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Original contribution

Heterogenous high-level HER-2 amplification in a small subset of colorectal cancers☆

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Summary HER-2 is the molecular target for antibody-based treatment of breast cancer (trastuzumab). The potential benefit of anti–HER-2 therapy is currently investigated in several other HER-2 amplified cancers. For example, trastuzumab was recently shown to be effective in HER-2 positive gastric cancer. To address the potential applicability of anti–HER-2 therapy in colorectal cancer, tissue microarray sections and colorectal resection specimens of 1851 colorectal cancers were analyzed for HER-2 overexpression and amplification using FDA approved reagents for immunohistochemistry and fluorescence in situ hybridization. HER-2 amplification was seen in 2.5% and HER-2 overexpression in 2.7% of 1439 interpretable colorectal cancers. Amplification was often high level with HER-2 copies ranging from 4 to 60 per tumor cell and was strongly related to protein overexpression. HER-2 amplification and overexpression were unrelated to histological tumor type, tumor localization, grading, pT, pN, pM or survival. As heterogeneity of drug target expression could represent a major drawback for targeted cancer therapy we next studied HER-2 heterogeneity in selected cases. Extensive evaluation of all available large sections from patients with HER-2 positive colorectal cancer revealed heterogenous findings in 3 of 4 cases. In summary, high-level HER-2 amplification occurs in a small fraction of colorectal cancers. Heterogeneity of amplification may limit the utility of anti-HER-2 therapy in some of these tumors and therefore, adequate clinical trials are needed to further evaluate this approach.

1. Introduction

The HER-2/neu protein, a transmembrane tyrosine kinase growth factor receptor is found on normal and

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malignant epithelial cells and is involved in the regulation of cell proliferation and differentiation [1,2]. HER-2 is a strong prognostic factor in patients with breast cancer and has been successfully used as a therapeutic target in this disease [3,4]. In 15% to 20% of breast cancer cases, the HER-2 protein is overexpressed as a consequence of gene amplification [4-6]. Overexpressed HER-2 protein serves as the therapeutic target for Trastuzumab, a humanized monoclonal antibody [7]. Trastuzumab is now used both in a metastatic setting and as an adjuvant therapy of HER-2 positive breast cancer. Several other tumor types can also express increased levels of HER-2, including gastric, lung, urinary bladder, pancreatic, and esophagus cancer [8-11] and evidence accumulates that trastuzumab may also be effective in HER-2 positive tumors other then breast cancer [12-15]. Data from a recent study of trastuzumab in combination with chemotherapy compared with chemotherapy alone in nearly 4000 patients with HER-2 positive advanced gastric cancer (ToGA trial, protocol number: BO18255) showed that adding trastuzumab to standard chemotherapy prolongs survival in advanced gastric cancer by a median of nearly three months to 13.8 months. This international phase III study also showed that trastuzumab reduces the risk of death in patients with HER-2-positive advanced stomach cancer by 26% compared to patients not receiving trastuzumab. Patients with tumors exhibiting high levels of HER-2 experienced even greater benefit from the addition of trastuzumab, their lives were extended to 16 months on average [16]. In colorectal cancer (CRC) patients, HER-2 overexpression has been described in 3 to 47% of the cases [13,17-19]. Some authors have reported an association between HER-2 overexpression and shorter survival [17,20], whereas other authors did not find such a correlation [18]. HER-2 gene amplification was found in about 1% to 3% of colorectal carcinomas [21,22]. Tumors with HER-2 overexpression due to gene amplification might potentially benefit from trastuzumab therapy. One important precondition for the success of trastuzumab in breast cancer is probably the striking homogeneity of amplification and expression within these cancers [23]. This can only be explained by an early development of HER-2 amplification in breast cancer progression. To what extent this remarkable homogeneity of HER-2 amplification also applies to other tumor types is unclear. For example, in bladder cancer, HER-2 amplification in considerably more heterogenous [24]. Heterogeneity of target expression within individual cancers and between primary tumors and metastases could represent a major drawback to targeted cancer therapy. To evaluate frequency and heterogeneity of HER-2 alteration, we analyzed a series of 1851 primary colorectal carcinomas. The US Food and Drug Administration (FDA) approved methods for immunohistochemistry (HercepTestTM; DAKO, Glostrup, Denmark) and fluorescence in situ hybridization (PathVysionTM; Vysis-Abbott, Wiesbaden, Germany) were used.

2. Materials and methods

2.1. Patients and tissue microarray construction

Two different tissue microarray (TMA) with a total of 1800 CRC samples were included in this study. The first TMA (TMA Basel) was manufactured from resection specimens of 1420 CRC patients at the Institute of Pathology of the University Hospital of Basel [25]. Raw survival data were obtained from the responsible physicians for all of the 1420 patients. The median follow up time was 38.4 month (range, 1-144 months). The second TMA (TMA Hamburg) included samples from 380 CRC patients, whose tumor resection specimens were examined at the Institute of Pathology of the University Medical Center Hamburg-Eppendorf. Also for this TMA, raw survival data were available for all of the 380 patients with a median follow up period of 42.8 month (range, 1-180 month). TMA construction was as described [26]. In brief, hematoxylin and eosin (HE)–stained sections were made from each block to define representative tumor regions. One tissue cylinder with a diameter of 0.6 mm was then punched from each tumor "donor" tissue block using a home made semiautomated precision instrument and brought into empty recipient paraffin blocks. Four μm sections of the resulting TMA blocks were transferred to an adhesive coated slide system (Instrumedics Inc, Hackensack, NJ). Consecutive sections were used for fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC). In addition, a total of 51 consecutive patients with CRC who underwent colorectal resection at the University Medical Center Hamburg-Eppendorf from November 1, 2008, to April 31, 2009, were included in this study. These tumors were screened for HER-2 amplification on one large section each. Patient information and clinical data such as age, sex, localization and type of the tumor, pTNM-stage and carcinoma grade were retrieved from clinical and pathological databases (Table 1).

2.2. Heterogeneity analysis

From four HER-2 positive cancers, large tissue sections of all available tissue blocks containing primary or metastatic cancer (9-14 blocks per patient) were examined by IHC and FISH to study HER-2 heterogeneity.

Heterogeneity was defined as less than 100% of tumor cells being positive for HER-2 FISH and/or IHC.

2.3. Fluorescence in situ hybridization

For proteolytic slide pretreatment a commercial kit was utilized (Paraffin pretreatment reagent kit, Vysis-Abbott). SpectrumOrange-labeled HER-2-probes were used together with SpectrumGreen-labeled CEP17 reference probes (PathVysion, Vysis-Abbott) for TMA and large section analysis.