Identification of allosteric modulators of KRas using second harmonic generation

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The Ras protein family consists of small GTPases that are critical signaling transducers regulating cellular processes including proliferation, differentiation, and survival. As monomeric GTPases, Ras proteins cycle between an inactive GDP-bound state and an active GTP-bound state. Oncogenic mutations in one of the Ras isoforms, H-, N-, and K-Ras, impair GTP hydrolysis, thus de-regulating Ras signaling. Ras mutations are found in one-third of human cancers, and tumors bearing these mutations are notoriously difficult to treat. Conventional approaches to inhibit Ras with small molecules have been largely unsuccessful leading many to characterize the Ras family as an “undruggable” target (Stephen et al, 2014). However, there is renewed enthusiasm towards Ras drug discovery in response to a recent surge of reports revealing structural complexities to Ras proteins that provide opportunity for innovative drug discovery efforts (Stephen et al, 2014; Ostrem et al, 2013).

In light of findings suggesting KRas contains transient and dynamic binding pockets, many of which are undetectable with traditional structural techniques, we used the Biodesy Delta second harmonic generation (SHG) platform to screen for conformational modulators of KRas. SHG is an exquisitely sensitive biophysical technique that detects real-time changes in protein conformation (Salafsky, 2007; Moree et al, 2015, Journal Biophysics). When a labeled protein tethered to a membrane-coated surface is pulsed with infrared light, the SHG probe converts a portion of the incident infrared into blue light, termed the 'second harmonic signal.' The intensity of the second harmonic signal is highly dependent on the orientation of the dye probe in relation to the surface, and is extremely sensitive to relative shifts and alterations, indicative of conformational change (Moree et al, 2015, Journal Biophysics; Moree et al, 2015, Journal of Biological Chemistry).

We implemented the Biodesy SHG platform to identify Ras modulators from a chemically diverse ~2700 fragment library. Using ±3σ criteria, our hit rate was 1.4%; however, we selected a larger collection of compounds for secondary screening at lower concentrations against both GDP KRas and 'GTP' KRas. 160 hit fragments were characterized in dose response SHG assays, and ~50 were for selected for orthoganol

validation by SPR. The top 20% of detectable binders are currently under evaluation in a series of HSQC NMR experiments to characterize the binding site(s) of each compound of interest. Concurrently, we are running classical biochemical assays in the presence of these compounds to determine how KRas conformational modulators affect its function. Moving forward we will use our structural and functional insights to guide medicinal chemistry on these fragments towards the development of lead targeted KRas therapies.

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