Chapter 1

Introduction

1.1 Background

Esophageal Squamous Cell Carcinoma (ESCC) is a malignant tumor arising from the squamous epithelial lining of the esophagus, most commonly affecting its upper and middle regions (Acharya et al., 2023). Globally, ESCC accounts for approximately 85% of all esophageal cancer cases, with over 500,000 diagnoses reported globally (Morgan et al., 2022). Despite advances in oncology, the 5-year survival rate remains around 20% because early-stage disease is often asymptomatic and there are no reliable early markers for ESCC, resulting in most patients being diagnosed only after the disease has progressed to an advanced stage (Chaber-Ciopinska et al., 2016; Yang & Hu, 2021). At this stage, curative options such as endoscopic eradication are no longer feasible, and patients require multimodal treatment strategies (Acharya et al., 2023), involving a combination of surgical resection, radiotherapy, and neoadjuvant chemotherapy using the Doxetacel, Cisplatin, 5-FU (DCF) regimen (Abbas & Krasna, 2017; Baladi et al., 2025). While this approach improves tumor response rates, treatment resistance remains a major clinical challenge, frequently leading to relapse and poor prognosis (Liao et al., 2025). This raises an important question of how certain tumor cells are able to survive chemotherapy and contribute to tumor regrowth after treatment. Furthermore, as the DCF regimen is predominantly only applied in locally advanced ESCC, it remains unclear whether earlier therapeutic intervention would enhance treatment response or whether treatment-resistant cellular populations are already established at the early stages of tumor development.

Growing evidence suggests that cancer stem cells (CSCs) contribute significantly to resistance cases in cancers (Lohan-Codeço et al., 2022). Although the existence of a distinct CSC population in the esophagus remains debated, several studies have proposed the presence of stem-like cellular populations that may contribute to ESCC pathogenesis and therapeutic resistance. Many CSC-associated properties are thought to originate from normal stem/progenitor cell programs, a

population of cells that is naturally present within the esophageal epithelium. These stem/progenitor cells normally will proliferate to maintain esophageal tissue, and once they begin to differentiate, they exit the cell cycle, move up from the basal layer, and are ultimately shed from the tissue surface (Grommisch et al., 2024). However, not limited in ESCC, as they can self-renew, survive chemotherapy, and regenerate tumor populations following treatment

The canonical Wnt pathway is a major regulator of esophageal basal stem/progenitor cell maintenance, proliferation, and differentiation, with leucine-rich repeat-containing G protein-coupled receptors (Lgr) such as Lgr6 serving as important upstream modulators (Feng et al., 2021). Our previous studies on mouse organoid models derived from advanced-stage ESCC indicated that cells derived from $Lgr6^{+}$ populations, which does not necessarily retained the Lgr6 expression, not only survived but also proliferated following chemotherapy, implying that these cells possess a special ability to resist chemotherapy-induced toxicity. Whether this behavior is restricted to advanced disease or is already present during the early stages of ESCC tumorigenesis remains unclear.

Interestingly, preliminary immunostaining analyses from early-stage ESCC and hyperplastic esophageal tissues revealed that $Lgr6^{+}$ cell pools were also present within the esophageal epithelium, despite the generally favorable prognosis associated with early-stage disease. This suggests that $Lgr6^{+}$ populations are not exclusive to advanced malignancy but may already exist within earlier epithelial lesions where tissue architecture and homeostasis are largely preserved. Nonetheless, it remains uncertain how $Lgr6^{+}$-derived cells respond to chemotherapy at earlier stages of tumorigenesis.

In addition to tumor stage, the duration and pattern of chemotherapy exposure are increasingly recognized as important determinants of treatment response. Therefore, systematically comparing dynamic responses of $Lgr6^{+}$ cells and their progeny towards short-term and continuous chemotherapy regimens, as well as after its withdrawal, is important to better reflect clinically relevant treatment scenarios. By understanding the role of $Lgr6^{+}$-derived cells during the earliest

stages of ESCC under different chemotherapy exposure conditions, this study hopes to uncover potential therapeutic vulnerabilities that could ultimately improve patient recovery outcomes.

1.2 Objective

This study aims to investigate the role of Lgr6+-derived cells in chemotherapy response in ESCC across different stages of tumor progression. Specifically, it seeks to (i) perform lineage tracing of $Lgr6^{+}$ cells with their progeny in early-stage ESCC organoid models, and (ii) evaluate the effects of different chemotherapy exposure regimens—immediate, continuous, and withdrawal—on $Lgr6^{+}$derived cell dynamics in both early- and advanced-stage ESCC models.

1.3 Hypothesis

Early-stage ESCC organoids are expected to recapitulate advanced-stage dynamics, where Lgr6+-derived cells ultimately dominate the organoid population following treatment. We further hypothesize that the survival and proliferative dynamics of these cells are regimen-dependent; short-term exposure will primarily trigger acute apoptosis, continuous exposure will establish a dynamic equilibrium between cell death and proliferation, and drug withdrawal will alleviate selective pressure, triggering robust compensatory proliferation to drive organoid repopulation.