Development of Monovalent Fluorescent Probes for Single Particle Tracking of ErbB Receptors

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ABSTRACT

The erbB, or Her, family of receptor tyrosine kinases require dimerization to begin their signaling cascades. It is challenging to study the diffusion of these receptors using conventional antibody methods because a single, divalent immunoglobulin can crosslink two receptors. To address this problem, we are using a multifaceted approach to generate monovalent fluorescent probes based on highly specific antibodies, including Fabs, single-chain antibodies (scFv), and Nanobody molecules for conjugation to quantum dots (QDs) for single particle tracking (SPT). These nanoprobes will serve as an important tool in studying erbB receptor dynamics and provide high spatiotemporal resolution.

EXTENDED ABSTRACT

The erbB, or Her, family of receptor tyrosine kinases regulate signaling involved in many cellular functions, including cell growth and motility. Moreover, aberrant signaling by erbB receptors is implicated in many cancers. The mechanisms of signal initiation are not completely understood, though hetero- and homodimerization appear to be required to initiate signaling. Despite biochemical and crystallographic data, direct evidence of erbB receptor interplay on living cells has remained elusive. Live cell microscopy can visualize receptor dynamics, however it is challenging to study the rapid diffusional dynamics of these receptors using conventional antibody labeling methods since a single, divalent immunoglobulin (IgG) can artificially crosslink two receptors. To address this problem and minimize artifacts, we are using a multifaceted approach to generate monovalent fluorescent probes based on highly specific antibodies; these include Fabs, single-chain antibodies (scFv), and Nanobody molecules for conjugation to quantum dots (QDs) for single particle tracking (SPT).

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Semiconducting nanocrystals (or QDs) are highly photostable and bright probes, characterized by wide excitation and narrow emission spectra [1]. The use of QDs allows for long-term and multicolor SPT. Epidermal Growth Factor (EGF), the ligand for erbB1, has been conjugated to QDs for use in live cell imaging to study liganded-receptor dynamics [2]. We are developing further monovalent probes to monitor the motion of unliganded receptors; these include an Fab prepared by papain digestion of an anti-erbB1 IgG antibody and Nanobodies raised against the extracellular domain of the receptor [4]. We have also coupled Herceptin, a humanized antibody commonly used to treat breast tumors with erbB2 overexpression, to QDs in order to track erbB2 diffusion. We perform multicolor SPT using two or more spectrally distinct QDs to visualize dimerization and diffusion events. This data is captured at video rate and we use novel analysis routines to quantify receptor interactions. Combinations of erbB1 and erbB2 probes will be used to monitor heterodimerization.

Ultimately, we plan to prepare monovalent probes specific to each of the four erbB family members. These nanoprobes will serve as an important tool in studying erbB receptor dynamics and provide high spatiotemporal resolution. This work takes advantage of many microscopy and analytic methods in order to elucidate the regulation of erbB signaling.

REFERENCES

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[4] Nanobodies are a kind gift of P.M.P. van Bergen en Henegouwen. Roovers et al., Cancer Immunol Immunother 56 (2007) 303-317.