and tissue samples were analyzed by cell-free DNA methylation immunoprecipitation sequencing to generate genome-wide 5-mC profiles. Information on genomic alterations (*i.e.*, somatic mutations/fusions and copy number alterations) determined in matched plasma samples were available from previous studies.

Results

We devised a strategy that identified tumor-specific 5-mC biomarkers by reducing 5-mC background signals derived from hematopoietic cells. This was followed by differential methylation analysis (cases *versus* controls) and biomarker validation using 5-mC profiles of *ALK*-positive tumor tissues. The resulting 245 differentially methylated regions were enriched for lung adenocarcinoma-specific 5-mC patterns in TCGA data and indicated transcriptional repression of several genes described to be silenced in NSCLC (*e.g.*, *PCDH10*, *TBX2*, *CDO1*, and *HOXA9*). Additionally, 5-mC-based tumor DNA quantification (5-mC score) was highly correlated to cell-free genomic alterations (Spearman, $\rho > 0.6$) and samples with high 5-mC

scores showed significantly shorter overall survival (log-rank p = 0.025). Longitudinal 5-mC scores reflected radiologic disease assessments and were significantly elevated at disease progression compared to the therapy start (p = 0.0023). In 7 out of 8 instances, rising 5-mC scores preceded imaging-based evaluation of disease progression.

Conclusion

We demonstrated a strategy to identify 5-mC biomarkers from plasma of cancer patients and integrated them to a quantitative measure of cancer-associated 5-mC alterations. Using longitudinal plasma samples of ALK-positive NSCLC patients, we highlighted the suitability of cfDNA methylation for prognosis and therapy monitoring.