Apoptosis and colorectal cancer. Studies on pathogenesis and potential therapeutic targets
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Chapter 1
Introduction and outline of the thesis.
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Colorectal cancer is the second cause of cancer related deaths in the western world. The development of colorectal cancer is characterised by a sequence of events during which normal colonic epithelium gradually transforms to carcinoma tissue, in most cases via the development of colorectal adenomas. This sequence of events is driven by an accumulation of molecular (epi)genetic alterations causing progressive disorders in cell growth, differentiation and apoptosis. Apoptosis, or programmed cell death, plays an important role in the development and maintenance of tissue homeostasis but also represents an effective mechanism by which abnormal cells, such as tumour cells, can be eliminated. Abnormalities in apoptotic function or resistance to apoptosis have been identified as important events in the pathogenesis of colorectal cancer and its resistance to chemotherapeutic drugs and radiotherapy.

During apoptosis, a complex death program is initiated that ultimately leads to the fragmentation of the cell. The death program can be either initiated by the cell itself or by certain external stimuli. These external stimuli may induce apoptosis by targeting one of two pathways. The ‘extrinsic’ pathway is initiated by triggering cell death receptors on the cell surface, leading to activation of the intracellular apoptotic machinery. The ‘intrinsic’ pathway of apoptosis is initiated via the mitochondria by cellular stress, such as chemotherapeutic drugs and radiation. The elucidation of the molecular mechanisms regulating these processes is of primary interest.

Tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a transmembrane protein belonging to the TNF superfamily. TRAIL triggers the extrinsic apoptotic pathway by binding to its membrane-bound death receptors DR4 and DR5, which transmit an apoptotic signal via their intracellular death domains. TRAIL can also bind to decoy receptors DcR1 and DcR2 that are unable to initiate an apoptotic signal. In vitro, TRAIL induces apoptosis in malignant cells but not in normal cells. Preclinical data in mice and nonhuman primates have shown that TRAIL inhibits tumour growth without serious systemic toxicity. Therefore, TRAIL is considered a promising new anti-cancer agent.

This thesis aims to gain insight into changes in apoptosis occurring during the development of colorectal cancer. In addition, the potential of TRAIL-mediated apoptosis as a therapeutic target in colorectal neoplasms is investigated. Finally, possible mechanisms behind apoptosis induction by non-steroidal anti-inflammatory drugs (NSAIDs), in particular sulindac, in colonic epithelial cells are explored, with emphasis on TRAIL mediated apoptosis.

Chapter 2 summarises current knowledge of mechanisms of apoptosis and the role of dysregulation of apoptosis in the pathogenesis of colorectal cancer. Although apoptosis has been extensively investigated in colon tissue at different stages of colorectal cancer development, results are conflicting and controversial. These studies were systematically reviewed.

Over the last years, new techniques have been developed to investigate apoptosis in human tissue. Chapter 3 describes a new technique of identification of apoptotic cells in colon cells by M30 immunoreactivity. The M30 antibody recognises caspase-cleaved cytokeratin 18 and has shown to be an early marker of apoptosis in epithelial cells. The technique was
compared to identification of apoptotic cells using morphological criteria, the method that is considered the gold standard.

To investigate the potential use of TRAIL or TRAIL receptor agonists as therapeutic agents in colon neoplasms, the expression of the four membrane bound TRAIL receptors was studied in colon tissue sections in different stages of colon cancer development, i.e. normal colon, adenomas and carcinomas. The results are described in Chapter 4. In the same study, correlations between the degree of apoptosis and expression of TRAIL and its receptors were explored. Finally, possible differences in apoptosis were studied between truly normal colon tissue and normal colon obtained from resection margins from surgical specimens of colorectal cancer patients.

Up to 10 % of all colorectal cancers occur in the setting of defined cancer predisposition syndromes. The remainder of cases are named sporadic disease. The two major entities known to be associated with a highly increased risk of developing colorectal cancer are familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC). Tumours from HNPCC patients are characterised by length alterations in repetitive sequences distributed throughout the genome, so-called microsatellite instability (MSI). The MSI phenotype is also found in 10-15 % of sporadic colorectal cancer cases. Expanding on the results described in Chapter 4, where patients with sporadic disease had been studied, the same experiments were performed in material obtained from patients with FAP and HNPCC and the results are described in Chapter 5. In addition, sporadic tumours with MSI were studied. Up to half of colorectal tumours with MSI contain mutations in the BAX gene. BAX, a pro-apoptotic member of the Bcl-2 family, has been reported to play a role in sensitivity to TRAIL-mediated apoptosis in vitro. The presence of BAX mutations in MSI positive tumours could potentially limit the use of TRAIL or TRAIL receptor agonists. Therefore, the relationship was investigated between the presence or absence of BAX mutations and the level of apoptosis and expression of TRAIL and its receptors in MSI positive tumours.

Although the majority of colorectal carcinomas is macroscopically resectable, 50 % of patients subsequently relapse. In patients with established lymph-node metastasis (stage III), adjuvant chemotherapy following surgical resection has proven benefit. However, intrinsic as well as acquired resistance to chemotherapeutic drugs is a major problem in the treatment of this disease. It is therefore of importance to identify prognostic factors that can help to develop new patient-tailored treatment strategies for colorectal cancer patients. In Chapter 6, the prognostic impact of TRAIL, DR4 and DR5 expression was investigated in tumours of 376 stage III colon cancer patients, who had received adjuvant chemotherapy in a previous nation-wide randomised trial.

An important strategy to prevent colorectal cancer is chemoprevention, i.e. the use of drugs that inhibit the development of colorectal adenomas or carcinomas. Chemoprevention is particularly important in patients who have an increased risk of developing colorectal neoplasia. The most promising chemopreventive agents are aspirin and other NSAIDs. Numerous studies have established the efficacy of the NSAIDs sulindac and celecoxib in patients with FAP, while chemoprevention studies in HNPCC patients are ongoing. The mechanisms by which NSAIDs inhibit neoplastic growth are not fully known. In Chapter 7, potential mechanisms behind the chemopreventive action of NSAIDs are briefly reviewed.
In the same chapter, two important recent randomised placebo-controlled trials showing a chemopreventive effect of aspirin in patients with previous colorectal adenomas or carcinomas are discussed.

The mechanisms behind the chemopreventive efficacy of NSAIDs partly involve the induction of apoptosis in neoplastic cells. Apoptosis induction by sulindac in colon cancer cells seems to involve the intrinsic, mitochondrial pathway, as well as the extrinsic, death receptor pathway. With respect to the TRAIL pathway, sulindac induced in vitro up-regulation of DR5 mRNA and protein levels, but not of DR4. Some studies have suggested that sulindac may mediate its chemopreventive effect through modulation of the APC-β-catenin-Wnt pathway, possibly mediated by the cell-cycle regulating protein p21. Activation of the APC-β-catenin-Wnt is considered the initial event in the neoplastic transformation of normal colon epithelium. In Chapter 8, the effects of sulindac were studied on apoptosis and expression of DR4 and DR5, β-catenin and p21 in normal appearing colon mucosa. For this purpose, tissue obtained before and after sulindac treatment during two chemoprevention trials in HNPCC and FAP patients respectively was investigated.

Finally, the results of the studies described in this thesis are summarised and future perspectives are discussed (Chapter 9).