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Clinical Cancer Research

Fluorescence Resonance Energy Transfer Biosensors for Cancer Detection and Evaluation of Drug Efficacy

Shaoying Lu[1] and Yingxiao Wang[1,2]

A sensitive and specific fluorescence resonance energy transfer (FRET) biosensor was developed by Mizutani and colleagues and applied to detect the activity of BCR-ABL kinase in live cells. This biosensor allowed the detection of cancerous and drug-resistant cells, and the evaluation of kinase inhibitor efficacy. Future biosensor development and imaging can increasingly contribute to cancer diagnosis and therapeutics. *Clin Cancer Res; 16(15); 3822–4. ©2010 AACR.*

In this issue of *Clinical Cancer Research*, Mizutani and colleagues report on the development and application of a sensitive and specific biosensor, based on fluorescence resonance energy transfer (FRET), for the quantification of BCR-ABL activity and its response to drugs in living cells (1). This work pioneers the application of FRET-based biosensors to evaluate the efficacy of the kinase inhibitors with a clinically relevant experimental design. FRET between two chromophores occurs when the donor chromophore in an excited state, through nonradiative dipole coupling, transfers energy to a correctly oriented acceptor at close proximity. Because FRET signals are independent of the chromophore concentration and excitation light intensity, the FRET mechanism has been widely applied to developing fluorescent biosensors for visualizing molecular activities in live cells with high spatiotemporal resolution.

Recently, progress in molecular therapy has highlighted the targeting of certain malfunctioning molecules and pathways in cancer (2). For example, the small molecule kinase inhibitor imatinib has achieved great success in treating chronic myeloid leukemia (CML) and gastrointestinal stromal tumors, whose growth is acutely dependent on the expression of specific kinase mutants (3). Ideally, the efficiency of a kinase inhibitor needs to be evaluated at the level of protein interactions, the living cells, and in animal models (4). At the protein level, the potential of kinase inhibitors to inhibit the phosphorylation of a substrate protein or peptide can be evaluated quite conveniently, even via commercially available services. However, there is a lack of technology to accurately evaluate the effect of kinase inhibitors in live cells. The genetically encoded FRET biosensors, based on fluorescent proteins (FP), can be ideal candidates for this purpose (5). The feasibility of applying FRET biosensors to screening kinase inhibitors is underscored by the recent development of numerous FRET-based biosensors, such as those for the detection of oncogene-related kinase activities including Src, FAK, PKA, EGFR, and ABL (6, 7), and of other molecules important for migration and cancer invasion (5).

In their report in this issue of *Clinical Cancer Research*, the authors used CrkL, a major substrate of the BCR-ABL kinase containing both tyrosine and SH2 domain, to be sandwiched between Venus (a variant of yellow FP) and enhanced cyan FP (ECFP). When the BCR-ABL kinase is active, the phosphorylated tyrosine site on CrkL can bind its intramolecular SH2 domain to cause a conformational change and, subsequently, FRET increase. The sensitivity of this biosensor was further improved by truncating the C terminus of the substrate CrkL sequence, circularly permutating the donor ECFP, and monomerizing the acceptor Venus. Although it is possible to further improve the biosensor sensitivity by replacing Venus with a bright yellow FP, which can form weak dimers with ECFP and hence increase the FRET efficiency of the activated biosensor (8), the final version of this FRET biosensor, termed Pickles, displayed a remarkable 80% increase of FRET ratio *in vitro* upon stimulation by BCR-ABL. The authors, then, carefully verified the specificity of the Pickles biosensor toward BCR-ABL by screening against an array of kinases. Mutations on the tyrosine site Y207 and the SH2 domains further confirmed that the FRET change of Pickles was indeed due to the designed intramolecular binding between the SH2 domain and phosphorylated tyrosine site within the CrkL sequence.

With the BCR-ABL FRET biosensor optimized, the authors further examined the efficacy of several established drugs for the treatment of CML, using cell lines and primary cells expressing this biosensor (Fig. 1). The results revealed that the FRET biosensor can detect the

Authors' Affiliations: Departments of [1]Bioengineering & Beckman Institute for Advanced Science and Technology, and [2]Molecular and Integrative Physiology, Neuroscience Program, Center of Biophysics and Computational Biology, Institute of Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois

Corresponding Author: Yingxiao Wang, University of Illinois, Urbana-Champaign, 4261 Beckman Institute, 405 N. Mathews Avenue, Urbana, IL 61801. Phone: 217-333-6727; Fax: 217-265-0246; E-mail: yingxiao@illinois.edu.

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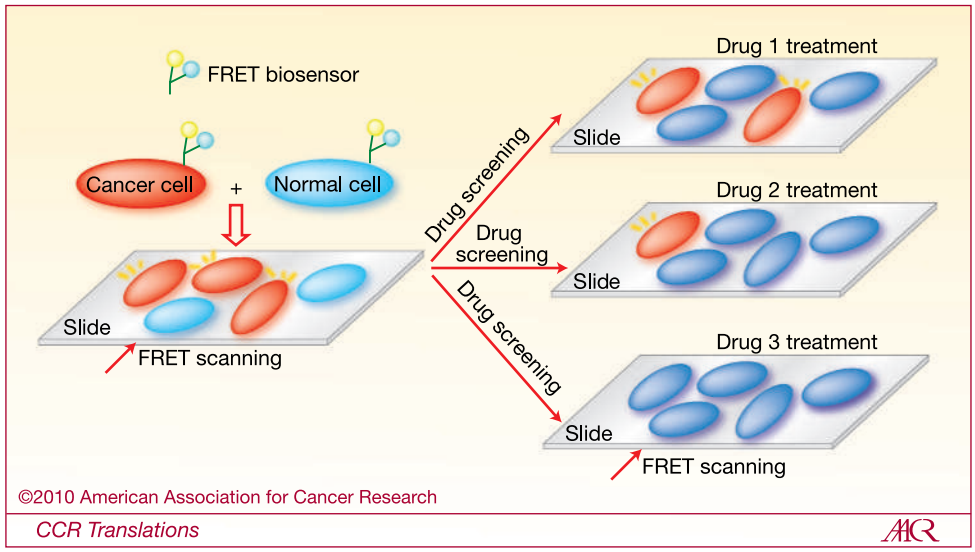

Fig. 1. The application of a FRET biosensor for drug screening. Cancer (red) and normal (blue) cells from biopsy samples can be introduced with FRET biosensors to detect cancerous molecular activities, for example, BCR_ABL kinase activity. FRET scanning can identify the cancer cells and quantify their cancerous activities on the basis of the FRET signals. The biopsy samples and cells expressing the biosensors can be subjected to different drug treatments to assess the efficacy of different drugs in inhibiting the target molecular activities.

inhibitory effect of imatinib at a concentration as low as 0.1 μmol/L. In contrast, established approaches based on western blotting or antibody staining, coupled with flow cytometry, can only detect the imatinib effect when it reaches a concentration level of 0.5 or 1 μmol/L, respectively. Although it would be a fairer comparison if the biosensor signals were also assayed using flow cytometry when comparing to antibody staining, these quantification results showed that the sensitivity of the FRET biosensor is outstanding and clearly superior over that of western blotting, which is widely used for inhibitor screening (9). Further results revealed and verified that two second-generation BCR-ABL inhibitors, nilotinib and dasatinib, were more potent than imatinib.

More importantly, the authors showed that the FRET biosensor in combination with flow cytometry can be used to detect a small percentage of drug-resistant cancer cells mixed in a large cell population. This finding is exciting because these drug-resistant cells may likely constitute the main reason for CML relapse and therapeutic failure. Therefore, the FRET biosensor developed here can provide a powerful tool to assess the biopsy samples from a particular patient and to predict the future probability of his or her resistance to specific drugs. This tool should provide invaluable information for clinicians and physicians to identify and design alternative therapeutic approaches with better efficiency and higher chances of success.

With the power shown by the current report, it is envisioned that numerous new FRET biosensors will be developed for cancer medicine. Although its future potential is tremendous, the broad application of FRET technology to cancer detection and drug screening is in need of further improvement in several areas. First, FRET biosensors, at their current stage, are generally introduced into live cells with methods involving liposome-based delivery, electroporation, or virus infection. The typical efficiency level of these methods is around 20 to 30%. This low level of efficiency of delivering biosensors into cells, particularly into primary cells, may constitute a major obstacle for the detection of rare cancer cells, such as those detected in circulation, for example, circulating tumor cells (10). Hence, new ways of delivering biosensors into cells with a high level of efficiency are needed to promote the field of FRET application in cancer detection. Second, advanced flow cytometry with high-resolution imaging capability will be helpful to overcome the problem of cell heterogeneity and allow the high throughput screening of drugs with high accuracy and subcellular resolutions. Third, the majority of the biosensors were developed semirationally, mostly in a trial-and-error fashion on the basis of personal experience and published literature. Systematic and high throughput screening approaches are, hence, greatly needed to automate biosensor development and optimization. Finally, FRET biosensors with distinctive colors for the simultaneous visualization of multiple cancer-related molecular events, such as those previously published to visualize Src and MT1-MMP activities in the same single cell, can be very useful to provide a precise assessment and prediction of cancer characteristics and drug efficacy (11).

In summary, FRET biosensors are ideal for screening for the efficacy of kinase inhibitors or drugs in live cells. In addition to drug screening, specific and sensitive biosensors can be developed for biomarkers with applications in early cancer detection, cancer prognosis, and

monitoring of therapeutic efficacy. More importantly, FRET biosensors can be applied to visualize subcellular molecular signaling events in real time, for the identification of novel targeting molecules and pathways, which may lead to a new era in clinical cancer research.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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