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TOPIC HIGHLIGHT

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Proteomic and metabolic prediction of response to therapy in gastric cancer

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Abstract

Several new treatment options for gastric cancer have been introduced but the prognosis of patients diagnosed with gastric cancer is still poor. Disease prognosis could be improved for high-risk individuals by implementing earlier screenings. Because many patients are asymptomatic during the early stages of gastric cancer, the diagnosis is often delayed and patients present with unresectable locally advanced or metastatic disease. Cytotoxic treatment has been shown to prolong survival in general, but not all patients are responders. The application of targeted therapies and multimodal treatment has improved prognosis for those with advanced disease. However, these new therapeutic strategies do not uniformly benefit all patients. Predicting whether patients will respond to specific therapies would be of particular value and would allow for stratifying patients for personalized treatment strategies. Metabolic imaging by PET was the first technique with the potential to predict the response of esophago-gastric cancer to neoadjuvant therapy. Exploring and validating tissue-based biomarkers are ongoing processes. In this review, we discuss the status of several targeted therapies for gastric cancer, as well as proteomic and metabolic methods for investigating biomarkers for therapy response prediction in gastric cancer.

Key words: Gastric cancer; Therapy; Response prediction; Positron emission tomography; Matrix-assisted laser desorption-ionization

Core tip: The prognosis of patients diagnosed with gastric cancer is still poor. Cytotoxic treatment and targeted therapies have improved the prognosis of patients. However, patients do not benefit equally from these treatment options. The ability to predict whether patients will respond to specific therapies would be of particular value and would allow for stratifying patients for personalized treatment strategies. In this review, we discuss the status of targeted therapies for gastric cancer, as well as proteomic and metabolic methods for investigating biomarkers for therapy response prediction in gastric cancer.

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INTRODUCTION

Gastric cancer morbidity ranks number four and mortality ranks number two with respect to worldwide cancer disease incidence and death[1]. Disease outcome depends on the tumor stage at the time of diagnosis; if not diagnosed early, prognosis is generally poor. Because most of the patients are asymptomatic during the early stages of gastric cancer, the diagnosis is often delayed and patients present with an unresectable locally advanced or metastatic disease. Current treatment protocols for gastric cancer are based primarily on results of clinical studies, and to a lesser degree on specific histological features. Treatment options for gastric cancer patients include surgery, chemotherapy and radiation therapy. The current prognosis for individuals with gastric cancer is grim, with fewer than 25% of patients surviving at 5 years after diagnosis[1,2]. Improved preoperative care and surgical techniques have produced clear benefits. However, real progress will only be achieved through the development of new treatment options that have reduced cell toxicity compared with that of standard therapeutic regimens. Recent studies are exploring approaches based on molecularly targeted therapeutics. This strategy personalizes the treatment therapy based on individual biomarkers, which can be used to select treatments that most effectively remediate cancers with specific biomarkers. Further work is needed to characterize early tumor responses to different neoadjuvant therapies.

Inter-individual variability of drug response or resistance but also individual tumor heterogeneity presents a challenge when treating gastric cancer. The identification of predictive tumor markers at the time of diagnosis that enable managers to develop more effective therapeutic strategies would be invaluable for patient treatment. Therefore, current research is focusing on indentifying novel, cancer- and patient-specific imaging and tissue- and blood-based biomarkers. Recent progress in gene sequencing and molecular diagnostics enables the identification of potentially useful biomarkers; however, many of these are controversial. Some studies are in apparent disagreement. Currently, the status of human epidermal growth factor receptor-2 (HER2) is used to select trastuzumab chemotherapy. However, no other biomarkers have been approved by medical consensus and governing agencies.

The quantification of molecular alterations correlating with heterogeneous gastric tumors at different stages of disease progression is technically challenging, and prevents the development of reliable biomarkers. Another obstacle is tumor heterogeneity, which is particularly evident in gastric cancer. New proteomic technologies are developing rapidly. Proteomic approaches promote large-scale sample screening and facilitate identification of proteins associated with disease and treatment. Metabolic changes associated with invasive cancers could be useful for predicting treatment responses; these changes could be tracked with specific metabolic tracers and molecular metabolic imaging during early assessment of patient-specific treatment strategies.

Gastric cancer is an active topic of clinical and basic research due to the high morbidity and mortality. A full understanding of molecular parameters that determine prognosis and how to predict and control therapeutic responses is lacking. Identification of specific biomarkers will elucidate the molecular, proteomic, and metabolic treatment responses and drug resistance mechanisms. This review discusses proteomic approaches for biomarker detection and metabolic imaging for early prediction of gastric cancer response to systemic and targeted therapies.

THERAPEUTIC STRATEGIES

Due to recent large scale randomized studies, a globally accepted standard medical treatment of gastric cancer can be defined[3]. Recent studies have demonstrated that preoperative and perioperative chemotherapy improves the clinical outcome for patients with gastric cancer[4-6]. Patients with potentially resectable tumors are treated with surgery and perioperative chemotherapy or postoperative chemoradiation[7]. In the metastatic disease setting, patients are treated with combination chemotherapy because exposure to cytotoxins prolongs survival and improves control of symptoms[8]. Recently, an international author team evaluated commonly used therapeutic strategies for gastric cancer[3]. Their analysis indicated that a combination of cisplatin and 5-fluorouracil was the preferred strategy. However, oxaliplatin efficacy was equivalent to that of cisplatin[3], and oral fluoropyrimidines such as S-1 and capecitabine could be substituted for 5-fluorouracil[3]. Combination chemotherapy was preferred for the majority of patients due to the balanced benefit-to-risk ratio[3]. For fit patients with high tumor burden and possible secondary resectability, triplet chemotherapy had greater efficiency and produced higher treatment response rates[3]. Docetaxel resulted in significantly higher side effects[3]. For elderly and infirm patients, monotherapy and dose modifications were considered as beneficial[3]. In general, approximately 50% of patients respond to chemotherapy containing cisplatin, fluorouracil and anthracyclines or taxanes, but median survival is less than 12 mo with these combinations[9]. The optimal approach for a given patient remains unclear and controversial. However, one consistent finding is that patients who exhibit a histopathological response to neoadjuvant therapy are more likely to receive a survival benefit. Cytotoxic therapy provides positive response rates ranging from 20%-60%[10]. There are a few studies that evaluate clinical or histopathological markers that predict response and prognosis for neoadjuvant-treated gastric cancer, and none of the potential markers have been validated in prospective studies[10-15]. Identifying predictive, pretherapeutic markers for treatment response would facilitate customization of individual care strategies. Due to this patient specific situation, there is a need

Table 1 Potential biomarkers for therapy response prediction in gastric cancer

Ref.	Biomarker	Sample	Patients	Chemotherapy
Huang *et al*[25], 2013	AMBP	Serum	17 GC patients	paclitaxel, capecitabine
Sekikawa *et al*[26], 2013	REG Iα	Tissue	70 GC patients	S-1, Cisplatin
Okada *et al*[27], 2013	FOXM1	Tissue	81 GC patients	docetaxel, 5-FU, cisplatin/5-FU, cisplatin
Sugita *et al*[28], 2010	BNIP3 DAPK	Tissue	80 GC patients	5-FU, irinotecan/docetaxel/cisplatin
Mitani *et al*[29], 2007	REG4	Serum	36 GC patients	5-FU, cisplatin

AMBP: Alpha-1microglobulin/bikunin precursor; REG Iα: Regenerating gene Iα; FoxM1: Forkhead box M1 transcription factor; BNIP3: Bcl-2/adenovirus E1B 19 kDa-interacting protein 3; DAPK: Death associated protein kinase; REG IV: Regenerating gene IV; GC: Gastric cancer; 5-FU: 5-fluorouracil.

to use clinical, genomic, transcriptomic, proteomic, and other information sources to plot the optimal course for an individual patient in terms of disease risk assessment, prevention, treatment, or palliation. This is the concept of personalized medicine. Therapeutic approaches for gastric cancer will become increasingly customized in future clinical practice.

Research efforts for gastric cancer have focused on improving prognosis and decreasing chemotherapeutic toxicity. The addition of molecularly targeted agents to treatment protocols may achieve both of these goals. The human HER family is one of the main targets in human cancer therapies[16,17]. The HER family contains four related members, HER1 (ErbB1 or EGFR), HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). HER2 is an important biomarker in gastric tumors and can be specifically targeted via a monoclonal antibody for trastuzumab therapy[18]. For patients with advanced gastric cancer or cancer of the gastro-esophageal junction, survival is improved with trastuzumab therapy combined with chemotherapy compared with that of chemotherapy alone[19]. Trastuzumab combined with capecitabine or 5-fluorouracil and cisplatin has been approved for treating patients with HER2-positive metastatic adenocarcinoma of the stomach or gastro-esophageal junction by the European Union, United States, and Japan.

Lapatinib, a tyrosine kinase inhibitor against both epidermal growth factor receptor (EGFR) and HER2, has had modest single activity[20]. Additionally, a statistically significant improvement in overall survival (primary endpoint) with the addition of lapatinib to capecitabine plus oxaliplatin (CapeOx) as the first-line treatment of advanced or metastatic gastric or gastro-esophageal adenocarcinoma could not be demonstrated (Logic trial)[21]. With regard to toxicity, lapatinib in combination with CapeOx showed an increased rate of grade 3 diarrhea and a higher rate of skin toxicity. In contrast to the success obtained with trastuzumab in advanced gastric cancer, monoclonal antibodies that target EGFR have failed to improve outcome in biologically unselected gastric cancer patients[22,23]. It remains to be elucidated from tumor tissue analyses if a small proportion of gastric cancer patients may benefit from anti-EGFR targeted therapy, *e.g.*, in the case of *EGFR* gene amplification[24]. The negative results obtained with cetuximab (EXPAND study) and panitumumab (REAL3 study) emphasize the need to have a biologically meaningful target before studying targeted agents in larger populations. But the development of trastuzumab in HER2-overexpressing gastric cancer raises hope that further progress may be achieved.

Developing new targeted and multimodal therapies for gastrointestinal cancer has improved patient prognosis. However, additional treatment choices add greater complexity to the therapeutic strategy, and selecting the correct regimen has become more challenging for clinical managers. Therefore, the identification of new therapeutic response biomarkers is crucial.

POTENTIAL BIOMARKERS

The selection of anticancer regimens based on individual patient biomarkers constitutes personalized cancer treatment. There is a strong need to identify parameters that can be used as reporters of tumor responsiveness during the early phases of neoadjuvant therapies. Current therapeutic management is based primarily on clinical data and histological features. Several new treatment options have been introduced recently, but variations in individual responses and drug resistance present challenges. Many promising markers for disease prognosis or therapeutic response have been identified, but the diagnostic value of many biomarkers is controversial. The only molecular marker currently in clinical use to tailor patient therapy is the HER2 status for treatment with trastuzumab. Identifying new cancer-specific biomarkers for predicting patient responses to different therapies is currently a focus of translational research. Table 1 presents studies that have identified potential biomarkers that could be used for predicting therapy response in gastric cancer patients[25-33]. These "discovery" studies provide some perspectives for establishment of further biomarkers. In this regard, in a recently published study it was demonstrated that high levels of serum AMBP (Alpha-1Microglobulin/Bikunin Precursor) could predict the poor response of gastric cancer patients treated with paclitaxel-capecitabine chemotherapy[25]. Also recently published was a study identifying REG Iα (Regenerating Gene Iα) expression in tissue biopsies of gastric cancer patients for predicting response to chemotherapy with S1 plus cisplatin[26]. Another potential biomarker, FoxM1 (Forkhead Box M1 Transcription Factor), was suggested as biomarker for resistance to

chemotherapy with docetaxel in addition to 5-fluorouracil derivate plus cisplatin when overexpressed gastric cancer tissue[27]. The methylation of the apoptosis-related genes, *BNIP3* (Bcl-2/Adenovirus E1B 19 kDa-interacting Protein 3) and *DAPK* (Death Associated Protein Kinase) was examined in tumor samples from patients treated with fluoropyrimidine-based chemotherapy for metastatic or recurrent gastric cancer and was found to indicate lower response to the chemotherapy[28]. High levels of REG Ⅳ (regenerating gene family, member 4) in the serum of gastric cancer patients were identified to predict resistance to 5-fluorouracil-based chemotherapy[29].

These new biomarkers have not yet progressed from basic research into clinical practice. A greater understanding of these biomarkers could reveal novel insights into the molecular changes underlying cancer progression, metabolic responses to treatments, and mechanisms leading to chemotherapy resistance. Metabolic and proteomic changes are features of invasive cancers, and may provide valuable information for assessing prognosis and response to treatment for patients with gastric cancer. Proteomics evaluates protein expression, post-translational modifications, and complex expression patterns in tissues, cells, and biological fluids[34,35].

PROTEOMICS ANALYSIS FOR PREDICTING THERAPEUTIC RESPONSES

Changes in protein profiles reflect changes in cellular metabolism and cellular responses to extracellular conditions. Proteins are key effector molecules that influence pathological conditions. The development of proteomics technologies enables screening of different samples such as fluids and clinical tissues, including fresh/frozen and formalin-fixed paraffin embedded (FFPE) materials. Fresh/frozen tissue is more suitable for proteomics analysis than chemically cross-linked material. However, archival FFPE clinical samples represent a rich source for proteomics investigations, and these are often linked with extensive follow-up patient information that report disease outcomes. Independent studies demonstrate that frozen samples are equivalent to chemically fixed samples after rehydration and heat-induced reversal of fixation[36-38]. Obvious advantages of FFPE samples are convenience of handling, storage, and archival follow-up information that often covers decades. Therefore, FFPE material has been used for cancer research by many groups[38-44].

Proteomics studies can provide information about general protein expression patterns, expression of individual proteins, post-translational protein modification, and protein-protein interactions. Proteins can be analyzed by electrophoresis, chromatography, visualization, and mass spectrometry. The development of advanced protein separation systems such as high-resolution chromatography and high-sensitivity mass spectrometry have facilitated development of proteomics technologies[45]. Proteomics studies using mass spectrometry techniques are discovering important data that can be used to predict therapy responses, particularly studies using specialized protein separation techniques, matrix-assisted laser desorption-ionization (MALDI), and time-of-flight mass spectrometry (TOF-MS)[46,47]. Mass spectrometry-based proteomics is generally performed on fresh/frozen tissues. Mass spectrometry of FFPE tissues requires proteolytic digestion of the samples to generate peptides that can be analyzed by liquid chromatography–mass spectrometry (LC-MS). Applications of LC-MS for analysis of FFPE samples have recently been reviewed by Steiner *et al*[48]. These identified protein profiles can be used for analysis of useful biomarkers for gastric cancer. A recently published workflow for analysis of FFPE samples of colon adenomas demonstrated that it is possible to analyze the proteome from microdissected tissue samples[49]. Recent progress in mass spectrometry techniques may lead to quantitative, reproducible, and highly multiplex proteomics analyses of FFPE samples in the future.

MASS SPECTROMETRY

Mass spectrometry detects and identifies the chemical composition of samples on the basis of their mass-to-charge ratio (m/z) after ionization, and can be used to determine protein molecular weight, structure, and post-translational modifications. In TOF-MS, ionic flight times are measured over a fixed distance and correlated with specific m/z values. The measured output counts the total number of ions at each m/z value. MALDI-TOF analysis is highly sensitive and accurate, even for proteins with molecular weights less than 200 kDa[50]. Coupling mass spectrometry with protein separation methods enables characterization of amino acid sequences and post-translational modification. Mass spectrometry is a powerful tool for proteomics analysis, and has been used to identify biomarkers in cancer proteomes for early diagnosis, to assess disease prognosis, and to predict therapeutic responses[51-53]. Several biomarker studies have been conducted using serum samples. The main obstacle for proteomic analysis of serum is the large variability in protein concentrations that can render identification of the low-abundance proteins of interest extremely challenging. Serum screening may identify non-specific markers associated with systemic responses or secondary processes unrelated to cancer. These can include effects from diet, smoking, alcohol consumption, or other factors that complicate analysis. These serum-related issues are not encountered when performing mass spectrometry analysis of tissue samples.

A novel method has recently been developed for cancer-specific biomarker screening of patient tissue specimens. The MALDI imaging mass spectrometry technology facilitates the application of MALDI mass spectrometry to the analysis of tissue sections (Figure 1)[50,54-59]. In MALDI imaging mass spectrometry, the molecular content identified by mass spectrometry is specifically localized within tissue sections and biopsies.

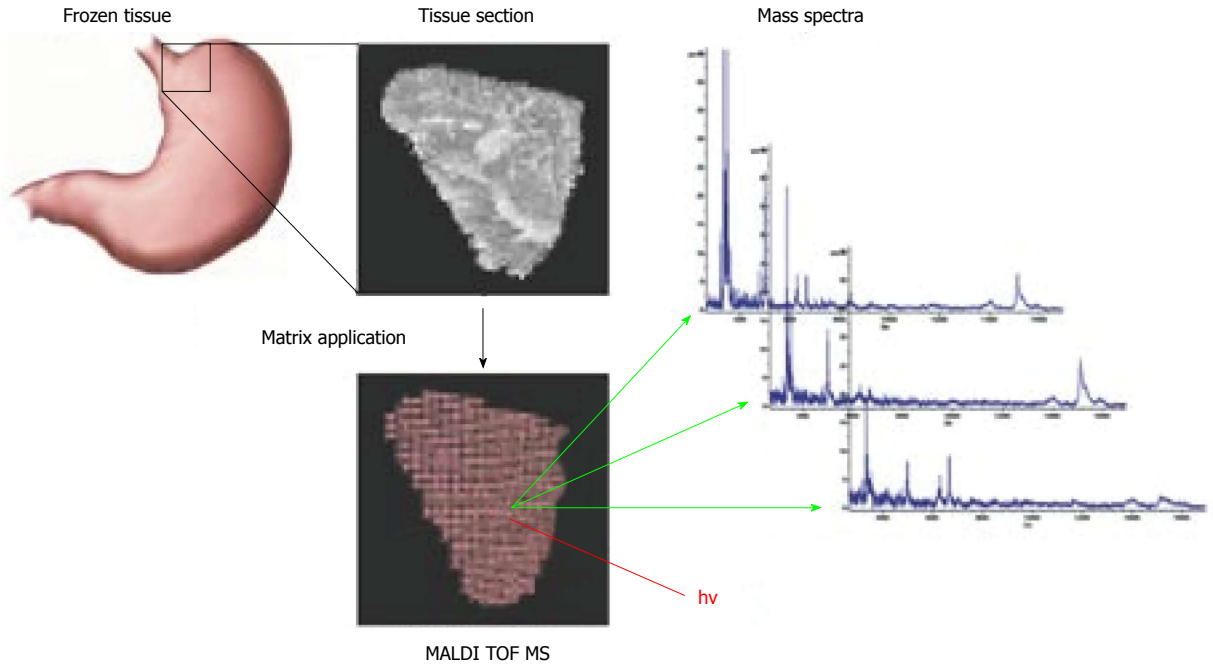

Figure 1 Principle of matrix-assisted laser desorption-ionization imaging mass spectrometry. A section is cut from frozen tissue and prepared for mass spectrometry by coating with matrix solution. Energy for desorption and ionization is supplied by a pulsed laser beam. For each point in the user-defined measurement grid, a mass spectrum is generated by MALDI-TOF MS[79]. Copyright © 2009, Rights Managed by Nature Publishing Group. MALDI: Matrix-assisted laser desorption-ionization; TOF: Time-of-flight; MS: Mass spectrometry.

Peptides, proteins, posttranslational modifications, therapeutic agents and their metabolites, lipids, cell metabolites, molecular tracers, contrast agents, and toxins can be identified and localized[58]. This technique correlates *in situ* molecular patterns with m/z distributions. Tissue sections are scanned and a mass spectrum is acquired for selected regions, which are then subjected to histomorphological analysis and visualization (Figure 1). MALDI imaging has been implemented in several studies to identify differential protein expression profiles for human glioblastoma cancer, non-small-cell lung tumors, and ovarian cancer[54,60,61]. One of the first MALDI Imaging studies in gastric cancer demonstrated that MALDI imaging in combination with hierarchical clustering of the m/z values allows the comprehensive analysis of the *in situ* cancer proteome[62]. This cluster analysis allowed for the classification of complex human tissues and facilitated specific and cancer-related *in situ* biomarker analysis and identification. Also, in the case of gastric cancer, MALDI Imaging could be obtained as a diagnostic tool to identify early-stage tumor. By histology-directed profiling of 63 gastric cancer and 43 healthy endoscopic biopsies, profiles for separating tumor from healthy tissue, and for distinguishing stage Ia from more advanced stages were identified[63]. The results from this study could be of clinical relevance, because stage Ia lesions are potential candidates for endoscopic treatment. For patients with more advanced-stage disease, clinically relevant information is related to improving risk stratification. A recent study analyzed 63 intestinal primary gastric cancer tissues using MALDI imaging[64] and identified 7 tumor-specific proteins that independently correlated with poor survival. A previously unknown protein, CRIP1, was identified and confirmed to be an independent prognostic factor for gastric cancer. A proof-of-principle MALDI imaging study demonstrated that the HER2 status of gastric cancer could be predicted accurately by specific protein patterns (Figure 2)[65]. A recently published MALDI Imaging study demonstrated that the clinical response to neoadjuvant chemotherapy with cisplatin and 5-FU in adenocarcinomas of the gastro-esophageal junction could be correlated to preexisting defects in mitochondria of the patients´ tumor cells[66]. Additionally in this study several mitochondrial proteins were identified which previously have not been recognized in the context of neoadjuvant chemotherapy treatment. In general, because of its practical simplicity and ability to gain reliable information, even from endoscopic biopsy sections, MALDI Imaging might have the potential to complement histopathological evaluation for assisting diagnostic, risk assessment, or response prediction to therapy.

METABOLIC ANALYSIS IN THERAPY RESPONSE PREDICTION

Molecular imaging of tumor metabolism using specific tracers could guide decisions about treatment strategies for cancer patients. Since the discovery regarding glucose metabolism in cancerous tumors by Warburg, there is consensus that malignant cell metabolism is crucial for pathogenesis and progression of cancer[67]. Changes in glucose metabolism are determined using fluorodeoxyglucose (FDG)-positron emission tomography (PET)

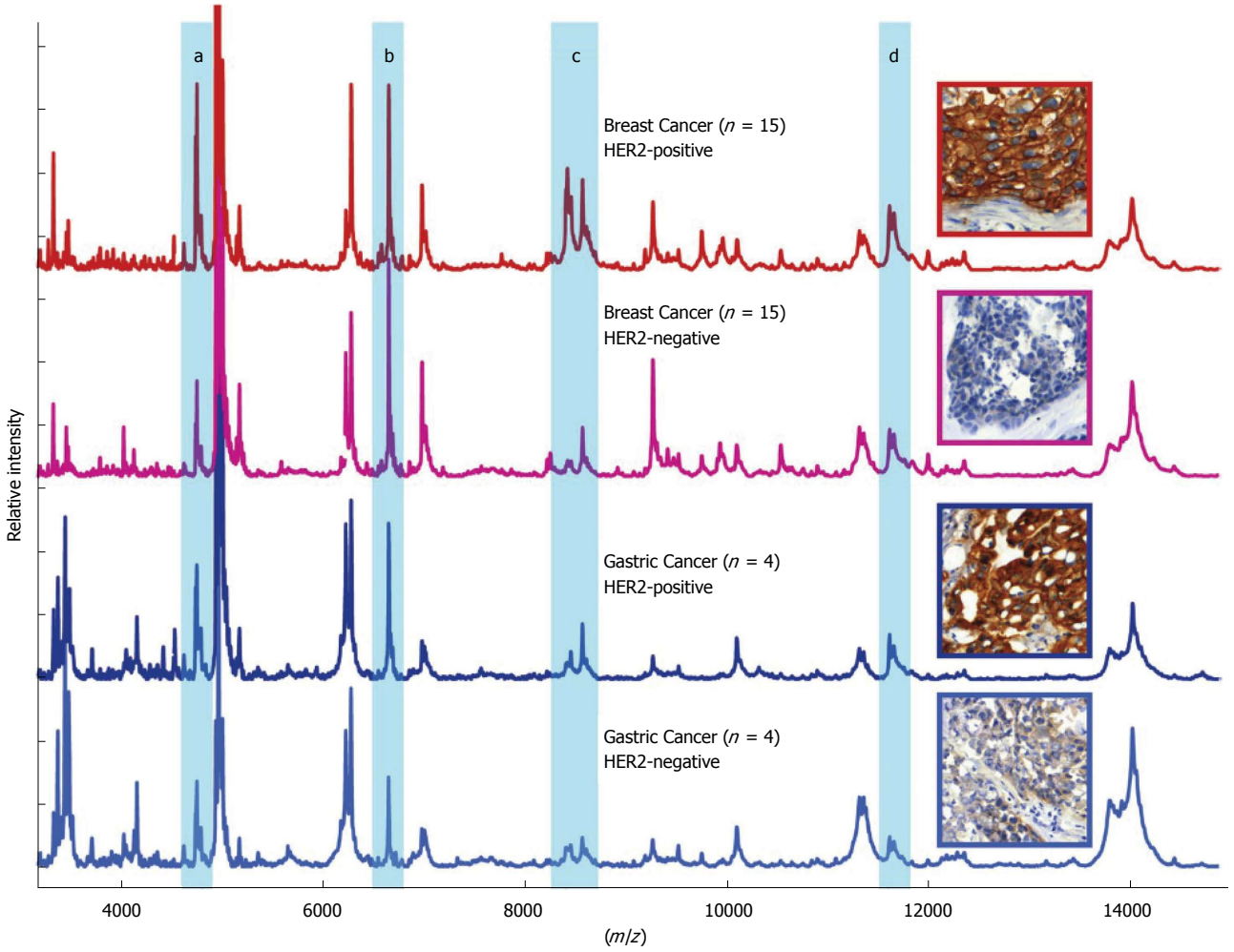

Figure 2 Matrix-assisted laser desorption-ionization imaging mass spectrometric profiles of breast and gastric cancer tissues. Human epidermal growth factor receptor-2 (HER2)-status can be identified on a proteomic level across different cancer types suggesting that HER2 overexpression may constitute a unique molecular event independent of the tumor site[65]. Reprinted with permission from [65]. Copyright © 2010 American Chemical Society.

and positron emission tomography-computed tomography (PET-CT) imaging. These changes can be used for tumor diagnosis. Measurement of tumor FDG uptake using PET can facilitate the assessment of tumor cell metabolic activity *in vivo*. ^{18}F-FDG-PET can be used to measure the response to therapy, tumor metabolism, and patient prognosis. PET imaging can determine the tumor response to treatment during the course of chemotherapy, radiotherapy, or chemoradiotherapy. Changes in ^{18}F-FDG uptake compared with that in pretherapeutic scans can be correlated with histopathological changes and/or survival. During the early course of treatment, tumor metabolic activity is significantly reduced in patients that positively respond to treatment compared with those that do not (Figure 3)[68,69].

Early assessment of treatment response for gastric cancer using FDG-PET is challenging because many primary tumors are not FDG avid[70-74]. If the tumor is FDG avid, prediction of response and prognosis using FDG-PET is feasible for gastric cancer[75]. Ott *et al*[75] established a standard for assessing if a treatment resulted in positive patient responses and improved patient prognoses; after two weeks of chemotherapy, approximately 35% of patients should have reduced FDG uptake compared with that of the pre-treatment FDG uptake. This standard was corroborated by a subsequent study with a larger patient cohort, in which approximately 33% of patients had insufficient FDG to monitor using FDG-PET[76]. Survival data identified three independent prognostic groups, including metabolic responders, metabolic non-responders, and non FDG-avid patients. Herrmann *et al*[77] investigated whether a marker of tumor cell proliferation, ^{18}F-fluorothymidine (FLT), could be used to detect locally advanced gastric cancer. Absolute uptake values for ^{18}F-FLT were lower than those for ^{18}F-FDG, but ^{18}F-FLT-PET exhibited higher sensitivity. Therefore, ^{18}F-FLT-PET may be a useful diagnostic tool for quantifying tumor cell proliferation. A recently published prospective study by Ott *et al*[78] reported that non-FDG-avid gastric tumors can be visualized with the proliferation marker FLT. This can expand the potential of molecular imaging for assessing responses to neoadjuvant therapy.

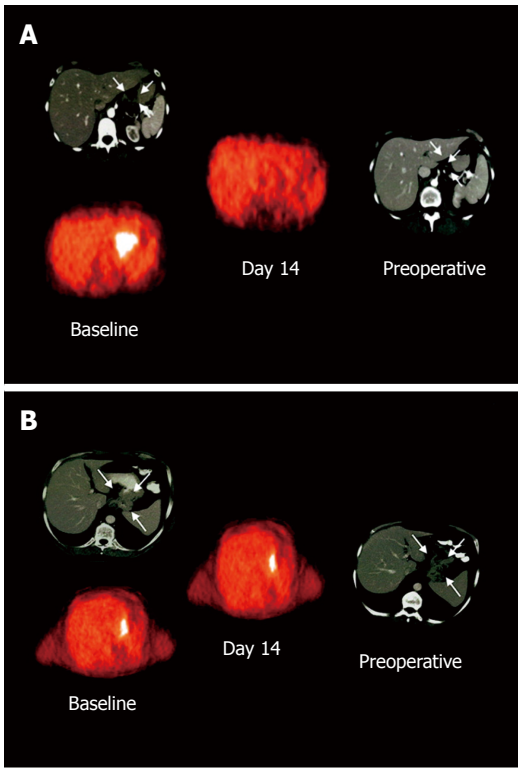

Figure 3 Positron emission tomography with the glucose analog fluorine-18 fluorodeoxyglucose studies in patients with clinically responding and nonresponding tumors[75]. A: In the responding tumor, fluorodeoxyglucose (FDG) uptake decreases to background level 14 d after initiation of chemotherapy; B: In contrast, FDG uptake is almost unchanged for the nonresponding tumor. Copyright © 2003 American Society of Clinical Oncology.

CONCLUSION

Gastric cancer is a biological heterogeneous disease; therefore, no single medical treatment is the best option for all types of gastric cancer. Even for the treatment with classical cytotoxic therapies, different sensitivities to specific agents probably exist in different gastric cancer subtypes. Current treatment protocols for gastric cancer are based primarily on clinical data and histological features. Therefore, new targeted agents are needed beside the already established anti-HER2 directed treatment with trastuzumab. Several potential biomarkers have been identified that can predict treatment responses, and can be used to customize therapeutic approaches with respect to specific tumor parameters.

With a better proteomic and metabolic characterization of gastric cancer, new and improved treatment options may become available in future. To identify patients that could benefit most from novel treatments, it is important to assess early patient responses. Molecular and proteomics analyses have identified a number of proteins that might be useful for predicting therapeutic responses. Most of these biomarkers require further validation in larger studies. Molecular imaging could facilitate early assessment of patient responses to treatments. MALDI imaging is a novel approach to identify new biomarkers. The combination of different approaches is necessary to identify new, cancer-specific, and patient-specific biomarkers that could be used to establish personalized treatment strategies and clinical management of patients with gastric cancer.

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