Chapter 9

Discussion and future perspectives

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In the past decades, the knowledge of the tumorigenic process at the level of genetics (including mutations and single nucleotide polymorphisms), gene transcription (regulated by epigenetic mechanisms), gene translation (including non-coding RNAs) and proteins (comprising biochemical pathways) has increased exponentially. There is a need for new prognostic (providing information on the likely clinical outcome on the basis of tumor characteristics) and predictive (predicting the response to therapy on the basis of tumor characteristics) biomarkers to advance the field of individualized medicine for colorectal cancer patients. To date, only a few biomarkers that show changes in colorectal tumor tissues as compared to normal tissues have been implemented in clinical practice, including measuring CEA levels for postoperative surveillance (1) and assessment of KRAS mutations to predict the response to anti-EGFR therapy (2). No other biomarkers have been recommended for use in clinical practice by the European Group on Tumor Markers (EGTM) (3).

In order to find clinically relevant biomarkers, understanding the underlying tumor biology is of uttermost importance. Until recently, studies on finding new biomarkers have been focused on genetic changes in tumor tissues. Now it is becoming increasingly clear that genetics does not solely determine the course of tumor development and progression. Epigenetic mechanisms, including DNA methylation and histone modifications, play a significant role in cancer development, progression, metastasis and drug resistance, and are therefore potential new biomarkers in colorectal cancer. In this thesis, epigenetic mechanisms were identified as prognostic biomarkers. In addition, the studied changes in epigenetic regulation provide information about the underlying tumor biology.

The dynamic nature of epigenetic mechanisms – new options for therapy

Epigenetic mechanisms are dynamic modifications on DNA and histone proteins that are added or removed depending on the demands of the cell under specific conditions. For example, in order for the cells to differentiate during embryonic development, changes in DNA methylation and histone modifications allow for genes to be switched on or off at the correct stage of the developmental process (4-7). Following cellular differentiation, epigenetic modifications ensure cell- and tissue-specific gene expression patterns and regulate gene expression in response to environmental stimuli (8). In cancer cells, numerous changes in epigenetic modifications occur that promote tumor development, progression and metastasis. Because of their dynamic nature, epigenetic mechanisms are potentially reversible and therefore present as attractive targets for therapeutic intervention, especially since epigenetic alterations might also be the cause of drug resistance in human cancer. Several cellular processes that could contribute to drug resistance have been described to be affected by aberrant epigenetic modifications in cancer, including enzymatic drug-metabolism, drug efflux, DNA repair and apoptosis (9-13). Reversion of these aberrant epigenetic patterns might sensitize the tumor to anti-cancer treatments.

Epigenetic therapies in a clinical setting

Several epigenetic drugs have been extensively studied and are now tested in clinical trials for treatment of hematological malignancies, including FDA-approved drugs 5-aza-2'-deoxycytidine

(decitabine; 14,15) and several histone deacetylase (HDAC) inhibitors (16,17). To date, epigenetic drugs are most effective in hematological diseases and have not shown any conclusive effectiveness of antitumor activity of any of these drugs in solid tumors, including colorectal cancer (reviewed in ref 9). This suggests that more knowledge is needed concerning the complex tumor biology and the epigenetic mechanisms involved in the tumorigenic process in solid tumors. In addition to the limited effect of epigenetic drugs in solid tumors, major concerns of using epigenetic drugs such as DNA methyltransferase (DNMT) or HDAC inhibitors include their non-specificity and therefore the possibility of unwanted side-effects of the drugs. The inhibitors might reactivate genes that are normally silent and that might promote tumor aggressiveness in addition to or instead of the expected reactivation of tumor suppressor genes (18). Despite these concerns, increasing evidence suggests that combination treatment with HDAC inhibitors and DNMT inhibitors results in a synergistic response, leading to re-expression of silenced genes, increased apoptosis and reduced tumorigenesis (19,20). In addition, combination of epigenetic drugs and current standard therapy regimens might provide a more effective way of treatment. More research is needed concerning the specific epigenetic changes in (colorectal) tumors, in order to provide targets for the development of specific epigenetic drugs. In this thesis new potential biomarkers, including DNA methylation on specific genes or repetitive sequences and histone modifications and -modifying enzymes, were identified that might provide new targets for future epigenetic therapies, as will be discussed below.

Genome-wide DNA methylation

In this thesis, genome-wide DNA methylation was studied using LINE-1 and Alu repetitive sequences. Low levels of LINE-1 methylation were found to be associated with poor prognosis, which can be explained by activation of its retrotransposon activity and hence potential random insertion of the sequences and/or changes in methylation of surrounding gene regions. In contrast, Alu methylation was not found to correlate with prognosis, indicating that our findings in rectal cancer were LINE-1 sequence-specific and not generally applicable to the whole genome. This might explain why epigenetic drugs do not yet function optimally in solid tumors, including colorectal cancer: not all regions of the genome are equally affected by changes in epigenetic modifications. Identification of the (gene) regions that are differentially regulated and have prognostic or predictive value in colorectal cancer is therefore of key importance in the search for new, clinically useful, biomarkers. The findings in this study also underline the importance of the development of more specific epigenetic drugs, targeting single epigenetic modifying proteins or maybe even sequence-specific epigenetic alterations. Furthermore, measuring LINE-1 methylation in biopsy tissues of cancer patients might provide a good risk assessment for the potential development of a distant recurrent tumor in individual patients.

Global histone modifications and –modifying enzymes

The studies concerning global histone modifications presented in this thesis have identified multiple prognostic biomarkers in colorectal cancer. Histone modifying enzymes including HDACs and EZH2 present as promising targets for epigenetic therapies, but most likely need to be combined with other – existing – therapies, as discussed above. The studies presented in this thesis showed that combining histone-modifying enzymes and histone modifications resulted

in better stratification of patients into different risk groups as compared to individual markers, underlining the importance of pathway-focused approaches. Considering this, future research should focus on combining multiple drugs targeting epigenetic enzymes. This might lead the way to success for epigenetic therapy in solid tumors. Risk assessment for individual patients based on the expression of histone modifications and –modifying enzymes might add to the current TNM staging system.

Gene-specific epigenetic modifications

The transcriptional status (including both DNA methylation and histone modifications) of specific genes, as shown for apoptosis genes in this thesis, has prognostic and predictive value in colorectal cancer. DNA methylation of combined intrinsic apoptosis pathway genes Apaf1, Bcl2 and p53 was found to be prognostic in our rectal cancer patient cohort. In literature, methylation of apoptosis genes has also been linked to drug resistance. For example, methylation of apoptosis effectors BNIP3 and DAPK has been shown to predict lower response rates to fluoropyrimidine-based treatment in gastric cancer (21). In addition to DNA methylation, we also studied histone modifications on apoptosis gene promoters. The transcriptional status of the respective apoptosis genes, on the basis of the presence of activating and/or silencing histone modifications, were shown to correlate with the response to anti-cancer treatments, including chemotherapy, irradiation or immunotherapy. Determining the transcriptional status of key genes in for example the apoptosis pathway in patient tissues (biopsies) may lead to specific decisions for therapy on the basis of the biological make-up of individual tumors. Continuing on the discussion above, current epigenetic therapies lack specificity and may thereby fail to be effective in several types of solid tumors, including colorectal cancer. New therapies should be developed focused on reversing epigenetic alterations on specific genes or sequences in individual tumors that show prognostic and/or predictive value, thereby getting one step closer to individualized medicine. For this purpose, more research is needed to understand the mechanisms of gene- or pathway specific epigenetic regulation by epigenetic modifiers such as DNMTs and histone-modifying enzymes. This knowledge will aid in the identification of clinically relevant biomarkers that can be used to develop new - epigenetic - therapies for solid tumors.

Future perspectives

Several epigenetic clinically prognostic biomarkers were identified in colorectal cancer in this thesis, including both genome-wide and gene-specific patterns of DNA methylation and histone modifications. Knowledge of tumor biology is of key importance in the development of new therapies and the making of informed, patient-specific, treatment decisions. Pathway-focused approaches, as presented in this thesis, provide information regarding possible synergistic interactions of biomarkers. This information will lead to identification of new combinations of treatment regimens that enhance the anti-cancer effects in individual patients. Multidisciplinary medicine, combining the knowledge provided by biomarker research and results of clinical trials, is therefore important to advance the field of individualized medicine. In addition, newly validated biomarkers will add crucial information to tumor characterization instruments currently used in clinical practice, such as the TNM staging system. Adding information on epigenetic

modifications, will aid in identifying high-risk patients and predicting response to therapy based on tumor characteristics, and will thereby provide opportunities for individualized medicine.

In conclusion, the work presented in this thesis underlines the importance of epigenetics in tumor development. The various studies presented show that epigenetic alterations have a profound impact on patient survival and tumor recurrence. Even though most cancer research has focused on the effect of gene mutations on the tumorigenic process during the last decades, it is becoming increasingly clear that epigenetic mechanisms are important mediators in this process. A delicate interplay between genetics and epigenetics determines the fate of each individual cell in the human body. Gene mutations cause changes in gene expression or stability/interactions of the resulting mutated proteins, which all have downstream effects within pathways that are accompanied by changes in the epigenetic regulation of these downstream factors. In addition, gene mutations in epigenetic modifiers will have direct effects on the transcriptional regulation of many genes or gene regions. Therefore, epigenetic mechanisms are unquestionably tied to the tumorigenic process and should be considered as a grand new source of information not only for identification of prognostic and predictive biomarkers, but also for the development of new, possibly tumor- and therefore patient-specific, anti-cancer therapies.

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