Thesis Outline
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This dissertation is divided into two sections. The first section introduces four studies that were performed to further develop and validate techniques for DNA methylation analysis. The second section shows application of the developed techniques and intends to demonstrate that the relatively new field of epigenetics can be utilized for better subclassification of gastrointestinal malignancies to aid their surgical treatment. The studies demonstrate DNA methylation analysis on primary GI tumor tissue to potentially hold value in clinical decision making. The four chapters of the second section focus on gastric cancer, colorectal cancer and on rectal cancer specifically.

Section One

The first chapter introduces a method that allows to control methylation-specific PCR reactions better, a technique widely used in DNA methylation analysis. In the study a protocol is described for synthesis of completely unmethylated whole genomic DNA that can be used as a negative control to include in MSP experiment set-ups. The sample DNA is cheaply and quickly synthesized and quantities of DNA are obtained. The introduced so-called universal unmethylated control (UUC) can be used as a standard negative, unmethylated reference sample for any human gene.

The second chapter reports on a protocol that dramatically reduces the number of steps needed to obtain sodium bisulfite modified sample DNA from formalin-fixed paraffin-embedded (FFPE) tissue DNA ready for PCR amplification. This protocol eliminates many purification and washing steps used in classic DNA isolation techniques and classic bisulfite treatment methods. The procedure was named on-slide sodium bisulfate modification (SBM) since the formalin fixed paraffin embedded (FFPE) tissue section on the glass slide is incubated directly into sodium bisulfite solution. The DNA is modified in situ, still being in the nucleus. Performance of DNA conversion efficiency is tested and compared with the classic SBM methods. Application is shown in that on-slide SBM enables methylation-specific PCR (MSP) experiments on small tissue areas (1-2 mm²) that previously were difficult to perform. For instance, direct comparison of specific gene promoter methylation status of tumor cells with adjacent adenoma cells to adjacent normal epithelial cells on the same tissue section is now possible.

The PCR technique used in the first two chapters was semi-quantitative and we wished to combine the on-slide protocol with a fully quantitative PCR technique for better detailed DNA methylation assessment. The third chapter introduces Absolute Quantitative Analysis of Methylated Alleles (AQAMA). The experiments show that AQAMA can be combined with on-slide SBM and constitutes a robust assay that enables new opportunities to study cancer progression in a better quantitative detail.

The fourth chapter describes a protocol that aims to further improve the detailed analytical capabilities of the in-situ DNA modification concept. We integrated in-situ modification with the existing laser capture microdissection (LCM) system. LCM can select and isolate and pick up individual cells or groups of cells from a paraffin tissue section. The developed so-called “on-cap SBM” technique can compare isolated cells from histologically different tissue areas. The studies show feasibility of performing on-cap SBM combined with AQAMA assessment of the
LINE-1 DNA repeat-sequence. Application is shown that LINE-1 methylation levels of primary tumor tissue can discern presence of lymph node metastasis or distant disease spread.

Section 2

After optimizing our analytical approaches for DNA methylation assessment we formulated translational research questions. Gastro-intestinal primary tumor FFPE tissue was available as patient study material from gastric, colon and rectal cancer. The TNM-staging system accurately predicts disease outcome and is used for most treatment decisions for patients with gastro-intestinal tumors. Further subclassification to assess disease aggressiveness, however, is needed to further improve tailoring (neo)adjuvant treatment to radical surgery. Therefore as a central objective of Section 2 DNA methylation analysis was evaluated for its potential to subgroup GI cancer patients and analyze prognostic.

In gastric cancer protein expression of cyclo-oxygenase-2 (COX-2) enzyme has been tested to provide additional prognostic information independent from TNM staging. Methods for protein expression assessment, however, are still difficult to standardize and allow for inter observer variation. In chapter 5 we looked into whether epigenetic status of the COX-2 gene promoter region in gastric cancer patients controls COX-2 expression and whether it can be utilized as a prognostic marker in gastric cancer. Identification of novel biomarkers with good diagnostic performance qualities for gastric cancer has become increasingly important especially since recent studies are showing effectiveness of chemoradiation regimens in addition to surgery where this was unsuccessful in the past.

The next three chapters test clinical utility of methylated-in-tumor loci (MINT) in rectal and colon cancer. MINT loci have been repeatedly shown to be aberrantly methylated in colorectal and gastric cancer, however, their prognostic utility has not been explored widely to date.

In chapters 6 and 7 we test quantitative MINT locus methylation for its ability to subclassify rectal large bowel adenocarcinomas specifically and whether they can serve as biomarkers to aid treatment decisions in the multimodality treatment approach of rectal cancer. Clearly separate subgroups of rectal cancer patients based on MINT locus methylation levels could be identified. Chapter 6 focuses on predictive value on the potential of early rectal cancer to distantly metastasize. Chapter 7 evaluates the ability of predicting rectal cancer local recurrence probability. The data provide evidence for MINT markers to be of potential help in indicating patients towards specific adjuvant treatment regimens aimed to reduce either local or distant recurrence.

In chapter 8 we test changes in MINT locus methylation quantitatively during CRC progression using our developed techniques AQAMA and on-slide SBM. We study these methylation changes in relation to microsatellite instability and DNA mismatch repair system sufficiency which is an important hallmark of genomic instability of a subgroup of CRCs. The relation between DNA methylation is used to epigenetically subgroup large bowel cancers and this is analyzed for its value predicting distant metastasis probability.