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Single Molecule and Single Cell Analysis of HER2 Receptors in Breast Cancer Cells Using Liquid Phase Scanning Transmission Electron Microscopy

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HER2 positive breast cancer is an aggressive form of cancer, diagnosed in about 20% of breast cancer patients [1]. HER2-targeted antibody drugs trastuzumab [2] and pertuzumab are the standard of care in combination with chemotherapy in the metastatic setting. These targeted drugs prevent activation of HER2 via dimerization and subsequent activation of growth signaling. In early and metastatic stages of the disease this treatment strategy significantly improves survival. However, many patients do not respond at all, and by far most patients have developed so-called acquired resistance by about a year of initial treatment [3]. But despite extensive research, effective remedies against drug resistance development are lacking. Our research is based on the hypothesis that particular subpopulations of cancer cells at the tumor site play a critical role in the development of drug resistance. These cells are characterized by different morphology and receptor expression levels. The problem of drug resistance development should thus be tackled by taking cancer cell heterogeneity into account [4]. However, this has been challenging until now due to limitations of the available analytical methods. Biochemical methods generally used to study the cellular responses to drugs are not capable of examining proteins in intact single cells and use pooled cellular material. Information is thus obtained only from average responses across a cell population, insufficient for studies including cell heterogeneity. A range of microscopy techniques exist but none of these provides the required localized stoichiometric information for endogenously expressed proteins.

SKBR3 cells, an HER2 overexpressing human breast cancer cell line, were studied with correlative direct interference contrast (DIC)-, fluorescence microscopy, and liquid-phase scanning transmission electron microscopy (STEM) [5]. In contrast to conventional electron microscopy studies, the cells were imaged as a whole and in liquid state, so that the membrane proteins remained in the intact plasma membrane. The locations of individual HER2 receptor molecules were detected using a specific label consisting of an anti-HER2-Affibody in combination with a quantum dot (QD) nanoparticle. Fluorescence microscopy revealed considerable differences of HER2 membrane expression between individual cells, and between different membrane regions of the same cell (Fig. 1A). Subsequent STEM images of the corresponding cellular regions (Fig. 1B) provided images of individually labeled HER2 receptors (Fig. 1C). The high spatial resolution of 3 nm, the 1:1 labeling stoichiometry, and the close proximity between the QD and the receptor allowed quantifying the stoichiometry of HER2 complexes as well as distinguishing between monomers, dimers, and higher order clusters via statistical analysis using the pair correlation function. HER2 distribution patterns were also determined for two distinct cellular sub-populations, flat/resting cells, and cancer stem cells, revealing a different functional state of the HER2 receptor in these sub-populations. The influence of the drug trastuzumab was studied, and the results analyzed per cancer cell sub-population. Our findings indicate that flat cells and cancer stem cells respond differently to the drug, which could be a factor driving the development of drug resistance. In general, the results show that our experimental approach is feasible and can be used to study the

influence of drugs on HER2 homodimerization at the single-cell level aiming to understand the molecular mechanism behind drug resistance in cancer [6].

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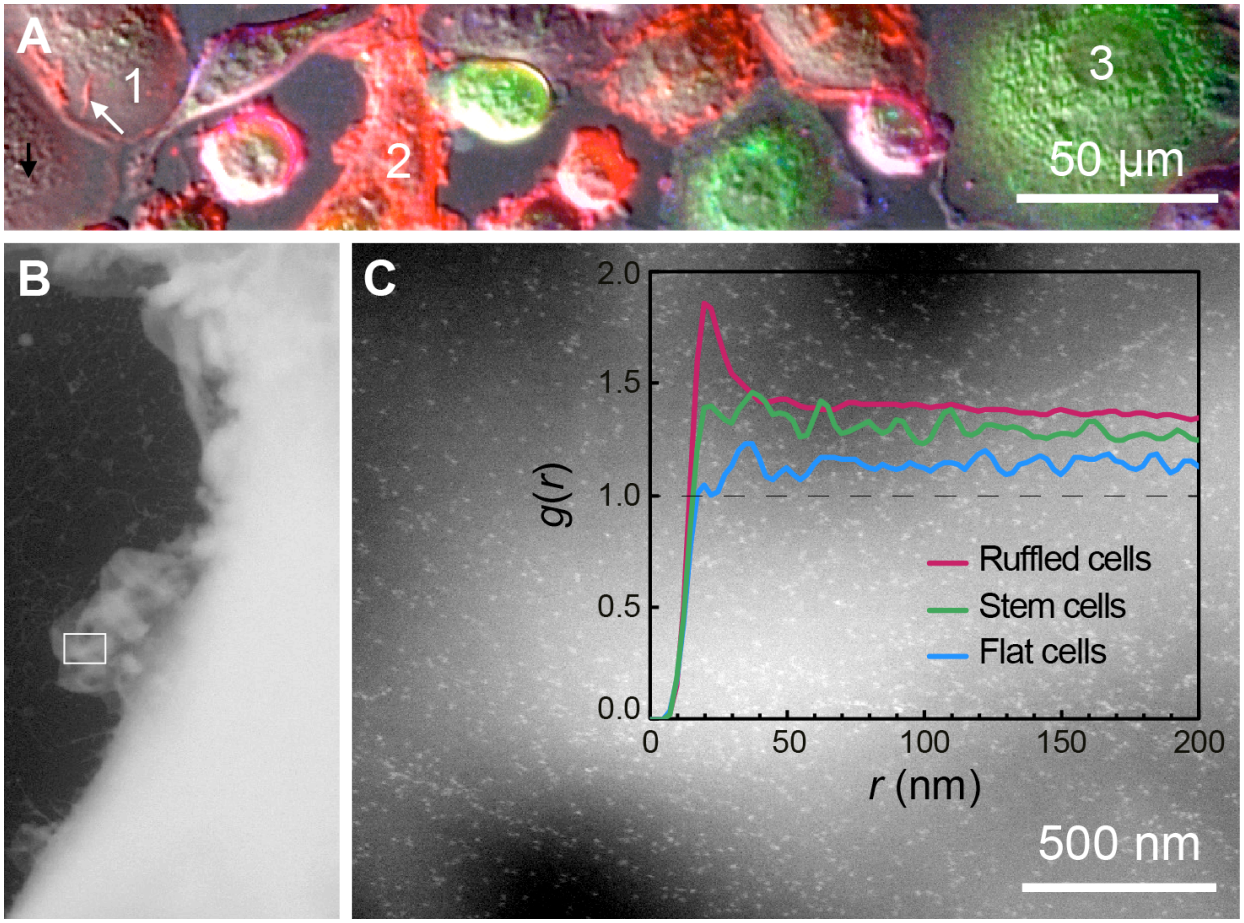

Figure 1. SKBR3 breast cancer cells studied with correlative microscopy. (A) Overlay of DIC- and fluorescence signals. HER2 proteins were labeled with QDs (red color). Cancer STEM cells are visible in green (CD44+); the absence of CD24 (blue) was also tested. Cell #1 is a ruffled cell (an example of a ruffle is indicated with an arrow) and a low abundance of HER2. Cell #2 is a ruffled cell with a high abundance of HER2. Cell #3 is a breast cancer stem cell. (B) Cell #2 was imaged as whole and in liquid state using environmental scanning electron microscopy with STEM detection. (C) STEM image recorded at the rectangle in B showing the individual QDs marking the locations of single HER2 proteins. The inset depicts the pair correlation function $g(r)$ measuring the probability of the pair distance (r). Three different cell types were studied, ruffled bulk cancer cells, cancer stem cells and flat/resting cells. HER2 was present as signaling active homodimer only in the ruffled cells.

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