



## **Samenvatting van het proefschrift**

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*"A closeup of colon cancer"*

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Understanding development of colorectal cancer requires knowledge on homeostasis of the normal intestinal epithelium as well as intestinal tumorigenesis. In the current thesis, a number of aspects of these two intricately connected subjects are further discussed. During development the mammalian intestinal epithelium undergoes major changes that allow a dietary transition from mother's milk to the adult diet. These developmental changes result from a genetic program intrinsic to the gut tube. In chapter 3, we show that transcriptional repressor B lymphocyte induced maturation protein 1 (Blimp1) is highly expressed in the developing and postnatal intestinal epithelium until the suckling to weaning transition. Intestine specific deletion of Blimp1 results in growth retardation and excessive neonatal mortality. Mutant mice lack all of the typical epithelial features of the suckling period and are born with features of an adult-like intestine. We conclude that the suckling to weaning transition is regulated by a single transcriptional repressor that delays epithelial maturation. In chapter 4 we take a closer look at stem cell to transit amplifying (TA) cell transition. Stem cells generate rapidly dividing TA cells that have lost the capacity for self renewal but cycle for a number of times until they undergo terminal differentiation. We know very little of the type of signals that trigger the earliest steps of stem cell differentiation and mediate a stem cell to TA cell transition. We show that in normal intestinal epithelium ER stress and activity of the unfolded protein response (UPR) is induced at the stem cell to transit amplifying cell transition. Induction of ER stress causes loss of stemness in a Perk-eIF2 $\alpha$  dependent manner. Inhibition of Perk-eIF2 $\alpha$  signaling results in stem cell accumulation in organoid culture of primary intestinal epithelium. Our findings show that the UPR plays an important role in the regulation of intestinal epithelial stem cell differentiation. In chapter 5 we further investigate the homeostatic role of intestinal Hedgehog signaling. Indian Hedgehog (Ihh) is expressed by the differentiated epithelial cells of the small intestine

and signals to the mesenchyme where it induces unidentified factors that negatively regulate intestinal epithelial precursor cell fate. Recently genetic variants in the Hh pathway have been linked to the development of inflammatory bowel disease. Deletion of Ihh from the intestinal epithelium initially resulted in a proliferative response of the intestinal epithelium with lengthening and fissioning of crypts and increased Wnt signaling. The epithelial proliferative response was associated with loss of Bmp and Activin signaling from the epithelium of the villus and crypts respectively. At the same stage we observed a substantial influx of fibroblasts and macrophages into the villus core with increased mesenchymal Tgf- $\beta$  signaling and deposition of extracellular matrix proteins. Prolonged loss of Ihh resulted in progressive leukocyte infiltration of the crypt area, blunting and loss of villi and the development of intestinal fibrosis. We conclude that loss of Ihh initiates several events that are characteristic of an intestinal wound repair response. Prolonged loss resulted in progressive inflammation, mucosal damage and the development of intestinal fibrosis. Ihh is a signal derived from the superficial epithelial cells that may act as a critical indicator of epithelial integrity. In the studies on development of colorectal cancer (CRC), we have mainly focused on gender disparity in development of CRC. Clinical data suggest that progestins have chemopreventive properties in the development of colorectal cancer. Examining a potential protective effect of progestins and progesterone signaling on colon cancer development in chapter 6, we found that the progesterone receptor (PR) is not expressed in normal and neoplastic intestinal tissue, but expression was confined to sporadic mesenchymal cells. To analyze the influence of systemic progesterone receptor signaling, we crossed mice which lacked the progesterone receptor (PRKO) to the ApcMin/+ mouse, a model for spontaneous intestinal polyposis. PRKO-ApcMin/+ mice exhibited no change in polyp number, size or localization compared to ApcMin/+. Examining effects of progestins on the intestinal epithelium that are independent of the PR, we treated mice with MPA and progesterone. We found no effects of either substance on gross intestinal morphology or epithelial proliferation. Also, in rats treated with MPA, injection with the carcinogen azoxymethane did not result in a difference in the number or size of aberrant crypt foci, a surrogate end-point for adenoma development. We conclude that expression of the progesterone receptor is limited to cells in the intestinal mesenchyme. We did not observe any effect of progesterone receptor signaling or of progestin treatment in rodent models of intestinal tumorigenesis. The fact that men are more likely than women to develop colonic cancers and precursor lesions, is likewise observed in ApcPirc/+ rats that harbor a mutation in the Apc gene and spontaneously develop intestinal polyps. In chapter 7 we set out to investigate the contribution of sex hormones in development of colorectal tumorigenesis. Using both ApcPirc/+ rats and a chemical model to assess ACF formation in the rat colon, we find that in female animals, depletion of endogenous hormones by means of ovariectomy does not affect prevalence of ACFs or polyps. Additionally, no effect is observed upon supplementation of one or a combination of female hormones. Depletion of male hormones by means of orchidectomy markedly protects from polyp development in

ApcPirc/+ rats, which by supplementation with dihydrotestosterone (DHT) are reverted to levels found in control animals. Interestingly, no protection from ACF formation was observed upon orchidectomy and in contrast to ApcPirc/+ animals, supplementation with DHT exhibits protective effects on ACF numbers. We thus find that gender disparity in development of intestinal tumors is due to tumor promoting effects of male hormones. Androgens influence progression of established lesions, but do not influence the rate by which ACFs develop. In chapter 8, we focus on the role of female hormones during cancer that develops in the context of intestinal inflammation, since hormone replacement therapy was found to increase the risk of developing ulcerative colitis in postmenopausal women. Chronic intestinal inflammation predisposes to colon cancer development, but effects of female hormones on colitis-associated cancer development have not been examined. We therefore investigated the role of female hormones in the dextran sodium sulfate (DSS)-azoxymethane (AOM) mouse model for colitis-associated cancer, performing ovariectomies, or sham operations, on mice, and supplementing these animals with indicated hormones. Additionally, we used oestrogen receptor  $\alpha$  or  $\beta$  (Er $\alpha$  or Er $\beta$ ) mutant mice. To study colitis or colitis-associated cancer, we used DSS only, or DSS and AOM, respectively. We find that ovariectomy protects female mice against colitis-associated tumour development. Hormone replacement in ovariectomised mice with either oestradiol (E2), medroxyprogesterone acetate or a combination of both suggests that oestrogens are the ovary-derived factor that promotes tumour development in the context of inflammatory damage. E2-treated animals showed increased clinical symptoms and Il-6 production upon DSS-induced colitis and enhanced epithelial proliferation. Treatment with E2 markedly increased the numbers of polyps in ovariectomised mice and also strongly promoted tumour progression with all E2-treated animals developing at least one invasive adenocarcinoma, whereas, placebo-treated animals developed adenomas only. Using Er mutant mice, we find that the protumorigenic effect of oestrogen depends on both Er $\alpha$  and Er $\beta$ . These results suggest that oestrogens promote inflammation-associated cancer development by impairing the mucosal response to inflammatory damage. Extrinsic factors that influence development of colorectal tumorigenesis include molecular patterns that are derived from either pathogens (PAMPs) or cellular damage (DAMPs). These molecules can promote tumourigenesis by activation of the innate immune system, but the individual contribution of ligands and their receptors remains elusive. In chapter 9, we investigate a role for the receptor for advanced glycation endproducts (Rage), a pattern recognition receptor that binds multiple ligands derived from a damaged cell environment such as Hmgb1 and S100 protein. Interestingly, upon rage activation, transcription of a number of Rage ligands is increased, thus constituting a feed-forward pro-inflammatory loop that activates a tissue damage response through Rage signalling. Mice that lack Rage fail to develop such a tissue damage response and they exhibit lower rates of tumor formation. Blocking DAMP signaling may thus diminish the activation of the innate immune system during tumor development and hence inhibit tumorigenesis.

Together these studies of both normal intestinal homeostasis as well as intestinal tumorigenesis may increase our understanding of the gut in the normal healthy situation and enhance understanding and eventually treatment after normal mechanisms have derailed and a tumor has formed. ◀