SOUTHERN RESEARCH

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High Throughout Screening For Wnt/β-Catenin Signaling Inhibitors

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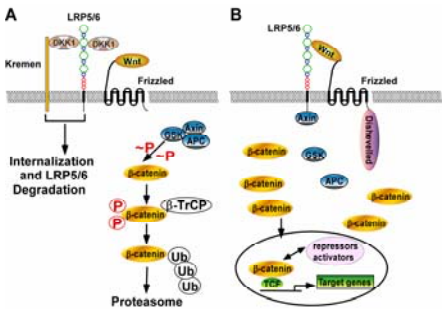
Abstract

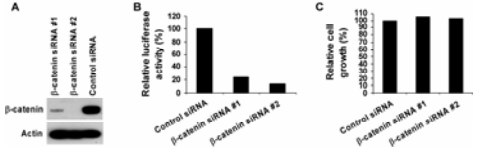
The canonical Wnt signaling pathway is involved in various differentiation events during embryonic development and when aberrantly activated can lead to tumor formation. Disruption of Wnt signal represents an opportunity for rational cancer therapy. In this study, we stably transfected Wnt signaling reporter construct Super8XTOPFlash into colorectal cancer SW480 cells, which exhibit a constitutive high level of Wnt signaling activation, and developed a cell-based HTS assay in a 384 or 1536-well plate format that allows for the identification of potential inhibitors of Wnt signaling from large compound libraries. The assay was validated by screening a diversity set of 3,200 compounds from Chembridge. With this validation screening, we identified 12 compounds that can significantly block the Super8XTOPFlash luciferase activity in SW480 cells. Among them, the activities of 7 compounds in Wnt signaling inhibition were confirmed by a secondary assay (the inhibition of alkaline phosphatase activity induced by Wnt3A in pluripotent C2C12 cells). None of these compounds or similar structures have been reported in the literature for their inhibitory activities in Wnt signaling. In addition, the inhibitory effects of three potent compounds on Wnt signaling were further verified by several secondary assays, and their anti-cancer activities were observed in several colorectal cancer cell lines. Finally, we have screened ~125,000 compounds from our institute's libraries and, identified several hundred small molecules that block the Super8XTOPFlash luciferase activity in SW480 cells.

The Wnt/β-catenin signaling pathway



The cell-based HTS assay for Wnt/β-catenin signaling inhibitors

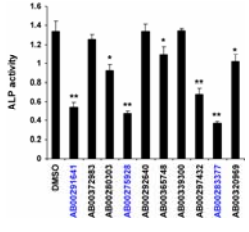
1. The Wnt/β-catenin signaling is monitored by luciferase activity with colon cancer SW480 cells stably transfected with Wnt signaling reporter construct Super8XTOPFlash. SW480 cells exhibit a constitutive high level of Wnt signaling activation due to mutations in *APC*.
2. Cell viability determination with the Alamar Blue Kit was performed as a counter-screen.
3. Primary hits: compounds that exhibit >80% inhibition of the Super8XTOPFlash luciferase signal but does not significantly affect cell growth with >75% viability, as inhibition of Wnt signaling do not immediately kill SW480 cells (see following figure and a study reported by Verma et al., Clin. Cancer Res. 9, 1291-1300, 2003).



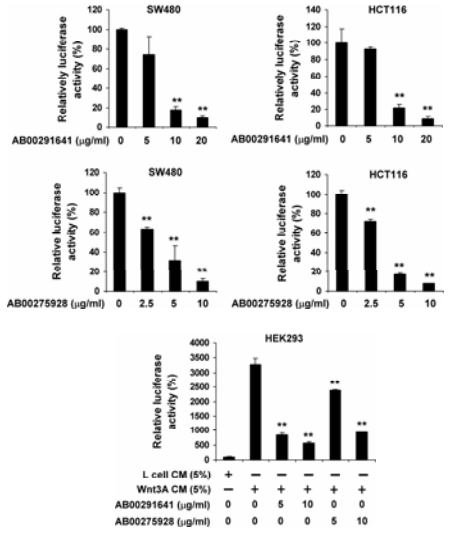
4. The assay is sensitive and robust, with Z values consistently > 0.6, signal to background (S/B) > 40, and signal to noise (S/N) > 9.
5. The assay was validated by screening a diversity set of 3,200 compounds from Chembridge.
6. With screening of ~125,000 compounds of our institute libraries, we have identified more than 250 highly active compounds that block the Super8XTOPFlash luciferase activity in SW480 cells with IC50 < 3 μM, and more than 300 moderately active compounds with 3 μM < IC50 < 60 μM.

Twelve hits identified by validation screening of a diversity set of 3,200 compounds from Chembridge at 10 μg/ml

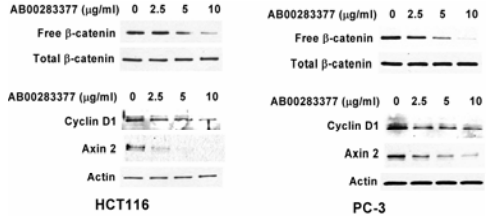
Object ID	Wnt Signaling Inhibition (%) Run 1	Run 2	Mean	Cell Viability (%) Run 1	Run 2	Mean
ABO00291641	99	100	99.5	81	79	80
ABO00372983	98	98	98	97	101	99
ABO00280303	98	94	96	84	90	87
ABO00356982	96	96	96	78	89	83.5
ABO00275928	95	95	95	86	94	90
ABO00292640	95	94	94.5	96	95	95.5
ABO00365748	93	94	93.5	91	95	93
ABO00339300	93	90	91.5	103	99	101
ABO00297432	90	90	90	93	93	93
ABO00275811	96	77	86.5	89	102	95.5
ABO00283377	91	80	85.5	79	84	81.5
ABO00320969	83	83	83	93	107	100



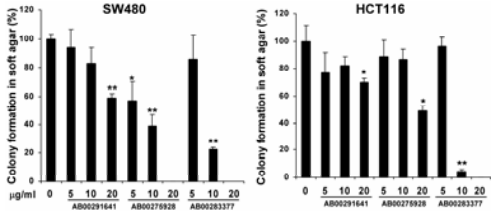
Wnt hits AB00291641 and AB00275928 block Wnt/β-catenin signaling in colon cancer cells and HEK293 cells



Wnt hit AB00283377 blocks Wnt/β-catenin signaling in colon cancer HCT116 cells and prostate cancer PC-3 cells



Wnt hits block colon cancer cell colony formation in soft agar



Future studies on hits identified from the screening of ~125,000 compounds

1. A clustering analysis of the hits and selection of chemically tractable scaffolds to pursue.
2. Various secondary assays to confirm the activity of selected hits on Wnt/β-catenin signaling.
3. Hit to lead chemistry.
4. Mechanism studies.
5. *In vitro* anti-cancer activities.
6. Animal studies.