Chapter 1

Introduction

1.1. Background

Cancer poses a significant burden on global health and is still one of the leading causes of mortality worldwide. Approximately 20 million new cases and 10 million deaths were attributed to cancer in 2022. The global incidence of cancer is projected to increase by 77% by 2050 (Bray et al., 2024). Conventional cancer treatments such as chemotherapy rely on non-specific cytotoxic agents that affect both cancerous and healthy cells. As a result, chemotherapy treatments often result in off-target toxicity and adverse effects (Anand et al., 2022). This highlights the urgent need for more effective and targeted cancer therapeutics.

To overcome these limitations, targeted cancer therapies have been developed to improve specificity while maintaining effective antitumor activity. Among these approaches, antibody-drug conjugates (ADCs) have emerged as a class of targeted cancer therapeutics that combine the antigen specificity of monoclonal antibodies (mAbs) with the potent cytotoxicity of small-molecule drugs. The design of ADCs allows them to selectively bind to specific antigens that are expressed on the cancer cell surface, enabling targeted and controlled release of highly potent cytotoxic agents directly into cancer cells, which minimizes off-target toxicity and improves therapeutic index (Fu et al., 2022; Pettinato, 2021).

The cytotoxic efficacy of ADCs relies on several key factors, including the level of antigen expression, antibody-antigen binding affinity, and the efficiency of intracellular delivery of the cytotoxic payload. Following antigen binding, the ADC-antigen complex must undergo internalization and intracellular trafficking through the endosomal-lysosomal pathway, where cleavage of the linker occurs to release the cytotoxic payload inside the cell. Therefore, ADC efficacy is influenced not only by target antigen expression but also by cellular processes that regulate ADC internalization and intracellular trafficking (Fu et al., 2022; Pettinato, 2021; Tsuchikama & An, 2016; Samantasinghar et al., 2023).

Carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6) is a glycosyl-phosphatidyl-inositol (GPI)-anchored cell surface glycoprotein that is overexpressed in various malignancies, including colorectal, gastric, breast, pancreatic, and lung cancers. Its overexpression has been correlated with cancer progression and poor clinical outcomes, which make CEACAM6 a promising therapeutic target for ADC-based therapies (Wu et al., 2024). To target CEACAM6-expressing cancers, an anti-CEACAM6 heavy-chain antibody-drug conjugate (hHCAb-ADC) has been developed. Compared with conventional IgG-based antibodies, heavy-chain antibodies have a smaller size and a single-variable domain structure, which enables binding to glycosylated-masked epitopes and improves tumor penetration. This anti-CEACAM6 hHCAb-ADC has demonstrated strong binding to glycosylated CEACAM6 and exhibited potent cytotoxic activity in CEACAM6-positive cancer cell models (Chiang et al., 2018; Tsai et al., 2026).

Despite its potency, the cellular processes underlying the internalization of this anti-CEACAM6 hHCAb-ADC have not been completely understood. CEACAM6, the target of the anti-CEACAM6 hHCAb-ADC, is a GPI-anchored protein that localizes to a cholesterol-rich membrane microdomain known as the lipid rafts, which plays a role in endocytosis (Zhao et al., 2024; Al-Khinji, 2025; Hammood et al., 2021). Therefore, the lipid rafts and its associated endocytosis pathways may play a role in the internalization and cytotoxic efficacy of the anti-CEACAM6 hHCAb-ADC. This study aims to explore the contribution of the lipid rafts and associated endocytosis pathway to the cytotoxic efficacy of an anti-CEACAM6 hHCAb-ADC.

1.2. Objective

To explore the contribution of the lipid rafts and associated endocytosis pathways to the cytotoxic efficacy of an anti-CEACAM6 heavy-chain antibody-drug conjugate (hHCAb-ADC) through cholesterol depletion by MβCD and knockdown of lipid raft scaffold proteins (caveolin-1 and flotillin-1).

1.3. Hypothesis

Null Hypothesis (H_0): Perturbation of the lipid rafts and associated endocytosis pathways does not significantly reduce the cytotoxic efficacy of anti-CEACAM6 hHCAb-ADC in HT29 cells.

Alternative Hypothesis (H_1): Perturbation of the lipid rafts and associated endocytosis pathways significantly reduce the cytotoxic efficacy of anti-CEACAM6 hHCAb-ADC in HT29 cells.