CHAPTER 11

PREDICTION OF RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN CARCINOMAS OF THE UPPER GASTROINTESTINAL TRACT

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Abstract: Multimodal treatment protocols are increasingly employed to improve the survival of patients with locally advanced adenocarcinomas of the upper gastrointestinal tract, however, only 30–40% per year of the patients respond to 5-FU and cisplatin-based neoadjuvant chemotherapy. The goal of our studies is the identification of reliable genetic markers, on the genomic DNA-level, mRNA, or protein level that could predict response of upper gastrointestinal carcinomas prior to neoadjuvant chemotherapy.

In esophageal carcinomas, a higher gene expression of methylenetetrahydrofolate reductase (MTHFR), an enzyme involved in folate metabolism, was more frequently found in responding patients. In addition high gene expression of *caldesmon* and of the two drug carrier proteins, MRP1 and MDR1 was associated with response to therapy. By performing a genome-wide profiling on the protein level in a small group of patients, new potential markers were identified, which have to be validated in ongoing studies.

In gastric carcinomas, mutations of the *p53* gene revealed no association with response or survival, but tumors with a high rate of loss of heterozygosity (LOH), determined by microsatellite analysis, showed a better response to a cisplatin-based chemotherapy. Analysis of expression of 5-FU-(e.g., *TS*, *DPD*, and *TP*) and cisplatin-(e.g., *ERCC1*, *ERCC4*, *GADD45A*, and *KU80*) related genes, demonstrated an association of *DPD* expression with response and survival. The combined consideration of *TP* and *GADD45* gene expression, showed the most obvious association with therapy response in this tumor.

Our studies point to promising markers with potential use for chemotherapy response prediction of adenocarcinomas of the upper gastrointestinal tract, but prospective studies for validation are necessary.

Key words: Neoadjuvant chemothepy, carcinomas, gastrointestinal tract, LOH

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1. INTRODUCTION AND OBJECTIVE

Multimodal treatment protocols are increasingly employed to improve the survival of patients with locally advanced adenocarcinomas of the esophagus and stomach. Neoadjuvant chemotherapeutic treatment, mainly based on cisplatin and 5-FU, has been used since 1989 in several clinical trials and recently, a statistically significant improvement in respect to resectability, progression-free and overall survival in operable gastric and lower esophageal cancer has been demonstrated in a large randomized, controlled phase III trial (MAGIC trial) [1]. However, only 30–40% of the patients respond to therapy and the majority of patients undergo several month of toxic, expensive therapy without survival benefit. In particular, in the case of esophageal carcinomas, it has been shown that patients with nonresponding tumors seem to have an even worse prognosis than patients treated by surgery alone, which may be related to therapy-induced side effects, selection of chemotherapy-resistant, more aggressive tumor cells and delay of surgery [2]. Thus, the identification of reliable genetic markers that could predict response is highly demanding.

Several molecular markers had been investigated as potential response predictors. Thymidylate synthase as the target enzyme for 5-FU has been widely studied for 5-FU-containing regimens in gastrointestinal cancer, but the results are inconsistent [3,4,5]. Dihydropyrimidine dehydrogenase (DPD) and thymidine phosphorylase (TP) are two other important regulatory enzymes involved in the degradation of 5-FU, and low levels of DPD have been shown to be associated with response in gastric carcinoma [5,6], whereas conflicting results have been reported for TP.

The other major component used for the treatment of carcinomas of the upper gastrointestinal tract is cisplatin, which supposedly directly damages DNA. A significant association of the gene expression of the nucleotide excision enzyme *ERCC1*, which is involved in DNA repair, with response to neoadjuvant chemotherapy has been reported [4].

Other markers such as glutathione *S*-transferase, vascular endothelial growth factor and apoptosis-related genes have been such as *bcl-2*, *bax*, and *p53* have mostly been studied by immunohistochemistry, and the results have been inconclusive, so that no markers has been found to be clinically relevant at present [3,7].

Thus, the goal of our studies is to identify effective molecular markers for response prediction for patients with esophageal and gastric carcinomas treated by a neoadjuvant chemotherapy. We are using different strategies based on one side, on targeted approaches to characterize pretherapeutic biopsies for tumor-specific molecular alterations on the genomic DNA and mRNA-level. We also analyze constitutional genetic factors, e.g., DNA-polymorphisms in therapy-related genes. On the other side, we perform a genome-wide profiling on the protein level, to identify new marker proteins.

2. RESULTS

2.1. Characterization of pretherapeutic biopsies of esophageal carcinomas

2.1.1. Analysis of m-RNA expression of therapy-related genes

In this study, paraffin-embedded, formalin-fixed endoscopic esophageal tumor biopsies of 38 patients with locally advanced esophageal adenocarcinomas (Barrett's adenocarcinoma) were included. All patients underwent two cycles of cisplatin and fluorouracil (5-FU) therapy with or without additional paclitaxel followed by abdominothoracal esophagectomy. RNA expression levels of 5-FU metabolism-associated genes, *thymidylate synthase*, *TP*, *DPD*, *MTHFR*, *MAP7*, *ELF3*, as well as of platinum and taxane-related genes *Caldesmon*, *ERCC1*, *ERCC4*, *HER2-neu*, *GADD45* and multidrug resistance gene *MRP1* were determined using real-time RT-PCR. Expression levels were correlated with response to chemotherapy histopathologically assessed in surgically resected specimens.

The results demonstrated that the responding patients showed significantly higher pretherapeutic expression levels of *MTHFR* ($p = 0.012$), *Caldesmon* ($p = 0.016$), and *MRP1* ($p = 0.007$). In addition, patients with high pretherapeutic *MTHFR* and *MRP1* levels had a survival benefit after surgery ($p = 0.013$ and $p = 0.015$, respectively) [8]. Additionally, investigation of intratumoral heterogeneity of gene expression of relevant genes (e.g., *MTHFR*, *Caldesmon*, *Her2-neu*, *ERCC4*, and *MRP1*) — verified in nine untreated Barrett's adenocarcinomas by examination of five distinct tumor areas — revealed no significant heterogeneity in gene expression indicating that expression profiles obtained from biopsy material may yield a representative genetic expression profile of total tumor tissue [8].

Thus in conclusion, the results indicate that determination of mRNA levels of few genes may be useful for the prediction of the success of neoadjuvant chemotherapy in individual cancer patients with locally advanced Barrett's adenocarcinoma.

2.1.2. Differential quantitative ProteoTope analysis of fresh frozen biopsies

A comprehensive protein profiling approach, using the ProteoSys platform, has been performed until now for a small group of patients. Quantitative and qualitative protein expression analysis was performed using 2D ProteoTope techniques after radioactive labeling of the protein extract with I-125 and I-131. The results so far point to an interesting group of proteins, which may be associated with response. Validation of specific proteins by immunohistochemical analysis in a high number of cases is now part of ongoing studies.

2.2. Characterization of pretherapeutic biopsies of gastric carcinomas

2.2.1. Microsatellite analysis and p53 mutation analysis

We evaluated microsatellite instability (MSI) and LOH in 53 pretherapeutic gastric carcinoma biopsies using 11 microsatellite markers. The entire coding region of the p53 gene (exons 2–11) was analyzed for mutations by denaturing high-pressure liquid chromatography (DHPLC) and sequencing. P53 protein expression was evaluated by immunohistochemistry. Patients were treated with a cisplatin-based, neoadjuvant chemotherapy regimen. Therapy response was evaluated by CT scan, endoscopy, and endoluminal ultrasound [9,10].

We identified p53 mutations in 19 of the 53 (36%) analyzed tumors. No significant association with response or survival was found for p53 mutation or for p53 protein expression. Microsatellite instability (either MSI-H or MSI-L) did not show a correlation with response. With respect to LOH, LOH at chromosome 17p13 showed a significant association with therapy response ($p = 0.022$), but did not reach statistical significance in terms of patient survival. The global LOH rate, expressed as fractional allelic loss (FAL) was assessed and tumors were classified into tumors with a high (>0.5), a medium (>0.25–0.5), and a low (0–0.25) FAL-value. A statistically significant association of FAL with therapy response was found ($p = 0.003$), with a high FAL being related to therapy response.

Thus, a high level of chromosomal instability (high FAL-value) defines a subset of patients who are more likely to benefit from cisplatin-based neoadjuvant chemotherapy. p53 mutation status is not significantly associated with therapy response and is not a useful marker for response prediction [9,10].

2.2.2. Methylation analysis

We investigated the methylation profile of six genes, which are frequently methylated in gastric cancer (e.g., *14-3-3σ*, *E-cadherine*, *HPP1*, *Lysyl oxidase*, *MGMT*, and *p16*) for an association with response and survival in a set of 61 neoadjuvant-treated gastric cancer patients by bisulfite/methylation-specific PCR using the TaqMan system. Only 46% of the patients showed tumor-specific methylation signals in four or more genes. There was no significant correlation of response with global methylation status or with any of the genes alone. Patients with a low methylation status showed a tendency for response to therapy and patients with no or only one methylated gene demonstrated a statistically significant better survival ($p = 0.027$). This interesting finding raises the question if the use of inhibitors of DNA methylation and/or histone deacetylase inhibitors might represent a therapeutic alternative for gastric cancer patients demonstrating a high methylation status in their tumors [11].

2.2.4. Analysis of mRNA expression of therapy-related genes

For gastric carcinomas we performed gene expression analysis, focusing on genes related to the effects of 5-FU or cisplatin. Pretherapeutic, formalin-fixed

and paraffin-embedded biopsies of 61 patients, who received a 5-FU and cisplatin-based chemotherapy were included. The expression of the 5-FU-related genes *TS*, *DPD* and *TP* and of the cisplatin-related genes *ERCC1*, *ERCC4*, *KU80* and *GADD45A* were analyzed by quantitative real-time PCR. The expression levels of single genes and of various combinations were tested for an association with response and overall survival [5]. High DPD levels were more frequently found in nonresponding patients and were associated with worse survival. *GADD45A* and *TP* levels demonstrated weak associations with response, but *GADD45A* expression correlated with survival. There was no association with response to TS expression, but tumors with a high TS level were associated with worse survival. The combination of *GADD45A* and *TP* revealed the strongest predictive impact. High expression values of *TP* and/or *GADD45A* were exclusively found in nonresponding patients ($p = 0.002$) and were associated with a significantly poorer survival ($p = 0.04$).

Thus, in conclusion, the combined gene expression levels of *TP* and *GADD45A* represent a new parameter to predict the clinical outcome after neoadjuvant chemotherapy in gastric cancer. The association of *DPD* expression with response and survival underlines a predominant role of *DPD* to predict 5-FU sensitivity. The association of *TS* expression levels with survival, but not with response, suggests an importance of this gene for tumor progression [5].

3. OUTLOOK

Although some of our studies point to promising markers with a potential use in chemotherapy response prediction for adenocarcinomas of the upper gastrointestinal tract, prospective studies for validation are necessary before they may be used in clinical practice. As chemotherapy response is considered to be highly complex, depending on tumor-specific characteristics as well as on the constitutional genetic makeup of the individual patient, integrative approaches for response prediction might be necessary. In addition the incorporation of early response evaluation by positron emission tomography (PET) for the therapeutic decision together with molecular markers, might result in superior sensitivity and specificity for a successful application of an individual therapy-strategy for patients with upper gastrointestinal malignancies.

REFERENCES

1. D. Cunnigham, W.H. Allum, S.P. Stenning, and S. Weeden, for the upper GI Cancer Clinical Study Group. Perioperative chemotherapy in operable gastric and lower oesophageal cancer: final results of a randomised, controlled trial (the MAGIC trial, ISRCTN 93793971). *ASCO Annual Meeting*, Abstract No. 4001 (2005).
2. J. Zacherl, A. Sendler, H.J. Stein, K. Ott, M. Feith, R. Jakesz, J.R. Siewert, and U. Fink. Current status of the neoadjuvant therapy for adenocarcinoma of the distal esophagus. *World J Surg* 27: 1067–1074 (2003).

3. K.H. Yeh, C.T. Shun, C.L. Chen, J.T. Lin, W.J. Lee, Y.C. Chen, and A.L. Cheng. High expression of thymidylate synthase is associated with the drug resistance of gastric carcinoma to high dose 5-fluorouracil-based systemic chemotherapy. *Cancer* 82: 1626–1631 (1998).
4. R. Metzger, C.G. Leichman, K.D. Danenberg, P.V. Danenberg, H.J. Lenz, K. Hayashi, S. Groshen, D. Salonga, H. Cohen, L. Laine, P. Crookes, H. Silberman, J. Baranda, B. Konda, and L. Leichman. ERCC1 mRNA levels complement thymidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and fluorouracil chemotherapy. *J Clin Oncol* 16: 309–316 (1998).
5. R. Napieralski, K. Ott, M. Kremer, K. Specht, H. Vogelsang, K. Becker, M. Müller, F. Lordick, U. Fink, J.R. Siewert, H. Höfler, and G. Keller. Combined GADD45A and TP expression levels predict response and survival of neoadjuvant treated gastric cancer patients. *Clin Cancer Res* 11: 3025–3031 (2005).
6. Y. Ishikawa, T. Kubota, Y. Otani, M. Watanabe, T. Teramoto, K. Kumai, T. Takechi, H. Okabe, M. Fukushima, and M. Kitajima. Dihydropyrimidine dehydrogenase and messenger RNA levels in gastric cancer: possible predictor for sensitivity to 5-fluorouracil. *Jpn J Cancer Res* 91(1): 105–112 (2000).
7. N. Boku, K. Chin, K. Hosokawa, A. Ohtsu, H. Tajiri, S. Yosihida, T. Yamao, H. Kondo, K. Shirao, Y. Shimada, D. Saito, T. Hasebe, K. Mukai, S. Seki, H. Saito, and P. Johnston. Biological Markers as a predictor for response and prognosis of unresectable gastric cancer patients treated with 5-fluorouracil and cis-platinum. *Clin Cancer Res* 4: 1469–1474, (1998).
8. R. Langer, K. Specht, K. Becker, E. Philipp, M. Beckisch, M. Sarbia, R. Busch, M. Feith, H.J. Stein, J.R.Siewert, and H. Höfler. Prediction of response to neoadjuvant chemotherapy in Barrett`s carcinoma by quantitative gene expression analysis. *Clin Cancer Res* in press (2005).
9. T. Grundei, H. Vogelsang, K. Ott, J. Mueller M. Scholz, K. Becker, U. Fink, J.R. Siewert, H. Höfler H, and G. Keller. Loss of heterozygosity and microsatellite instability as predictive markers for neoadjuvant treatment in gastric carcinoma. *Clin Cancer Res* 6: 4782–4788 (2000).
10. K. Ott, H. Vogelsang, J. Mueller, K. Becker, M. Müller, U. Fink, J.R. Siewert, H. Höfler, and G. Keller. Chromosomal instability rather than p53 mutation is associated with response to neoadjuvant cisplatin-based chemotherapy in gastric cancer. *Clin Cancer Res* 9: 2307–2315 (2003).
11. R. Napieralski, K. Ott, M. Kremer, K. Becker, H. Vogelsang, F. Lordick, J.R. Siewert, H. Höfler, and G. Keller. Hypermethylation phenotype and association with response and survival in neoadjuvant treated gastric carcinoma, submitted.