Chapter 1

Introduction

1.1 Background

Lung cancer remains the leading cause of cancer-related mortality globally, with 85% of cases classified as non-small cell lung carcinoma (NSCLC) (Su et al., 2025). The majority of this burden falls on Asia, accounting for approximately 60% of new diagnoses and 62% of deaths worldwide (Triphuridet et al., 2023). Notably, declining smoking rates have revealed a growing burden of lung cancer in never-smokers (LCINS), now estimated as the fifth leading cause of cancer mortality worldwide, with incidence rates 2.3 times higher in Asian compared to non-Asian never-smokers and over 60% of cases occurring in women (LoPiccolo et al., 2024; Tan & Tan, 2022; Triphuridet et al., 2023; Loh et al., 2022; Triphuridet et al., 2024). In Asian never-smokers, approximately 97% of LCINS cases manifest as lung adenocarcinoma (LUAD), with a five-year survival rate below 26% (Ren et al., 2025; Triphuridet et al., 2024). This poor prognosis is closely tied to oncogenic driver mutations in the epidermal growth factor receptor (EGFR) gene, which occur in 50–63% of Asian never-smoker LUAD patients — predominantly as L858R point mutations ($EGFR^{L858R}$, ~45%) and exon 19 deletions ($EGFR^{Ex19Del}$, ~40%) (Luo et al., 2023; Torasawa et al., 2025; Blechter et al., 2024; Khan et al., 2023; Wang et al., 2025; Wang et al., 2023).

While first- and second-generation EGFR tyrosine kinase inhibitors (TKIs) effectively target EGFR^L858R and EGFR^Ex19Del mutations, acquired T790M-mediated resistance develops in 66–74% of patients within 9–14 months (He et al., 2021; Khaddour et al., 2021; Ohara et al., 2021; Passaro et al., 2020; Wu et al., 2020). The third-generation TKI osimertinib overcomes T790M resistance and has become the standard first-line treatment for advanced EGFR-mutant LUAD (Cho et al., 2019; Takeyasu et al., 2024). Nevertheless, acquired resistance to osimertinib remains inevitable, underscoring the

critical need to identify the mechanisms and survival pathways driving therapeutic failure (Criscione et al., 2022; Liu et al., 2025; Yin et al., 2023).

Beyond genomic resistance mechanisms such as secondary EGFR mutations and bypass kinase activation, an emerging and clinically significant mode of osimertinib resistance involves phenotypic plasticity: the capacity of cancer cells to transcriptionally and epigenetically reprogram their differentiated identity (Quintanal-Villalonga et al., 2020). In EGFR-mutant LUAD, this plasticity manifests along a spectrum of lineage transitions, ranging from epithelial-to-mesenchymal transition (EMT) to overt histological transformation into small-cell or squamous cell carcinoma (Shaurova et al., 2020; Quintanal-Villalonga et al., 2020). Of particular relevance is the basal-like shift: a partial squamous transdifferentiation characterized by the hybrid co-expression of LUAD-associated alveolar markers alongside squamous/basal cell markers including TP63 and KRT5 (Quintanal-Villalonga et al., 2021; Shinozaki et al., 2025). Critically, this basal-shift phenotype has been identified in EGFR-TKI-resistant LUAD organoids lacking conventional resistance mutations, and is mechanistically linked to loss of the alveolar lineage transcription factor NKX2-1 (TTF-1), with sensitivity to CDK4/6 inhibition emerging as a potential therapeutic vulnerability (Shinozaki et al., 2025). Single-cell profiling of pre-transformed LUAD further confirms that KRT5, KRT6A, and TP63 are enriched in cells poised for lineage transition, suggesting the basal-like state represents an early and potentially reversible step in the resistance trajectory (Rakhade et al., 2024; Zatzman et al., 2025).

While cell-intrinsic mechanisms of plasticity are increasingly characterized, the role of the tumor microenvironment (TME) in initiating or accelerating basal-like transition remains poorly understood. The TME comprising stromal cells, immune cells, cytokines, and extracellular matrix components, is now recognized as an active driver of EGFR-TKI resistance through mechanisms including cancer-associated fibroblast (CAF)-mediated EMT, macrophage-derived paracrine signaling, and hypoxia-induced transcriptional reprogramming (Zhang et al., 2024; Lim et al., 2025; Lu et al., 2020).

Importantly, TME-induced plasticity may precede overt drug resistance, acting as an early survival strategy that primes cells for subsequent clonal evolution (Meng et al., 2025; Jeong et al., 2022). However, dissecting the specific TME signals that trigger basal-like transition in EGFR-mutant LUAD cells has been hampered by the lack of tractable experimental models that faithfully recapitulate distinct microenvironmental niches. Lung organoid culture systems offer a powerful solution: by culturing cancer cells in defined media formulations that differentially activate growth factor, cytokine, and niche signaling axes, it is possible to model distinct TME-like states in a controlled and reproducible manner (Kobayashi et al., 2024; Tan et al., 2024). In this study, we exploit four commercially defined and custom organoid media each encoding a distinct signaling milieu as proxies for different TME conditions, to systematically interrogate which microenvironmental contexts are sufficient to induce a basal-like transition in parental EGFR-mutant PC-9 cells.

1.2 Objective

This study aims to characterize the basal-like phenotype switch in EGFR-mutant LUAD cells induced by distinct TME-mimicking organoid media, and to determine how this transition influences osimertinib sensitivity and transcriptional reprogramming using 4 defined organoid media.

1.3 Hypothesis

Based on preliminary observations of morphological and phenotypic changes in PC-9 cells cultured in Media 1 and 4, we hypothesize that media 1 and 4 will induce a basal-like transition in parental PC-9 cells, leading to upregulation of basal markers (e.g., TP63, KRT5) and reduced osimertinib sensitivity, while media 2 and 3 will preserve EGFR-mutant alveolar identity and maintain TKI sensitivity, reflecting their distinct signaling compositions relative to Media 1 and 4.