Wnt Signaling Drug Discovery System

Time Bioscience proudly announce that the Wnt drug discovery system is available for you to run Wnt-related drug screening. The system consists of purified recombinant mouse and human Wnt3a, HEK293, HCT116, and SW480 Wnt reporter cell lines as well as their control cell lines with TCF binding site mutations. The cell lines have been stably integrated with a plasmid containing Wnt gene promoter with wild type or mutant TCF binding sites and luciferase gene. The cell lines also constantly express GFP as a control of cell numbers and viability.

The WNT gene family consists of structurally related genes that encode secreted signaling proteins. These proteins have been implicated in oncogenesis, adipogenesis, and in several other developmental processes, including regulation of cell fate and patterning during embryogenesis. Wnt3a is a protein that is encoded by the WNT3A gene, a member of the WNT gene family. Mouse Wnt3a shows 96% amino acid identity to human Wnt3a protein. Activity of the canonical Wnt signaling pathway by Wnt3a leads to nuclear translocation of β-catenin and the formation of TCF transcription factor complex. The TCF complex interacts with Wnt gene transcriptional response elements and leads to the expression of Wnt-responsive genes.

Most colorectal carcinomas harbor genetic alterations that result in stabilization of β-catenin, leading ultimately to cancer. Colorectal carcinoma cell line HCT116 harbors both wild-type and mutated β-catenin genes (deletion of codon 45). Whereas, colorectal carcinoma cell line SW480 expresses a truncated form of adenomatous polyposis coli (APC) that is a key player in β-catenin destruction complex. The mutation results in the accumulation of β-catenin. Both accumulation and direct mutation of β-catenin results in the expression of oncogenes regulated by canonical Wnt signaling.

We evaluated our Wnt drug discovery system using both Wnt3a proteins and pivotal canonical Wnt inhibitor.

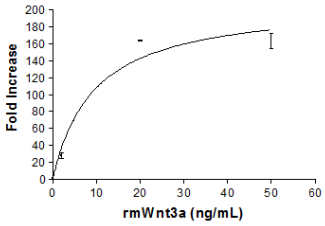

Figure 1
rmWnt3a Dose Response

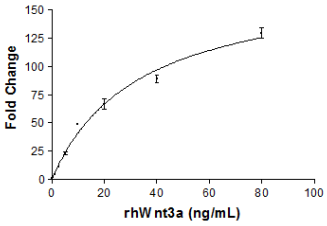

Figure 2
rhWnt3a Dose Response

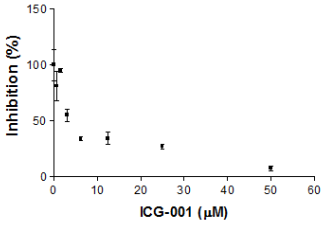

Figure 3
Inhibition of rhWnt3a Response

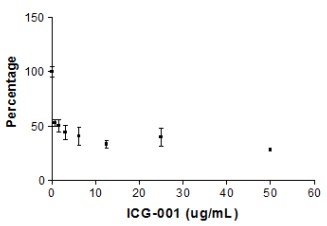

Figure 4
Inhibition of Endogenous Wnt Signaling

The luciferase activity from HEK293 Wnt reporter cell line (Cat: WRCL293A, Clone: 2A9D4E5C) increases 100 or 50 fold after 6- to 8-hour treatment with 10 ng/mL of recombinant mouse Wnt3a (Fig. 1) or recombinant human Wnt3a (Fig. 2), respectively. The response to rhWnt3a can be inhibited by ICG-001 with an IC_{50} of 3 μM (Figs. 3). Using different clone, the endogenous Wnt signaling can be inhibited by ICG-001 with an IC_{50} of 3 μM (Figs. 4).

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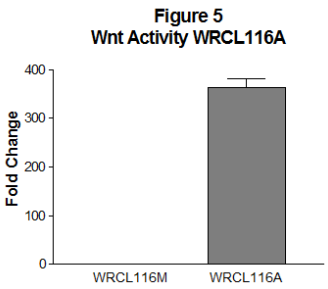
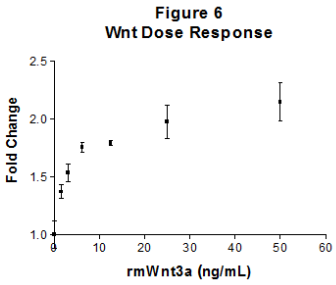
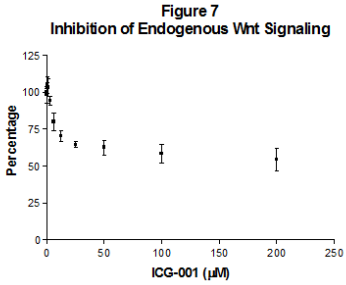
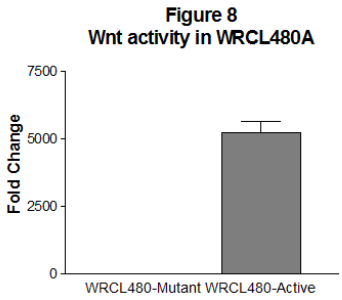
Since HCT116 cells harbor mutated β-catenin gene, resulting in accumulation of β-catenin, the HCT116 Wnt reporter cell line shows more than 300 times higher luciferase activity compared to that from mutant HCT116 Wnt reporter cell line (Fig. 5). However, the luciferase activity from the HCT116 Wnt reporter cell line increases 2 fold after 6- to 8-hour treatment with 50 ng/mL of recombinant mouse Wnt3a (Fig. 6). A half of endogenous Wnt signaling can be inhibited by ICG-001 with an IC_{50} of 3 μM (Figs. 7).

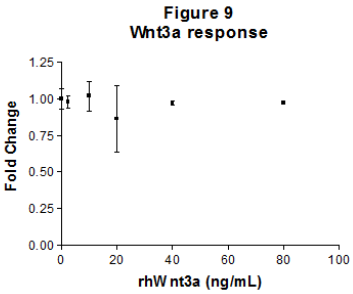


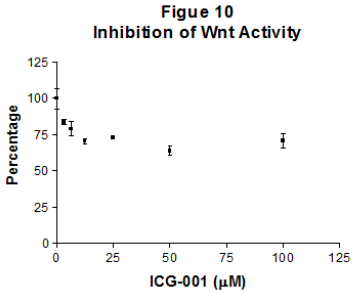




SW480 Wnt reporter cell line shows more than 5000 times higher luciferase activity compared to that from Mutant SW480 Wnt reporter cell line (Fig. 8). However, SW480 Wnt reporter cell line doesn't response to Wnt3a stimulation (Fig. 9) and only a fraction of luciferase activity can be inhibited after 16-hour treatment with ICG-001 (Fig. 10)







In summary, our Wnt drug discovery system responds to Wnt3a stimulation with a super high sensitivity and specificity. Since HCT116 cells harbor beta-catenin mutant, whereas, SW480 cells contain APC mutation, it is a valuable tool for Wnt drug screening and the research of mechanism of actions.