Abstract

The Wnt signaling pathway represents the principal evolutionarily conserved signaling cascade found in all multicellular organisms. It plays a key role not only in many processes during embryogenesis, but also in maintaining tissue homeostasis and regeneration. By contrast, mutations in genes encoding components of the pathway often result in increased activation of Wnt signaling and underlie onset of many human diseases, particularly cancer.

The canonical Wnt signaling pathway is essential for proliferation and maintenance of the pluripotent state of intestinal stem cells and thus for homeostatic renewal of the intestinal epithelium. However, aberrant (hyper)activation of the Wnt signaling pathway is the initial step in development of intestinal neoplasia. Understanding the causes and identifying the consequences of the Wnt signaling hyperactivation is crucial for deciphering mechanisms leading to malignant transformation. Although the canonical Wnt signaling pathway has been the subject of scientific studies for several decades, all regulatory mechanisms and consequences of its hyperactivation have not been completely elucidated yet. During my PhD studies, I focused on understanding function(s) of some components and target genes of this signaling cascade. In this theses, results of my first author and one co-author publication are presented, which deal with two genes directly linked to Wnt signaling.

In my first-author publication, we studied the function of the msh homeobox 1 (MSX1) transcription factor in mouse and human intestines and tumors. We used a mouse model of the human disease Familial adenomatous polyposis (the $Apc^{+/min}$ mice) and mouse models harboring conditional knock-out alleles of the tumor suppressor gene adenomatous polyposis coli (*Apc*) and *Msx1*. These mice were intercrossed with mice expressing (regulated) Cre recombinase throughout the intestinal epithelium (*Villin-Cre* and *Villin-CreERT2*) or in the intestinal stem cells *(Lgr5-EGFP-IRES-CreERT2*), i.e. strains which enable spatiotemporal inactivation of the specific gene(s). We found that Msx1 is essential during formation of the so-called ectopic crypts, which are pouches of proliferating cells aberrantly occurring in the otherwise differentiated villous compartment after inactivation of the tumor suppressor gene adenomatous polyposis coli (*Apc*). Ectopic crypts have been described as a typical morphological feature of human serrated adenomas, which represent an aggressive type of intestinal polyps. Moreover, we suggest that Msx1 inactivation leads to a morphological conversion of intestinal tumors from tubular to villous adenomas, which is in humans associated with more advanced stages along the path towards fully developed carcinoma and a worse survival prognosis. We also found out that Msx1 represents a robust marker of human

colorectal carcinomas with the most elevated expression in the early stages of tumorigenesis. In the second publication, we described the role of a tumor suppressor hypermethylated in cancer 1 (*Hic1*) in mouse intestines. Using mice harboring conditional alleles of the *Hic1* gene and expressing Cre recombinase throughout the intestinal epithelium, we described that *Hic1* loss leads to increased numbers of differentiated intestinal epithelial cells and elevated levels of toll-like receptor 2 (*Tlr2*). Consequently, Tlr2 activates the NF-κB signaling pathway, which promotes intestinal tumorigenesis.

Key words: Wnt signaling, MSX1, HIC1, colorectal cancer, ectopic crypts