Improving diagnostic, prognostic and predictive biomarkers in colorectal cancer: the role of proteomics and stromatogenesis

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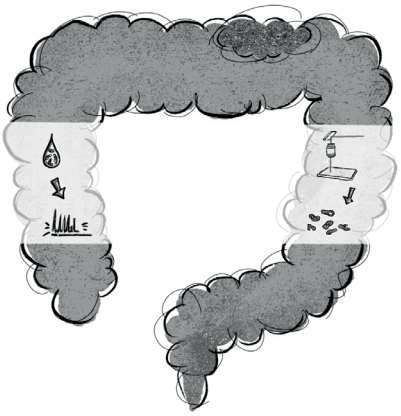

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Introduction and Outline

INTRODUCTION

Colorectal cancer is one of the most common diagnosed cancers, with incidence rates ranking second in women and third in men. Moreover, it is the third and fourth leading cause of cancer-related deaths in women and men, respectively [1].

Most colorectal cancers originate from normal colonic mucosa through an adenoma-carcinoma sequence. The prognosis of colorectal cancer mainly depends on the tumor stage. Therefore, early diagnosis is of great importance to reduce disease-related mortality [2, 3].

Despite histologic tumor staging, stage-independent outcome variability occurs, which probably reflects tumoral molecular heterogeneity. Even patients with early stage colorectal cancer may show disease relapse and cancer progression despite initial surgical management. This indicates a need for biomarkers that contribute to risk stratification of colorectal cancer beyond conventional clinicopathological staging [4].

This thesis analyses the pathologic and molecular characterizations of colorectal cancer. It thereby focuses on the role of biomarkers to improve disease specific survival. A biomarker – or biological marker – is a measurable indicator that reflects normal biological processes, pathogenic processes or responses to an exposure or intervention [5]. Biomarkers are often defined in accordance with how they are applied. This research focuses on three particular subtypes of biomarkers: diagnostic, prognostic and predictive biomarkers and their role on improving colorectal cancer outcome.

First, a *diagnostic* biomarker detects or confirms the presence of a disease or medical condition of interest [6]. Second, a *prognostic* biomarker is used to identify the likelihood of a clinical event, disease recurrence, or disease progression in patients with a disease or medical condition of interest [6]. And third a *predictive* biomarker is present or changes to indicate that an individual or group of individuals is more likely to experience a favourable or unfavourable effect from the exposure to a medical product or environmental agent [6].

This doctoral thesis consists of two parts. In the first part, the research analyses the role of proteomics as a *diagnostic* biomarker for early colorectal cancer detection. This research is relevant because through its use as a diagnostic biomarker, proteomics may improve screening applications.

The second part of this research examines the role of stromatogenesis as a *prognostic* and *predictive* biomarker. This part of the research promotes the development and selection of personalised therapies based on the pathological and molecular findings that result from analysing stroma tissue.

This research as a whole thereby offers new insights into colorectal cancer diagnostic biomarkers that could ultimately help improve and simplify early detection and prognostic and predictive biomarkers that define the optimal personalized treatment for patients in routine clinical practice; thereby improving their survival.

OUTLINE

Part 1 Proteomics as a Diagnostic Biomarker

Early diagnosis of cancer is of pivotal importance to reduce disease-related mortality. Therefore, non-invasive screening methods can offer a vital improvement for colorectal cancer survival. However, existing screening protocols have limited sensitivity and specificity [7-10].

The use of serum biomarkers to distinguish cancer patients from healthy persons may be a tool to improve screening programs. Serum is an ideal sample type for early detection markers since samples can be obtained in a straightforward, standardised manner at minimal cost, minimal risk and, most importantly, in a less-invasive manner compared to existing detection methods, such as colonoscopy [11]. i. Mass spectrometry-based proteomics is widely applied for mapping and identifying peptides and proteins in body fluids [12-16].

Chapters 2 and 3 focus on a possible role of proteomic serum biomarkers in screening programmes. Specifically, **chapter 2** provides an overview of and reviews profiling methods that have the potential to be implemented in a clinical setting and national screening programs. **Chapter 3** offers a case-controlled study that identifies proteomic profiles and their potential for colorectal cancer screening. In that study, serum samples were obtained from 126 colorectal cancer patients (CRC) before treatment and 277 healthy control individuals. An additional group of samples from 50 CRC patients and 82 controls was used for validation. Peptide and protein profiles were acquired on a matrix-assisted laser desorption/ionization time-of-flight system and the results were validated on an identical patient set. This resulted in a relatively simple and cheap test that could be promising for improving current CRC screening and reducing the number of necessary colonoscopies.

Part 2 Stromatogenesis as a Prognostic and Predictive Biomarker

Chapters 4-6 focus on stromatogenesis. Stromatogenesis is the formation of new specific types of tumor stroma. Tumor stroma is mainly composed of fibroblasts and extracellular matrix, at sites of active tumor cell invasion. There are many types of stromal formation, such as the usual reactive fibrosis that surrounds benign neoplasms (fibrous capsule), or the formation of avascular connective tissue that fills the gap of a wound (scar tissue). However, this type of stroma in malignant tumors is of a different kind. It facilitates tumor cell invasion and migration. Therefore, tumor-stroma and cancer cell interactions may be key elements in the puzzle of tumor survival, growth, invasion and metastasis [17].

Tumor stroma as a prognostic factor for stage II and III colon cancer patients

The pathologic variables that are commonly used nowadays as indicators of outcome and therapy response can be improved. Therefore, more specific markers are necessary that i) contribute to unravelling the molecular heterogeneity of colon cancer, ii) that discriminate between high- and low-risk groups, and iii) that can possibly even predict therapy response. A prognostic biomarker is the tumor stroma ratio (TSR), which is based on microscopic pathological analysis on conventional hematoxylin an eosin (H&E)-stained paraffin sections. Assessment is fast, cheap and reliable. Previous research by our group demonstrated that the TSR in colon cancer patients is a strong independent prognostic parameter [18, 19]. Patients with a high stroma percentage within the primary tumor have a poor prognosis.

Chapter 4 provides a validation of the TSR based on a large group of 710 patients with stage II and III colon cancer that participated in the VICTOR trial [20, 21]. The VICTOR trial was a randomized clinical trial where patients with stage II and III CRC after complete potential curative treatment were randomized for adjuvant rofecoxib or placebo. In our study, tissue samples from the most invasive part of the tumor were used for analysis of the TSR, using conventional microscopy. We also investigated the possible additional prognostic value of the TSR next to current parameters such as the ASCO high-risk criteria (T4 tumor stage, lymph node yield<10 nodes in the resection specimen, poor tumor differentiation, vascular invasion or perforation of the bowel wall at presentation) and microsatellite instability status that are used in routine pathology reporting.

Bevacizumab for high-risk stroma-high colon cancer patients.

TSR distinguishes between aggressive and non-aggressive tumors. However, it is not yet entirely clear why TSR makes this distinction since the underlying mechanism driving stromatogenesis is still not fully understood. Nevertheless, we do know that tumor

stroma plays an important role in tumor formation and progression [22]. Moreover, one of the factors of tumor progression is angiogenesis, which is facilitated by the tumor stroma. The tumor stroma environment contains multiple different cells including (cancer-associated) fibroblasts, angiogenic vascular cells, and infiltrating immune cells [23]. The prognostic value of TSR has been demonstrated previously [18,19], but its use in therapy selection is a promising new approach to improve TSR-high patients' survival. The stromal environment contributes to tumor angiogenesis, which supplies the oxygen and nutrients needed for tumor growth and progression [24]. Anti-angiogenic therapy, for example with bevacizumab, a monoclonal antibody against vascular endothelial growth factor, can therefore play an important role in treating patients with increased angiogenesis.

Chapter 5 provides our investigation into the added value of bevacizumab to standard chemotherapy for high-risk patients in the QUASAR2 trial. The QUASAR 2 trail was a large phase III randomized trial of adjuvant capecitabine (CAP) ± bevacizumab (BEV) after complete surgical resection of high-risk stage II and stage III colorectal cancer [24]. Tissue samples of the primary tumor of 965 colon cancer patients were analysed for TSR. The study analysed the relation between TSR and the presence of vascular invasion.

Evaluation of the molecular architecture of the tumor associated stroma in colon cancer patients

To effectively address the improvement of survival of colorectal cancer patients, it is of great importance to understand why patients with a high stromal percentage have a poor prognosis, what causes the aggressiveness of high stromal formation, and what pathways are involved in this process. To evaluate the architecture of the tumor-associated stroma tissue, **chapter 6** provides a pilot study that used laser capture microdissection (LCM) that was coupled to broad-scale protein pathway activation mapping, and subsequently reverse phase protein microarrays (RPMA). This technique uses cellular enrichment of specific tissue cells via LCM for tissue biomarker discovery and might contribute to future selection criteria for personalized treatment [15, 16, 26, 27]. It is a high throughput multiplex proteomic platform which can measure hundreds of analytes in a large number of samples with only a small amount of biological material [28-30]. This technique could identify new stromal-based targeted information that offers insights for treatment options because it helps identify activated pathways within the tumor stroma of patients with aggressive colon cancer.

Patients with histologically proven stage II and stage III colon cancer were selected from a LUMC database. TSR was defined and patients were grouped in a stroma-high

or stroma-low group. For this feasibility study, we analysed only evident cases with ≤30% stroma or ≥70% stroma. We performed reverse phase protein microarray that used microdissected material to generate multiplexed pathway profiling for both groups. For this study, we selected 58 proteins and phosphoproteins. The analytes were chosen based on their involvement in key aspects of epithelial mesenchymal transition, extra-cellular matrix composition and remodeling, angiogenesis, inflammation, and transcription. We compared the results in activation/phosphorylation and expression levels of the different analytes between both groups. Furthermore, we did correlation analysis to analyse the possible interactions between different analytes.

Conclusions and Future Perspectives

Finally, **chapter 7** includes a summary of this thesis as well as conclusions and discussion on future perspectives. **Chapter 8** provides a summary in Dutch.

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