Duolink®

APPLICATION NOTE 0763, v1.0

Studies of dimerization and activation of receptors in the EGFR-family using Duolink®

INTRODUCTION

Our aim was to investigate the dimerization and activation pattern of two receptors in the EGFR-family using breast cancer cell lines and selected colon tissues as model systems. The importance of the human epidermal growth factor receptor (HER) family in the development and progression of various cancers is widely recognized. Depending on the interaction partner for a specific HER receptor, different signaling cascades are activated. The Duolink® assay allows precise detection and quantification of proteins, protein interactions and modifications in fixed cells and tissue samples in their correct cellular context at physiologically relevant expression levels[1]. At least 11 different ligands are known that regulate HER-family receptor activation and dimerization[2]. Here we give an example of how Duolink can be used to study epidermal growth factor (EGF) receptor (EGFR) and HER2 receptor dimerization and activation patterns in cells and tissue samples. Measuring HER-family interaction patterns may have great potential as a companion diagnostic tool for the new-generation anti-cancer therapies aimed at inhibiting specific receptor dimerizations[2].

THE DUOLINK REAGENTS AND *IN SITU* PLA TECHNOLOGY

The Duolink kit series, developed by Olink Bioscience, allows the user to combine any pair of immunofluorescence or immunohistochemistry validated antibodies. Duolink read-out is performed either with a fluorescent label, for fluorescence microscopy, or HRP for brightfield detection. The resulting distinct spots are derived from single-molecule protein-interaction events, which are visualized using a standard microscope.

STUDYING HER-FAMILY RECEPTOR DIMERIZATION AND ACTIVATION IN CELLS

To validate EGFR-HER2 dimerization and activation we used EGF, which exclusively binds and phosphorylates EGFR. Two different cell lines, SKBR3 (known to express EGFR and high levels of HER2) and MDA-MB-468 (known to express EGFR and very low levels of HER2), were stimulated with the ligand EGF. Both cell lines responded to EGF stimulation by phosphorylation of EGFR (Fig 1), which was measured using an anti-phosphorylated EGFR (pEGFR) antibody (Tyr-1068). However, the interaction between pEGFR and HER2 was detectable only in the SKBR3 cell line after EGF-stimulation.

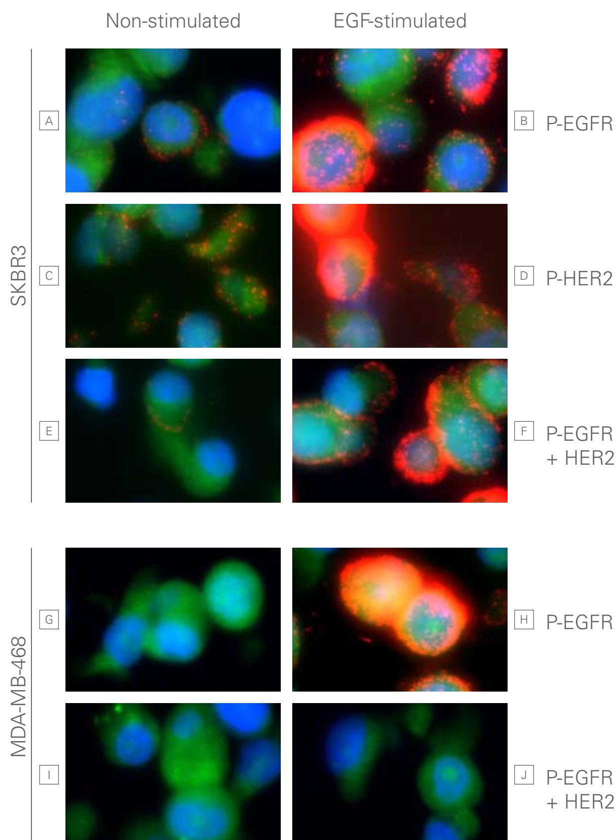

Figure 1. Two breast cancer cell lines, SKBR3 and MDA-MB-468, are assayed with Duolink before and after EGF treatment for three targets: pEGFR alone, pHER2 alone and the complex pEGFR-HER2. Red: PLA signals. Green: Sample autofluorescence (to visualize the cell morphology). Blue: nuclei (DAPI). Images were taken pair wise with the same exposure time of EGF-stimulated and non-stimulated samples for each assayed analyte.

This was expected, given that the MDA-MB-468 cell line has very low levels of HER2 expression. To further confirm that EGFR-HER2 interactions took place, phosphorylated HER2 (pHER2) was measured using an anti-pHER2 (Tyr1221/1222) antibody. EGF does not bind as a ligand to HER2; therefore, HER2 can be activated after EGF stimulation only via a complex with EGF-stimulated EGFR. The result showed a

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strong phosphorylation of HER2 in SKBR3. The use of an antibody targeting pEGFR instead of total EGFR ensured that it was an activated EGFR-HER2 complex that was measured and not an inactive, preformed complex that does not elicit downstream signaling[3,4]. The cells were formalin fixed and paraffin embedded.

STUDYING TYROSINE KINASE RECEPTOR DIMERIZATION AND ACTIVATION IN TISSUE

The Duolink assay validated on cells (Fig 1) were transferred and applied directly to tissue samples, one case of serrated adenoma from colon and one case of colon primary tumor. Both cases were known to contain high levels of pEGFR (Fig 2A and 2B) and were assayed for pEGFR-HER2 complexes using the same assay as shown on cells in Figure 1. Of these two samples, only the serrated adenoma exhibited high levels in the pEGFR-HER2 assay. A complex between activated EGFR and HER2 should lead to phopshorylation also of the HER2 receptor. The Duolink assay measuring pHER2 presented high levels of pHER2 in the same tissue compartment in serrated adenoma that displayed high levels of pEGFR-HER2 complexes (Fig 2E). The case of primary tumor had low expression of pHER2 (Fig 2F), thus displayed low levels of the complex pEGFR-HER2 (Fig 2D) as well. The formalin fixed and paraffin embedded tissue were anonymized, only the pathological anatomical diagnosis were known.

Another example of how Duolink can be used to study HER-family interactions was recently published for the HER2-PTK6 complex detected in FFPE material[5]. The result indicates prognostic relevance of the HER2-PTK6 complex, showing the importance of investigating protein-interaction events for diagnostic use.

CONCLUSIONS

The Duolink reagents offer a unique possibility to decipher HER-family receptor interactions. It holds great promise to further fine-tune companion diagnostics for HER-family receptor targeted therapies as well as a means to measure effect of new drugs targeting specific interactions. *In situ* PLA, using Duolink reagents, is a straightforward process for reporting protein interactions with very high specificity in their natural context at physiological expression levels, also of homodimerization events. This enables an efficient and simple way for both validation and characterization of protein interactions. The Duolink PLA probes ensure that any immunofluorescence or immunohistochemistry validated antibody can be used to fit the unique application of the user.

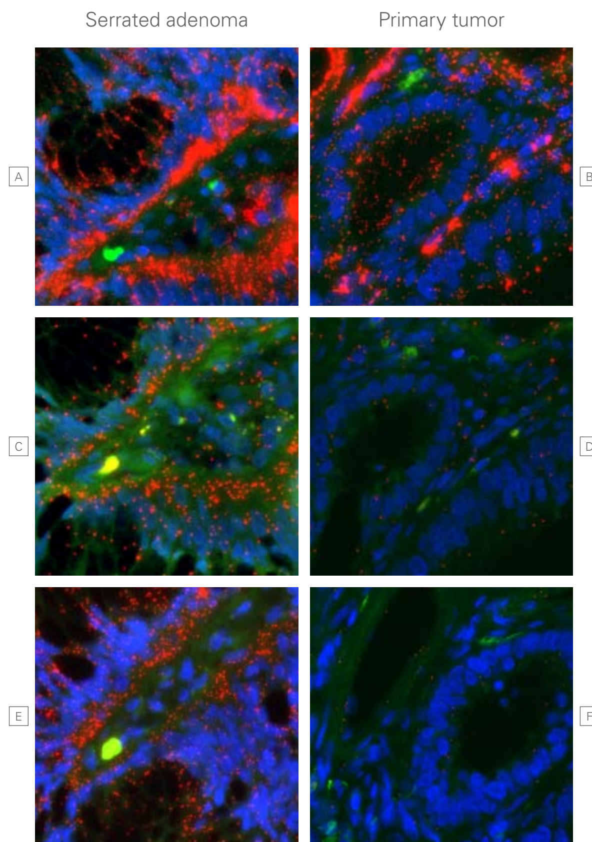

Figure 2. Duolink assay of HER-family dimerization and activation in colon tissue samples. Both samples contained high levels of pEGFR (A,B) but only the serrated adenoma showed high levels of pEGFR-HER2 complexes (C,D) and of pHER2 (E,F). Red: PLA signals. Green: Sample autofluorescence (to visualize the cell morphology). Blue: nuclei (DAPI).

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