Small Molecule Fusion Compounds for the Optimization of MASPIT, a High Throughput Three-Hybrid Target Deconvolution Assay

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Methods that allow high throughput identification of cellular targets of bioactive small molecules are invaluable assets in pharmaceutical research. They are useful in mechanism of action studies of hits identified via phenotypic screening. Alternatively, they may uncover 'off-target' proteins of established drugs, that may contribute to their therapeutic efficacy or unwanted side effects (polypharmacology). Finally, such methods also allow to identify potential novel therapeutic applications of existing drugs within the scope of drug repositioning projects.

MAmmalian Small molecule-Protein Interaction Trap (MASPIT) is a three-hybrid system that enables high throughput proteome-wide screening for intracellular targets of small molecules in intact human cells, based on the JAK-STAT signaling pathway of the cytokine receptor (CR).[1],[2] MASPIT employs *E. coli* dihydrofolate reductase (DHFR), fused to the CR to present a methotrexate (MTX) fusion compound (MFC) to the intracellular environment. This allows the screening of a small molecule bait against a collection of chimeric prey proteins. As a result of the interaction between the bait and a prey protein, the JAK-STAT pathway is activated, resulting in the expression of the luciferase reporter gene.

A *conditio sine qua non* for successful MASPIT analysis is the availability of appropriate synthetic probes.[3] In this presentation we will discuss the evaluation of two alternative chemical dimerizer approaches aimed at increasing the sensitivity of MASPIT.[4] To circumvent the potential limitations related to the binding of MTX to endogenous human DHFR, we explored trimethoprim (TMP) as an alternative prokaryote-specific DHFR ligand.[5] MASPIT evaluation of TMP fusion compounds with tamoxifen, reversine, and simvastatin as model baits, resulted in dose-response curves shifted towards lower EC_{50} values than those of their MTX congeners. Furthermore, we present a scalable synthesis of a versatile TMP-azido reagent that displayed a similar improvement in sensitivity, possibly owing to increased membrane permeability relative to the MTX anchor. Applying the SNAP-tag approach[6] to introduce a covalent bond into the system, on the other hand, produced an inferior readout than in the MTX or TMP-tag based assay. Therefore, further optimization efforts so as to stabilize the ternary complex in order to capture the transient and possibly low-affinity bait-prey interactions, were initiated by implementing photoactivatable crosslinkers.

References

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