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Investigating the Intracellular Fate of Gelonin Immunotoxins

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Engineering of antibody based therapeutics has led to the development of a class of drugs called immunotoxins. Immunotoxins are potent fusion proteins that combine binding and toxin domains to facilitate targeted cytotoxicity. Here we describe a number of immunotoxins based on the plant toxin gelonin, incorporating ds-scFv and fibronectin binding scaffolds, targeting the carcinoembryonic antigen (CEA) and epidermal growth factor receptor (EGFR). At the cellular level, specific antigen binding, receptor mediated endocytosis, and access to the cytoplasm each represent a potentially limiting step in achieving cytotoxicity. We strive to investigate and quantify these sequential transport events for immunotoxins to identify control points for protein engineering. We used affinity titration on antigen expressing tumor cell lines, quantum fluorescence correlation flow cytometry, and canonical in vitro cytotoxicity assays to measure affinity, dose dependence, and when combining dose matched data, the limits of endosomal escape. Results suggest that immunotoxins against CEA and EGFR can induce specific cytotoxicity through significant antigen internalization but that the rate limiting step, endosomal escape, is independent of binding affinity, target, and cell type. Improving our understanding of the kinetics of cellular events will allow for informed engineering of future immunotoxins and other tools to enhance their targeted delivery.