[BIOINFO 2023]

Unveiling resistance mechanisms of non-small cell lung cancer against third-generation EGFR tyrosine-kinase inhibitors through single-cell DNA sequencing

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Non-small cell lung cancer (NSCLC) is a leading cause of cancer-related deaths globally. The primary treatment for NSCLC with EGFR activating mutations is EGFR tyrosine kinase inhibitors (TKI). However, most patients who initially respond to this therapy eventually develop resistance due to additional genetic mutations. While previous studies reported resistance to third-generation TKI, the underlying genetic mechanisms are not fully understood. This study aims to explore the genetic mutations that impact the effectiveness of third-generation TKI, using single-cell DNA sequencing of tumors that have developed resistance. We sequenced tumor tissues from five patients who had been treated with either lazertinib or osimertinib. The sequencing was performed using Illumina HiSeq2000 and Mission Bio's Tapestri® THP-V2 single-cell library preparation. Our analysis, conducted using both in-house and vendor-distributed pipelines, identified 237 SNVs (an average of 59.25 per sample) and 50 CNVs (an average of 12.5 per sample). By analyzing clonality, we identified potential mechanisms through which minor resistant clones might survive and persist. These include missense mutations in EGFR and TP53, as well as copy number alterations in EGFR, DDR2, HRAS, CDK4, and STK11. We reconstructed the lineage to map the genetic evolution within tumors towards acquired resistance to reveal a synergistic effect of the acquired mutations and their co-existence with the original driver mutations. Our findings contribute valuable insights to the fight against resistant clones to third-generation TKIs and may aid in the discovery of new therapeutic targets in NSCLC.