Abstract

Lung adenocarcinoma (LUAD) driven by EGFR mutations remains a therapeutic challenge due to acquired resistance to osimertinib. The tumor microenvironment (TME) can induce phenotypic plasticity, including a basal-like transition associated with therapy resistance, but the specific TME signals that trigger this transition remain unclear. This study investigated whether four distinct TME-mimicking organoid media are sufficient to induce a basal-like transition in EGFR-mutant PC-9 cells, and how this influences osimertinib sensitivity and transcriptional reprogramming. Longitudinal IC_{50} assays revealed divergent trajectories: progressive resistance in Media 1 (5.5× increase), progressive sensitization in Media 2 (5.6× decrease), transient sensitization followed by rebound in Media 3, and modest resistance in Media 4. KRT5 was robustly upregulated across all media at mRNA and protein levels. However, TP63 was completely absent across four independent assays (RNA-seq, RT-qPCR, western blot, immunofluorescence), indicating that KRT5 induction does not reflect a TP63-driven basal-like transition. LUAD lineage markers were consistently low across all conditions. The hypothesis that TME-mimicking conditions drive a basal-like transition is partially supported: initiating events (KRT5) are present, but full basal commitment (TP63) was not achieved within P0–P3. Rather than a single lineage switch, different media select for distinct non-genetic adaptive states along a phenotypic plasticity continuum.

Keywords: EGFR mutation, NSCLC, TKI resistance, Basal-shift, 3D culture