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Original contribution

Fibroblast growth factor receptor 1 gene amplification in gastric adenocarcinoma☆,☆☆

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Summary Gastric adenocarcinomas are associated with a poor prognosis due to the fact that the tumor has often metastasized by the time of diagnosis. Thus, identification of novel therapeutic targets is highly desirable. Here, we examined gene copy number of fibroblast growth factor receptor 1 (*FGFR1*), a potential target for tyrosine kinase inhibitors, and clinicopathologic parameters in a large cohort of gastric adenocarcinomas. We performed fluorescence in situ hybridization analysis of 293 gastric adenocarcinomas using tissue microarrays. Amplification of the *FGFR1* gene is a rare but noticeable event that can be found in 2% (6/293) of cases and was associated with poor 10-year survival (median 15.3 months in *FGFR1*-amplified cases versus 36 months in nonamplified cases, $P = .047$) and a higher rate of distant metastasis ($P = .025$). *FGFR1* appears to represent a potential new therapeutic target in a subset of patients with gastric carcinoma. Identification of gastric cancers harboring *FGFR1* amplification may be important in preselecting patients and/or interpreting clinical studies using tyrosine kinase inhibitors.

1. Introduction

Gastric cancer is the third leading cause of cancer-related death worldwide (723 000 deaths in 2012, 8.8% of total) [1]. According to GLOBOCAN 2012 of the International Agency for Research on Cancer (IARC), 951 000 new cases of gastric cancer were estimated to have occurred in 2012 (6.8% of total cancer burden) [1]. This finding ranks gastric cancer as the fifth most common malignancy in the

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world, showing a relative decrease in frequency overall, mainly reflecting changes in lifestyle and *Helicobacter pylori* eradication [2]. More than 70% of cases (677 000 cases) occur in less-developed regions [1]. Although diagnostic methods, surgical skills, and chemotherapy have improved, the overall prognosis remains poor in advanced stage III/IV and metastatic disease. Therefore, novel therapeutic options are urgently needed.

Human fibroblast growth factors belong to a family of 23 ligands specific to 4 tyrosine kinase receptor proteins: fibroblast growth factor receptor 1 to 4 (FGFR1-4) [3,4]. Fibroblast growth factor signaling is able to promote carcinogenesis by altering angiogenesis, proliferation, migration, and cell survival [4]. This pathway has been observed to be aberrant in several solid organ cancers so far [5]. Recently, *FGFR1* amplification has been observed in squamous cell lung cancer (21%) [6], oral squamous cell carcinoma (17.4%) [7], breast carcinoma (14.5%) [8], ovarian carcinoma (7.8%) [9], bladder carcinoma (3.4%) [10], and pancreatic adenocarcinoma (2.6%) [11]. Small molecule inhibitors have been used to successfully target *FGFR1* in breast carcinoma [12], urothelial carcinoma [13], pancreatic adenocarcinoma [11], and non–small cell lung cancer [14] as well as squamous cell lung cancer [6] in vitro. Previous studies have demonstrated in 4.1% to 5.0% of gastric cancers *FGFR2* gene amplification depending on patient selection and techniques used for detection [15–17], and responses to FGFR inhibitors in vivo using xenograft models of gastric cancer have been reported [16].

In this study, we assessed by fluorescence in situ hybridization (FISH) and high-throughput tissue microarray (TMA) analysis whether, in addition to *FGFR2, FGFR1* is also amplified in a subset of gastric adenocarcinoma.

2. Materials and methods

2.1. Patient cohort

Two hundred ninety-three (293) human tissue samples of patients with gastric adenocarcinoma who underwent partial or total gastrectomy between 1992 and 2011 were retrieved from the archives at the Institute of Pathology, University Hospital Bonn, in accordance with the local ethics committee. Clinical follow-up data were collected by reviewing medical records and were available for 145 patients. The overall survival (OS) was determined from the time of surgery until time of death or end point of analysis.

2.2. Construction of TMAs

Formalin-fixed, paraffin-embedded tissue samples were used for the construction of TMAs as previously described [18]. Three 0.5-mm cores from tumor-containing donor blocks were inserted into the TMA block. Sufficiently fixed paraffin blocks containing different areas of the tumor (intramucosal, lamina propria, and muscularis propria invasion) were used for sampling, and 3 different areas containing dense tumor areas were chosen for punch biopsies. Subsequently to FISH, 2 to 3 cores per case were scored and analyzed for *FGFR1* amplification depending on the number of tumor cells available per core. All interpretable cores were analyzed, individual FISH scores compared, and an average score calculated. Cases with less than 50 tumor nuclei within all 3 cores were omitted from analysis. All *FGFR1*-amplified carcinomas with mixed histology were additionally analyzed for tumor heterogeneity by standard paraffin sections.

Centralized review by I. Gütgemann and M. Schäfer was performed regarding diagnosis, grading, and staging according to the *UICC TNM Classification of Malignant Tumours*, seventh edition [19] (Table 1). Histologic types and grades were determined according to the original Laurén classification [20] and according to the WHO classification [21] (Table 2). Image capture of hematoxylin and eosin–stained slides was performed using a DM5500 B microscope (Leica, Wetzlar, Germany) and Diskus software (Hilgers, Königswinter, Germany).

2.3. Fluorescence in situ hybridization

TMAs were hybridized with the BAC clone RP11-148D21 [6] combined with the commercial probe XCE8 green (MetaSystems, Altlussheim, Germany). The reference probe is located on the centromeric region of chromosome 8, and the target probe is located on the *FGFR1* locus spanning 8p11.23 to 8p11.22.

For TMAs, 3- to 4-μm tissue sections were mounted on silanized slides. Deparaffinization, protease treatment, and washes were performed on the half-automated VP2000 processor system (Abbott Molecular, Wiesbaden, Germany) according to manufacturer's conditions. After pretreatment, the slides were denatured in the presence of 10-μl probe for 5 minutes at 75°C and hybridized at 37°C overnight. Post-hybridization saline sodium citrate washes were performed at 72°C, and the slides were stained with DAPI before analysis.

Images were taken with a fluorescence microscope (Zeiss, Jena, Germany) equipped with appropriate filters and the FISH imaging and capturing software Isis V 5.4 (MetaSystems).

2.3.1. Scoring

FISH signals were counted in at least 50 cells per TMA core. Amplification was defined as previously described by Weiss et al [6]. When 2 normal centromeric signals (XCE8) were detected, 3 to 8 *FGFR1* probe signals (FGFR1/XCE8 average signal ratio between 1.5 and 4) indicated low-level amplification and more than 8 signals indicated high-level amplification, as previously described [6,11,22].

2.4. Statistical analysis

For comparison of amplification status with tumor stage, nodal status, resection status, and grade,