Abstract

Antibody-drug conjugates (ADCs) require efficient antigen binding, internalization, intracellular trafficking, and payload release to exert cytotoxic activity. CEACAM6 is a GPI-anchored cell surface protein that localizes to cholesterol-rich lipid raft domains, suggesting that lipid raft-associated endocytosis may play a role in the cytotoxic activity of CEACAM6-targeting ADCs. This study aimed to evaluate whether perturbation of cholesterol-rich lipid rafts and associated endocytosis pathways affects the cytotoxic efficacy of an anti-CEACAM6 heavy-chain antibody-drug conjugate (hHCAb-ADC) in HT29 cells. MβCD-mediated cholesterol depletion, caveolin-1 and flotillin-1 knockdown, and exploratory Pitstop-2 co-treatment were used to perturb lipid raft-associated, caveolin-mediated, flotillin-mediated, and clathrin-mediated endocytosis pathways, respectively. Cytotoxic efficacy was evaluated using MTT assay and IC_{50} analysis. Overall findings suggest that anti-CEACAM6 hHCAb-ADC cytotoxic efficacy in HT29 cells was not strongly reduced by the tested perturbations and therefore may not be strongly dependent on the cholesterol-rich lipid rafts, caveolin-mediated, flotillin-mediated, and clathrin-mediated endocytosis pathways. However, the specific internalization pathway for anti-CEACAM6 hHCAb-ADC remains unresolved and requires direct ADC internalization and intracellular trafficking studies. Methodological insight and preliminary evidence from this study may help future research to optimize experimental conditions and prioritize investigation of potential endocytosis pathways for anti-CEACAM6 hHCAb-ADC.

Keywords: anti-CEACAM6 hHCAb-ADC, lipid rafts, endocytosis