Wnt Proteins in Intestinal Epithelial Progenitor Cells

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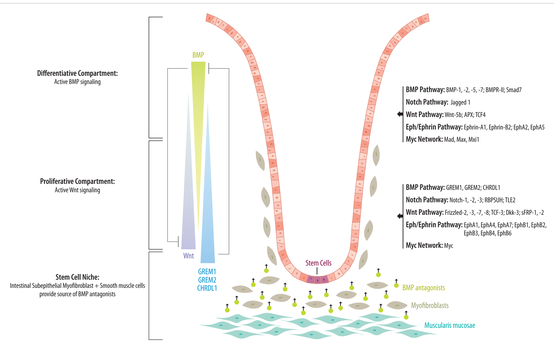
ABSTRACT

Wnt ligands, receptors, and inhibitors are differentially expressed along the intestinal crypt-villus axis and function as major stem cell niche factors that regulate stem/progenitor cell self-renewal, proliferation, differentiation, and migration. Our study shows that recombinant Wnt-3a, Wnt-5a, Wnt-5b, Wnt-7a, and Wnt-10b are able to promote proliferation and survival of small intestinal epithelial cells (IECs). Recombinant Wnt-11 stimulates cell proliferation but fails to promote cell survival at the same concentrations. The Wnt inhibitors WIF-1, Dkks, and sFRPs induce IEC cell death and antagonize the survival activity of Wnts in a dose-dependent manner. We find that Wnt-3a activates the β-Catenin/TCF-dependent canonical pathway in IECs, while the promotion of growth and survival by Wnt-10b is β-Catenin/TCF-independent. Our data suggest that both canonical and non-canonical Wnts provide survival and proliferation signals in intestinal epithelium. The mechanisms and the signaling pathways underlying these functions will be further investigated.

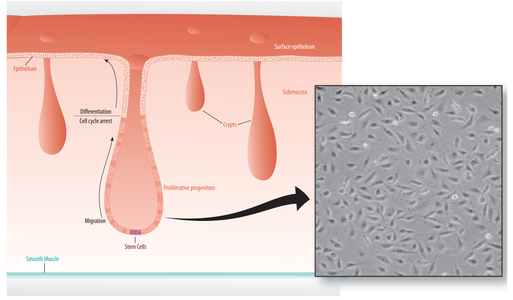
Wnt signaling in intestinal epithelial cell development



The mammalian intestinal epithelium is an anatomically highly organized single layer of cells. These cells are renewed every 4–5 days in mouse throughout life. Stem cells at the base of the crypts divide, differentiate, and migrate up the villi to replace shedded epithelium. This transit is controlled by regulators including members of the bone morphogenetic protein (BMP), Notch, and Wnt families. Wnt proteins, their receptors and inhibitors play important roles in the renewal of intestinal epithelium. This study, enabled by a large collection of R&D Systems recombinant proteins, provides information about different functions of Wnts on the survival and proliferation of intestinal epithelial cells. However, the role of individual Wnts in intestinal stem cells remains largely unclear.

Picture is adapted from Kosinski C. *et al.* (2007) PNAS **104**:15418.

IEC-18 cell



IEC-18 cells are a non-transformed small intestinal cell line derived from native rat ileal crypts. These cells are commonly used as an *in vitro* model of intestinal epithelium because they have the following characteristics:

- epithelioid morphology
- grow in a monolayer in culture
- contact-inhibited cell growth
- do not form colonies in soft agar
- are not tumorigenic when injected in mice
- retain the potential to differentiate into various cell lineages

RESULTS

R&D Systems Wnt Proteins

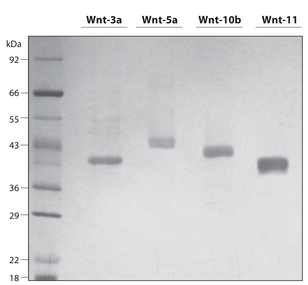


FIGURE 1. SDS-PAGE analysis of R&D Systems recombinant Wnt-3a, Wnt-5a, Wnt-10b, and Wnt-11. Recombinant Mouse Wnt-3a (Catalog # 1324-WN; Lane 1), Recombinant Human/Mouse Wnt-5a (Catalog # 645-WN; Lane 2), Recombinant Mouse Wnt-10b (Catalog # 2110-WN; Lane 3), and Recombinant Human Wnt-11 (Catalog # 6179-WN) were loaded at 1 µg/lane under reducing conditions onto an SDS-PAGE gel and detected by silver staining. Molecular weight markers are shown on the left.

Wnt-3a, Wnt-5a, Wnt-5b, Wnt-7a, and Wnt-10b inhibit serum withdrawal-induced apoptosis

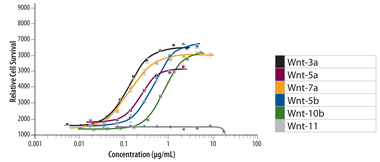


FIGURE 2. Recombinant Wnt-3a, Wnt-5a, Wnt-5b, Wnt-7a, and Wnt-10b prevent apoptosis. IEC-18 cells were cultured in serum-free media for 16 hours in the presence of serial dilutions of the indicated Wnt proteins. At the end of the culture period, living cells were quantified by a fluorometric assay using the redox-sensitive dye Resazurin (Catalog # AR002). Recombinant Mouse Wnt-3a (Catalog # 1324-WN), Human/Mouse Wnt-5a (Catalog # 645-WN), Mouse Wnt-5b (Catalog # 3006-WN), Human Wnt-7a (Catalog # 3008-WN), and Mouse Wnt-10b (Catalog # 2110-WN) prevent serum withdrawal-induced apoptosis, but Recombinant Human Wnt-11 (Catalog # 6179-WN) is unable to support IEC survival under the same conditions.

Wnt-11 stimulates IEC cell proliferation but fails to promote cell survival

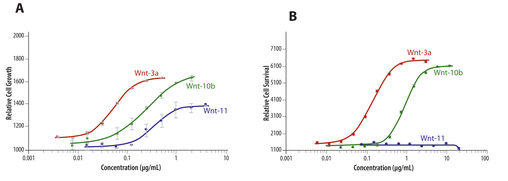


FIGURE 3. Differences in survival and/or proliferative potencies of recombinant Wnt-3a, Wnt-10b, and Wnt-11. Intestinal epithelial cells were cultured in low fetal bovine serum (0.1%) for 6 days (**A**), or in serum-free media for 16 hours (**B**), in the presence of serial dilutions of Wnt proteins. Cell viability was assessed using Resazurin. Recombinant Human Wnt-11 (Catalog # 6179-WN) promotes proliferation (**A**), but does not sustain survival of the cells (**B**). Recombinant Mouse Wnt-3a (Catalog # 1324-WN) and Recombinant Mouse Wnt-10b (Catalog # 2110-WN) promote proliferation and survival of the cells (**A** & **B**).

Wnt inhibitors induce IEC cell death

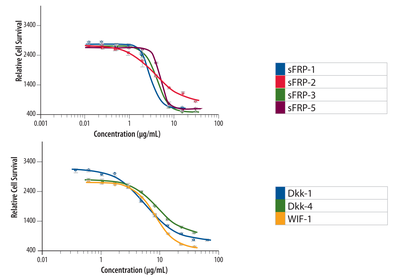


FIGURE 4. WIF-1, Dkks, and sFRPs induce IEC-18 cell death. Cells were cultured in DMEM with 0.1% serum and the indicated concentrations of one of the following Wnt inhibitors, Recombinant Human sFRP-1 (Catalog # 5396-SF), Recombinant Mouse sFRP-2 (Catalog # 1169-FR), Recombinant Mouse sFRP-3 (Catalog # 592-FR), Recombinant Mouse sFRP-5 (Catalog # 7195-SF), Recombinant Mouse Dkk-1 (Catalog # 5897-DK), Recombinant Mouse Dkk-4 (Catalog # 3105-DK), or Recombinant Mouse WIF-1 (Catalog # 135-WF) for 16 hours. The effect of the Wnt inhibitors on cell viability was measured using Resazurin. In the presence of all of the Wnt inhibitors tested, survival of the IEC-18 cells was abolished in a concentration-dependent manner.

Wnt inhibitors antagonize the survival activity of Wnts in IECs

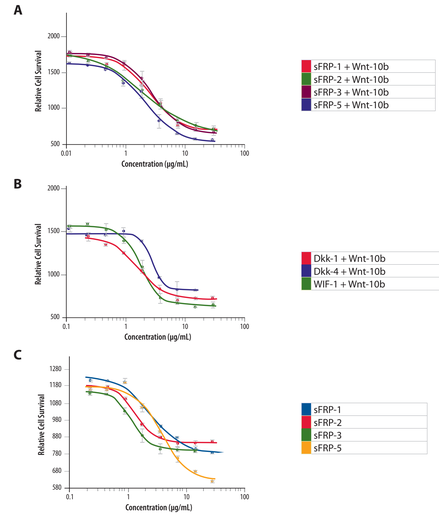


FIGURE 5. WIF-1, Dkks, and sFRPs antagonize Wnt activity in IECs. Cells were cultured in serum-free media in the presence of 1 µg/mL Recombinant Mouse Wnt-10b (Catalog # 2110-WN; **A** & **B**) or 0.3 µg/mL Recombinant Mouse Wnt-3a (Catalog # 1324-WN; **C**) with the indicated serial dilutions of one of the following Wnt inhibitors, Recombinant Human sFRP-1 (Catalog # 5396-SF), Recombinant Mouse sFRP-2 (Catalog # 1169-FR), Recombinant Mouse sFRP-3 (Catalog # 592-FR), Recombinant Mouse sFRP-5 (Catalog # 7195-SF), Recombinant Mouse Dkk-1 (Catalog # 5897-DK), Recombinant Mouse Dkk-4 (Catalog # 3105-DK), or Recombinant Mouse WIF-1 (Catalog # 135-WF). Cell viability was assessed using Resazurin. The data show the mean values and standard deviations from at least three independent experiments.

Wnt-3a activates the β-Catenin/TCF-dependent canonical pathway in IECs

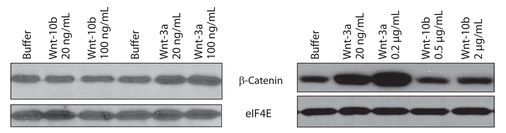


FIGURE 6. Activation of the canonical Wnt signaling pathway in IEC-18 and HEK293 cells. Wnt signaling was stimulated in IEC-18 and HEK293 cells with Recombinant Mouse Wnt-3a (Catalog # 1324-WN) for 16 hours, and was assessed based on the level of β-Catenin accumulation compared to samples treated with Recombinant Mouse Wnt-10b (Catalog # 2110-WN). The levels of β-Catenin accumulation were determined by Western blot using a Mouse Anti-Human/Mouse/Rat β-Catenin Monoclonal Antibody (Catalog # MAB1329), and a Mouse Anti-Human/Mouse/Rat eIF4E Monoclonal Antibody (Catalog # MAB3228) as a total protein loading control. The data shown are typical results observed in three independent experiments.

Wnt-10b induces phosphorylation of STAT5a/b and STAT6 in IECs

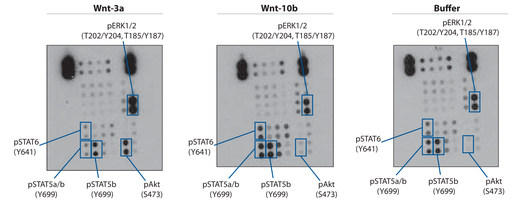


FIGURE 7. Assessment of phosphorylation on intracellular signaling molecules in Wnt-treated IEC-18 cells. Cells were exposed to 0.25 µg/mL of Recombinant Mouse Wnt-3a (Catalog # 1324-WN) or Recombinant Mouse Wnt-10b (Catalog # 2110-WN) for 20 minutes after 16 hours of serum-free starvation. The phosphorylation of various kinases and signaling molecules was analyzed in cell lysates using the Proteome Profiler™ Human Phospho-Kinase Antibody Array (Catalog # ARY003).

Wnt-3a and Wnt-10b support IEC cell proliferation/survival via different downstream signaling pathways

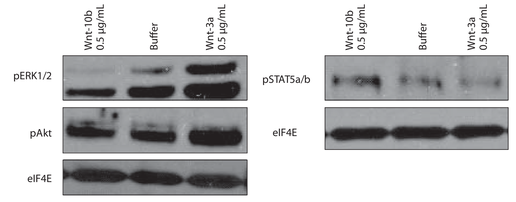


FIGURE 8. Effects of Wnts on the MAPK, PI 3-Kinase/Akt, and STAT signaling pathways. Cells were serum starved for 16 hours, then exposed to 0.5 µg/mL of the indicated Wnt proteins for 20 minutes. Whole cell extracts were prepared and analyzed by Western blot using a Rabbit Anti-Human/Mouse/Rat Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1018), a Rabbit Anti-Human/Mouse/Rat Phospho-Akt (S473) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF887), and a Rabbit Anti-Human Phospho-STAT5a/b (Y694/Y699) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4190). A Mouse Anti-Human/Mouse/Rat eIF4E Monoclonal Antibody (Catalog # MAB3228) was used as a total protein loading control. The data shown are representative results obtained from three independent experiments.

CONCLUSION

- Wnt proteins, irrespective of their ability to stimulate canonical Wnt signaling, promote proliferation (Wnt-11, Wnt-3a, and Wnt-10b) and prevent serum withdrawal-induced apoptosis (Wnt-3a, Wnt-5a, Wnt-5b, Wnt-7a and Wnt-10b) in IEC-18 newborn rat small intestinal epithelial stem/progenitor cells.
- The Wnt inhibitors WIF-1, Dkks, and sFRPs induce IEC-18 cell death by neutralizing the survival activity of Wnts in a dose-dependent manner.
- Wnt-3a and Wnt-10b support IEC-18 cell proliferation and survival through different signaling pathways:
  - *Wnt-10b promotes β-Catenin/TCF-independent signaling and functions partially through STAT signaling.*
  - *Wnt-3a activates the β-Catenin/TCF-dependent canonical pathway, and the Src/ERK and PI 3-Kinase/Akt signaling cascades.*

These data suggest that both canonical and non-canonical Wnts provide survival and proliferation signals in intestinal epithelium through different downstream pathways.

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