A Rapid, Cell-Based based High-Throughput Assay for Small Molecule Inhibitors of RBP–RNA Interactions

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Background: Epigenetic RNA modifications direct the expression or repression of target genes during development and disease, particularly oncogenesis. There is a growing interest in the development of therapeutic modulators for epigenetic pathways that drive disease phenotypes. Importantly RNA-binding proteins (RBPs) form a major class of proteins that influence epigenetic processes and represent a novel class of drug target. To study RNA-protein interactions, well-established proximity assays such as AlphaLISA require high-quality purified protein and are significantly limited in their ability to demonstrate an in-cell target engagement. Current assays used to assess disruption of RNA-protein interactions in cell lysates, such as EMSA or RNA immunoprecipitation, are typically semi-quantitative and labor-intensive, making them poorly suited for high-throughput screening. A homogenous sensitive cell-based assay is therefore needed to complement these existing methods and support the development of inhibitors of RNA-protein interactions. Method: In this study, we adapted NanoBRET (bioluminescence resonance energy transfer) technology by tagging an endogenous RBP with NanoLuc luciferase. A stable cell line was generated that constitutively expresses robust NanoLuctagged RBP, eliminating the variability associated with transient transfection protocols. The interacting RNA oligonucleotide was synthesized with an Alexa Fluor 594 label to serve as the fluorescent acceptor of bioluminescence energy transferred from the C-terminally fused NanoLuc-RBP donor. An unlabeled version of the oligonucleotide served as a competitive binder to monitor ligand displacement. The BRET ratio was calculated by dividing the acceptor emission value by the donor emission value. Results: Our RNA-to-RBP binding assay successfully provided measurements of binding affinities and inhibitor-mediated disruption in an endogenous cell system. EC50 values for novel RBP inhibitors, determined using existing and sensitive methods, were consistent with those obtained from the NanoBRET assay. Conclusion: The successful demonstration in utility as a robust platform for high-throughput discovery of small-molecule modulators of RNA-to-RBP interactions