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Quantification of cellular internalization and cytosolic release of antibody in living cells by a split-GFP complementation assay

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Considering the number of cytosolic proteins associated with many diseases, development of cellular internalization molecules from outside of living cells is highly in demand. To reach the cytosol after cellular uptake, cell-penetrating molecules should be escaped from early endosomes prior to the lysosomal degradation. However, it is very challengeable to separate the pool of cytosolic localized molecules from those trapped in the endocytic vesicles. In this study, we described a method to directly demonstrate the cytosolic localization and quantification the cytosolic amount of a cytosol-penetrating IgG antibody, TMab4, based on enhanced split GFP complementation system. We generated TMab4 genetically fused with one GFP11 fragment and separately established HeLa cells expressing the other GFP1-10 fragment in the cytosol such that complemented GFP fluorescence is observed only when the extracellularly-treated TMab4 reaches the cytosol after cellular internalization. The high affinity interactions between streptavidin-binding peptide 2 (SBP2) and streptavidin (SA) was employed as respective fusion partner to the GFP fragments to enhance the sensitivity of GFP complementation. With this method, cytosolic concentration and endosomal escape efficiency of TMab4 was estimated to be about 170 nM and 1.3-4.3% after extracellular treatment of HeLa cells with 1μM of TMab4 for 6h. Our enhanced split GFP complementation assay provides a useful tool to directly quantify cytosolic amounts of cytosol-penetrating agents and allows cell-based high-throughput screening for cytosol-penetrating agents with increased endosomal-escaping activity.

Biography

Ji-Sun Kim has developed a passion for antibody engineering to generate the extremely effective cancer drug. She is a second year PhD student in Antibody Engineering lab at the Ajou University in South Korea. She focuses on research for generating cytosol penetrating antibody targeting intracellular antigen and evaluating the developability of the cytosol penetrating antibody therapeutics. Also, she evaluated *in vivo* efficacy of antibody to overcome the limitation of current therapeutics and therapy approach. She is also interested in the development of new-generation cytotoxic fusion protein using cytosol penetrating antibody platform technology. Her research achieves results including papers and intelligence properties in Korea and abroad.

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