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The use of optical assays in the development of receptor kinase specific antitumor therapy

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A variety of human cancers have been associated with over-expression or mutations of the tyrosine kinase receptors. Specifically, the mechanism of activation of Vascular Endothelial Growth Factor Receptors (VEGFRs) and Epidermal Growth Factor Receptor (EGFR), which involve numerous ligands, multiple dimerization partners and a variety of downstream signaling components, are interesting therapeutic drug targets.

Application of Bimolecular Fluorescence Complementation (BiFC) and Fluorescence Resonance Energy Transfer (FRET) imaging techniques, specifically developed and tested along with control sets for the VEGFR1 and VEGFR2 proteins, allowed to monitor the dimerization between different VEGFR subtypes. Both optical assays confirmed the occurrence of VEGFR1 homodimers and VEGFR1/VEGFR2 heterodimers in intact single cells. In addition, the relation between VEGF and VEGFR1 homodimer formation was confirmed. VEGFR1/VEGFR2 heterodimers formed independently from VEGF stimulation. Inhibition using a VEGF antibody did impair the formation of VEGFR1 homodimers but not the formation of heterodimers, suggesting the existence of preformed VEGFR1/VEGFR2 heterodimer complexes. Consequently, FRET assay of the dimerization has revealed that the ligand-independent dimerization exclusively occurs in the presence of VEGFR2. This finding leads to a better understanding of the selective roles of VEGF ligands and their corresponding receptors in control and maintenance of angiogenic system. The experimental techniques developed in this work will further allow the investigation of similar receptor kinase proteins. Moreover the techniques can be improved further for monitoring the dynamics of activation processes of the signaling pathways. This may in the future help to develop angiogenesis-targeted drugs for therapeutic purposes in oncologic therapy.

EGFR is another well-established target in tumor therapy. The use of EGFR targeted therapies is growing rapidly for the treatment of solid tumors. Using optical techniques such as FRET and FACS, the therapeutic effect of EGFR tyrosine kinase inhibitor AG1478 and the cytotoxic agent 5-FU were described. The application of a FRET assay to measure the phosphorylation level of EGFR showed a strong correlation with the respective ELISA outcomes which underlines the utility of antibody-based FRET assays to detect the EGFR phosphorylation in single cells. Neither assay indicated any influence of 5-FU on EGFR phosphorylation. Neither apoptosis could be increased, nor could cell proliferation be inhibited using combinations of AG 1478 and 5-FU. Therefore, this experimental data argues against the clinical combination of 5-FU with EGFR-tyrosine kinase inhibitors.