Pharmacogenetics of advanced colorectal cancer treatment

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Outline of the thesis

Colorectal cancer is one of the leading causes of cancer related deaths.[1] Surgery with curative intent is indicated for patients without distant metastases and in a subset of patients with resectable distant metastases.[2] For irresectable metastatic colorectal cancer, only palliative treatment options remain. Current standard treatment consists of chemotherapeutic drugs (the fluoropyrimidines, oxaliplatin and irinotecan) and antibodies against vascular endothelial growth factor (VEGF; bevacizumab)[3] and the epidermal growth factor receptor (EGFR; cetuximab and panitumumab).[4-6] Even though the optimal use of these agents has not been defined, the most commonly applied first-line treatment consists of a fluoropyrimidine as monotherapy, or combined with oxaliplatin or irinotecan, plus bevacizumab, while the other drugs are used as salvage treatments.[7,8] With the currently available regimens, the median overall survival of metastatic colorectal cancer patients is approximately two years.[2] Despite the improvement of prognosis of metastatic colorectal cancer patients from roughly 12 to 24 months in the past fifteen years[2], the efficacy of these expensive and potentially toxic treatments remains limited and unpredictable. It is therefore desirable to develop predictive markers to aid better selecting patients for these treatments.

In order to select patients for treatment, germline genetic variation between patients, as well as somatic mutations in their tumors can be used. As anti-cancer treatment exerts its effect in the tumor, it is reasonable to correlate the genetic mutations in the tumor to the anti-tumor response. Indeed, some of these mutations are used in routine clinical practice, such as *EGFR* mutation testing for the selection of non-small cell lung cancer patients for treatment with the small-molecule tyrosine kinase inhibitors against EGFR gefitinib and erlotinib[9,10] and *KRAS* mutation testing for the selection of metastatic colorectal cancer patients for cetuximab or panitumumab treatment.[11] A disadvantage of the use of somatic mutations, is that the tumor is genetically unstable, resulting in different genetic composition over time. Moreover, discordance in mutational status may be present between the primary tumor and corresponding metastatic lesions for some genetic variants, as well as discordance within one tumor sample.

Heritable germline variation in DNA derived from peripheral blood or other normal tissue is studied in the field of pharmacogenetics. Genetic polymorphisms may be present in drug target proteins, or in enzymes involved in the pharmacokinetics of the drug of interest. The presence of a genetic polymorphism in a gene can result in increased or decreased expression, or altered function of the protein. As a result, drug response – either efficacy or toxicity – may be altered. Advantages over tumor-derived genetic variation are that germline genotypes remain constant over time, and that the collection of blood or saliva is only mildly invasive. Moreover, the germline genetic variation is the same as in tumor tissue, but not vice-versa: somatic mutations that originate in tumor tissues cannot be detected in germline material.[12]

The aim of this thesis is to identify germline pharmacogenetic markers for predicting the response to palliative treatment of metastatic colorectal cancer.

The first part of the thesis focuses on predictive germline markers for the efficacy of cetuximab. A review of pharmacogenetic studies for EGFR and VEGF targeted therapy is given in **chapter 2**. Germline DNA was obtained from patients in the CAIRO2 trial of the Dutch Colorectal Cancer Group (DCCG). In this randomized phase III study, patients with previously untreated metastatic colorectal cancer were treated with capecitabine, oxaliplatin and bevacizumab or the same regimen plus cetuximab. Surprisingly, the addition of cetuximab resulted in decreased median progression-free survival (PFS).[13] The influence of five different germline polymorphisms on the efficacy of cetuximab was investigated in patients of the CAIRO2 study **(chapter 3)**. To further explore the mechanism underlying the results of this pharmacogenetic analysis, *in vitro* research on the influence of the *FCGR3A* Phe158Val polymorphism was performed. As a model for tumor-associated macrophages, type 2 macrophages were cultured from monocytes of healthy donors harboring the different *FCGR3A* genotypes. The activation of these type 2 macrophages under the influence of cetuximab was studied **(chapter 4)**.

In the second part of the thesis, predictive germline variation for the efficacy of capecitabine, oxaliplatin and bevacizumab – the treatment in the control arm of the CAIRO2 study – was studied. The literature on pharmacogenetics of cytotoxic therapy is reviewed in **chapter 5**.

In the previous CAIRO study[7], an exploratory study was performed with candidate polymorphisms in DNA repair genes.[14] Polymorphisms in the *ATM* and *ERCC5* genes were associated with the efficacy of an oxaliplatin-based regimen. To confirm these preliminary findings, the effects of these polymorphisms on treatment response were investigated in the control arm of the CAIRO2 study **(chapter 6)**.

In classic pharmacogenetic studies, each polymorphism is correlated with the clinical end-point. A limitation to this method is that the complexity underlying drug response is not fully taken into account. It is therefore not surprising that inconsistent results have been published for most pharmacogenetic markers.[15,16] Since drug response involves many different proteins – such as therapeutic targets, molecules in the signaling pathway, metabolic enzymes or drug transporters – it is likely that the impact of polymorphisms in the corresponding genes exert their influence only in the presence of other polymorphisms. This concept is known as non-linear interaction, or epistasis.[17]

To investigate epistasis in relation to drug response, novel methods such the multifactor dimensionality reduction (MDR) and classification and regression tree (CART) techniques can be applied. The technical aspects of these techniques are

described and illustrated using sunitinib induced toxicity data from a previous study[18] **(chapter 7)**. The MDR method was applied to explore the association and interaction of 17 frequently studied polymorphisms in different candidate genes in the control arm of the CAIRO2 study **(chapter 8)**.

Currently, most pharmacogenetic studies include polymorphisms in so-called candidate genes. A limitation of this approach is that only mechanistically related genes and polymorphisms are studied, which is by definition restricted by our current understanding of the mechanism of action of the drugs of interest. To identify novel polymorphisms – and genes – that are associated with response to capecitabine, oxaliplatin and bevacizumab, a hypothesis-free genome wide association study was performed with an array including more than 700,000 polymorphisms **(chapter 9)**.
The results from these studies are summarized **(chapter 10)** and put into perspective in the general discussion **(chapter 11)**.

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