Smoothened Cilial Translocation: Translating Mechanistic Understanding into a Novel Effective Strategy for Drug Discovery

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Abstract

The Hedgehog (Hh) signaling pathway has emerged as a therapeutic target of opportunity given the pathway's contribution to a variety of diseases, notably several neurodegenerative diseases and a spectrum of cancers. Early drug leads identified Smoothened (Smo), a membrane protein essential for signaling, as an attractive target for drug development. Accumulation of Smo in the primary cilium is a critical regulatory event in vertebrate Hh signaling. Using a novel post-translational labeling technique, we demonstrated that cilial Smo mainly originates from an intracellular source. Surprisingly, cyclopamine, a widely used Hh antagonist, induces a cilial accumulation of Smo similar to that induced by Hh ligand and several Hh agonists. In contrast, other antagonists abrogate Smo cilial accumulation. Cyclopamine's action of promoting Smo accumulation in the primary cilium correlates with prolonged hypersensitivity to pathway stimulation after drug removal, raising potential concerns in using antagonists that harbor this property in cancer therapy. Therefore, the potential for unfavorable actions of some of the existing clinical candidates argues for the need for understanding pharmacological regulation of Smo translocation to the primary cilium, and further, a focused effort to selectively identify antagonists of Smo cilial accumulation. To this end, we developed a novel high-content screen methodology based on directly observing Smo translocation to the primary cilium.

Using the system, we selectively screened pathway antagonists that block Smo cilial translocation. As we had hoped, novel hits identified in this screen can effectively block Hh response without inducing rebound hyperactivity. While this approach provides an effective new strategy for Hh targeted cancer drug development, a counterpart screen to identify drugs promoting cilial accumulation of Smo revealed potentially dangerous cross-talking of some drugs in clinic with Hh signaling. Most strikingly, we discovered that a large number of anti-inflammatory drugs stimulate Smo translocation to the primary cilium. The use of anti-inflammation treatment in conjunction with cancer therapy is common given that tumor is often surrounded by an inflammatory environment. Our findings of this unforeseen cross-talking raise concerns about ramifications of anti-inflammation drugs and potential drug-drug interactions in Hh-targeted cancer therapy.