High-throughput single-molecule tracking technology streamlines drug discovery

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Abstract

We have successfully developed a method for screening drugs that inhibit the function of biomolecules by applying single-molecule imaging to observe the behavior of intracellular biomolecules. In this novel drug screening approach, the effects of drugs are evaluated by observing molecular behaviors that were not utilized in conventional drug discovery, such as changes in diffusion motion, oligomer formation, and internalization associated with biomolecular signal transduction. It is expected to be applied to the development of drugs with mechanisms of action different from existing drugs and to the repositioning of approved drugs.

Background & Results

Single-molecule imaging enables observation of diffusion changes and multimer formation during biomolecule activation/inactivation, making it promising for drug screening. For this reason, its application to drug screening has been anticipated. However, its reliance on skilled manual techniques limited efficiency for large-scale applications. In 2018, we developed an automated large-scale system using machine learning and robotics, achieving over 100-fold faster throughput than manual methods. This enabled practical high-volume measurements, previously infeasible, and we applied it to screening targeting the epidermal growth factor (EGF) receptor.

The EGF receptor plays key roles in signal transduction for cell proliferation, differentiation, and apoptosis - essential for homeostasis. It's a prime anticancer target due to overexpression or mutations in many cancers. Using the developed method to observe the behavior of the EGF receptor, we conducted drug screening on a library containing over 1,000 approved drugs. Observing EGF receptor behavior with our method, we screened a library of over 1,000 FDA-approved drugs. We accurately identified all known inhibitors (tyrosine kinase inhibitors) and uncovered drugs with previously unknown EGF receptor effects from the library. This approach can detect efficacious compounds overlooked in conventional screening, opening avenues for novel drug discovery and repositioning of approved medications.

Significance of the research and Future perspective

By applying large-scale single-molecule imaging to drug screening, we obtained drugs that act on multiple events, such as changes in the diffusion motion, multimer formation and internalization of signal transduction molecules. Since this method can select compounds that would be overlooked in conventional screening, it is considered to be one of the effective options for new drug discovery or for exploring drugs for diseases that have been difficult to evaluate until now. Furthermore, the automated large-scale single-molecule imaging, which forms the basis of this method, provides an effective technique for basic research to elucidate the mechanisms of functional expression of membrane proteins such as receptors and ion channels present on the cell membrane.

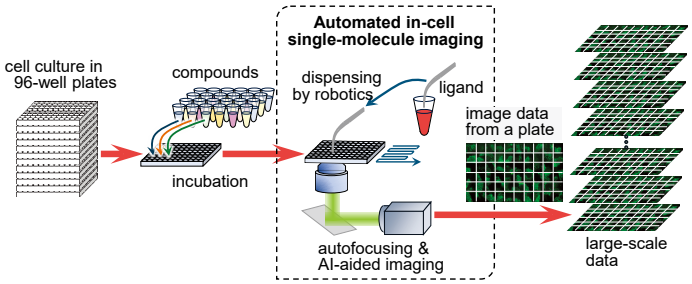

Fig 1. Overview of drug screening via large-scale single-molecule imaging

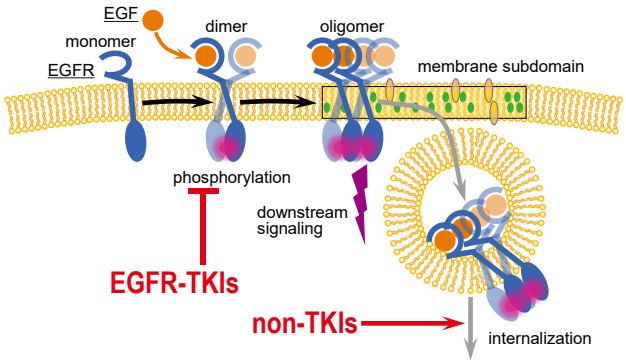

Fig 2. Compounds showing different effects on EGFR obtained from the single-molecule tracking-based screening

Patent	Japanese Patent No.6952300, No.7226825, US11002728B2, US11567293B2, Japanese Patent Application No.2023-031358
Treatise	Yasui, Masato; Hiroshima, Michio et al. Automated single-molecule imaging in living cells. Nature Communications. 2018, 9, 3061. doi: 10.1038/s41467-018-05524-7 Watanabe, Daisuke; Hiroshima, Michio et al. Single molecule tracking based drug screening. Nature Communications. 2024, 15, 8975. doi: 10.1038/s41467-024-53432-w
URL	https://www.fbs.osaka-u.ac.jp/en/research_group/detail/2
Keyword	single-molecule imaging, large-scale automated measurement, cell membrane receptor, drug screening