CHAPTER 1

General introduction and thesis outline

Colon cancer is a major contributor to cancer-related mortality worldwide. Death from colon cancer occurs in the majority of the cases from widespread metastatic disease. Surgery alone can cure a large proportion of colon cancer patients presenting with non-metastasized disease (1;2). Without any adjuvant treatment, up to fifty percent of these patients will not develop disease recurrence or metastasis following surgery (2-4).

Despite optimal surgical therapy, between 30-50% of initial stage II or III colon cancer patients will still suffer from metastatic disease (2-4). This risk can significantly be reduced by applying postoperative or adjuvant chemotherapy. Studies have however shown that only a selected proportion of the stage II and III patients will actually benefit from adjuvant treatment because not all patients within this cohort will develop a distant metastasis and because of low response rates to therapy, in the case of stage II disease this might even only be 15% of the patients (5;6). The decision to offer adjuvant therapy, especially for stage II disease, should therefore be balanced against the possible risks of treatment-related toxicity (6). This makes it essential for the clinician to be able to precisely identify the high risk patient cohort.

Nowadays, treatment allocation is based on tumor staging using only the TNM (Tissue Node Metastasis) criteria developed by the IUCC (Union Internationale Contre le Cancer) and the AJCC (American Joint Committee on Cancer) that are applied worldwide (7). The application of the TNM criteria, based on tumor morphological characteristics, falls short in the identification of the high risk patient population. Prognostic biomarkers that provide additional information on patient outcome might improve staging criteria. Therefore, increasing attention is now being directed towards the discovery of prognostic biomarkers to identify high risk colon cancer patients. Several methods have been used, but two main approaches can be distinguished.

A genomic approach that involves the simultaneous examination of a large number of genes using complex platforms, including multigene-based mutation assays and gene expression microarray technologies. The power of this approach lies within its capacity to tackle tumor heterogeneity with the goal to develop a unique genetic signature to provide the opportunity to match therapy to the characteristics of the individual patient's tumor (8). So far, despite numerous studies reporting on different prognostic gene sets, only two genomic profiles have been validated in independent patient cohorts. These are the ColoPrint and the 12-gene colon cancer recurrence score (9-11). The colon cancer recurrence score has been offered by the Genomic Health company since January 2010 for clinical use as the Oncotype DX® Colon Cancer Assay and is now available to support treatment planning for stage II and stage III colon cancer patients (12). A point of discussion in the use of these signatures is the lack of strong biological basis (13). As only a small number of the genes from the different gene expression signatures actually overlap and only a weak interaction was observed between the gene expression signatures and tumor stage (13-16). This lack of interaction between gene expression signatures and tumor stage has been found in many studies, for example, in a large validation

study of the colon recurrence score developed by the NSABP (National Surgical Adjuvant Breast and Bowel Project) but also in the second validation study of the ColoPrint (9;11). Furthermore, in this last study the results for risk recurrence, based on the Coloprint and those based on several clinical tumor biology-based factors such as, T-stage and tumor grade were disconcordant in 50% of the patients indicating a lack of interaction of the signature with actual tumor biology (9). Probably in the future of the clinical decision making process besides gene expression signature, other clinico-pathology-based markers may be used to achieve the most accurate risk assessment.

The second approach is a more tumor biology-directed approach, with a focus on biological determinants of the tumor's metastatic potential. The biological hallmarks of cancer are six biological capabilities a cell acquires during the process of tumorigenesis (17). They include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. Recently, two 'emerging hallmarks', features that tumors gain during their development, were added: reprogramming of energy metabolism and evading immune destruction (17;18). In this thesis we focus especially on three hallmarks of tumor development in colon cancer in order to indentify biomarkers of disease recurrence and to develop new treatment strategies based on this biological approach. In part 1 the focus is on markers of apoptosis and proliferation, and in part 2 on tumor-immune interactions.

AIMS AND OUTLINE

Part 1 Biomarkers of apoptosis and proliferation

A key factor in colonic tissue homeostasis is the balance that exists between cellular apoptosis, or programmed cell death, and the level of cellular proliferation (19-21). Several hallmarks of cancer development such as sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality together determine a balance between cell proliferation and cell death. The outcome of this balance determines tumor growth (17). Therefore, biomarkers representing these pathways might harbor the potential to identify high risk stage II colon cancer patients. Previously, we have been able to determine a prognostic value of the level of apoptosis in rectal cancer patients (22;23). In colon cancer this value has been debated (20;24-27). The aim of the first part of this thesis was to determine the clinical prognostic value of apoptosis and proliferation in colon cancer patients and to develop biomarkers with the potential to be used in a clinical setting to identify high risk stage II colon cancer patients. Chapter 2 provides a review of the current literature of biomarkers of apoptosis in colorectal cancer in order to identify any biomarker that has already been proven to be of clinical prognostic value. In chapter 3 a combined analysis of biomarkers of proliferation and apoptosis, determined with immunohistochemistry in a large cohort of colon

cancer patients, is evaluated for its prognostic quality. Chapter 4 describes the development and identification of a new prognostic, biochemically determined, biomarker based on tumor cell proliferation in stage II colon cancer patients: the CDK1 SA (Cyclin Dependent Kinase 1 Specific Activity). Finally in chapter 5 a biomarker combination consisting of two biochemical assays reflecting the level of apoptosis and proliferation in colon cancer tissue is studied for the clinical identification of high risk stage II colon cancer patients.

Part 2 Tumor immune interactions

In part 2 the focus is on one of the emerging hallmarks in cancer biology: evading immune destruction (18). Studying tumor immune interactions not only provides us with new biomarkers of tumor aggressiveness, it will also open a door to new cancer treatment strategies. Besides focusing on chemotherapy as adjuvant treatment strategy for high risk stage II patients, exploiting possibilities of activating the patient's immune system as a therapeutic modality is becoming an emerging modality as well. Chapter 6 gives an overview of the colorectal cancer vaccines that have been studied in clinical trials until now including the first studies with the p53-SLP vaccine which is used in a clinical phase I/II trial as described in chapter 7. Although the results of these studies are hopeful, as we were capable of eliciting vaccine-specific T-helper responses, optimal patient selection in advance may make real clinical breakthroughs possible. Therefore, in chapter 8 we focus on the determination of key factors in tumor-immune interactions such as tumor expression of HLA class I and local presence of Foxp3- and CD8-positive cells and their prognostic value. In chapter 9 we combine this knowledge into a clinical prognostic tumor-immune phenotype that might eventually aid us in the identification of high risk stage II colon cancer patients and select those that might benefit from adjuvant vaccination or chemotherapy treatment strategies.

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