Epigenomic Profiling of Liquid Biopsy to Predict Immunotherapy Resistance

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Immune checkpoint inhibitors (ICIs), such as anti-PD1, have revolutionized cancer treatment. However, only a small proportion of patients benefit from this therapy, and effective biomarkers to predict primary and secondary resistance are lacking. A key limitation is the difficulty in obtaining sufficient tumor samples for clinical testing. Liquid biopsy (LB) provides a non-invasive alternative, which enables the detection of tumor-derived epigenetic alterations, such as DNA methylation and noncoding RNAs (ncRNAs). It is expected that epigenomic profiling of LB will allow the identification of novel non-invasive biomarkers and mechanisms of resistance to ICIs in several types of tumors, such as non-small cell lung cancer (NSCLC), providing critical insights into the mechanisms driving resistance and help refine patient selection for immunotherapy. In line with this, our group has identified that cell-free DNA (cfDNA), extracellular vesicles (EVs), and specific subtypes of peripheral leukocytes exhibit distinct epigenetic profiles that can predict the response to immunotherapy before initiating treatment in patients with metastatic NSCLC. These previous results have recently enabled us to launch the EPILUNAR Project, which aims to discover novel non-invasive epigenetic biomarkers and resistance mechanisms associated with ICIs in mNSCLC to personalize immunotherapy management and improve treatment outcomes. In this project, 250 mNSCLC patients before initiating first-line anti-PD1-based therapy will be recruited and monitored by four spanish groups from CIBERONC. Therapy response will be assessed during the follow-up. We will integrate epigenomics, artificial intelligence (AI), and LB to identify key biomarkers and mechanisms driving resistance. By combining genome-wide methylation profiling of cfDNA with AI, we will develop non-invasive cfDNA methylation signatures to predict ICI resistance. Additionally, whole-genome DNA methylation profiling of single CTCs will be performed to uncover novel resistance mechanisms. The molecular phenotype of circulating immune cells will also be characterized. Moreover, MPE-derived organoids will be generated as tumor models to validate the identified resistance mechanisms. The role of stable intronic sequence RNAs (sisRNAs), a specific lncRNA subtype, in ICI resistance will also be explored. In conclusion, our group and collaborators are working to identify non-invasive epigenetic biomarkers for predicting immunotherapy resistance, uncover novel mechanisms impairing treatment efficacy, and pave the way for innovative strategies to overcome this resistance in mNSCLC, ultimately contributing to more effective and personalized therapeutic approaches.