Impact of Genetic Targets on Cancer Therapy in Esophagogastric Cancer

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Abstract The treatment of esophagogastric cancer has been rapidly evolving in the past decade. New cytotoxic drugs and targeted agents have been integrated in the therapeutic paradigm. To better understand the tumor biology and to better utilize targeted agents, genetic alterations in esophagogastric cancer have been actively explored. For example, Her2/Neu amplification and expression were observed in gastric and gastroesophageal (GE) junction cancers. Combination of trastuzumab with cytotoxic chemotherapy has demonstrated a survival advantage in patients with Her2/Neu positive gastric cancer. However, the prognosis of advanced esophagogastric cancer remains poor. This is largely attributed to the tumor heterogeneity and poorly understood tumor biology. This article provides a summary of potential genetic targets and the role of novel targeted agents in the treatment of esophagogastric cancer.

Keywords Esphageal cancer • Gastric cancer • EGFR • Her2/Neu • Angiogenesis • Cetuximab • Trastuzumab • Bevacizumab

Introduction

Esophagogastric cancer is a heterogeneous disease. Histologically, it includes squamous cell carcinoma and adenocarcinoma. Traditionally, these two histologies are treated very similarly. Most clinical protocols include both histologies. However, the etiology and prognosis are very different between squamous cell carcinoma and

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adenocarcinoma. Anatomically, adenocarcinoma is usually located at the distal one-third of the esophagus and frequently involves GE junction with extending into the stomach. The disease is mainly caused by acid reflux, Barrett's esophagus, high fat diet and obesity [1–3]. Squamous cell carcinoma, however, involves the upper third of the esophagus and is usually related to tobacco and alcohol consumption. Epidemiologically, adenocarcinoma is more prevalent in Western world [4, 5]. The incidence of squamous cell carcinoma is declining while the incidence of adenocarcinoma is rising [6]. Thus, these two histologies should be considered two distinct disease entities. With new targeted agents available on the market, better understanding the molecular pathologenesis of squamous cell carcinoma and adenocarcinoma of the esophagus is at urge. Incorporating targeted agents to the therapeutic paradigm would potentially allow us to have better patient selection and to tailor therapy based on tumor genetics.

Targeting Epidermal Growth Factor Receptor (EGFR) Family Pathway

Epidermal growth factor receptor family consists of EGFR (ErbB1), Her2/Neu (ErbB2), ErbB3 and ErbB4 [7–9]. Upon binding of the ligand, the receptor undergoes dimerization and activation of its intrinsic tyrosine kinase activity resulting in initiation of down stream signaling cascade [10]. The activation of EGFRs ultimately leads to cell proliferation, differentiation or even transformation. Targeting EGFR with monoclonal antibody or small typrosine kinase inhibitors has been demonstrated clinically efficacious in solid tumors such as non-small cell lung cancer, head and neck cancer and colorectal cancers either alone or in combination with chemotherapy or radiotherapy. In head and neck cancers, cetuximab potentiates the effect of radiotherapy with a significant survival benefit [11]. More importantly, targeted agents allow us better understanding the tumor biology and tailoring therapy to individual patient. For example, non-small cell lung cancer harboring specific EGFR mutations are particularly sensitive to gefitinib therapy [12]. Patients with such mutations usually demonstrated a higher response rate and longer survival. Like conventional chemotherapy, drug resistance remains a problem. Such resistance to gefitinib was identified [13].

Expression or overexpression of EGFR family members has been described in esophageal and gastric cancers. Overexpression of EGFR has been detected in majority of esophagogastric cancers ranging from 18 to 90% [14–16]. This overexpression is generally associated with a more clinically aggressive disease and a poor prognosis [17–20]. In addition, the EGFR overexpression may cause treatment resistance such as radio resistance in esophageal cancer [21]. Cetuximab, a chimeric monoclonal antibody against the extracellular domain of EGFR, was evaluated in esophageal and gastric cancers. Single agent cetuximab has minimal activity in patients with metastatic esophageal and gastric cancers [22, 23]. However, addition cetuximab

to chemotherapy may augment the efficacy of cytotoxic drugs. Pinto and colleagues investigated the activity of cetuximab in combination with cisplatin and decetaxel in patients with advanced GE junction or gastric cancers for first line therapy [24]. The authors showed that a 41.2% response rate was achieved. The median overall survival was 9 months and median time to progression was 5 months. Comparing to the historically data, the addition of cetuximab may have a small improvement of response rate but not overall survival.

Several studies have assessed cetuximab in the preoperative chemoradiation setting and demonstrated feasibility and safety. Ruhstaller et al. published a phase IB/II study (SAKK75/06) using cetuximab with chemotherapy and chemoradiation in both squamous cell carcinoma and adenocarcinoma of the esophagus [25]. A total of 28 patients entered the study. All patients were treated with 2 cycles of induction chemotherapy with cisplatin and docetaxel. Subsequently, patients were allocated into two cohorts. One cohort received weekly cisplatin and cetuximab with radiation while the other cohort received weekly decetaxel, cisplatin and cetuximab with radiation. R0 resection was performed in 25 patients. The study demonstrated that addition of cetuximab with chemoradiation is feasible. An impressive 86% (95% CI, 57–98%) pathological complete response (pCR) or near complete response were achieved in patients with adenocarcinoma. A 64% (95% CI, 31–89%) pCR or near pCR were observed in squamous cell carcinoma. De Vita and colleagues reported another approach using oxaliplatin and cetuximab in a single arm phase II study [26]. Patients in the study were treated with induction chemotherapy of FOLFOX4 and cetuximab followed by cetuximab concurrent with radiotherapy (50 Gy). A 27% pCR was reached. RTOG phase III study is under the way to evaluate cisplatin, paclitaxel and radiation with or without cetuximab in adenocarinoma or squamous cell carcinoma of the esophagus (www.clinicaltrials.gov). The role of cetuximab in the preoperative chemoradiation will be further delineated.

Erlotinib is a small molecule inhibiting EGFR function. Ilson et al. reported a phase II study of erlotinib in patients with advanced previously treated adenocarcinoma and squamous cell carcinoma of the esophagus [27]. Eighty percent of patients in the study had tumors overexpressing EGFR. The partial response rate is low (8%). Time-to-progression was longer in squamous cell histology than that of adenocarcinoma. Thus, it is worthwhile to further evaluate this agent in squamous histology.

Her2/neu is a receptor tyrosine kinase that belongs to EGFR family. Her2/neu gene amplification (FISH) and protein overexpression are found in approximately 20–25% of breast cancers and are predictive of poor prognosis [28]. Trastuzumab, a humanized IgG1 monoclonal antibody to Her2/neu, showed clinical activity when used as a single agent or in combination with chemotherapy in Her2/neu positive breast cancers. Similar to breast cancer, roughly 19–22% of gastric or GE junction cancers have amplification and overexpression of Her2/neu [29–31]. Unlike in breast cancers, Her2/neu as a prognostic marker is less consistent. For example, some studies showed that the expression and amplification were frequently associated with nodal metastasis, advanced stages, distant metastasis and intestinal histology [31, 32]. On the other hand, Shah and co-workers found that Her2/neu expression is

a favorable prognostic factor in an univariate analysis but not in the multivariate analysis [33]. Hence, Her2/neu is not an independent prognostic biomarker.

ToGA study is by far the largest phase III study evaluating the activity of trastuzumab in advanced gastric and GE junction cancer [30]. A total of 3,803 patients were screened for the study. The study tested 3,665 patients for Her2/neu expression by both fluorescence in-situ hybridization (FISH) and immunohistochemistry staining (IHC). Eight hundred and ten patients were positive for either FISH or IHC. Finally, a total of 594 patients were randomized 1:1 to receive either chemotherapy (cisplatin, 5FU) alone or trastuzumab with chemotherapy. The median overall survival (OS) was 13.8 months in the trastuzumab arm and 11.1 months in the chemotherapy arm (HR 0.74; 95% CI 0.6–0.91; p=0.0046). Progression-free survival was also superior in the trastuzumab arm (6.7 months vs. 5.5 months; HR 0.71; 95% CI 0.59–0.85; p=0.0002). Further analysis showed that nearly all patients with FISH positive/IHC 1+ above tumors benefited from trastuzumab. However, a small proportion of patients that are FISH positive but IHC negative had minimal benefit from trastuzumab (HR 0.92; 95% CI 0.48–1.76). About 15 patients whose tumors were IHC 3+ but FISH negative had no benefit from trastuzumab. The discrepancies of these results could be due to testing error or bias. In addition, these two groups contained very small number of patients that did not have sufficient statistical power to draw further conclusions. The ToGA study is the first phase III study to incorporate a targeted agent in treating gastric and esophageal cancers. Assessing Her2/neu expression in patients with metastatic gastric or GE junction cancers became a new standard practice. Transtuzumab in combination with different chemotherapy regimens such as oxaliplatin and capecitabine (CAPOX) is also under investigation. Currently, RTOG has launched a phase III study using carboplatin, paclitaxel with or without trastuzumab concurrent with radiotherapy in the preoperative setting (www.clinicatrial.gov). Disease-free survival and pCR will be assessed. The results of these clinical studies are awaited.

Lapatinib, a small molecular inhibitor to Her2/neu and EGFR, has demonstrated good clinical activity in breast cancer. Recently, Iqbal et al. published a Southwest Oncology Group (SWOG) study using lapatinib as a single agent in unselected metastatic gastric cancer patients [34]. The study enrolled a total of 47 patients. A 11% partial response rate and 23% stable disease were reported. Combination of lapatinib with various chemotherapies is being explored.

Targeting Angiogenesis Pathway

Anti-angiogenesis therapy has been proven to be effective in many solid tumors. Bevacizumab, a humanized monoclonal antibody against vascular endothelial growth factor (VEGF), has been used to treat many solid tumors including colorectal cancer, breast cancer, renal cell carcinoma and hepatocellular carcinoma [35–39]. In colorectal cancer, addition of bevacizumab to chemotherapy results in a better response rate, progression-free survival and overall survival [36].

Like in other solid tumors, a higher level of VEGF was also found in the surgical specimen of esophagogastric cancer specimens [40–43]. The higher level of VEGF expression is associated with a more aggressive clinical course, nodal metastasis, higher TNM staging and poor prognosis. Based on these preclinical findings and clinical success in other tumors, bevacizumab was first evaluated in several small single arm studies. Shah and co-workers showed that combination of bevaizumab, irinotecan and cisplatin resulted in 65% response rate and 12.3 overall median survival rate [44]. Recently, Shah and colleagues reported another single arm phase II study using bevacizumab with modified DCF (decetaxel, cisplatin and 5FU) [45]. The study demonstrated a median overall survival of 16.8 months. During the subgroup analysis, a 85% response rate was achieved in GE junction and proximal gastric cancers. A 56% response rate was reached for distal and body gastric cancers. However, diffuse type gastric cancer has much lower response rate (38%). Despite these encouraging data, the efficacy of bevacizumab was disappointing in the phase III study. AVAGAST (avastin for advanced gastric cancer) study is a double-blind, randomized, placebo-controlled phase III study of cisplatin and capecitabine with or without bevacizumab as first line therapy in advanced gastric or GE junction cancers [46]. A total of 774 patients were enrolled in the study. The study subjects were randomized to chemotherapy with placebo or chemotherapy with bevacizumab. The overall response rate was significantly higher with the addition of bevacizumab (38% vs. 29.5%, $p=0.0121$). The progression-free survival is significantly longer in the bevacizumab arm (6.7 months in bevacizumab arm vs. 5.3 months in the placebo arm; $p=0.0037$). However, there was no difference in overall survival between the two study arms (HR = 0.87, $p=0.1002$).

Bevacizumab was also evaluated in preoperative chemoradiation to explore the feasibility, safety and preliminary efficacy. Ilson et al. presented their study of bevacizumab, irinotecan and cisplatin with radiotherapy in localized gastroesophageal cancer [47]. Preliminary analysis showed that it is safe and feasible. Delayed wound-healing was not observed. Other approaches such as using bevacizumab in the perioperative chemotherapy are under evaluation. MAGIC study offered preioperative chemotherapy ECF in operable gastric and GE junction cancer. The study demonstrated survival benefit for this approach [48]. Because of the success of MAGIC trial, integrating bevacizumab in perioperative chemotherapy is currently being evaluated in a phase III study (ST03). Preliminary data showed reasonable safety without increasing surgical risks significantly [49]. Other anti-angiogenesis agents (ramucuryumab and apatinib) in the second or third line therapy are under the way (www.clinicaltrials.gov). Multi-tyrosine kinase inhibitors with anti-angiogenic effects including sorafenib and sunitinib are being actively evaluated [50–52]. To better select patients and to stratify patients in future clinical trials, several biomarkers have been explored to predict the efficacy of bevacizumab. These markers include VEGF level, circulating endothelial progenitor cells or circulating endothelial cells [38, 53–58]. However, the results are rather disappointing. More researches are needed to better understand the role angiogenesis in tumor development and progression. Furthermore, the mechanism of these anti-angiogenic agents needs to be further defined.

Other Potential Targets

In addition to these two major pathways, other potential molecular targets were assessed. Cyclooxygenase-2 (COX-2) pathway has been the center of attention in cancer development and progression several years ago. It has been shown that COX-2 inhibitors can reduce the risks for esophageal, gastric and colorectal cancers [59–61]. COX-2 is frequently upper regulated in gastrointestinal tumors [62, 63]. Through cross talk with other signaling pathways, COX-2 has been shown to activate NFκB and EGFR or to inactive tumor suppressor gene, APC [64–67]. Celecoxib, a COX-2 inhibitor, has been assessed with chemotherapy in both advanced and localized disease [68, 69]. However, it is difficult to obtain conclusive results in these small studies. Because of the recently recognized cardiac complications of COX-2 inhibitors, further utilization of celecoxib in gastroesophageal cancers and other tumor types has been placed on hold.

PI3 kinase/AKT/mTOR pathways are important in regulating cell proliferation and transformation. These pathways frequently cross talk with other receptor tyrosine kinase mediated signal cascade. Hilebrandt and co-workers from M.D. Anderson examined the genetic variations of PI3 kinase/PTEN/AKT/mTOR pathway in 210 patients with respectable esophageal cancer [70]. The authors demonstrated that certain single nucleotide polymorphism (SNP) is associated with higher risk of recurrence and resistance to chemoradiation. For example, patients with SNP AKT1:rs892119 variation has much poor prognosis comparing to wild type AKT (median survival of 12 months for one or two AKT variant; median survival of 42 months for the wild type). The poor survival for the AKT1:rs892119 was not affected by different chemotherapy agents such as 5FU or cisplatin. Clinically, everolimus, a mTOR inhibitor, is being actively assessed in advanced gastric cancer [71].

Perhaps, the most intriguing target is "cancer stem cells" that was first explored in hematological malignancies. The esophageal stem cells have similar characteristics to other cancer stem cells. They are slow growing, self-renewal and a high proliferative potential triggered by wound healing process [72]. Characterization of esophageal stem cells, however, is rather inconsistent in the literature. Kalabis et al. described that the esophageal basal epithelial cells with self-renewal properties are $CD34^{+}$ [73]. Other markers including CD133, adenosine triphosphate-binding cassette superfamily G 2 (ABCG2), CD44 and Musashi-1 have been described in the literature [74–76]. It is not clear what the clinical implications are when these markers are detected in clinical specimens or cell lines. Although there is a great interest to target stem cells in cancer therapy, such approach is largely hindered by poorly defined biology of these cells.

Conclusions

Esophagogastric cancer remains a public health problem worldwide. Over the past decade, the treatment of esophagogastric cancer has been evolving rapidly. These include surgical techniques and development of new cytotoxic agents as well as

targeted therapy. Recent introduction of trastuzumab in advanced disease brought a new era of personalized medicine in managing esophagogastric cancers. However, more researches are required to better understand the tumor biology and the mechanism of action of targeted agents. Only through these mechanistic explorations, potential predictive and prognostic biomarkers could be identified. These biomarkers will allow us to have better patient selection, better stratification and better trial designs.

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