Probes and Assays For Measuring E3 Ligase Activity

Published date: June 19, 2018

Technology description

Compositions and methods for studying protein ubiquitination and developing therapeutics to modulate protein ubiquitination. #biomedical #screening #assay #researchtool #method

BACKGROUND

Protein ubiquitination is a highly conserved post-translational modification that regulates fundamental cellular processes. Ubiquitin conjugation is controlled by the sequential action of three enzymes: ubiquitin activating enzyme 1 (E1, 2 known variants), ubiquitin conjugating enzyme 2 (E2, ~37 known variants), and ubiquitin ligase enzyme 3 (E3, ~600 known variants). Among these, E3 enzymes stand out due to the astonishing complexity and diversity of biochemical reactions they catalyze. Such complexity makes it difficult to study their biochemical properties and design assays to help discover and characterize pharmacological modulators of E3s. Typical biochemical assays to study E3 enzymes require at least three enzymes E1/E2/E3, ubiquitin, and ATP in the simplest case. As such, studying protein ubiquitination and developing therapeutics targeting protein ubiquitination are difficult due to the complexity of the E1→E2→E3 ubiquitination cascade.

ABSTRACT

Northwestern inventors have developed a two-component ubiquitination system for high throughput screening for pharmacological inhibitors/activators of ubiquitin ligases. The first component enables E3 ubiquitin ligases that contain catalytic cysteines to undergo auto-ubiquitination and substrate auto-ubiquitination in the absence of ATP, E1 and E2 enzymes, substantially simplifying the reaction conditions. In a second component, they designed and created a fluorescent C-terminal ubiquitin thioester named UbiFlu for their low-cost, sensitive, and simple high throughput-screening assay for discovering small molecule inhibitors or activators of HECT E3, RBR E3, or NEL E3 ubiquitin ligases. These enzymes are emerging drug targets to treat cancers, neurodegenerative diseases, pathogenic infections. In the developed assay, UbiFlu reacts with the target E3 ligases and releases the fluorophore. Thus, the enzymatic reaction progress can be continuously monitored using either an in-gel fluorescence scanning method or fluorescent polarization assay. The developed method provides substantial advantages over existing methods of E3 ligase inhibitor screening. Since the developed assay obviates the need for ATP, E1, and E2 enzymes in the reaction mixture, it reduces the cost of the assay as well reduces the number of false positives caused by off target inhibition of E1 or E2 enzymes. Given that E3s are deregulated in many human diseases, this assay and the UbiFlu probes are powerful

tools to discover new drug leads to treat cancers, neurodegenerative diseases, hypertensive disorders, autoimmune disorders, and pathogenic infections.

Publications

Park S, Krist, DT, Statsyuk, AV (2015) Protein Ubiquitination and Formation of Polyubiquitin Chains without ATP, E1 and E2 Enzymes. Chemical Science. 6: 1770-1779.

Application area

High throughput screening assay for pharmacological inhibitors/activators of HECT E3s, RBR E3s, and NEL E3 ubiquitin ligases

Advantages

Economical
Sensitive
High throughput-screening
Reduced likelihood of false positive readouts

Institution

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