A Live Cell Imaging Assay for siRNA Enhancers and Suppressors of Inhibitors of Mitotic Kinesin-5

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Abstract

Kinesin-5 inhibitors (K5I) are a class of potential anti-cancer drugs targeting the Kinesin-5 motor protein. Cells treated by K5I undergo sustained mitotic arrest with monopolar spindles, and, ultimately, apoptosis. To study the underlying mechanisms of arrest and death in the response to K5I, we performed two genome-wide RNA interference screens.

An enhancer screen was carried out at low inhibitor concentration to identify siRNAs that enhance the cell response to K5I; a suppressor screen was carried out at high KI5 concentration to identify siRNAs that suppress the response. Using HeLa cells expressing a histone H2B-GFP fusion, we developed a live-cell imaging assay to track cell response to K5I over 72 hours. A screening microscope was used to acquire single wavelength images at 24 hour intervals after gene knockdown and drug addition in order to track phenotypic changes. CellProfiler and CellVisualizer analysis software allowed us to identify key cellular phenotypes such as monopolar spindles and normal bipolar spindles in a high throughput, automated manner.

We have identified 20 KI5 enhancer and 100 KI5 suppressor siRNAs. As expected, we found some spindle assembly checkpoint and cell cycle checkpoint proteins, but we have also identified several novel genes. These results not only enable us to better understand the cell response to K5Is, but also provide new tools/targets for studying the action of anti-mitotic drugs.