Abstract

Beyond its essential roles in embryonic development, the Wnt-mediated signal transduction cascade is critically implicated in homeostasis of adult tissues. In the gastrointestinal epithelium, the threshold of active Wnt signaling is kept in a physiological range by a spectrum of regulatory networks and loops, thereby balancing the opposing processes of cell fate determination, proliferation and stem cell self-renewal. Furthermore, compelling evidence undoubtedly link an aberrant Wnt activity to the onset of bowel cancer. Understanding the principle causes and effects secondary to excessive Wnt signaling can provide valuable insights into the pathology of the malignant transformation of the colorectum. The proposed thesis attempts to focus on novel modes of the Wnt pathway modulation; both general and context-specific nuances of the Wnt level adjustment are thereby delineated. The results are presented in three distinct research publications and one review article.

The first study examines the contribution of the distinct post-translational modifications, which the Wnt proteins undergo, to their proper processing, secretion and signaling activity. First, we investigated the sequential order and mutual interdependence of cysteine and serine-linked fatty acylation and N-linked glycosylation of murine Wnt1 and Wnt3a proteins. Our data indicate that the attachment of palmitoleic acid to a conserved serine residue precedes and is mandatory for the subsequent S-linked palmitation. Regarding the sequence of the acylation-glycosylation events, initial linkage of the oligosaccharide chains most likely conditions the succeeding double-acyl modification. Lastly, linkage of the fatty acyls presumably underlies the protein´s ability to associate with extracellular matrix; a critical feature to the signaling competency of the Wnt ligand as revealed.

The second scientific report delivers a thorough characterization of two novel gene-targeted alleles of the murine *Hic1* gene; a recognized tumor suppressor gene that encodes for a negative regulator of the Wnt pathway. A conditional *Hic1* gene knock-out allele and a citrine (a monomeric derivative of the enhanced yellow fluorescent protein (EYFP)) reporter knock-in mouse strain greatly facilitate the research of Hic1 physiological roles and visualize Hic1 endogenous distribution, respectively.

The third article identifies TROY, a member of the Tumor Necrosis Factor Receptor family, as a novel negative modulator of the Wnt pathway and, in addition, confirms its status as a context-specific Wnt target gene. Importantly, in the intestinal epithelium the Troy expression is demonstrated to be restricted to fast cycling stem cells, thereby defining Troy as a novel marker of these unique cells. In there, Troy likely interacts with the prominent stem cell specifier leucine-rich repeat-containing G-protein coupled receptor (Lgr5) to reduce the local level of the Wnt pathway activity. Lastly, elevated amounts of Troy were observed in lesions arising upon genetically defined, the Wnt-pathway driven mouse models of hereditary or induced bowel cancer. On the contrary, collected samples of human sporadic colorectal cancer indicated a different pattern of *TROY* tumoral deregulation in humans.

Finally, the review article summarizes the principal signaling pathways that govern the architecture and homeostasis of the gastrointestinal tract. Ranging from the Wnt/β-catenin and Notch pathways to circuits triggered by Hedgehog, EGF or BMP ligands, all cascades are discussed in detail with respect to their fundamental roles both in physiology and malignant transformation of the gut.