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Investigating the mechanism of action of novel smoothened antagonists discovered by *in silico* screening of the homodimer interface

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Small molecules targeting the transmembrane domain (TMD) of the smoothened (Smo) receptor, the main transducer of the Hedgehog (Hh) signaling pathway, are in clinical trials for the treatment of basal cell carcinoma and medulloblastoma, which is one of the most aggressive brain tumors in childhood. The clinical efficacies of these inhibitors are hampered by the occurrence of point mutations located at the TMD binding site leading to resistance. Thus, identification of novel drugs with original pharmacological properties and targeting regions of the Smo protein exhibiting few mutations, would provide a new strategy for potent cancer therapies. Multiple genetic, biochemical and crystallographic data suggest that Smo functions as a homodimer. We have now identified two potentially druggable cavities at the interface of the homodimer that have been screened *in silico* to identify putative dimer-biased antagonists. From an in-house library of 5 million commercially drug-like compounds, we selected and purchased 64 compounds that we have tested for their inhibition properties using already developed cellular assays aimed at measuring Hh-induced activation through Smo. Among the positive hits identified, and after their in house synthesis, one compound (DR90) inhibited mouse cerebellar granule cell proliferation induced by Hh (IC_{50}=2.5 μM). DR90 was found to be an allosteric competitor of the Smo agonist SAG towards this response. Binding experiment analysis of DR90 to wild type human Smo receptor and to various mutants using [3H] MRT-92 and bodipycyclopamine indicated a non-conventional mode of binding for DR90. Further experiments are in progress to identify the binding mode of DR90 and related molecules to Smo. Our studies should help to design more effective therapeutic strategies for treating Hh-linked cancers and associated chemoresistance and better understanding the transduction mechanism of Smo, an atypical member of the G protein-coupled receptor superfamily.

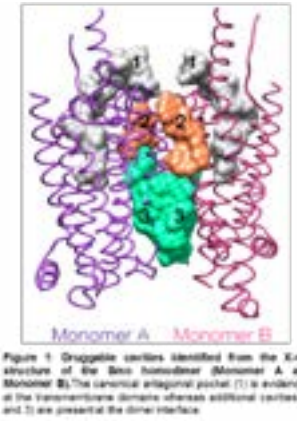

Figure 1: Druggable cavities identified from the X-ray structure of the Smo homodimer (Monomer A and Monomer B). The canonical antagonist pocket (1) is evidenced at the transmembrane domains whereas additional cavities (2 and 3) are present at the dimer interface.

Biography

P Banerjee has expertise in cellular assays for evaluating and screening pharmacological compounds stems from her thorough training in cancer biology and signal transduction mechanisms. During her earlier work for PhD, she worked with geriatric human patients to assess the efficacy of a phase IV medication for alleviating the biochemical signatures of senescence. Later she has identified the key signaling pathway of presumed antioxidants that paradoxically triggered oxidative stress, particularly in fast-growing cancer cells. Her most recent work extends to structural biology and aims to identify and characterize compounds that target a novel interfacial binding pocket in the dimeric GPCR, smoothened (Smo), mutation of which is causally associated with medulloblastoma in humans.

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