Introduction

Antibodies against cell surface antigens can be internalized through their specific interactions with these proteins. The anti-cancer efficacy of antibody-associated therapeutics like antibody-drug conjugates is thought to rely on their uptake by cancer cells expressing the surface antigen. Therefore, the measurement of antibody internalization efficiency of an antibody is critical for its success. Based on years of experience in antibody-drug conjugates (ADC) development and characterization, Creative Biosciences provides internalization analysis service to help clients verify the internalization of their antibody and ADC samples, and this service can be also very useful in helping clients select appropriate internalizing antibody for their development.

Presented here is a case study for the antibody internalization detection via the confocal microscopy and flow cytometry strategies.

Internalization: An important process during antibody-based therapeutics

The efficacy of certain antibody-based therapeutics depends on internalization process. Once internalization, such as the ADC molecules are transported to subsequent intracellular compartments where the toxic payload drug is released to accomplish the cytotoxicity of the ADC. Thus, the rate and extent of antibody internalization is of crucial importance to its effectiveness and need to be characterized precisely.

CBL has adopted and perfected several techniques to study antibody/ADC internalization. Confocal microscopy and flow cytometry are suitable methods to detect the internalization efficiency.

Case 1: Internalization and intracellular trafficking of anti-HER2 mAb-vc-Toxin (NGL-Z1 ADC)

**Measurement method:**
1. Cells were seeded per plate and incubated with NGL-Z1 ADC and positive control antibody (30 min at 41°C) and incubated (24 h) respectively.
2. For internalization: NGL-Z1 ADC, the cells were incubated with Alexa Fluor 488-labeled goat anti-human IgG, then incubated with MDA-MB-231 unlabeled anti-human (CD314) antibody, respectively.
3. The nucleus compartment was stained with DAPI Fluorescence images were taken using confocal microscope.

**Results:**
NGL-Z1 ADCs or positive control antibody was shown as green, and lysosome markers were shown as red, and DAPI-stained nuclei was shown in blue. Compared to incubation at 4°C for 24 h, the significant yellow signal appeared in both ADC, whereas control antibody groups after 37°C incubation for 24 h, and the positive signal was no co-localization of signals to ADC or positive antibody (green+lysosome markers) pair, indicating that ADC and positive control antibody were internalized and transported to the lysosome successfully.

Case 2: Cellular internalized and lysosomal trafficking of anti-HER2 mAb and anti-HER2 mAb-vc-MMAE

**Measurement method:**
1. Cells were seeded per plate and incubated with mAb or mAb-MMAE for incubation for 24h and were incubated at 37°C.
2. For internalization mAb-vc-MMAE, the cells were incubated with mouse anti-human IgG (Alexa Fluor 488-labeled goat anti-human IgG), then incubated with Alexa Fluor 488-labeled goat anti-human IgG and Alexa Fluor 555-labeled goat anti-mouse IgG (green+lysosome markers) pair, indicating that mAb and positive control antibody were internalized and transported to the lysosome successfully.

**Case 3:** Internalization kinetics and intracellular trafficking of anti-Trop mAb-vc-aauristatin analogue

**Measurement method:**
1. Cells were cultured, harvested, primary antibody, was labeled with Dylight680 antibody labeling kit.
2. The labeled antibody was added to the cells and incubated at 41°C.
3. To initiate internalization, cells were pre-warmed binding buffer supplemented with unlabeled potential target antigen incubated in 37°C.
4. Internalization was stopped by the quench buffer. Cells were treated with tycon-EDTA supplemented with quench and incubated in a 37°C to remove an internalized antibody on the surface.
5. Samples were collected to measure the maximal amount of surface-bound fluorescent signals.
6. Co-localization of mAb-vc-aauristatin analogue with (lysosomal marker LAMP-2) was detected with confocal microscopy.

Conclusions

ADC Case Study
Antibody Internalization Assay

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