Brain Metastases of Gastro-oesophageal Cancer: Evaluation of Molecules with Relevance for Targeted Therapies

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Abstract. Background: Brain metastases (BM) of gastro-oesophageal cancer are exceedingly rare and only limited data exist on their pathobiology. Materials and Methods: We identified tissue samples of BM of gastro-oesophageal cancer and analyzed the expression of human epidermal growth factor receptor-2 (HER2), phosphorylated signal transducer and activator of transcription-3 (pSTAT3), epithelial growth factor receptor (EGFR), V600E point mutation of the v-raf murine sarcoma viral oncogene homolog-B1 (BRAF V600E), cluster of differentiation molecule-34 (CD34), hypoxia inducible factor-1α (HIF 1-α) and Ki-67 by immunohistochemical methods. Results: Our series comprised of twenty adenocarcinomas and one oesophageal squamous cell carcinoma. Three (14%), 7 (33%), 9 (43%), 18 (86%) and 0 BM specimens were scored positively for HER2, EGFR, pSTAT3, HIF1-α and BRAF V600E expression. The median Ki-67 index was 59%. The microvascular density was moderate-to-high and active intratumoral microvascular sprouting was evident in 20/21 (95%) of BMs. The HER2 and EGFR expression status were consistent between primary tumors and BM in all three assessable cases. HIF1-α and pSTAT3 expression were significantly higher in HER2-positive cases. Conclusion: Therapeutic use of agents targeting HER2, pSTAT3, EGFR and angiogenesis may be feasible for selected BM of gastro-oesophageal cancer. HER2 positivity does not seem to predispose to brain colonization in gastro-oesophageal cancer.

Gastro-oesophageal cancer is a major cause of cancer-related mortality and is the second leading cause of death worldwide. The risk of local and distant recurrence is high, even for resectable disease at initial presentation. In approximately two-thirds of patients, metastatic disease is present at first diagnosis, and for such patients, palliative chemotherapy results in 5-year survival rates of below 15% (1, 2).

Brain metastases (BM) are rare in patients with gastro-oesophageal cancer, affecting fewer than 2% of patients (3). Since the incidence of gastro-oesophageal junction (GEJ) cancer has seen a significant rise within the last decades, the total number of patients with this disease and BM is also expected to increase. BM are generally associated with high morbidity, high mortality and poor prognosis. Current treatment options are mainly based on local therapy approaches such as neurosurgery, radiosurgery and radiotherapy and symptomatic therapy with steroids (4-7). For some tumor types and molecular tumor sub-types, targeted agents have recently shown favourable efficacy and led to shrinking of BM, e.g. the v-Raf murine sarcoma viral oncogene homolog-B1 (BRAF) inhibitors vemurafenib and dabrafenib in BRAF V600E-mutated melanoma, the antibody against cytotoxic T-lymphocyte-associated antigen-4 (CTLA4) ipilimumab in melanoma, epithelial growth factor receptor (EGFR) inhibitors in non-small cell lung cancer, and human epidermal growth factor receptor (HER2) tyrosine kinase inhibitor lapatinib in HER2-overexpressing breast cancer (8).

However, owing to the rarity of the disease, only very limited data on the pathobiology and particularly the expression of potential targets for novel drug compounds in BM of gastro-oesophageal carcinoma are available. We undertook the present study in order to analyze the expression of molecules relevant for targeted therapies, as well as angiogenic patterns and tumor cell proliferation, in a relatively large series of 21 cases of BM of gastro-oesophageal cancer.

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Materials and Methods

Patients. The bio-banks of the Institute of Neurology (Neuropathology), Medical University of Vienna, and the Department of Neuropathology, University of Heidelberg, were searched from 1994-2012 for BM originating from gastro-oesophageal cancer. Cranial bone, dural or spinal metastases were not included in this study. If available, tissue specimens of corresponding primary tumors were also retrieved. The study was approved by the local Ethics Committee.

Methods. Immunohistochemistry and in situ hybridization were performed on 1 to 3-µm-thick slides of the paraffin-embedded specimens fixed in 4% buffered formalin.

Expression of HER2 protein, tyrosine-705 phosphorylated signal transducer and activator of transcription-3 (pSTAT3), EGFR, cluster of differentiation molecule-34 (CD34), BRAFV600E, hypoxia-inducible factor-1 (HIF1)-α and Ki-67 was detected applying the antibodies shown in Table I. A Dako (Dako, Glostrup, Denmark) autostainer was used for immunohistochemistry in the case of Ki-67 antibodies shown in Table I. A Dako (Dako, Glostrup, Denmark) autostainer was used for immunohistochemistry in the case of Ki-67 antibodies shown in Table I. A Dako (Dako, Glostrup, Denmark) autostainer was used for immunohistochemistry in the case of Ki-67 antibodies shown in Table I. A Dako (Dako, Glostrup, Denmark) autostainer was used for immunohistochemistry in the case of Ki-67 antibodies shown in Table I. A Dako (Dako, Glostrup, Denmark) autostainer was used for immunohistochemistry in the case of Ki-67 antibodies shown in Table I. A Dako (Dako, Glostrup, Denmark) autostainer was used for immunohistochemistry in the case of Ki-67 antibodies shown in Table I. A Dako (Dako, Glostrup, Denmark) autostainer was used for immunohistochemistry in the case of Ki-67 antibodies shown in Table I. A Dako (Dako, Glostrup, Denmark) autostainer was used for immunohistochemistry in the case of Ki-67 antibodies shown in Table I. A Dako (Dako, Glostrup, Denmark) autostainer was used for immunohistochemistry in the case of Ki-67 antibodies shown in Table I. A Dako (Dako, Glostrup, Denmark) autostainer was used for immunohistochemistry in the case of Ki-67 antibodies shown in Table I. A Dako (Dako, Glostrup, Denmark) autostainer was used for immunohistochemistry in the case of Ki-67 antibodies shown in Table I. A Dako (Dako, Glostrup, Denmark) autostainer was used for immunohistochemistry in the case of Ki-67 antibodies shown in Table I. A Dako (Dako, Glostrup, Denmark) autostainer was used for immunohistochemistry in the case of Ki-67 antibodies shown in Table I. A Dako (Dak...
as 3+ (Table II, Figure 1), and one case as 2+. Subsequent in situ hybridization in the HER2 2+ case revealed a trisomy of chromosome 7, but no gene amplification was found, so it was scored as HER2-negative. Of one of the three HER2-positive BM cases, a tissue specimen of the primary tumor was available, which was also scored as HER2 3+ at immunohistochemistry. The two other primary tumors available were negative for HER2 expression, as were their corresponding BM.

A total of 7/21 (33%) BM were scored as being positive for EGFR expression. Primary tumor specimens were available from three EGFR-negative BM cases, which also showed no EGFR expression.

pSTAT3 expression was observed in 9/21 (42.9%) patients and HIF1-α expression in 18/21 (86%) samples (Table I). None of the cases showed expression of the BRAF V600E protein. The median MIB-1 index was 59% (range 2-89%).

Figure 1. Examples of immunostaining results for brain metastases of gastro-oesophageal cancer. A: Strong (3+) anti-human epidermal growth factor receptor-2 (HER2) immunostaining; B: prominent anti-epithelial growth factor receptor (EGFR) immunostaining; C: prominent nuclear expression of phosphorylated signal transducer and activator of transcription-3 (pSTAT3); D: anti-Ki67 immunostaining showing labeling of a high fraction of tumor cell nuclei; E: prominent nuclear expression of hypoxia induced factor-1α (HIF1-α); F: marked microvascular sprouting with microvascular clustering, proliferation and activated endothelial cells.
Among the BM specimens, six showed high, 13 cases moderate and two cases low MVD. Silent tumor microvessels were found in all 21 BM cases and in 20/21 (95%) BMs, intratumoral microvascular sprouting was evident. Correlation of protein expression. In HER2-positive cases, the HIF1-α score was significantly higher (median=90, range=0-110) than in HER2-negative ones (median 30, range 0-90) \( (p=0.024, \text{ Fisher's exact test}) \). In addition, the pSTAT3 score was higher in HER2-positive cases (median=30, range=5-40 vs. median=0, range=0-40; \( p=0.047, \text{ Fisher's exact test} \)). Furthermore, a significant correlation between HIF1-α and pSTAT3 scores \( (p=0.028, \text{ Spearman's coefficient of correlation 0.48}) \) was observed.

Discussion

In the present study, we investigated HER2 status and expression of pSTAT3, EGFR, BRAF V600E, CD34, HIF1-α and Ki-67 in BM of gastro-oesophageal cancer. The tyrosine kinase receptor HER2 is overexpressed in about 15% of gastro-oesophageal carcinomas and is an important novel drug target for this tumor type. The antibody against HER2, trastuzumab has shown clinically meaningful activity in HER2-positive advanced gastric and GEJ cancer in a recent phase III trial (9). Interestingly, in breast cancer, HER2 expression is associated with an increased incidence of BM. However, it is unclear whether HER2 expression itself or an effect of HER2-targeted therapy with trastuzumab leads to the increased frequency of central nervous system (CNS) involvement. Trastuzumab does not cross the blood-brain barrier due to its high molecular weight and exiled of HER2-positive tumor cells into the ‘sanctuary’ of the brain has been suggested as a potential reason for the brain-tropism of HER2-positive disease (9). We found HER2 positivity in only three cases of our series of 21 patients with gastro-oesophageal cancer who developed BM without having been treated with anti-HER2 therapy, which corresponds to a similar rate of HER2-positive cases described for primary tumors. However, we found HER2
status to be consistent in the three available cases with matched primary tumors and BM, as is also evident between primary tumors and lymph node and non-brain visceral metastases (18). Thus, our data provide indirect evidence against brain-tropism of gastro-oesophageal cancer cells conferred by HER2 expression-alone. However, a recent smaller study reported a higher frequency of HER2 overexpression, with 5/9 BM samples of oesophageal cancer showing HER2 3+ positivity (19). A selection bias may explain the differing results between our study and the investigation by Hejle et al., and the characterization of a larger independent series would be of interest to clarify the definite proportion of HER2-expressing gastro-oesophageal cancer BM. In any case, HER2-targeted therapeutics crossing the blood-brain barrier such as lapatinib, or other novel drugs may be a feasible treatment option for selected patients with BM of gastro-oesophageal cancer. Furthermore, future studies will show whether the use of trastuzumab leads to an increase of BM in patients with gastro-oesophageal cancer patients, as has been described for breast cancer (8).

STAT3 is a transcription factor involved in physiological cellular response to cytokines and growth factors. Following activation by phosphorylation, dimerized STAT3 translocates to the cell nucleus and regulates transcription of several genes involved in growth, differentiation and apoptosis including cyclin-D1, B-cell lymphoma/leukemia-2 (BCL2), vascular endothelial growth factor (VEGF), nuclear factor kappa-B, interleukin-6 and metalloproteinases, among others (20). Activation and overexpression of STAT3 is in involved in malignant transformation and has been found in various tumor types such as breast, prostate, gliomas, bladder, ovarian and head and neck cancer (21-23). A recent study by our group documented pSTAT3 expression in approximately 45% of esophageal carcinomas and found overexpression to be associated with an unfavorable prognosis; STAT3 signaling also correlates with epigenetic aberrance during gastrocarcinogenesis (24, 25). In line with our previous findings, pSTAT3 overexpression was observed in 43% of BM in our current series of BM from gastro-oesophageal cancer (24). The similar rate of pSTAT3 overexpression between primary tumors and BM seems to suggest that this factor is not per se involved in brain colonization. However, as discussed previously, it may serve as a therapeutic target as some agents such as sunitinib, BP-1-102, STA-21 and S31-201 have shown significant antineoplastic activity via STAT3 inhibition and novel STAT3 inhibitors are currently under development (26-30). In good correlation with our findings in primary esophageal cancer, we found a significant association of pSTAT3 expression with HER2 expression in BM from gastro-oesophageal carcinoma. Our results thus support our previous data suggesting a relevant pathobiological connection between these two molecules and their potential feasibility as targets for multi-targeted drugs (24).

EGFR is overexpressed in many cancer types and has been effectively targeted by monoclonal antibodies (e.g. cetuximab, panitumumab) and tyrosine kinase inhibitors (e.g. gefitinib, erlotinib) in several tumor types including colorectal and lung cancer. EGFR overexpression is found in more than half of patients with gastro-oesophageal cancer and several clinical trials are investigating anti-EGFR drugs for this indication, although it must be noted that some agents such as panitumumab have not shown favorable antitumor activity in oesophageo-gastric cancer (31-33). We found EGFR overexpression in one-third of our BM cases. While most monoclonal antibodies are too large to cross the blood–brain/blood–tumor barrier, small molecules such as tyrosine kinase inhibitors may be effective. Of note, EGFR tyrosine kinase inhibitors have been shown to induce tumor regression in BM of non-small cell lung cancer (NSCLC), particularly in cases with activating EGFR mutations, with erlotinib achieving higher cerebrospinal concentrations and allegedly higher response rates than gefitinib (8). However, while inhibition of EGFR-alone may not be sufficient in halting tumor growth due to redundant pro-neoplastic signaling pathways, novel multi-tyrosine kinase inhibitors targeting several signaling cascades in parallel may show more therapeutic efficacy. In any case, more research is needed to fully understand the role of the EGFR pathway in oesophageo-gastric cancer and to develop effective inhibiting compounds.

We found a positive correlation of HER2 and HIF1-α expression, an observation which is well in keeping with previous findings in breast and ovarian cancer (34, 35). HER2 has in fact been shown to up-regulate HIF1-α expression by downstream signalling via the AKT pathway and may induce angiogenesis, even independently of hypoxia (36, 37). We found evidence for significant angiogenic activity in BM from gastro-esophageal cancer, with moderate-to-high microvascular density and active intravascular sprouting in most cases. Thus, antiangiogenic agents such as bevacizumab and cediranib, for which emerging data from large trials have shown high antitumor and anti-edematous symptomatic efficacy in primary brain tumors, may be a feasible treatment option for patients with BM of gastro-oesophageal cancer (38, 39). Antiangiogenic agents have been shown to be safe for patients with BM (40) and results of a large phase III study have shown evidence of bevacizumab activity in advanced gastric cancer, particularly in patients identified as having high plasma-VEGF levels and tissue neuropilin-1 expression (41).

BRAF V600E mutations are common in some cancer types, such as melanoma, papillary thyroid cancer and hairy cell leukemia and clinically active inhibitors do exist, which are also effective for mutation-bearing BM (42). Among gastrointestinal tumors, BRAF mutations have been detected in a fraction of colorectal carcinoma cases and the
V600E mutation status seems to be associated with metastatic patterns (43). In our series of BM from gastro-esophageal cancer cases, we detected no BRAF V600E aberrations, a finding which is in line with previous studies showing a low frequency of BRAF mutations in oesophago-gastric cancer (44-48). Our data support the notion that BRAF mutations are not a predisposing factor for CNS spread to this tumor type.

In conclusion, our study provides some insight into the pathobiology of BM of gastro-oesophageal carcinoma and may help to define treatment strategies and further research possibilities for this rare cancer manifestation.

Conflicts of Interest

Andreas von Deimling and David Capper declare shared inventorship of BRAF antibody clone VE1. A patent for diagnostic application of VE1 has been applied for. All terms are being managed by the German Cancer Research Center in accordance with its conflict of interest policies. All other authors have no conflicts of interest.

References


