Chapter 8

Summary

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Colorectal cancer is one of the most common diagnosed cancers worldwide, and is the second most important cause of cancer mortality in Europe. Despite careful assessment of tumor stage to decide on treatment strategies using the tumor, nodes and metastasis (TNM) staging system, large differences in patient survival and tumor recurrence are observed among patients with tumors with the same TNM classification. The general aim of the work presented in this thesis was to identify new clinically prognostic biomarkers in colorectal cancer in order to better classify patients, which might aid in the decision-making process concerning anti-cancer therapies in the future. The work presented in this thesis is a starting point for finding new clinically prognostic biomarkers by studying epigenetic mechanisms regulating gene expression. Epigenetic mechanisms were studied at different levels: both DNA methylation and histone modifications were studied genome-wide and at specific gene promoter regions.

Chapters 2 and 3 report on DNA methylation studies that were performed in rectal cancer tissues from patients enrolled in the Dutch multicenter TME clinical trial. **Chapter 2** describes the prognostic value of DNA methylation of repetitive retrotransposon sequence long interspersed element 1 (LINE-1), but not of Alu repetitive sequences, in early-stage rectal cancer. Low methylation at LINE-1 sequences was shown to correlate with shorter patient survival and a higher probability of tumor recurrence, which can be attributed to activation of LINE-1 retrotransposons which can reintegrate at random sites into the genome and can thereby interrupt regular gene sequences or influence DNA methylation of neighboring genes. In addition, results suggested that high methylation at histone H3 lysine 27 (H3K27me3) when DNA methylation at LINE-1 was low could have a "protective" function in the cells by preventing deregulated gene expression when DNA methylation is absent. In contrast, high acetylation of H3K9 in combination with high levels of DNA methylation at LINE-1 sequences could be associated with a poor disease outcome through activation of aberrant gene expression. In **Chapter 3**, DNA methylation was studied at promoter regions of apoptosis genes functioning in both the intrinsic and extrinsic apoptosis pathway routes. Combined survival analyses of intrinsic apoptosis pathway genes Apaf1, Bcl2 and p53 showed shorter survival and recurrence-free periods when an increasing number of markers showed high methylation (all low, 1 high, 2 high or all high). The shortest survival, however, was observed for patients showing low methylation of all markers, which – as was expected - correlated with high apoptosis (M30), but also with high proliferation (Ki-67).

In addition to DNA methylation, histone modifications were studied in colorectal cancer tissues, both globally and at gene-specific promoter regions. **Chapter 4** describes the prognostic value of global nuclear expression of histone deacetylases SIRT1, HDAC1 and HDAC2 combined with histone modifications H3K56Ac and H4K16Ac. High expression of histone deacetylases prevents aberrant gene expression, high levels of H4K16Ac are associated with silenced repetitive sequences and high levels of H3K56Ac are essential for proper non-homologous end-joining. Indeed, better patient survival and less tumor recurrence were observed when more markers showed high nuclear expression in combined marker survival trend analyses. **Chapter 5** shows

the prognostic value of Polycomb-group proteins EZH2, BMI1 and SUZ12 with associated histone modification H3K27me3 in colorectal cancer tissues. As would be expected from the transcriptional silencing function of H3K27me3, high expression of the histone modification and the three Polycomb-group proteins showed better patient survival and longer recurrence-free survival in combined marker survival trend analyses. Better stratification of patients was obtained by combining the expression data of the investigated biomarkers as compared to the individual markers, underlining the importance of investigating multiple markers simultaneously. In **Chapter 6**, the prognostic value of histone methylation at several histone tail residues in early-stage (TNM stage I and II) colon cancer was investigated. Low nuclear expression of H3K4me3, and high expression of H3K9me3 and H4K20me3 were associated with good prognosis, both in individual marker analyses as well as in combined marker analyses. **Chapter 7** reports on the specific chromatin environment at apoptosis gene promoter regions that can be used to predict sensitivity of colorectal cancer cell lines to cisplatin, anti-Fas or radiation therapy. The results presented in this study indicate that the apoptotic response of individual cell lines is indeed correlated with the transcriptional status of the apoptotic genes, as measured by the balance between activating and silencing histone modifications.

In conclusion, the study of epigenetic mechanisms as presented in this thesis indeed resulted in the identification of clinically relevant prognostic biomarkers. On a genome-wide level, deregulated DNA methylation on repetitive sequences (LINE-1) and deregulated expression of histone modifications and -modifying enzymes showed strong correlations to clinical outcome. At specific gene promoter regions, all related to the process of apoptosis, DNA methylation was correlated to clinical outcome and the transcriptional status based on histone modifications was predictive for the response to therapy. Taken together, these results emphasize the importance of deregulated epigenetic mechanisms in tumorigenesis. In the future, the described potentially reversible epigenetic mechanisms might provide a starting point for the identification of important epigenetic biomarkers and might eventually lead to the development of tumor-specific treatment and hence individualized medicine.