Supplementary material to

Binding of trastuzumab to ErbB2 is inhibited by a high pericellular density of hyaluronan

Tímea Váradi, Tamás Mersich, Päivi Auvinen, Raija Tammi, Markku Tammi,

Ferenc Salamon, István Besznyák Jr, Ferenc Jakab, Zsolt Baranyai, János Szöllősi, Peter Nagy

SUPPLEMENTARY MATERIALS AND METHODS

1. Cells, antibodies

SKBR-3 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and grown according to their specifications. For flow cytometric FRET experiments cells were harvested by trypsinization. The anti-ErbB2 antibody, trastuzumab (Herceptin®), was purchased from Roche (Basel, Switzerland) and pertuzumab (Omnitarg®) was a kind gift from Genentech (South San Francisco, CA). The OP15 mAb against an intracellular epitope of ErbB2 was obtained from Calbiochem-Merck Biosciences (Schwalbach, Germany). Antibodies were labeled with AlexaFluor546 or AlexaFluor647 (Invitrogen, Carlsbad, CA) according to the instructions of the manufacturer. Secondary labeling was carried out using AlexaFluor647 goat anti-mouse IgG (Invitrogen).

2. Labeling of cells with fluorescent antibodies

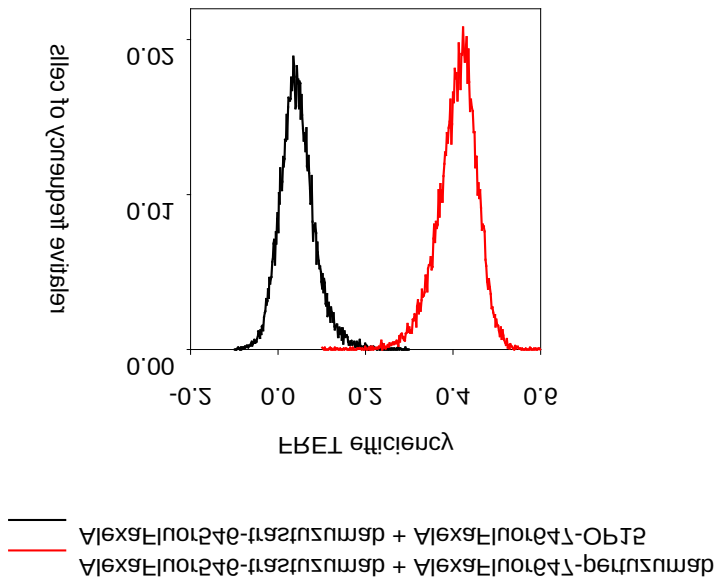
Trypsinized cells were fixed in 4% formaldehyde for 30 min and washed twice in Tris buffer. ~10^6 cells were suspended in PBS-BSA-TX (PBS containing 0.1% BSA and 0.1% Triton X-100) and samples were incubated in the presence of primary antibodies for 30 min followed by

washing and staining with secondary antibodies for 60 min. Finally, samples were fixed in 1% formaldehyde.

Fluorescence resonance energy transfer (FRET) was measured with a FacsArray flow cytometer (Becton Dickinson, Franklin Lakes, NJ). Antibodies labeled with AlexaFluor546 and AlexaFluor647 were used as donor and acceptor, respectively. The donor, FRET and acceptor fluorescence intensities were measured in the Yellow, Far Red and Red channels, respectively. The Yellow and Far Red intensities were excited with a 532 nm solid state laser and detected using a 585/42 nm bandpass and a 685 nm longpass filter, respectively. The Red intensity was excited at 635 nm using a diode laser and measured using a 661/16 nm bandpass filter. The necessary controls, calibration samples and evaluation principles have been described elsewhere (Nagy et al. 2006). The FRET efficiency was calculated on a cell-by-cell basis using the ReFlex software (www.freewebs.com/cytoflex) (Szentesi et al. 2004).

SUPPLEMENTARY FIGURES

Figure S1 Intramolecular FRET measurements between ErbB2 epitopes: trastuzumab and OP15 are outside FRET distance from each other.



SKBR-3 cells were labeled with AlexaFluor546-trastuzumab and OP15 secondarily stained with AlexaFluor647-goat anti-mouse IgG (black curve). As a positive control SKBR-3 cells were dual-stained with a mixture of AlexaFluor546-trastuzumab and AlexaFluor647-pertuzumab to measure the strong intramolecular FRET usually observed between these two epitopes (Nagy et al. 1998). Cells (20000/sample) were measured by flow cytometry and FRET was calculated on a cell-by-cell basis and is displayed as decimal fraction. The FRET value of 0.04 obtained for the trastuzumab-OP15 donor-acceptor pair (as opposed to the strong FRET of 0.4 for the positive control) implies that FRET does not take place between the extracellular trastuzumab and intracellular OP15 epitopes.

SUPPLEMENTARY REFERENCES

Nagy P, Bene L, Balázs M, Hyun WC, Lockett SJ, Chiang NY, Waldman F, Feuerstein BG, Damjanovich S, Szöllősi J (1998) EGF-induced redistribution of erbB2 on breast tumor cells: flow and image cytometric energy transfer measurements. Cytometry 32:120-131

Nagy P, Vereb G, Damjanovich S, Mátyus L, Szöllősi J (2006) Measuring FRET in flow cytometry and microscopy. In Robinson JP, ed. Current Protocols in Cytometry. New York, John Wiley & Sons, 12.18.11-12.18.13

Szentesi G, Horváth G, Bori I, Vámosi G, Szöllősi J, Gáspár R, Damjanovich S, Jenei A, Mátyus L (2004) Computer program for determining fluorescence resonance energy transfer efficiency from flow cytometric data on a cell-by-cell basis. Comput Methods Programs Biomed 75:201-211